Food Chemistry 119 (2010) 886–895

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae)

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ARTICLE INFO

Article history:
Received 18 June 2009
Received in revised form 17 July 2009
Accepted 22 July 2009

Keywords:
Flavonoids
Phenolic acids
Anthocyanins
Antioxidant activity
Aristotelia chilensis
Elaeocarpaceae

ABSTRACT

From ethanolic, water extracts and their fractions of mature fruits of wild black-berry *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae), different phenolic compounds were identified by chromatographic (HPLC) and unequivocally assignments by spectroscopic (UV, NMR) data analysis. Anthocyanidins, flavonoids and phenolic acids fractions were obtained using flash and open column chromatography. The main compounds gentisic acid, ferulic acid, gallic acid, *p*-coumaric acid, sinapic acid, 4-hydroxybenzoic acid, delphinidin, cyanidin, vanillic acid, delphinidin gallate, gallocatechin gallate, quercetin, rutin, myricetin, catechin and epi-catechin as mixture 1:1, and several glycosides of anthocyanidins (delphinidin-3-sambubioside-5-glucoside, delphinidin-3,5-diglucoside, cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, delphinidin-3-sambubioside, delphinidin-3-glucoside, cyanidin-3-sambubioside, and cyanidin-3-glucoside), and proanthocyanidin B were detected. In addition to phytochemical analysis the antioxidant activities of extracts, partitions and fractions were strongly correlated with the highest polyphenol contents. The most active samples were the ethanolic and acetone extracts in all bioassays used and all samples were compared for activity against butylated hydroxy toluene (BHT), quercetin and tocopherol used as pattern samples. The juice (**E**), EtOH extract (**A**) and acetone partition (**B**) were found to have IC_{50} values of 4.7, 1.7 and 7.4 ppm, respectively against DPPH and 5.9, 2.1 and 3.9 ppm, respectively against TBARS formation. Additionally, the fraction **F-4** showed a strong activity with IC_{50} of 4.9 and 6.5 ppm, against DPPH and TBARS respectively. Consistent with this finding, EtOH extract had the greatest ORAC and FRAP values as percentage of activity. On the other hand the IC_{50} values for the inhibitory activity against $O_2^{\cdot-}$ of extract **B**, **F-3** and **F-4** were 9.7, 13.2 and 10.7 ppm, respectively and against $OH^{\cdot-}$ were 29.1, 7.0 and 6.3 ppm, respectively. The EtOH extract protects against stress oxidative reducing the concentration of the MDA a lipid peroxidation index. These results shows that this fruit could be useful as antioxidant and nutraceutical sources.

1. Introduction

Antioxidants are substances that delay the oxidation process, inhibiting the polymerisation chain initiated by free radicals and other subsequent oxidising reactions (Halliwell & Aruoma, 1991). This concept is fundamental to biomedical, nutraceutical, food chemistry and phytochemical sciences, where synthetic antioxidants like butylated hydroxy toluene (BHT) have long been used to preserve quality of food by protecting against oxidation-related deterioration. A growing body of literature points to the importance of natural antioxidants from many plants, which may be used to reduce cellular oxidative damage, not only in foods, but also in the human body. This may provide protection against chronic diseases, including cancer and neurodegenerative diseases, inflammation and cardiovascular disease (Prior, Wu, & Schaich, 2005).

The increasing interest in the measurement of the antioxidant activity of different plant samples is derived from the overwhelming evidence of the importance of Reactive Oxygen Species (ROS), including superoxide ($O_2^{\cdot-}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$), hydroxyl ($OH^{\cdot-}$), and nitric oxide ($NO^{\cdot}$) radicals in aging and chronic disease

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doi:10.1016/j.foodchem.2009.07.045

(Fernandes, Costa, Toste, Lima, & Reis, 2004). Several methods have been developed to measure the antioxidant activity in biological samples, including the oxygen radical absorption capacity (ORAC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picryl-hydrazil (DPPH) radical scavenging and inhibition of formation of thiobarbituric acid reactive species (TBARS) (Prior et al., 2005; Taruscio, Barney, & Exon, 2004).

The use of traditional medicine is widespread and plants still present a large source of novel active biological compounds with different activities, including anti-inflammatory, anti-cancer, anti-viral, anti-bacterial and cardioprotective activities. Antioxidants may play a role in of these health promoting activities (Yan, Murphy, Hammond, Vinson, & Nieto, 2002).

The numerous benefic effects attributed to phenolic products has given rise to a new interest in finding vegetal species with high phenolic content and relevant biological activity. Berries constitute a rich dietary source of phenolic antioxidant and bioactive properties (Pool-Zobel, Bub, Schröder, & Rechkemmer, 1999; Smith, Marley, Seigler, Singletary, & Meline, 2000). Chilean wild black-berry *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae), an edible black-coloured fruit, which reach its ripeness between December and March, have a popular and very high consume during these months in Central and South Chile and western of Argentina. Previously, we have reported the alkaloid composition of the leaves of *A. chilensis* and the botanical characteristics were reported previously (Cespedes, El-Hafidi, Pavon, & Alarcon, 2008; Silva, Bittner, Cespedes, & Jakupovic, 1997).

Up-to-date some studies reports that the juice (an aqueous extract) from fruits of *A. chilensis* has a good antioxidant activity against FRAP analysis but not reduce endogenous oxidative DNA damage in human colon cells (Pool-Zobel et al., 1999), an effective capacity to inhibit the cooper-induced LDL oxidation *in vitro* and the induction of intracellular oxidative stress induced by hydrogen peroxide in human endothelial cells culture (Miranda-Rottmann et al., 2002), other study report only the partial composition of anthocyanidins constituents of the juice (Escribano-Bailon, Alcalde-Eon, Muñoz, Rives-Gonzalo, & Santos-Buelga, 2006), and recently was reported the inhibitory activity against aldose reductase by an extract rich in anthocyanins of this fruit (Kraft et al., 2007). Subsequently, we have a recent report about the effects of MeOH extract from ripe fruits of *A. chilensis* on isquemic/reperfusion system, several antioxidant activities of that extract and its relationship between total phenolic levels and the cardioprotective effect. In that work, the antioxidant activities determined for MeOH extract were ORAC, FRAP, DPPH and TBARS as an index of lipid peroxidation in liposomes and heart homogenate (Cespedes et al., 2008).

In spite of those data, there are not previous ethno-botanical reports or a more complete phenolic content of the aqueous and ethanolic extracts and fractions or a more complete phytochemical study of this fruit and neither of the mechanisms involved has been evaluated until now.

In the continuation of our general screening program of Chilean flora with biological activities a re-examination of the EtOH extract of fruits of *A. chilensis* (Elaeocarpaceae) has been initiated. Thus, in the present investigation, we designed to re-investigate the antioxidant activities (TBARS now from brain homogenate, crocin, DPPH, superoxide dismutase through hypoxanthine–xanthine oxidase system, hydroxyl anion radical through hydrogen peroxide–peroxidase system) of the EtOH, aqueous extracts, fractions, mixtures of phenolic compounds and its relationship to the occurrence of phenolic compounds, together with a phytochemical contents (HPLC and NMR analyses) of these extracts and fractions from ripe fruits of *A. chilensis*. Continuously, we are working in a more complete metabolomic profile of this plant.

2. Material and methods

2.1. Biological material

In this opportunity the fruits of *A. chilensis* (Mol) Stuntz (Elaeocarpaceae) were collected from fields at foothills of Los Andes Mountains (900 m, 38°38′57″S, 71°52′32″W) on track at shore of "Quepe" lake very near of "Conguillio" National Park, inside of Cherquenco district, a little town near to Temuco City, Chile, in January, 2006. The samples of plants and fruits were identified botanically by Professor Fernando Perich, M.Sc. (Departamento de Ciencias Químicas, U. de La Frontera, Temuco, Chile) and voucher specimens were deposited at the Herbarium (CONC) of Departamento de Botánica, Facultad de Ciencias Naturales y Oceanograficas, Universidad de Concepción, Concepción, Chile. Voucher: R. Rodríguez and C. Marticorena. The collected plants were air-dried and prepared for extraction.

2.2. Sample preparation

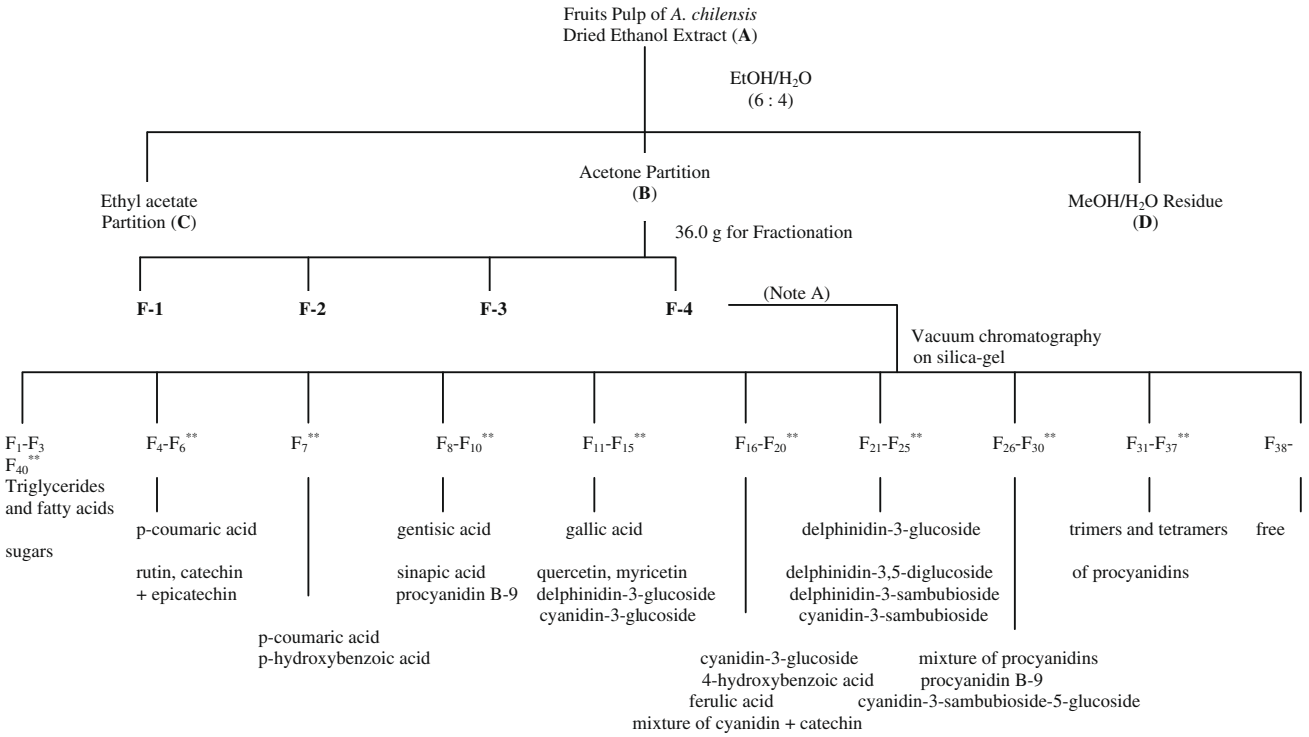
Fruits were separated in their main morphological parts (seed and pulp), the pulp was dried and then milled (450 g). One portion (300 g) was extracted with ethanol/water (6:4) containing 0.1% HCl and other portion (150 g) was extracted with bi-distiled water (juice). Thus was obtained two main extracts (**A** (EtOH/H_20) and **E** (water)). The EtOH/H_2O extract was then partitioned with acetone (**B**) and ethyl acetate (**C**), according to Cespedes et al. (2008), see Scheme 1 (Cespedes et al., 2008).

Based on popular use as ethno-medicines and beverages, juice (water extract) and EtOH extract from the pulp were studied. Because most of the ethno-medicinal use and biological activity was associated with the ethanol extract, and that a beverage similar to wine made with EtOH is used for the treatment of different illness, this extract was used for antioxidant evaluation and phytochemical analyzes. The ethanol extract (**A**) was concentrated and redissolved in ethanol:water (6:4), then partitioned into acetone (**B**) and ethyl acetate (**C**), leaving an EtOH-H_2O residue (**D**), as shown in Scheme 1. The acetone partition (**B**) showed an excellent antioxidant activity and was further fractionated into four fractions (**1**–**4**). These extracts, partitions and all fractions were analyzed by TLC as antioxidant bioautographic assay against DPPH (Cespedes et al., 2008; Dominguez et al., 2005). The ethyl acetate, and acetone partitions and their four fractions were analyzed for phenolics, flavonoids and oligomeric anthocyanidins contents.

The above-mentioned extracts (**A**–**E**) and partitions (**1**–**4**) were submitted to a number of analyses, including crocin, radical anion superoxide ($O^{\cdot-}$), the radical hydroxyl ($OH^{\cdot-}$), ORAC, FRAP, DPPH, and TBARS, and were evaluated for total phenolic content using the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999).

2.3. Chemicals and solvents

All reagents used were either analytical grade or chromatographic grade, AAPH, DPPH, BHT, THQ, BHA, TBH, EDTA, bovine serum albumin, Percoll, Trolox, quercetin, Folin–Ciocalteu reagent, TBA, TPTZ, ABTS, H_2O_2, peroxidase, $FeCl_3{\cdot}6H_2O$, hypoxanthine, xanthine oxidase, dihydroethydium (DHE), fluorescein disodium (FL), TMP, TEP, tris-hydrochloride buffer, phosphate buffered saline (PBS), $FeSO_4$, trichloroacetic acid, gentisic acid, gallic acid, *p*-coumaric acid, o-coumaric acid, propil-gallate, quercetin, myricetin, kaempferol, (±)-catechin hydrate, (−)-catechin gallate, (−)-gallocatechin, gallocatechin-gallate, α-carotene, saffron, crocin, sorbitol, tricine, and trizma-hydrochloride were



Scheme 1. Method of obtaining extracts, partitions, fractions. Fraction **1** (hexane 100%), fraction **2** (hexane:ethyl acetate 1:1), fraction **3** (ethyl acetate:methanol 1:1), fraction **4** (methanol 100%). Extract **E** correspond water 100%. Note A: **F-3** together with **F-4** were collect up and chromatographed on silica gel by vacuum chromatography, solvent system starting with n-hexane, ethyl acetate and increasing MeOH–H_2O. Furthermore F_4–F_{30} were chromatographed on Sephadex LH-20 column, solvent system starting with EtOH and going to 100% acetone.

purchased from Sigma–Aldrich Química, S.A. de C.V., Toluca, Mexico, or Sigma, St. Louis, MO. Glycosides of anthocyanidins (cyanidin 3,5-diglucoside, delphinidin 3,5-diglucoside, cyanidin, delphinidin) were purchased from Fluka, (Fluka-Sigma–Aldrich Química, S. A. de C. V., Toluca, Mexico), samples of proanthocyanidins were a gift from Prof. D. Seigler (University of Illinois, Urbana-Champaign).

Methanol, CH_2Cl_2, $CHCl_3$, NaCl, KCl, KH_2PO_4, $NaHPO_4$, NaOH, KOH, HCl, sodium acetate trihydrate, glacial acetic acid silica gel GF_{254} analytical chromatoplates, Sephadex LH-20, silica gel grade 60, (70–230, 60A°) for column chromatography, n-hexane, and ethyl acetate were purchased from Merck-Mexico, S.A., Mexico.

2.4. Apparatus

^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz, and ^{13}C NMR spectra were recorded at 75 MHz, respectively, on Varian VXR-300S and on Varian Mercury-300; chemical shifts (ppm) are related to $(CH_3)_4Si$ as the internal reference. CD_3OD, $CDCl_3$ and acetone-d_6 from Aldrich Chemical Co. were used as solvents. Coupling constants are quoted in Hertz. IR spectra were obtained on a Perkin–Elmer 283-B and a FT-IR Nicolet Magna 750 spectrophotometers. A UV Spectronic model Genesys 5 spectrophotometer was used for biological and spectrophotometric analyses. Optical rotation was measured on a JASCO DIP-360 spectropolarimeter. Melting points were obtained on a Fisher–Johns hot-plate apparatus and remain uncorrected. Fluorimetric measurements were determined with Turner Barnstead-Thermolyne, model Quantech S5 Fluorometer, with 420, 440, 470, 550, and 650 Turner filters. The electron impact mass spectrum was taken on a GC/MS Agilent 5975-C Series instrument (70 eV) equipped with a HP-5MSi inert column. Method: T_{max} 318 °C, ramp 50 °C/min, 130.672 Kpasc pressure, 1 μL volume of injection.

2.5. General experimental procedures

Preliminary, high-performance liquid chromatography (HPLC) was performed on a WATERS model 600E, equipped with Bondapack RP-18 column, 250 mM × 8 mM, flow rate 1.5 mL/min; UV detector (254, 235, 280, 520 nm), mobile phase MeOH/H_2O 7:3 v/v. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} Merck plates, and the spots were visualised by spraying with a 10% solution of H_2SO_4, followed by heating at 110 °C for 3 min and with DPPH at 1% MeOH solution. Additionally, was used a HPLC Hewlett–Packard, Series 1050, with diode array detector, and UV detector at 254, 280, 365 and 520 nm, column YMC C18-Pack ODS-AM-303, AM12S05–2546 WT, 250 × 4.6 mM, ID S-5um, 12 nm; movil phase water/methanol/acetonitrile (50:35:15), isocratic, pressure 212 bar; it was prepared 300 μL of each sample in amber vials and injected 20 μL of each sample. For comparative and separation procedures, an HPLC Hitachi Model LaChrome Elite L-2300 with a Kromasil column KR100 – 5C18 (250 × 4.6 mM), UV detector at 254, 280, 365 and 520 nm, movil phase water/methanol/acetonitrile (50:35:15), isocratic, pressure 212 bar was used for preparative isolation of compounds.

2.6. Bioactivity-guided isolation and purification of phenolic acids, flavonoids, anthocyanins and proanthocyanins

Elution of acetone partition (**B**) was carried out with hexane:hexane 100% (**F-1**); hexane:ethyl acetate, 1:1 (**F-2**); ethyl acetate:methanol, 1:1 (**F-3**); and methanol 100% (**F-4**), (see Scheme 1), by open column chromatography using silica gel (type G, 10–40 μM, Sigma–Aldrich) as solid phase,

The most active fractions **F-3** and **F-4** were collected and submitted to open column chromatography using SiO_2 (G 60, Merck) as solid phase and for final purification through Sephadex LH-20.

Elution of each one of these samples with n-hexane:ethyl acetate mixtures and ethyl acetate followed by methanol afforded a total of 40 subfractions (SF) [20 subfractions from **F-3** (**SF1**–**SF20**) and 20 subfractions from **F-4** (**SF21**–**SF40**)] that after evaporation of the solvent to dryness were analyzed by TLC, HPLC and bioautographic assay (Veglioglu, Mazza, Gao, & Oomah, 1998) using different solvent systems (n-hexane:ethyl acetate and DCM:MeOH mixtures) against pattern samples of phenolic acids, flavonoids and anthocyanins. Repeated TLC of these fractions led to the isolation of the secondary metabolites, some of which were purified by prep-TLC and prep-HPLC. All compounds were collected and identified by TLC (*Rf*), HPLC, GC/MS, IR, UV, ^{1}H NMR, and ^{13}C NMR data, compared with authentic samples (see Scheme 1).

On the other hand, an independent sample (45.0 g) of milled pulp was extracted with 0.1% HCl (v/v) in methanol for 20 h at room temperature, in darkness according to Longo and Vasapollo (2005). Briefly, the solution was filtered on a Buchner funnel, and the solid residue was washed with 0.1% HCl in methanol. The filtrate was dried using a rotatory evaporator at 30 °C. The remaining solid was redissolved on 0.01% HCl (v/v) in deionized water and successively purified through a C-18 Sep-Pack cartridge (Waters Corporation, Milford, MA), previously activated with methanol followed by 0.01% aqueous HCl (v/v). Anthocyanins and polyphenolics were adsorbed on the Sep-Pack column while sugars, acids, and other water soluble compounds were removed by washing the minicolumn with two volumes of 0.01% aqueous HCl (v/v). The anthocyanins were eluted with methanol containing 0.01% HCl (v/v), solvent was evaporated and the solid containing anthocyanins was stored at −20 °C, until used for successive analysis. Five millilitres of 2 N HCl was added to 1 mL of above solution in a screw–cap test tube, flushed with nitrogen, and capped. This solution was saponified with 5 mL of 10% KOH for 8 min in dark at room temperature, after neutralised and used for HPLC analysis and anthocyanins determination (Fig. 1).

In summary, the isolation, purification and identification of individual flavonoids, phenolic acids, anthocyanins and proanthocyanins was made by open column chromatography (silica gel G 60 (70–230, 60A°), Merck), Sephadex LH-20 (Sigma–Aldrich), preparative TLC (silica gel GF_{254} analytical chromatoplates, Merck and Machery-Nagel), HPLC procedures (were carried out with three different equipments, included – HPLC/UV and diode array detection), UV, ^{1}H NMR and ^{13}C NMR spectra were used for chemical structure determination, for NMR were used a Varian Mercury-400, a Varian VXR-300S and VXR-500S spectrometers, and direct comparison with authentic samples.

Only for comparative effects some antioxidant bioassays were made with samples of phenolic acids, flavonoids and anthocyanins whose antioxidant potency is reported in an ample body of literature.

2.7. Oxygen radical absorbance capacity estimation

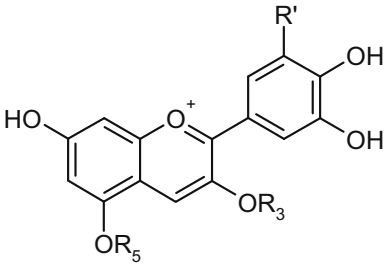
ORAC measures the antioxidant scavenging activity of a sample or standard against peroxyl radicals generated from AAPH at 37 °C using FL. All procedures were in according with Dominguez et al., 2005, Trolox was used as standard (Ou, Hampsch-Woodill, & Prior, 2001). Results are expressed as micromole of Trolox Equivalents (TE) per gram. All tests were conducted in triplicate.

2.8. Ferric reducing antioxidant power estimation

The FRAP assay was performed as previously described by Dominguez et al. (2005). Results are expressed as micromoles of catechin equivalents (Cat E) per gram of sample. All tests were conducted in triplicate.

2.9. Estimation of lipid peroxidation

As an index of lipid peroxidation, TBARS levels were measured using rat brain homogenates according to the method described by Ng with some modifications (Ng, Liu, & Wang, 2000), and as is described in Dominguez et al. (2005). Results are expressed as nanomoles of TBARS per milligram of protein, with percent inhibition after 30 min calculated as the inhibition ratio (IR), where (*C*) absorbance of the control and (*E*) absorbance of the test sample. These values were plotted against the log of the concentrations of individual extracts and fractions, and a decrease of 50% in peroxidation was defined as the EC_{50} (Dominguez et al., 2005).



	R'	R3	R5	Name	Content[a]	%	Peak number
18	OH	Xyl-Glc	Glc	Delphinidin-3-sambubioside-5-glucoside	101.05	35.1	1 (8.2)
19	OH	Glc	Glc	Delphinidin-3,5-diglucoside	49.80	17.3	2 (9.8)
20	H	Xyl-Glc	Glc	Cyanidin-3-sambubioside-5-glucoside	20.73	7.2	3a (11.9)
21	H	Glc	Glc	Cyanidin-3,5-diglucoside	18.71	6.5	3b (12.2)
22	OH	Xyl-Gluc	H	Delphinidin-3-sambubioside	30.51	10.6	4 (13.9)
23	OH	Glc	H	Delphinidin-3-glucoside	32.53	11.3	5 (15.3)
24	H	Xyl-Glc	H	Cyanidin-3-sambubioside	17.37	6.03	6 (17.6)
25	H	Glc	H	Cyanidin-3-glucoside	17.20	5.97	7 (18.4)
				Total anthocyanins	287.9		

[a] Content in mg/100g of dried weight

Fig. 1. Anthocyanidins isolated from fruits of *Aristotelia chilensis*. Xyl = Xylose, Glc = glucose. Expressed in mg-equivalents of delphinidin-3-glucoside. (HPLC-DAD, and UV using λ = 254, 280, 365 and 520 nm).

2.10. Generation of the radical anion superoxide ($O_2^{\cdot-}$) with the system hypoxanthine–xanthine oxidase

For the specific generation of radical anion superoxide a chemical system was used that involved hypoxanthine and the specific enzyme, xanthine oxidase, whose reaction subsequently to release the radical anion superoxide (El-Hafidi & Baños, 1997).

Briefly, in 1 ml of KH_2PO_4 (10 mM, pH 7.4) containing. 5 mM of hypoxanthine, 23 mg μg of xanthine oxidase and 1 mM μM of dihydroethydium (DHE), an indicator of radical anion superoxide

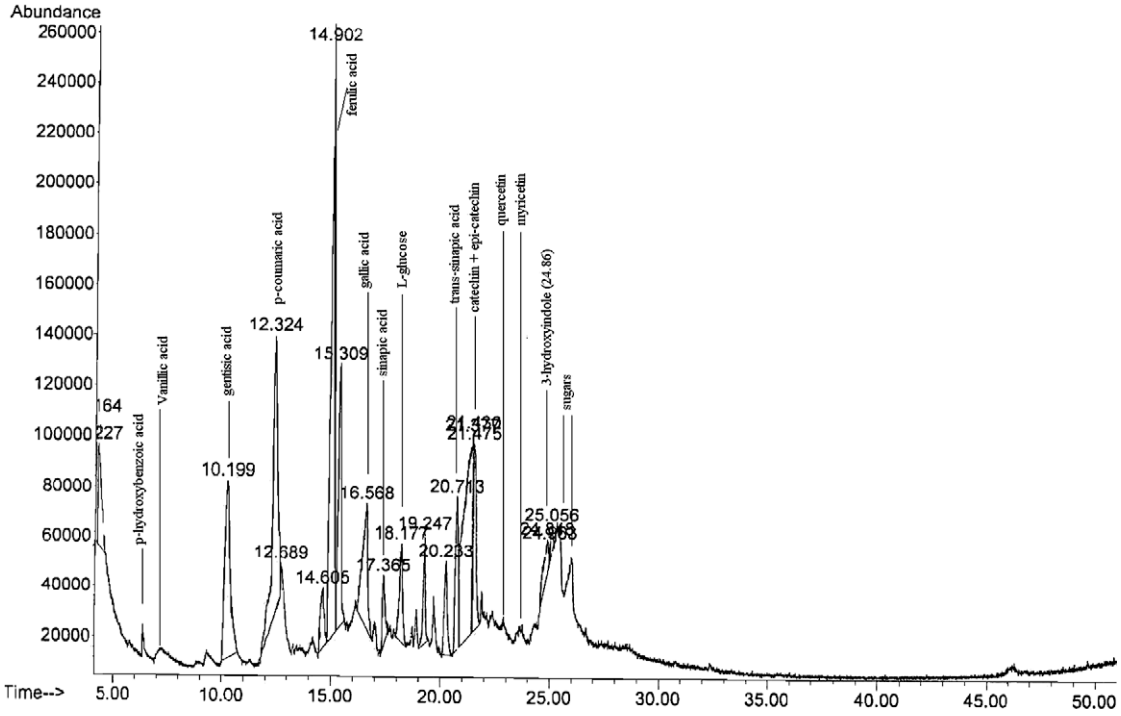


Fig. 2. HPLC-DAD of extract B (EtOH). All peaks were identified comparing with data bases, patterns and authentic samples.

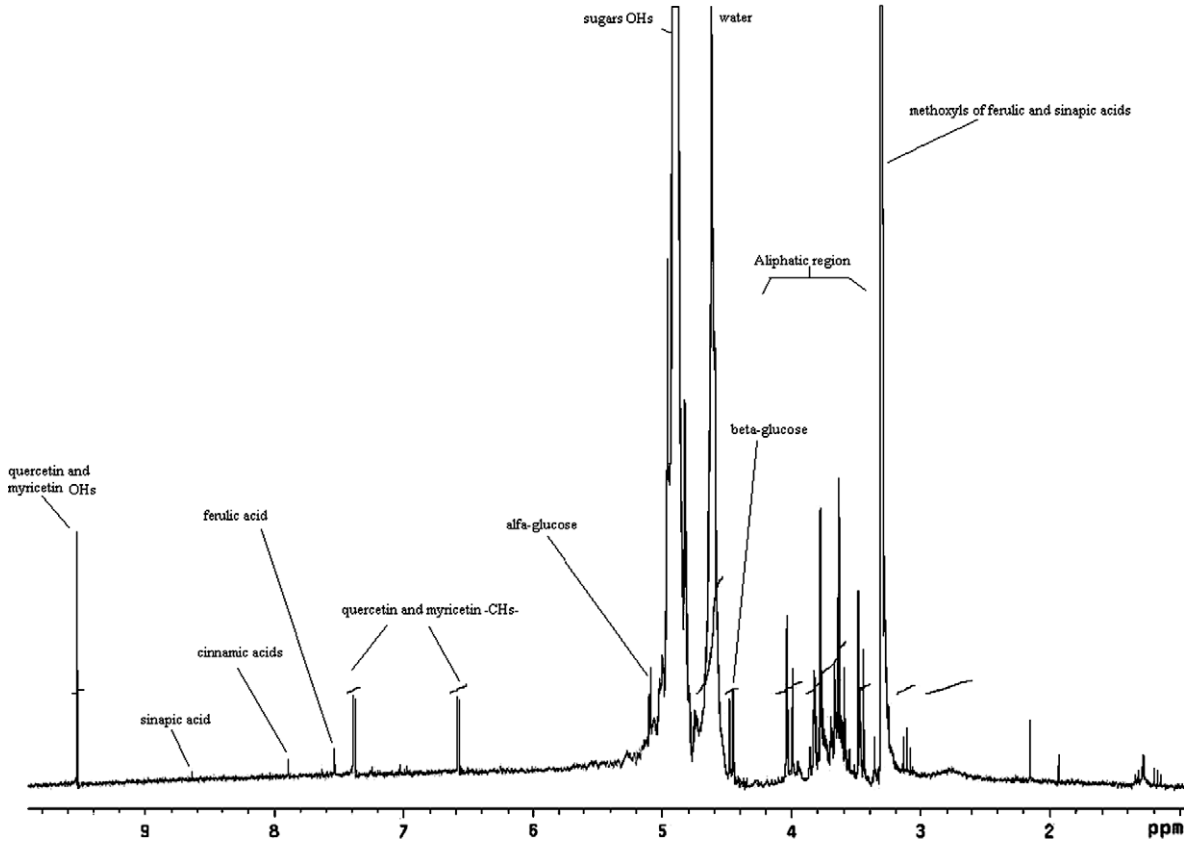


Fig. 3. H NMR (400 MHz) of EtOH (**A**) extract.

and it is detected by fluorescence which was carried out in the same Fluorometer described above (Turner Barnstead-Thermolyne, Quantech S5) at wavelength of 488 nm excitation and 620 nm emission. The reaction was performed at 37°C in the presence and absence of plant extracts, allowing them to incubate previously for 2 min and then the reaction began when adding the xanthine oxidase (Rahman, Ichiyanagi, Komiyama, Hatano, & Konishi, 2006; Wang & Jiao, 2000).

2.11. Generation of the radical hydroxyl ($OH^{\cdot -}$) by means of the system hydrogen peroxide-peroxidase

Briefly, the reaction of the H_2O_2 with the peroxidase generates radical hydroxyls ($OH^{\cdot}$) that they react with the ABTS (2,2-azinobis(3-ethylbenzothyazolin-6-sulphonic ammonium salt)), Sigma. In the presence of radical hydroxyl ABTS forms a radical cation $ABTS^{+}$, a soluble and a green colour end product which is detected by spectrophotometry at λ 414 nm. Each chemical reaction was carried out in 1 mL of sodium phosphate buffer (30 mM, pH 7.0) containing, ABTS 20 mM, H_2O_2 200 mM and 25 mg of peroxidase. The effect of different plant extracts on the generation of radical hydroxyl was analyzed in a spectrophotometer Perkin–Elmer Lambda 18 UV–visible at λ 414 nm. The results were expressed as enzymatic activity in mol/mg/min μmol/mg/min taking in account the extinction coefficient of the ABTS (36.8; Sigma–Aldrich product information; Keesey, 1987).

2.12. Reduction of the 2,2-diphenyl-1-picrylhydrazyl radical

Extracts and partitions were chromatographed on TLC and examined for antioxidant effects by spraying the TLC plates with DPPH reagent. Specifically, the plates were sprayed with 0.2% DPPH in methanol. Quercetin and α-tocopherol were used as standards (Dominguez et al., 2005).

2.13. Bleaching of crocin

The solutions were placed under UV_{254} light. Following the decrease of absorbance, bleaching of crocin and fluorescence emission at 440 and 470 nm were monitored with time each 5 min (Cespedes et al., 2008; Dominguez et al., 2005).

2.14. Estimation of total polyphenol content

The total phenolic content of extracts was determined using the Folin–Ciocalteu reagent: 10 μL sample or standard (10–100 μM Catechin) plus 150 μL diluted Folin–Ciocalteu reagent (1:4 reagent:water) was placed in each well of a 96 well plate, and incubated at RT for 3 min. Following addition of 50 μL sodium carbonate (2:3 saturated sodium carbonate:water) and a further incubation of 2 h at RT, absorbance was read at 725 nm. Results are expressed as μmol Cat E per gram. All tests were conducted in triplicate (Singleton et al., 1999).

2.15. Statistical analysis

Data were analyzed by one-way ANOVA followed by Dunnett's test for comparisons against control. Values of $p \leqslant 0.05$ (*) and $p \leqslant 0.01$ (**) were considered statistically significant and the significant differences between means were identified by GLM Procedures. In addition, differences between treatment means were established with a Student–Newman-Keuls (SNK) test. The I_{50} values for each analysis were calculated by Probit analysis. Complete statistical analyses were performed using the MicroCal Origin 7.0 statistical and graphs PC program. Data are expressed as a means ± SEM of five different experiments; when $P < 0.05$, the difference was considered to be statistically significant. Multiple comparisons between the experimental groups were performed by one-way ANOVA with a Tukey post hoc test.

3. Results and discussion

3.1. Phytochemical profile

Phenolic acids, some flavonoids, anthocyanins and a proanthocyanidin were identified from acetone partition (**B**), from which were obtained four main fractions (**F-1** to **F-4**). **F-3** and **F-4** were collected obtaining 40 subfractions (**SF**) from which were isolated and identified the active compounds (Scheme 1). From **SF-4** to **SF-6** were obtained *p*-coumaric acid, rutin and a mixture of catechin and epi-catechin; from **SF-7** was isolated a mixture of *p*-coumaric acid and *p*-hydroxybenzoic acid; **SF-8** to **SF-10** were collected and chromatographed on Sephadex LH-20 from this column were isolated gentisic acid, sinapic acid and procyanidin B; from **SF-11** to **SF-15** was isolated through Sephadex LH-20 gallic acid, quercetin, myricetin, delphinidin-3-glucoside and cyanidin-3-glucoside; from **SF-16** to **SF-20** was isolated through Sephadex LH-20 cyanidin-3-glucoside, 4-hydroxybenzoic acid, ferulic acid, a mixture of cyanidin and catechin; from **SF-21** to **SF-25** was isolated through Sephadex LH-20 delphinidin-3-glucoside, delphinidin-3,5-diglucoside, delphinidin-3-sambubioside and cyanidin-3-sambubioside; from **SF-26** to **SF-30** was isolated through Sephadex LH-20 a mixture of proanthocyanidins (probably trimers and tetramers) and a dimer identified as proanthocyanidins B, together with cyanidin-3-sambubioside-5-glucoside and delphinidin-3-sambubioside-5-glucoside, and finally from **SF-31** to **SF-37** through Sephadex LH-20 were isolated trimers and tetramers of proanthocyanidins unidentified, from **SF-38** to **SF-40** was isolated a mixture of free sugars (see Scheme 1). All known structures. These compounds were identified by comparison of their retention times (R_t) in the HPLC with those from our library HPLC data (see Fig. 2) and some structures were confirmed by their spectral features (UV, IR, H′ NMR, and GC/MS) with our data base and with those reported in the literature and by comparison with authentic samples (see Fig. 3), (Dugo, Mondello, Morabito, & Dugo, 2003; Hillebrand, Schwarz, & Winterhalter, 2004; Lee, 2002; Mabry, 1980; Mata-Bilbao, Andres-Lacueva, Jauregui, & Lamuela-Raventos, 2007; Merken & Beecher, 2000).

The anthocyanin composition of Maqui berries was determined by means of HPLC, UV, and GC/MS analysis. The HPLC chromatogram of the anthocyanins extract revealed that all peaks correspond to different pigments that coeluted under the conditions employed in this work, indicating the presence of eight different anthocyanins in Maqui berries. The structures the anthocyanins are shown in Fig. 1. All compounds were identified by comparison of HPLC retention times, and additionally with GC/MS, NMR and photodiode array (DAD/UV/VIS) spectroscopic data analysis.

3.2. Features of ^{1}H NMR spectra analyses of metabolites present in EtOH extract and fractions of A. chilensis

A completely dried and lyophilised EtOH extract and their three fractions from *A. chilensis* were redissolved with CD_3-OD; these were analyzed by ^{13}C and ^{1}H NMR. The H NMR spectra of total extract EtOH (Fig. 3) show common patterns at aliphatic region, carbohydrate region and aromatic region. In the sugar region, signals from glycosidically bound glucose were found apart from ubiquitous carbohydrates such as α-, β-glucose and sucrose. Based on its chemical shift (on the region from δ 3.1 to δ 5.2), the type of glycosides present could be deduced (Cazor, Deborde, Moing, Rolin, & This, 2006). The chemical shift for the anomeric glucose hydrogens

in aliphatic glycosides appears at δ 4.48 and 5.15 for α- and β-glucose, respectively, independently of structure of the side chain (Pereira et al., 2005). On the other hand, in the aromatic region of this ^{1}H NMR spectrum (Fig. 3) show a singlet at δ 9.53, presumably due to carboxylic proton or proton bond of flavonoid with hydroxyl group at C-5, and two doublet at δ 7.38 (J = 3.6 Hz) and δ 6.58 (J = 3.7 Hz), typical of quercetin and myricetin flavonoids. Other signal are a strong singlet at δ 3.34 assignable to methoxyl groups (ferulic acid), this signal is corroborate in everywhere fraction of the chromatograms (Fig. 2). The ^{13}C NMR of EtOH extract correlate very well these shifts with ^{1}H NMR, where is possible to observe aromatic, carbohydrate and aliphatic carbons.

In general the NMR spectra obtained show a dominance of signals in the carbohydrate region of the spectrum. In addition to these signals, well-defined signals in both the aromatic and aliphatic regions of the spectra were present (Fig. 3). The sharp singlet at δ 3.34 was identified as ferulic acid. Similarly, other signals in relatively clear areas of the trace could be assigned to particular carbohydrates (e.g. α- and β-glucose anomeric hydrogens at δ 5.15 and 4.48), and for polyphenols region where can be observed typical signals for anthocyanins and flavonoids (δ 6.4 to 9.5).

Interestingly, in the H NMR of **F-4** (spectra not show) do not appear the shifts to 7.9; 8.65 and 9.65 ppm, but is so very clear the shifts to 4.4 and 5.1 ppm for anomeric protons of β- and α-glucose, respectively, and the strong signal for O–CH_3 (ferulic acid), all additional signals are very similar to H NMR of EtOH extract.

Due to methodology procedures both NMR spectra do not showed signals under 3.0 until 0.0 ppm, where is possible to observe the signals for amino acids, we are working in the elucidation of these components.

In the extracts and fractions evaluated is possible to observe the presence of 4-hydroxyflavanones (flavonols), being quercetin, myricetin, the aglicone of rutin and ferulic acid the most abundant, together with several anthocyanins. A total of eight anthocyanins were identified and quantified.

On the other hand, the GC/MS of **F-4** show a peak at 24.86 min (data not shown) that cannot be compared with neither of known compounds in our data base. However, its mass spectra showed a molecular ion peak at *m/z* 133 and the fragmentation patterns lead us to a 3-hydroxyindole (Cespedes, Alarcon, Valdez, & Paredes-López, 2009). This compound has not been reported previously in this fruit, and the presence of this compound, although in minute amounts, show that the indole derivatives occurs also in the fruits of this plant; until now indole derivatives has been isolated only from leaves and stems (Silva et al., 1997).

3.3. Antioxidant activities

3.3.1. DPPH and TBARS evaluation

The DPPH radical scavenging assay was used first as a screen for antioxidant components within the primary extracts (Cespedes et al., 2008; Dominguez et al., 2005). As shown in Table 1, the juice, methanol and acetone partitions (**E**, **A** and **B**, respectively) had the highest inhibitory activity against DPPH radical formation compared to the other partitions with IC_{50} values of 4.7, 1.7 and 7.4 ppm, respectively. For partitions **C** and **D** the IC_{50} values were 30.6 and 17.9 ppm, respectively. Almost all these samples exhibited a concentration-dependence manner in their DPPH radical scavenging activities, particularly **A**, which showed the highest activity (100% inhibition) at a concentration of 8.6 ppm (data not shown). This action was greater than that of α-tocopherol, which at 31.6 ppm caused only 53.8% quenching and very similar to ferulic and *p*-coumaric acids with IC_{50} values of 5.1 and 7.8 ppm, respectively (data not shown). Of the four fractions only **F-3** and **F-4** were the most active against DPPH, with an IC_{50} of 7.1 and 4.9 ppm, respectively (Table 1).

Table 1
Amounts of phenolic content (mg/L ± Standard error) from **A**, **B**, **C**, **D**, **E** and fractions **F-1**, **F-2**, **F-3**, **F-4** from acetone partition **B** of *A. chilensis* needed to inhibit oxidative damage by 50%.[A]

Sample[B]	DPPH[C]	TBARS[D]	Crocin[E]
A	1.70 ± 0.3b	2.1 ± 0.1b	0.97 ± 0.1a
B	7.40 ± 1.4b	3.9 ± 0.2b	1.1 ± 0.5a
C	30.6 ± 13.6a	20.1 ± 0.8	22.9 ± 2.1b
D	17.9 ± 9.1a	11.2 ± 3.2c	15.8 ± 2.4b
Juice (E)	4.7 ± 0.7b	5.9 ± 0.9b	3.3 ± 0.5a
F-1	109.9 ± 9.2a	131.2 ± 12.5a	89.9 ± 10.2c
F-2	79.8 ± 5.5b	90.5 ± 9.9a	66.8 ± 8.8c
F-3	7.1 ± 1.4c	9.9 ± 2.7b	12.5 ± 2.1b
F-4	4.9 ± 0.5c	6.5 ± 1.3c	1.9 ± 0.2a
Quercetin	19.98	2.90	21.0
α-tocopherol	11.9	3.92	10.1

[A] Values expressed as μg/mL (ppm), Mean ± SD, n = 3. Different letters show significant differences at (P < 0.05), using Duncan's multiple-range test.
[B] See Scheme 1 for an explanation of extracts and partitions.
[C] IC_{50} for inhibition of DPPH radical formation.
[D] IC_{50} for inhibition of peroxidation of lipids, estimated as thiobarbituric acid reactive substances. Values are expressed as μg/mL (ppm), see Section 2 for details. Mean ± SD, n = 3. Different letters show significant differences at (P < 0.05), using Duncan's multiple-range test.
[E] IC_{50} for bleaching of crocin.

In addition to samples **A**–**D**, fractions **F-3** and **F-4** showed considerable activity, quenching DPPH· radical reduction completely (100% of inhibition, data not show). Nevertheless, **F-1** and **F-2** showed a moderate activity their IC_{50} values were 109.9 and 79.8 ppm, respectively (Table 1). The lowest I_{50} value for partition **A** (1.7 ppm) than for any of the other partitions, might be due to a synergistic effect of the components due to extraction procedures (mainly hydroxycinnamic acid derivatives, anthocyanidins and flavonoids) inside this extract, similar to that reported for components of *Vaccinium corymbosum* and *Vaccinium angustifolium* fruits (Ehlenfeldt & Prior, 2001; Lo & Cheung, 2005; Smith et al., 2000), where the acetone and MeOH partitions were the most active extracts.

Of the many biological macromolecules, including carbohydrates, lipids, proteins, and DNA, that can undergo oxidative damage in the presence of ROS, membrane lipids are especially sensitive to oxidation from this physiological process (Diplock et al., 1998). For this reason, brain homogenates were used for the investigation of lipid peroxidation as an assessment of oxidative stress. The capacity for plant extracts to prevent lipid peroxidation was assayed using malondialdehyde formation as an index of oxidative breakdown of membrane lipids, following incubation of rat brain cortical and hearth homogenates with the oxidant chemical species Fe^{2+}. Ferrous ion both stimulates lipid peroxidation and supports decomposition of lipids peroxides once formed, generating highly reactive intermediates such as hydroxyl radicals, perferryl and ferryl species (Ko, Cheng, Lin, & Teng, 1998). Against TBARS **F-4** was most effective, **F-3** was least effective, but none were as effective as **A** or **B** extracts, quercetin or BHT in inhibiting lipid peroxidation. Extract **A** had the greatest activity and reduced lipid peroxidation in a dose-dependent manner, and proved to be an excellent antioxidant, reflected by its low IC_{50} value (2.1 ppm) when analyzed by both TBARS and DPPH (Table 1), at the same level than quercetin and α-tocopherol whom shows IC_{50} of 2.9 and 3.92 ppm, against TBARS formation, respectively (Table 1).

When the relative contribution of each fraction to the total antioxidant activity was evaluated using TBARS, all fractions except **F-1** showed some protective effect, the IC_{50} values of partition **B, F-1** and **F-2** were 3.9, 131.2 and 90.5 ppm, respectively (Table 1). **F-3** and **F-4** were the most active, with IC_{50} values of 9.9 and 6.5 ppm, respectively, being **F-4** substantially more active than

other fractions. It is noteworthy that the value for **F-4** is very low compared with values for flavonoids and anthocyanins in general, as well as for myricetin or quercetin (data not shown) (Lo & Cheung, 2005; Makris & Rossiter, 2001).

It has been reported that the antioxidant activity of many compounds of botanical origin is proportional to the phenolic content (Rice-Evans, Miller, & Paganga, 1997), suggesting a causative relationship between total phenolic content and antioxidant activity (Veglioglu et al., 1998). Halliwell (Halliwell & Aruoma, 1991; Halliwell & Gutteridge, 1990) has defined antioxidants as substances that, when present at low concentrations compared with an oxidizable compound (e.g. DNA, protein, lipid, or carbohydrate), delay or prevent oxidative damage due to the presence of ROS. These ROS can undergo a redox reaction with phenolics, such that oxidant activity is inhibited in a concentration-dependent manner. In the presence of low concentrations of phenolics or other antioxidants, the breaking of chain reactions is considered to be the predominant mechanism (Pokorny et al., 1988), and the presence of phenolics have been suggested to be the most active substances from natural sources (Rice-Evans, 2000). Thus, in addition to total acidity, pH, and total sugars, we measured total phenolic content in each one of the extracts, partitions and fractions (data not shown). Extract **A**, which had the greatest DPPH and TBARS activities reduced MDA generation, had a significantly greater phenolic content than other extracts (17879 ± 335.7 of μmol Cat Equiv/g) and extract B showed a value of 16554 ± 501 of μmol Cat Equiv/g. The total phenolic content showed a small but significant increase for **F-4** (18987 ± 755.9 of μmol Cat Equiv/g) over **F-3** (13555 ± 310 of μmol Cat Eqiv/g), and **F-1** had significantly lower phenolic content. These findings correlate well with **F-4** having one of the greatest activities against DPPH and TBARS. Because **F-4** had one of the greatest activities against DPPH and TBARS formation, it could be that this fraction has the most active components.

3.3.2. ORAC and FRAP evaluations

The capacity for a compound to scavenge peroxyl radicals generated by spontaneous decomposition of AAPH was estimated in terms of Trolox Equivalents, using the ORAC assay (Dominguez et al., 2005). A wide variety of different phytochemicals from edible plants, purified or as an extract or fraction, have been found active in this assay, including alkaloids, coumarins, flavonoids, phenylpropanoids, terpenoids and phenolic acids (Aruoma, 2003; Dominguez et al., 2005; Ng et al., 2000; Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Among the extracts assayed here, the values were found to be in the range of 1740–31926 μmol TE/g extract for ORAC and from 710 to 13937 μmol Cat E/g extract for the FRAP assay, respectively. The ORAC and FRAP values for *A. chilensis* extracts are given in Table 2. As with our earlier measurements, extract **A** had the highest activity in both trials, with values of 31926.5 μmol TE/g extract and 13937.9 μmol Cat E/g extract for ORAC and FRAP assays, respectively. In similar form extract **B** show a very good potency with values of 29590.3 μmol TE/g and 10113.9 μmol Cat E/g for ORAC and FRAP assays, respectively. The other extracts (**C, D** and **E**) showed values from intermediate to good potency, 8941.5, 15641.1 and 22669.4 μmol TE/g extract in the ORAC assay with significantly differing ($P < 0.05$) and 6928.9, 5154.9 and 9930.8 μmol Cat E/g for FRAP assay with significantly differing ($P < 0.05$), respectively (Table 2). Among the fractions, **F-4** was significantly more than twice as active as any other fraction (Table 2).

The FRAP assay showed greater variability. Several extracts had very low values and only extracts **A**, **B**, **D** and **F-4** showed substantial activity. Again, **A** was significantly more active than any other samples. **F-3** and **F-4**, those with substantial phenolic content, were the fractions showing good activity in the FRAP assay. One possible explanation for the values obtained, is that for these samples, the reaction of the ferric-TPTZ complex was only partially completed within the 10 min reaction period. In agreement with the ORAC assay, it was extract **A**, partitions **B**, **C** and **F-4** that showed the greatest values. Those data correlate well with the ORAC values.

Table 2
Antioxidant Capacity of *A. chilensis* extracts **A, B, C, D, E** and **F-1, F-2, F-3**, and **F-4**, measured with the ORAC Assay and the FRAP Assay.

Sample[A]	ORAC[B]		FRAP[C]	
	[μg/mL]	μmol TE/g	[μg/mL]	μmol Cat E/g
A	10.0	31,926.5 ± 109.2a	25.0	13,937.9 ± 35.1b
B	10.0	29,590.3 ± 105.1a	25.0	10,113.9 ± 12.4a
C	10.0	8941.5 ± 20.2c	25.0	6928.9 ± 15.8c
D	10.0	15,641.1 ± 107.1b	25.0	5154.9 ± 10.9b
F-1	1.0	1740.2 ± 99.2c	2.5	N.D.
F-2	1.0	9631.3 ± 59.9c	2.5	710.6 ± 10.9d
F-3	1.0	16,750.5 ± 192.6b	2.5	1050.9 ± 10.3d
F-4	1.0	29,992.7 ± 135.9d	2.5	12,925.9 ± 104.5b
E	10.0	22699.4 ± 114.8d	25.0	9930.8 ± 12.8b

[A] Extracts **A** (methanol, 100%); **B** (acetone partition); **C** (ethyl acetate partition); **D** (MeOH/H2O residue); from *A. chilensis*. Fractions **1**–**4** from partition **B**. For detail see methods and Scheme 1.

[B] Expressed as μmol TE/g extract, (μmol of Trolox Equivalents/gram extract). Mean ± SD, $n = 3$. Different letters show significant differences at ($P < 0.05$), using Duncan's multiple-range test. In two different experiments, one for extracts and other for fractions.

[C] Expressed as μmol CatE/g extract, (μmol of Catequin Equivalents/gram extract). Mean ± SD, $n = 3$. Values with the same letter are not significantly different ($P < 0.05$).

Antioxidant activities bore a direct relationship with the phenolic content of the extracts and fractions. As with DPPH and TBARS activities, extract **A** was the most active in both the ORAC and FRAP assays. Among the fractions, **F-4** was the most active in both assays. These facts can be correlated very well between ORAC and total polyphenolic composition of all extracts and partitions and between FRAP and total phenolic composition of fractions, which are shown in Table 1. The phenolic characterisation suggests that the different phytochemicals antioxidants components in the active extract and fractions, mainly anthocyanins, cinnamic derivatives and flavonoids, may be involved in the antioxidant mechanism of action and the ORAC method gives us a direct measure of hydrophilic chain-breaking antioxidant capacity against peroxyl radical of our samples. Thus, the highest ORAC numbers of our extracts and fractions shows an excellent antioxidant potential (Table 2) as in the extracts **A**, **B** and **F-4**, for instance**.** In addition, the ORAC numbers of fractions showed a very high correlation with polyphenols content ($R > 0.95$) (data not shown), the same level of correlation was observed between the FRAP numbers and phenolic composition of the extracts and fractions. In the case of the extracts **A** and **B**, there is a similar level of correlation ($R > 0.98$) between FRAP numbers and its polyphenolic content (data not shown).

3.3.3. Superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical ($OH^{\cdot-}$) evaluations

In this study a hypoxanthine-XOD system was used for superoxide radical generation. Recently was confirmed that anthocyanins directly scavenged superoxide radicals even when a hypoxanthine-xod system is used (Rahman et al., 2006). Our results confirmed the findings reported by Rahman about the synergistic effects of mixtures of anthocyanins on $O_2^{\cdot-}$. Moreover, the extracts and fractions from *A. chilensis* fruits showed very good potency compared with other anthocyanins mixtures against $O_2^{\cdot-}$ and $OH^{\cdot-}$ (Wang & Jiao, 2000). The IC_{50} values for the inhibitory activity against $O_2^{\cdot-}$ of extract **B**, **F-3** and **F-4** were 9.7, 13.2 and 10.7 ppm, respectively and against $OH^{\cdot-}$ were 29.1, 7.0 and 6.3 ppm, respectively (Table 3).

Table 3
Scavenging capacity of extracts, fractions, compounds and mixtures from *A. chilensis* fruits on active oxygen species ($O_2^{\cdot-}$ and $OH^{\cdot-}$). Relative scavenging rate (% of inhibition).[a]

Sample[a]	$O_2^{\cdot-}$ [b]	$OH^{\cdot-}$ [c]
A	72.5	63.1
B	9.7	29.1
C	40.1	37.1
D	65.9	53.4
E	45.3	62.4
1	75.7	71.9
2	66.9	55.8
3	13.2	7.0
4	10.7	6.3

[a] Extracts **A** (methanol, 100%); **B** (acetone partition); **C** (ethyl acetate partition); **D** (MeOH/H2O residue); from *A. chilensis*. Fractions **1–4** from partition **B**. For detail see methods and Scheme 1.

[b] Data expressed as percent inhibition of radical ($O_2^{\cdot-}$ and $OH^{\cdot-}$) production in the presence of 0.1 mL of sample equivalent to 100 μg/mL (ppm) extract. Mean ± SD, $n = 3$. Different letters show significant differences at ($P < 0.05$), using Duncan's multiple-range test. In two different experiments, one for extracts and other for fractions.

[c] Expressed.

Among the different extracts and fractions assayed, extract **B** and **F-4** had the highest antioxidant capacity of inhibition of the free radical activity against $O_2^{\cdot-}$ and $OH^{\cdot-}$ (Table 3).

Except for proanthocyanidin B and gallocatechin gallate, the antioxidant activities were also evaluated against the radical anion superoxide and the radical hydroxyl (data not shown). In addition to **F-4**, the mixture of anthocyanins and the mixture of flavonoids showed the most potent antioxidant activity against $OH^{\cdot-}$ with an EC_{50} of 1.29 ± 0.31 and 4.35 ± 1.1 ppm, respectively, followed by hydroxycinnamic acids with a range between 3.96 and 10.5 ppm (data not shown). The antioxidant activities of the two mixtures were comparable to the activity of quercetin and tocopherol used as standards in this study. Against $O_2^{\cdot-}$ the most active was the anthocyanins mixture with an EC_{50} of 15.5 ± 2.5 ppm (data not shown). All antioxidant activities measured in this work for the isolated compounds and its mixtures are very similar to reported in an ample body of literature.

Finally, the extracts **A** and **B** of fruits of *A. chilensis* and some of its fractions exhibited substantial potency in scavenging DPPH-radical and inhibiting lipid peroxidation. Two of the four fractions isolated from **B**, **F-3** and **F-4** showed potency in scavenging against DPPH-radicals, as well as a strong inhibitory effect against lipid peroxidation, particularly **F-4**. The antioxidant activities, total phenolic content and ORAC and FRAP assays all correlated, suggesting but not proving a causative relationship. That it was the acetone partition **B** that showed this activity suggests that the phenolic compounds present are probably low or medium molecular weight, with relative high polarity. Additionally, the *in vitro* strong scavenging activity of EtOH (**B**) and **F-4** seems to be increased probably due to the presence of a 3-hydroxyindole derivative as in other samples (Fernandes et al., 2004). The phytochemical analysis of these extract, partitions and fractions have shown been rich in phenolic composition and is the identification of its chemical structures of bioactive components that may have a future role in human health maintenance and nutrition.

4. Concluding remarks

Many cellular components are sensitive to oxidative damage, caused by the presence of nitrogen or oxygen reactive species, including a myriad of different free radicals and rat brain homogenates, rich in lipids such as polyunsaturated fatty acids that can undergo peroxidation. Additionally, plants which have a strong capacity to synthesise proanthocyanidins generally do not metabolize substantial quantities of esters of gallic and hexa-hydroxydiphenolics acids and vice versa (Haslam, 2007). Our findings show that the extract **A** and acetone partition **B** of *A. chilensis* and several fractions of that extract (**F-3** and **F-4**), contain antioxidants that can inhibit lipid peroxidation, and that they have a high phenolic content. The relationship between total phenolics with DPPH, crocin, $O_2^{\cdot-}$, $OH^{\cdot-}$, ORAC and FRAP values in all extracts and fractions were similar to those found in other methanol and ethyl acetate plants extracts, and that values are similar with those for different known fruits and vegetables as carrots, berries, prunes, raisins, blueberries, spinach and broccoli (Cespedes et al., 2008; Dominguez et al., 2005 and references there in; Jeffery & Araya, 2009; Prior et al., 1998).

Acknowledgments

This work was supported in part by internal grant of Departamento Ciencias Basicas, Universidad del Bio Bio, Chillan, Chile. The authors thank to Roberto Rodriguez for botanical identification of the plant, Facultad de Ciencias Naturales y Oceanograficas, Universidad de Concepción, Concepción, Chile. We are indebted to Dr. Alfonso Romo de Vivar R., Antonio Nieto, Teresa Ramírez-Apan and Luis Velasco, Instituto de Química-UNAM, Juan Rodriguez-CINVESTAV-IPN, and Natalia Pavon-Department of Biochemistry, Instituto Nacional de Cardiología-Mexico for technical assistance.

References

Aruoma, O. I. (2003). Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutation Research, 523–524*, 9–20.

Cazor, A., Deborde, C., Moing, A., Rolin, D., & This, H. (2006). Sucrose, glucose, and fructose extraction in aqueous carrot root extracts prepared at different temperatures by means of direct NMR measurements. *Journal of Agricultural and Food Chemistry, 54*, 4681–4686.

Cespedes, C. L., El-Hafidi, M., Pavon, N., & Alarcon, J. (2008). Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis* (Elaeocarpaceae), Maqui. *Food Chemistry, 107*, 820–829.

Cespedes, C. L., Alarcon, J., Valdez, M., & Paredes-López, O. (2009). Antioxidant activity of an unusual 3-hydroxyindole derivative isolated from fruits of *Aristotelia chilensis* (Mol) Stuntz. *Zeitzchrift fur Naturforschung C*, 64c.

Diplock, A. T., Charleux, J. L., Crozier-Willi, G., Kok, F. J., Rice-Evans, C., Robefroid, M., Stahl, W., & Viña-Ribes, J. (1998). Functional food science and defence against reactive oxidative species. *Britain Journal of Nutrition, 80*(Suppl. 1), S77–S112.

Dominguez, M., Nieto, A., Marin, J. C., Keck, A-S., Jeffery, E., & Céspedes, C. L. (2005). Antioxidants activities of extracts from *Barkleyanthus salicifolius* (Asteraceae) and *Penstemon gentianoides* (Scrophulariaceae). *Journal of Agricultural and Food Chemistry, 53*, 5889–5895.

Dugo, P., Mondello, L., Morabito, D., & Dugo, G. (2003). Characterization of anthocyanins fraction of Scicilian blood orange juice by Micro-HPLC-ESI/MS. *Journal of Agricultural and Food Chemistry, 51*, 1373–1376.

Ehlenfeldt, M. K., & Prior, R. L. (2001). Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *Journal of Agricultural and Food Chemistry, 49*, 2222–2227.

El-Hafidi, M., & Baños, G. (1997). In vivo plasma lipid oxidation in sugar-induced rat hypertriglyceridemia and hypertension. *Hypertension, 30*, 624–628.

Escribano-Bailon, M. T., Alcalde-Eon, C., Muñoz, O., Rives-Gonzalo, J. C., & Santos-Buelga, C. (2006). Anthocyanins in berries of Maqui (*Aristotelia chilensis* (Mol) Stuntz). *Phytochemical Analysis, 17*, 8–14.

Fernandes, E., Costa, D., Toste, S. A., Lima, J. L. F. C., & Reis, S. (2004). *In vitro* scavenging activity for reactive oxygen and nitrogen species by nonsteroidal anti-inflammatory indole, pyrrole and oxazole derivative drugs. *Free Radical Biology and Medicine, 37*, 1905–1985.

Halliwell, B., & Aruoma, O. I. (1991). DNA damage by oxygen derived species. Its mechanism and measurement in mammalian systems. *FEBS Letters, 281*, 9–19.

Halliwell, B., & Gutteridge, J. M. C. (1990). Role of free radicals and catalytic metal ions in human disease. *Methods in Enzymology, 186*, 1–85.

Haslam, E. (2007). Vegetable tannins – lessons of a phytochemical lifetime. *Phytochemistry, 68*, 2713–2721.

Hillebrand, S., Schwarz, M., & Winterhalter, P. (2004). Characterization of anthocyanins and pyranoanthocyanins from blood orange [*Citrus sinensis* (L.) Osbeck] juice. *Journal of Agricultural and Food Chemistry, 52*, 7331–7338.

Jeffery, E. H., & Araya, M. (2009). Physiological effects of broccoli consumption. *Phytochemistry Review, 8*, 283–298.

Keesey, J. (1987). In *Biochemica information* (1st ed., p. 58). Indianapolis, IN, USA: Boehringer Mannheim Biochemicals.

Ko, F. N., Cheng, Z. J., Lin, C. N., & Teng, C. M. (1998). Scavenger and antioxidant properties of prenylflavones isolated from *Artocarpus heterophyllus*. *Free Radical Biology and Medicine, 25*, 160–168.

Kraft, T., Grace, M., Yousef, G., Rogers, R., Raskin, I., & Lila, M. (2007). Phytochemical composition and aldose reductase inhibitory activity of *Aristotelia chilensis* (maqui) berries. *FASEB Journal, 21*(5), A732.

Lee, H. S. (2002). Characterization of major anthocyanins and the color of red-fleshed Budd blood orange (*Citrus sinensis*). *Journal of Agricultural and Food Chemistry, 50*, 1243–1246.

Lo, K. M., & Cheung, P. C. K. (2005). Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. alba. *Food Chemistry, 89*, 533–539.

Longo, L., & Vasapollo, G. (2005). Determination of anthocyanins in *Ruscus aculeatus* L. berries. *Journal of Agricultural and Food Chemistry, 53*, 475–479.

Mabry, T. (1980). *Structural determination of flavonoids by UV, IR and NMR spectral data*. Academic Press.

Makris, D. P., & Rossiter, J. T. (2001). Comparison of quercetin and non-orthohydroxy flavonol as antioxidants by competing in vitro oxidation reactions. *Journal of Agricultural and Food Chemistry, 49*, 3370–3377.

Mata-Bilbao, M. L., Andres-Lacueva, C., Jauregui, O., & Lamuela-Raventos, R. M. (2007). Determination of flavonoids in a citrus fruit extract by LC-DAD and LC-MS. *Food Chemistry, 101*, 1742–1747.

Merken, H. M., & Beecher, G. R. (2000). Measurement of food flavonoids by high performance liquid chromatography: A review. *Journal of Agricultural and Food Chemistry, 48*, 576–599.

Miranda-Rottmann, S., Aspillaga, A. A., Perez, D. D., Vasquez, L., Martınez, A. L. F., & Leighton, F. (2002). Juice and phenolic fractions of the berry *Aristotelia chilensis* inhibit LDL oxidation in vitro and project human endothelial cells against oxidative stress. *Journal of Agricultural and Food Chemistry, 50*, 7542–7547.

Ng, T. B., Liu, F., & Wang, Z. T. (2000). Antioxidative activity of natural products from plants. *Life Sciences, 66*, 709–723.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4626.

Pereira, G. E., Gaudillere, J. P., Van Leeuwen, C., Hilbert, G., Lavialle, O., Maucourt, M., et al. (2005). ^{1}H-NMR and chemometrics to characterize mature grape berries in four wine-growing areas in Bordeaux, France. *Journal of Agricultural and Food Chemistry, 53*, 6382–6389.

Pokorny, J., Davidek, J., Tran, H. C., Valentova, H., Matejicek, J., & Dlaskova, Z. (1988). Reactions of oxidized lipids with protein. Part 15. Mechanism of lipoprotein formation from interactions of oxidized ethyl linoleate with egg albumin. *Nahrung, 32*, 343–350.

Pool-Zobel, B. L., Bub, A., Schröder, N., & Rechkemmer, G. (1999). Anthocyanins are potent antioxidants in model systems but do not reduce endogenous oxidative DNA damage in human colon cells. *European Journal of Nutrition, 38*, 227–234.

Prior, R. L., Cao, G. H., Martin, A., Sofic, E., McEwen, J., O'Brien, C., et al. (1998). Antioxidant capacity as influenced by total phenolics and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry, 46*, 2686–2693.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Rahman, M. M., Ichiyanagi, T., Komiyama, T., Hatano, Y., & Konishi, T. (2006). Superoxide radical and peroxynitrite-scavenging activity of anthocyanins; structure-activity relationship and their synergism. *Free Radical Research, 40*, 993–1002.

Rice-Evans, C. (2000). Measurement of total antioxidant activity as a marker of antioxidant status in vivo: Procedures and limitations. *Free Radical Research, 33*, 559–566.

Rice-Evans, C., Miller, N. J., Bolwell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Research, 22*, 375–383.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Silva, M., Bittner, M., Cespedes, C., & Jakupovic, J. (1997). The alkaloids of the genus *Aristotelia. Aristotelia chilensis* (Mol.) Stuntz. *Boletin de la Sociedad Chilena de Quimica, 42*, 39–47.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols an d other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Smith, M. A. L., Marley, K. A., Seigler, D. S., Singletary, K. W., & Meline, B. (2000). Bioactive properties of wild blueberry fruits. *Journal of Food Sciences, 65*, 352–356.

Taruscio, T. G., Barney, D. L., & Exon, J. (2004). Content and profile of flavonoids and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. *Journal of Agricultural and Food Chemistry, 52*, 3169–3176.

Veglioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Wang, Sh. Y., & Jiao, H. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *Journal of Agricultural and Food Chemistry, 48*, 5677–5684.

Yan, X., Murphy, B. T., Hammond, G. B., Vinson, J. A., & Nieto, C. C. (2002). Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *Journal of Agricultural and Food Chemistry, 50*, 5844–5849.