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Nutraceutical Properties of Herbal Infusions from Six Native Plants of Argentine Patagonia

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Abstract

Six native plants of South America traditionally consumed in the Patagonian region (southern Argentina and Chile), namely: *Adesmia boronioides* Hook. *f.*, *Apium australe* Thouars, *Buddleja globosa* Hope, *Drimys andina* (Reiche) R. Rodr. & Quezada, *Dysphania multifida* L. and *Solidago chilensis* Meyen were investigated to determine the nutraceutical properties of infusions of their aerial parts. The infusions were characterized in terms of their antioxidant activity, phenolic and flavonoid content, profile of phenolic compounds, general toxicity and cytotoxicity on two different human cell lines: T84 (derived from colon cancer) and HTR8/SVneo (not derived from cancer). Twenty-nine compounds, mainly phenolic acids and flavonoids, were identified. This is the first analysis of phenolic compounds in infusions from native plants of Patagonia. *D. andina*, *B. globosa* and *S. chilensis* showed high levels of antioxidants, even higher than those of Green Tea. The content of phenolic compounds correlated significantly with the antioxidant activity of the samples analyzed. The toxicity test indicated that the use of *A. australe*, *B. globosa* and *D. multifida* seems safe, but a moderate consumption is suggested for *A. boronioides*, *D. andina* and *S. chilensis* until more exhaustive and long-term results are available. Moreover, *A. boronioides* and *S. chilensis* showed anticancer potential due to their antiproliferative activity on human cancer cell lines.

Keywords Antioxidant activity · Antiproliferative activity · Infusions · Native plants · Argentine Patagonia · Phenolic compounds

Abbreviations

ATCC	American Type Culture Collection
BCB	β-carotene-linoleic acid method
DPPH	2,2′-diphenyl-1-picrylhydrazyl
EC50	Efficient concentration 50
GAE	Gallic acid equivalent
HTR8/SVneo	Cancer cell line from placental tissue
LC50	Lethal concentration 50
LC-DAD-MS	Liquid chromatography with diode array detection with tandem mass spectrometry
MTT	Methylthiazolyldiphenyl-tetrazolium bromide
QE	Quercetin equivalent
T84	Cancer cell line from colon
TPC	Total phenolic compound content
TF	Total flavonoid content
VCEAC	Vitamin C equivalent antioxidant capacity

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Introduction

The term nutraceutical is widely used to define a food or parts of a food, which, in addition to their basic nutritional value, provide health benefits, including the prevention and treatment of diseases [1]. Therefore, nutraceuticals have been considered the link between nutrition and medicine. Herbal plants infusions are considered to have

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nutraceutical properties due to their content of phenolic compounds. At present, there is a broad consensus in considering that the antioxidant activity, the content of phenolic compounds and the antiproliferative activity on cancer cells are nutraceutical properties [2]. South America, Argentina and Chile have a long tradition in the use of native plant species for food and medicinal purposes. The southern region of Argentina, *i.e.,* Argentine Patagonia, is a vast territory with a great diversity of native plants (see Online Resource 1). Despite the growing interest in health benefits through diet and natural care, the phytochemical and nutraceutical properties of herbal infusions of native plants from Patagonia have been little studied [3]. Some of the native plants widely consumed in the Patagonian region are: *Adesmia boronioides* Hook. *f.* (Fabaceae), *Apium australe* Thouars (Apiaceae), *Buddleja globosa* Hope (Buddlejaceae), *Drimys andina* (Reiche) R. Rodr. & Quezada (Asteraceae), *Dysphania multifida* L. (Chenopodiaceae) and *Solidago chilensis* Meyen (Asteraceae). *A. boronioides* is an aromatic plant widely consumed as an infusion and tincture. They have several medicinal uses and significant anti-inflammatory activity [4]. Their essential oil has a characteristic sweet-woody, licorice-spicy odor with potential utility in the fragrance industry. Recently, an experimental culture of this species has been started with the purpose of conserving the wild populations. *A. australe* is an edible plant widely distributed in humid environments of Patagonia. It is consumed as an infusion and the leaves are used in salads or as flavoring in soups [5]. This plant is closely related to the horticultural species *A. graveolens* (celery). *B. globosa*, commonly known as "matico" or "pañil", is a native shrub with presence in Argentina and Chile. The infusion of its leaves has a pleasant taste and is consumed by the indigenous Mapuche communities for the treatment of ulcers and wounds. The hydroalcoholic extract of its aerial parts has been evaluated *in vitro* to heal wounds and as anti-inflammatory [6]. In addition, some fermented cold drinks are prepared from its flowers. Experimental crops of *B. globosa* are currently being carried out in Chile. *D. andina* is an endemic spice, which grows from 1400 masl [7]. Its fruits are edible and were used as an anti-scurvy agent in expeditions of Spanish conquerors to the South Pacific Ocean. *D. multifida* (syn. *Chenopodium multifidum* L.), commonly known as "paico", is an aromatic plant widely used for medicinal purposes due to its digestive and anti-parasitic properties. It is mainly consumed as an infusion and in some cases also as a condiment. The toxicity and anthelmintic properties of this herb are due to the presence of the monoterpene peroxide ascaridole [8]. The toxicity of this herb in humans is a subject of current debate. *S. chilensis* is a native herb widely distributed in South America. Most of the biological activities of this plant are attributed to its high content of flavonoids and diterpenes [9]. It is consumed as an infusion and tincture. Its leaves have been used in traditional medicine for the treatment of pain, inflammation, bruising, swelling and wounds. In South America, *S. chilensis* is cultivated in Brazil, mainly with pharmacological purposes [9]. The aim of this study was to characterize the infusions made from the aerial parts of these six native plants of the Patagonian region, in terms of their antioxidant activity, phenolic and flavonoid content, profile of phenolic compounds, and general toxicity and cytotoxicity against the T84 (derived from human colon cancer) and HTR8/SVneo (derived from human placenta) cell lines.

Materials and Methods

Plant Material

The aerial parts of the six native species studied were harvested from wild populations of the province of Chubut, Argentina, between October 2016 and January 2017. The plants were collected at the following locations: *A. boronioides* [42° 51′ 20" S, 71° 17′ 12" W], *A. australe* [42° 05′ 21" S, 71° 36′ 23" W], *B. globosa* [42° 54′ 58" S, 71° 20′ 1 8" W], *D. andina* [41° 08′ 06" S, 71° 34′ 13" W], *D. multifida* [42° 55′ 51 "S, 71° 21' 54" W], *S. chilensis* [42° 55′ 53" S; 71° 21′ 51" W]. A voucher specimen of each plant was deposited at the Herbarium of the Miguel Lillo Foundation (https://plants.jstor.org/partner/LIL) under the codes LIL615497 (*A. boronioides*), LIL615498 (*A. australe*), LIL615500 (*B. globosa*), LIL615501 (*D. andina*), LIL615502 (*D. multifida*) and LIL615504 (*S. chilensis*). The plant material was identified by Dr. Nora Muruaga (Cryptogamic Herbarium Miguel Lillo, Argentina). Green loose leaf tea was purchased in a chinese local market.

Preparation of Infusions and Lyophilizates

The plant material was dried and powdered. All the infusions were prepared as 5% aqueous extracts as follows: 100 mL of boiling water was added carefully to 5 g of plant material (dry powder) and the mixture was left at room temperature for 20 min and then filtered through Whatman No. 1 filter paper [10]. A portion of the total aqueous extract (15 mL) was stored at 4 °C and used within 24 h for the determination of antioxidant activity and content of phenols and flavonoids [10]. The remaining aqueous extract was lyophilized and the residues were weighed and stored at −40 °C for further phytochemical characterization by chromatography and for assays of antiproliferative activity and general toxicity [11].

Standards and Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, ascorbic acid, potassium persulfate, and methylthiazolyldiphenyl-tetrazolium bromide (MTT), β-carotene and linoleic acid were purchased from Sigma-Aldrich (Gillingham, UK). Dulbecco's Modified Eagle Medium (DMEM) were purchased from Life Technologies, Gibco BRL (Maryland, USA). Fetal bovine serum (FBS) was obtained from Natocor Biotechnology (Buenos aires, Argentina). Phosphate buffered saline (PBS), L-glutamine solution (200 mM), trypsin-EDTA solution (170,000 U/L trypsin and 0.2 g/L EDTA) and penicillin-streptomycin solution (10,000 U/mL penicillin and 10 mg/mL streptomycin) were purchased from Invitrogen (California, USA). All other materials and solvents were of analytical grade.

Antioxidant Activity

To evaluate the antioxidant activity of the infusions, we used the DPPH and the β-carotene bleaching (BCB) assays. In the DPPH assay, the radical scavenging activity of the aqueous extracts was determined by measuring the reduction of an ethanolic solution of DPPH at 517 nm after adding the infusion of the plant. Ascorbic acid (vitamin C) was used as a reference compound [10–12]. Briefly, various dilutions of the extract (0.1 mL) were added to 3.9 mL of DPPH (30 mg/L, in ethanol) in 4.5 mL spectrophotometric cuvettes. The mixture was left in the dark for 30 min before reading the absorbance with ethanol as blank. Two parameters were calculated: I) % DPPH inhibition, which is the maximum radical scavenging activity for 0.1 mL of pure infusion. The percentage of inhibition was calculated using the following equation: % DPPH inhibition = $(Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$, where high percentages of DPPH inhibition indicate greater antioxidant activity [13]. II) Vitamin C equivalents *per* cup, where the amount of decrease in absorbance was calibrated against ascorbic acid standards and reported as milligrams of ascorbic acid equivalents per 200 mL (mg VCEAC/200 mL) [10, 11]. In the BCB assay, the antioxidant activity was evaluated by measuring the inhibition of the bleaching of β-carotene by the peroxides generated during the oxidation of linoleic acid [13, 14]. A stock solution of the β-carotene/linoleic acid mixture was prepared by mixing 0.5 mg of β-carotene, 1 mL of chloroform, 25 μL of linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated and then 100 mL of distilled water saturated with oxygen was added with vigorous shaking. Then, 3 mL of stock solution was placed in 4.5 mL spectrophotometric cuvettes and 0.5 mL of sample was added. The mixture was allowed to react for 48 h at room temperature. Absorbance values were measured at 470 nm. The results are shown as percentage of inhibition of bleaching against the blank according to the following equation: % Inhibition of bleaching = $[1- ((Abs_{\text{initial of sample}} - Abs_{\text{at 48 h of sample}})) / (Abs_{\text{initial of control}} - Abs_{\text{at 48 h of control}})] \times 100$, where high percentages of inhibition of bleaching indicate greater antioxidant activity [13, 14].

Total Phenolics and Flavonoid Content

The total phenolic compound content (TPC) was determined using the Folin–Ciocalteu colorimetric method [12, 15–17]. Each extract (20 μL) was mixed with 0.2 mL of 25% Folin-Ciocalteu's reagent and 0.8 mL of 16% (*w*/*v*) sodium carbonate. The mixture was kept in the dark for 20 min before measuring the absorbance at 765 nm. A calibration curve was constructed using gallic acid and results were expressed as mg of gallic acid equivalent (GAE) *per* cup (200 mL) of infusion and *per* gram of dry plant material [10, 11, 17]. The total flavonoid content (TF) was measured with the colorimetric method of aluminum trichloride reagent [18]. Each extract (100 μL) was mixed with 1 mL of 5% (*w*/*v*) aluminum trichloride. The mixture was kept in the dark for 20 min before measuring the absorbance at 425 nm. A calibration curve was constructed using quercetin and results were expressed as mg of quercetin equivalent (QE) *per* cup (200 mL) of infusion and *per* gram of dry plant material [10, 11, 17].

Liquid Chromatography Analysis of Phenolic Compounds

Phenolic compounds were analyzed by liquid chromatography with diode array detector and tandem mass spectrometry (LC-DAD-MS) [15, 19]. To this end, 5 mg of lyophilized infusion was resuspended in 1 mL of methanol:ethanol (1:1), filtered and injected. Reference standards of caffeic acid, chlorogenic acid, quercetin and rutin were solubilized in methanol, filtered and injected. The chromatographic equipment was an Ultimate 3000 RSLC Dionex model from Thermo Scientific with a UV-Vis detector model VWD-3400RS and mass detector TSQ Quantum Access Max. The separation was performed on a C18 Hypersil-GOLD column (50 × 2.1 mm; 1.9 μm particle size) kept at 30 °C, at a flow rate of 0.20 mL/min for 63 min. Gradient elution: solvent (A) H_2O (containing 2% AcOH), solvent (B) MeOH; gradient elution program: from 85 to 60% A for 30 min, from 60 to 25% A for 10 min, from 25 to 15% A for 5 min, 15% A isocratic for 10 min, from 15 to 85% A for 3 min, ending at 85% isocratic, 5 min. Total run: 63 min. The analysis was monitored at 254, 280, 330 and 365 nm and by electrospray ionization in the positive mode at a probe

temperature of 360 °C and a probe voltage of 4.5 kV [15]. In addition, the complete UV spectrum was recorded for each peak. The tentative identification was based on the retention time, UV spectral maxima and MS fragmentation, reference standards, database and bibliographic data [15, 19]. The main compounds of each infusion were also detected in a thin layer chromatography system [20]. The adsorbent was silica gel 60 F_{254}, whereas the chromatography solvents were ethyl acetate-formic acid-glacial acetic acid-distilled water (100:11:11:26). Spray reagent: natural products followed of polyethylene glycol. Physical detection: UV-365 nm.

General Toxicity

Eggs of brine shrimp *Artemia salina* (100 mg) were incubated for 48 h in a culture vessel (15 cm × 15 cm × 15 cm) containing sea water (38 g NaCl/L) at 25 °C; the saltwater solution was aerated continuously during incubation with an aquarium air pump until larvae hatched [13]. The larvae were collected from the culture vessels using an aquarium artemis and a micropipette. In 96-well culture plates, *A. salina* individuals were exposed for 24 h to 1.25–25 mg/mL final concentrations of the lyophilizate. Plates were observed using a stereomicroscope and the LC_{50} (lethal concentration that corresponds to 50% dead larvae) was determined for each lyophilized plant infusion. Wells with only brine shrimp in saltwater and wells with potassium dichromate were used as controls [13, 21].

Antiproliferative Activity

The HTR-8/SVneo trophoblast cell line and the T84 colon adenocarcinoma cell line were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA) [18]. Monolayers were grown in T75 flasks and maintained in DMEM supplemented with 10% (*v*/v) FBS, 2 mM L-glutamine, 1% antibiotic solution and incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. The cells were dispersed with 0.25% trypsin-EDTA and incubated in 96-well culture plates in DMEM/F12 medium with 10% FBS and grown until confluence. Cell viability was assessed using the MTT assay [18]. The dried residues of individual lyophilized infusions were diluted in DMEM/F12 (50 mg/mL) and added into the culture plates at different final concentrations (0.25–5 mg/mL), after filtration through a 0.2-μm filter. After 72 h, 20 μL of MTT (5 mg/mL in PBS) was added to each monolayer well and incubated for an additional 4 h at 37 °C. At the end, the MTT formazan precipitate was dissolved in 100 μL of DMSO. Optical density was measured at 570 nm (OD570nm) on an ELISA plate reader in a microplate photometer (Thermo Scientific Multiskan EX microplate photometer) and viable cells were calculated as a percentage of control (treated cultures OD570nm/control cultures OD570nm × 100). As a background value, a well containing only culture medium was used. Cells treated with no infusion were used as control. The half maximal effective concentration (EC50) parameter, which represents the concentration corresponding to a response midway between the lower and upper plateaus in a concentration-response curve, was calculated by regression analysis.

Statistical Analysis

All analyses were performed in triplicate and the results are the average of three independent experiments. The results were evaluated by one-way analysis of variance (ANOVA), considering a confidence level of 95% by Tukey's test. Spearman correlation test was performed between VCEAC and TPC.

Results and Discussion

Antioxidant Activity, Total Phenolic and Tlavonoid Content

The antioxidant capacity of a natural product is an important parameter to establish its possible health benefits. Since the antioxidant capacity of a plant extract is determined by a mixture of several different types of compounds, it is convenient to evaluate this property by means of more than one method [22]. Consequently, here we used two methods to evaluate the primary antioxidant activity: the DPPH scavenging assay and the BCB assay. The DPPH assay was used to test the ability of the aqueous extracts examined to donate H• and thus neutralize the corresponding reactive radicals (DPPH•), resulting in the decrease of the absorption band at 517 nm [22], whereas the BCB assay was used to measure the ability of an antioxidant to inhibit lipid peroxidation. In this assay, a system composed of β-carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant, which can be monitored spectrophotometrically at 470 nm [13, 14]. The results of the antioxidant activity of infusions made from native plants of Patagonia by using the DPPH and BCB assays are summarized in Fig. 1. In the DPPH assay, *B. globosa* and *D. andina* displayed strong antioxidant activity. It is worth noting that the scavenging ability of *D. andina* was comparable to that of Green Tea, a rich source of antioxidants [23]. *D. multifida* showed the lowest antioxidant activity. The order of antioxidant capacity for the infusions analyzed in the DPPH assay was as follows: Green Tea = *B. globosa* = *D. andina* > *S. chilensis* > *A. australe* > *A. boronioides* > *D. multifida* (Fig. 1a). In the

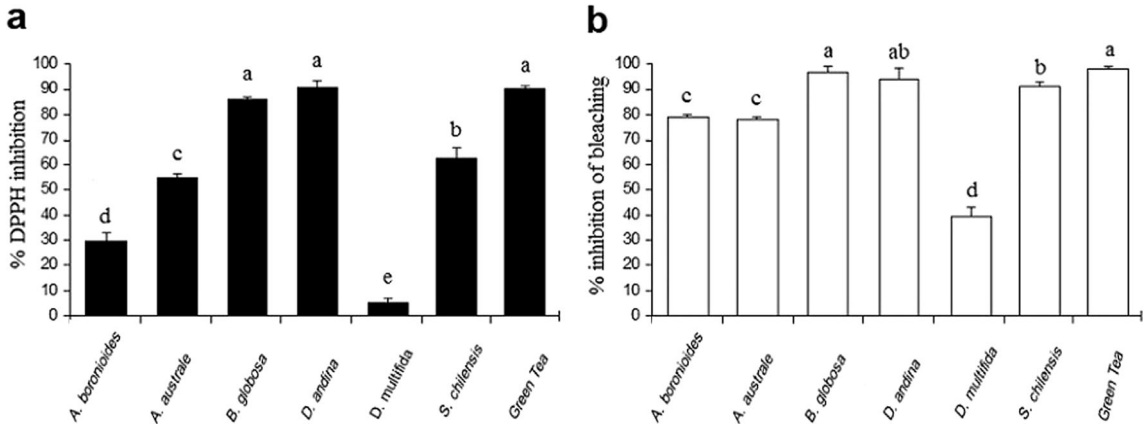

Fig. 1 Antioxidant activity of infusions in the DPPH and β-carotene bleaching assays. % DPPH inhibition **a** shows the maximum radical scavenging activity for 0.1 mL of infusion. % inhibition of bleaching **b** shows the antioxidant activity in the β-carotene/linoleic acid system for 0.5 mL of infusion. Green Tea (Chinese trademark) was included in the trial for comparative purposes

BCB assay, *B. globosa* and *D. andina* also showed the highest antioxidant values, similar to Green Tea (Fig. 1b), whereas *D. multifida* showed the lowest. In this assay, the antioxidant activity decreased in the following order: Green Tea = *B. globosa* ≥ *D. andina* ≥ *S. chilensis* > *A. boronioides* = *A. australe* > *D. multifida*. Notoriously, as shown, *B. globosa* and *D. andina* presented the highest values of antioxidant activity in both assays. Previously, a high antioxidant activity had been reported for *B. globosa* [6], but this is the first time that *D. andina* is analyzed. *S. chilensis* also showed high values of antioxidant activity, as could be expected due to its high content of flavonoids such as quercetin [9]. In relation to the antioxidant activity of *S. chilensis*, *A. australe*, *A. boronioides* and *D. multifida*, the BCB assay showed higher values than the DPPH assay. This result suggests that plants with a medium-low capacity to directly neutralize free radicals (DPPH assay) can be good lipid oxidation protective agents. These extracts have the ability to interact with the micelle that constitutes the lipid substrate and prevent direct oxidation. Table 1 shows the content of antioxidants (VCEAC), phenols (GAE) and flavonoids (QE) in one cup (200 mL) of infusion and the amount of phenols (GAE) and flavonoids (QE) *per* gram of dry plant material. Green Tea leaves and values reported in the literature were included for comparative purposes [10, 11, 23–25]. VCEAC is useful to evaluate the total antioxidant content in terms of ascorbic acid equivalents in foods products, particularly in infusions [12]. *B. globosa* and *D. andina* showed the highest VCEAC values, even higher than those of Green Tea. Besides, *B. globosa* and *D. andina* showed the highest GAE value. Spearman analysis for VCEAC and GAE values indicated a significant positive relationship ($r_s = 0.94$; $p < 0.001$), which means a strong correlation between these parameters. Therefore, the antioxidant activity of these plants could be attributed to the phenolic compounds present in the infusions. The correlation between antioxidant activity and phenol content was previously reported for other herbal infusions [10, 17]. The values of QE for *D. andina* and *S. chilensis* were considerably higher than those of Green Tea, and these values were the highest of all the plants analyzed. No correlation between VCEAC and QE content was found for the species analyzed ($r_s = 0.32$; $p = 0.498$). In summary, in comparison with Green Tea, *D. andina* showed higher values for the three parameters: VCEAC, GAE and QE; *B. globosa* presented higher values for VCEAC and GAE; and *S. chilensis* showed a higher value only for QE. Since these plants are traditionally consumed as an infusion, only the aqueous extracts were analyzed. The nutraceutical properties of lipophilic extracts (hexane, methylene chloride) were not addressed in this work.

In conclusion, the results obtained highlight the nutraceutical importance of these Patagonian plants and their infusions. Additionally, the use of the 'per cup' (200 mL) expression for antioxidants, phenols and flavonoids would be useful to compare the nutritional content of phytonutrients with other food sources [10, 11, 15]. Accordingly, a cup of infusion of *B. globosa* would provide an amount of phenols similar to that of 50 g of raspberries or 56 mL of red wine [26]. The application of this expression would help to merge results between different research groups around the world.

Liquid Chromatography Analysis of Phenolic Compounds

As mentioned above, the main phenolic compounds were identified by LC-DAD-MS. This powerful technique is suitable for fast identification of constituents in complex mixtures and to obtain tentative structures of phenolic compounds in fruits, crop plants, plant extracts and

Table 1 Content of antioxidants, phenols and flavonoids in one cup (200 mL) and *per* gram of dry weight (DW)

Herbal infusion	VCEAC (mg/200 mL)	GAE (mg/200 mL)	GAE (mg/g DW)	QE (mg/200 mL)	QE (mg/g DW)
A. boronioides	27.67 ± 1.53 e	40.67 ± 0.58 e	4.70 ± 0.1 e	24.33 ± 2.08 f	2.4 ± 0.2 f
A. australe	145.67 ± 2.08 d	88.33 ± 1,15 cd	8.9 ± 0.1 cd	37.33 ± 1.15 de	3.7 ± 0.1 de
B. globosa	294.33 ± 1.53 ab	121.67 ± 2.52 ab	12.2 ± 0.3 ab	31.67 ± 0.58 e	3.2 ± 0.1 e
D. andina	303.67 ± 2.08 a	121.69 ± 3,79 ab	12.2 ± 0.4 ab	67.00 ± 1.73 a	6.7 ± 0.2 a
D. multifida	22.67 ± 1.53 e	28.33 ± 2.08 e	2.8 ± 0.2 e	46.33 ± 4.93 c	4.6 ± 0.5 c
S. chilensis	156.33 ± 2.52 d	77.33 ± 2.08 d	7.7 ± 0.2 d	58.33 ± 2.08 b	5.8 ± 0.2 b
Green Tea *	225.67 ± 3.21 c	108.00 ± 7.21 bc	10.8 ± 0.7 bc	42.33 ± 2.08 cd	4.2 ± 0.2 cd
Green Tea **	273.33 ± 25.17 b	135.70 ± 22.05 a	13.5 ± 2.2 a	data not found	data not found

VCEAC Vitamin C equivalents, GAE Gallic acid equivalents, QE Quercetin equivalents. Values represented as mean ± SE. Different lowercase letters indicate a statistically significant difference at $p \leq 0.05$ (Tukey's test). Letter a denotes the highest value for each column (method) independently. * Indicates a reference value for a chinese trademark of Green Tea leaves that was included in the study. ** Indicates bibliographic reference value for Green Tea [10, 11, 23–25]

infusions [15, 19]. Online Resource 2 shows the phenolic compounds identified by LC-DAD-MS in the six plants analyzed. Twenty-nine compounds were identified. Quercetin and its glycosylated derivatives quercetin 3-O-glucoside and quercitrin along with 7-O-methylated apigenin and 3'-O-rutinoside of 7-methylated flavone were also found in all the infusions analyzed. Some phenolic acids widely distributed in the kingdom Plantae were also identified in some of the infusions analyzed: chlorogenic acid, caffeic acid, ferulic acid and gallic acid. Cyanidin glucoside was detected only in *A. australe* (II) and 3-O-rhamnoside of 3′,4′,2′,6′ tetrahydroxy, 4′-methoxy dihydrochalcone was only present in *D. multifida* (IV). An unidentified compound was found in *D. andina* (V). The phenolic compounds identified in the six plants analyzed suggest that the infusions of any of these herbs provide a wide variety of antioxidant compounds, which may contribute with healthy benefits [27].

General Toxicity and Antiproliferative Activity

Artemia salina was used to evaluate the general toxicity of the infusions analyzed according to previous researches [13, 21]. The information that can be obtained by this assay is very useful in two aspects: I) it provides initial and preliminary toxicity information on natural products and II) it is as an indicator of bioactivity, prior to testing a compound in cell lines [21, 28]. The usefulness of the *A. salina* bioassay to design pharmacological tests has been evaluated in mice, where it showed a very good correlation with acute toxicity studies [29]. However, additional studies would be advisable to evaluate the toxicity and long-term effects of infusions in other experimental models. According to the most accepted criteria, aqueous extracts present a risk of toxicity when $LC_{50} \leq 1$ mg/mL [29, 30].

The results of the *A. salina* bioassay for the infusions analyzed are shown in Table 2. *A. australe* is a plant widely cited as edible [5] and *D. multifida* is used as an infusion for medicinal purposes [8]. In the present study, neither species showed toxicity in the *A. salina* bioassay, which suggests that, in principle, the use and consumption of these plants throughout the range tested (0–50 mg/mL) appear to be safe. The infusions of *A. boronioides*, *B. globosa*, *D. andina* and *S. chilensis* showed toxic effects within the range analyzed, with LC_{50} between 5 and 17 mg/mL, and although these values are well above the toxicity threshold level of 1 mg/mL, additional studies would be desirable to have a more complete picture on the safety and long-term effects of these species. Currently, the generation of new information on plant foods and phytochemicals for the prevention and management of colon cancer is a very interesting topic [31]. Studies about the effects of herbal infusions on colon cancer cell lines have increased in the last years as their consumption has been highly associated with cancer-preventive properties [15]. Phenolic compounds can act in the epithelial tissues of the intestinal lumen [32] and flavonoids could inhibit the proliferation of cancer cells by the mechanism of cell cycle arrest through p53 [33] or by apoptosis of the intrinsic pathway (activation of caspase-3 and caspase-9) [2]. The antiproliferative effect of phenolic compounds here observed may be in direct correlation with the structural oxidation state and a greater activity due to the greater number of hydroxyl groups in the phenolic structure [34]. It has been found that flavone-type flavonoids have greater antiproliferative activity than flavanone-type flavonoids. The presence of at least three adjacent hydroxyl groups in a single phenolic compound has been strongly pointed out as possibly responsible for the antiproliferative activity [34]. Here, the anticancer potential (antiproliferative activity) of herbal infusions from plants of Argentine Patagonia was evaluated for the first

Table 2 General toxicity and antiproliferative activity

	Artemia test	Antiproliferative assay - EC_{50} (mg/mL)	
Herbal infusion	LC_{50} (mg/mL)	T84	HTR8-SVneo
A. boronioides	5.17 ± 2.16 b	1.36 ± 0.45 b	2.44 ± 0.41 a
A. australe	No toxic value	No cytotoxic value	No cytotoxic value
B. globosa	17.20 ± 2.55 a	1.37 ± 0.17 b	0.29 ± 0.09 c
D. mulfida	No toxic value	No cytotoxic value	No cytotoxic value
D. andina	9.88 ± 3.47 b	1.23 ± 0.16 b	0.28 ± 0.06 c
S. chilensis	8.53 ± 2.73 b	0.16 ± 0.07 c	0.24 ± 0.03 c

Values represented as mean ± SE. Different lowercase letters indicate a statistically significant difference at $p \leq 0.05$ (Tukey's test). Letter a denotes the highest value for each test

time. Following the results obtained by Gentile et al. [2], we decided to test the antiproliferative activity of the infusions by exposing two different cell lines to different concentrations of lyophilized extracts up to a maximum concentration of 5 mg of lyophilizate *per* mL of culture medium. These concentrations are consistent with the quantities that can be obtained at the intestinal level, after dietary ingestion of 1–2 cups of herbal infusions [15]. Figure 2 shows the results of the antiproliferative activity test of the six herbal infusions, performed by the MTT assay on the T84 (tumoral) and HTR-8/SVneo (non-tumoral) cell lines. As can be seen, the percentage of viable cells decreased with the increase in the concentration of lyophilized extract of *A. boronioides*, *B. globosa*, *D. andina* and *S. chilensis* in both cell types. The EC_{50} values for T84 cells indicate greater antiproliferative potency for the lyophilized extract of *S. chilensis* than for those of *A. boronioides*, *B. globosa* and *D. andina* (Table 2). For HTR-8/SVneo cells, *B. globosa*, *D. andina* and *S. chilensis* had a significant antiproliferative activity, higher than that of *A. boronioides*. The comparison of the EC_{50} values for both cell lines indicated that the lyophilized extracts had different specificity on the antiproliferative activity. *A. boronioides* had a higher antiproliferative effect on T84 cells, *B. globosa* and *D. andina* had a higher antiproliferative on HTR-8/SVneo cells, and *S. chilensis* affected the proliferation of both cell lines in a similar way. In the particular case of *A. boronioides*, this would indicate a selective action mechanism affecting cancer cells differentially. On the other hand, *A. australe* and *D. multifida* showed no antiproliferative effects on T84 or HTR-8/SVneo cell lines (Table 2). These results highlight the importance of using the *A. salina* bioassay prior to cell line research, since this test is faster, inexpensive and correlates well with antiproliferative assays. According to the structure-activity study for phenolic compounds carried out by Yanez et al. [34], the antiproliferative activity shown in Fig. 2 could be attributed to quercetin; however, this compound is also present in two species that showed no antiproliferative activity (*A. australe* and *D. multifida*). Therefore, it cannot be the only compound responsible for the activity observed. Gallic acid was detected only in *D. andina* and *S. chilensis* and could be responsible for the activity observed for these infusions; gallic acid was the only compound identified that has three adjacent hydroxyl groups in its structure, which confers high antiproliferative power [34].

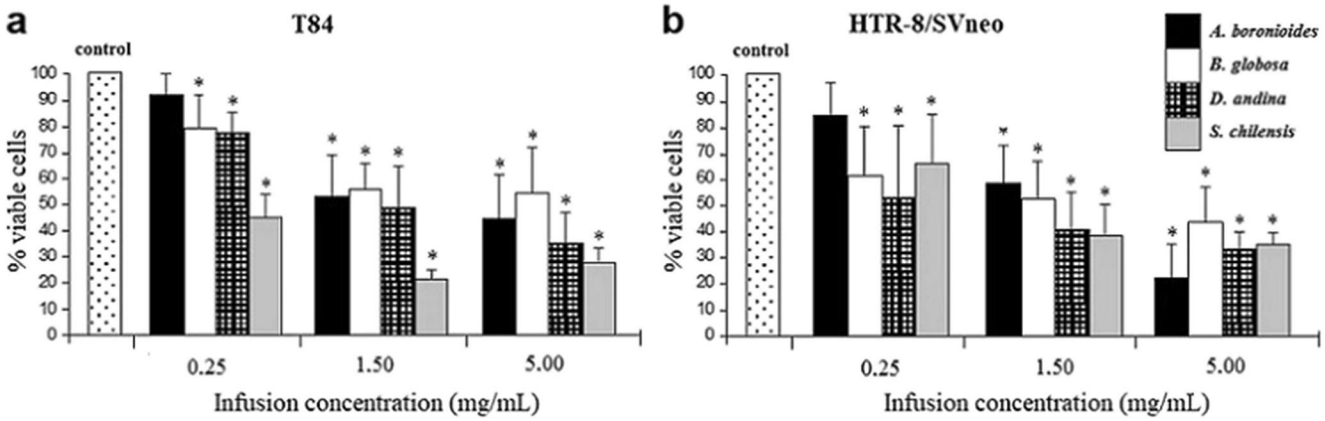

Fig. 2 Antiproliferative activity of infusions (lyophilized extract) on T84 and HTR-8/SVneo cells. Cell viability results (%) by the MTT assay after 72 h were relative to the control (untreated cells normalized to 100%). Each column represents the different plant species. Species without cytotoxic effect are not shown. Results are means ± SE. Asterisk indicates significant difference with respect to control ($p < 0.05$, Tukey's test)

Conclusions

We here presented an integrated study of nutraceutical properties like antioxidant activity, phenol content and antiproliferative activity on colon cancer cells of six herbal infusions from native plants consumed in Argentine Patagonia. This is the first report of phenolic compounds and potential anticancer activity (antiproliferative activity) for these species. Analysis of the infusions by using LC-DAD-MS allowed the identification of 29 compounds, most of which were phenolic acids (caffeic, ferulic, and chlorogenic), flavonoids (rutins, quercetin, 6-methoxyapigenin) and several flavonoid glycosides. The antioxidant activities of *B. globosa* and *D. andina* were similar to that of Green Tea, and even higher in terms of VCEAC content. On the other hand, the general toxicity test suggested that *A. australe*, *B. globosa* and *D. multifida* appear to be safe if consumed as tea, whereas a moderate consumption of *A. boronioides*, *D. andina* and *S. chilensis* is suggested. The antiproliferative activity of *A. boronioides* and *S. chilensis* indicated that these two plants are highly promising in cancer prevention.

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Compliance with Ethical Standards

Not applicable.

Human or Animal Studies This article does not contain any studies with human or animal subjects.

Conflict of Interest The authors declare that there is no conflict of interest regarding the publication of this paper.

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