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Extracts of Maqui (*Aristotelia chilensis*) and Murta (*Ugni molinae* Turcz.): Sources of Antioxidant Compounds and α-Glucosidase/α-Amylase Inhibitors

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Supporting Information

ABSTRACT: The objective of this work was to evaluate the antioxidant and antihemolytic activities of crude, aqueous, and organic-aqueous extracts of maqui (*Aristotelia chilensis*) and murta (*Ugni molinae* Turcz.), together with their inhibiting effect on enzymes involved in the metabolism of carbohydrates. Radical scavenging activity, inhibition of linoleic acid oxidation in a micellar system, antihemolytic activity, and inhibition of α-amylases and α-glucosidases were analyzed. Crude extracts of maqui leaves and fruits were found to be important sources of polyphenolic compounds, showing 69.0 ± 0.9 and 45.7 ± 1.1 mg GAE/g dm, respectively. Polyphenols from maqui leaves were active as antioxidants and antihemolytic compounds ($p < 0.05$), showing a noncompetitive inhibiting effect on α-glucosidase. Flavan-3-ol polymers and glycosylated flavonols, such as quercetin glucoside and kaempferol glucoside, were tentatively identified in extracts. This preliminary observation provides the basis for further examination of the suitability of polyphenol-enriched extracts from maqui and murta as nutritional or medicinal supplements with potential human health benefits.

KEYWORDS: Murta (*Ugni molinae* Turcz.), maqui (*Aristotelia chilensis*), antioxidant, antihemolytic effect, digestive enzymes inhibitor, polyphenols

INTRODUCTION

Murta (*Ugni molinae* Turcz.) and maqui (*Aristotelia chilensis* (Mol.) Stuntz) are native Chilean shrubs that have been used by Mapuche, Puelche, and Pehuenche people in folk medicine to treat various digestive disorders such as diarrhea and dysentery.[1] Traditionally, infusions prepared with murta leaves and branches have been consumed to treat urinary tract infections and diabetes in Chile.[2−4] In addition, it has been reported that murta presents anti-inflammatory characteristics.[5,6] Murta has also been cultivated in Australia and New Zealand for a few years, because of the market value of its berries, which possess a pleasant flavor and aroma.[7] Likewise, the leaves and also the small, edible, although astringent, purple/black berries of maqui have been used in Chilean folk medicine as anti-inflammatory, antihemorrhagic, and antipyretic agents, and also their use was considered as helpful for cardiac disorders and migraine.[1,8] Maqui berries can also be useful as a natural colorant due to the presence of anthocyanin pigments.[9]

Recent studies have focused on these native plants and include reports on the phenolic composition of murta leaf extracts, which consist of hydroxybenzoic acids, flavan-3-ols, and glycosylated flavonols;[10] descriptions of the anti-inflammatory effects attributed to active compounds, which were identified as triterpene acids;[5] analgesic properties associated with both flavonoid glucosides and triterpenoids;[6] demonstration of the protective properties against oxidative damage of human erythrocytes using hypochlorous acid as oxidant;[5] and antimicrobial activity. Regarding the latter, leaf extracts were particularly active in preventing the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* bacteria.[11]

Chemical analyses of maqui leaves have shown the presence of quinoline[12] and indole-type alkaloids.[13] Maqui fruits are a potential source of anthocyanins, delphinidin and cyanidin glucosides,[9] and phenolics showing high antioxidant capacity. Miranda-Rottmann et al.[14] suggested that maqui fruit may have antiatherogenic properties. Recently, increasing attention has being paid to the phytochemical profile of berry extracts and their in vitro antioxidant activity, which was strongly correlated with total polyphenol content.[15]

Polyphenolic compounds are known to be useful in formulating nutritional or medicinal supplements for the treatment of several diseases.[16] An important activity of polyphenols is the inhibition of digestive enzymes, especially carbohydrate-hydrolyzing enzymes

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such as α-amylase and α-glucosidase.[17] Inhibitors of these enzymes are able to retard carbohydrate digestion, thus causing a reduction in glucose absorption rate.[18] Effective α-amylase and α-glucosidase polyphenol-type inhibitors from natural resources have been reported to be useful in reducing postprandial hyperglycemia.[19,20] To our knowledge, the inhibition of α-glucosidase and α-amylase by polyphenolic compounds from murta and maqui has not been reported yet.

The focus of this study was to perform a screening of different activities of polyphenol-enriched extracts and fractions from the above-mentioned Chilean native plants. Therefore, the objective of this research was to evaluate the antioxidant and α-amylase and α-glucosidase inhibiting activities of extracts obtained from murta and maqui stems, leaves, and fruits. The antioxidant capacity, measured by radical scavenging activity (using the DPPH method), and the lipid oxidation inhibiting activity, using linoleic acid as substrate, were evaluated for aqueous and organic-aqueous extracts from these plants. The ability of polyphenolic compounds to prevent peroxidative hemolysis of human erythrocytes (antihemolytic activity) was also determined.The inhibitory activity on digestive enzymes was assessed by measuring the decrease of reducing sugars released by either α-amylase or α-glucosidase on specific substrates. Finally, a tentative identification of polyphenolic compounds from murta and maqui extracts was done using high-performance liquid chromatography−mass spectrometry (HPLC-MS).

MATERIALS AND METHODS

Crude Extract. Plant material (fruit, leaves, and stems) from murta and maqui was collected in January 2008 from the Andes Mountains near the town of Villarrica in the Araucanía Region (Chile). The collected material was dried (at 35 °C) to constant weight, ground in a coffee grinder, and sieved (Retsch, Germany). The fraction with particle size between 250 and 500 μm was used for the extract. Samples (5 g) were macerated with ethanol (50% v/v in water, solvent-to-solid ratio of 5:1) using a mortar and pestle at room temperature and then filtered through Whatman no. 1 filter paper (Whatman International Ltd., Maidstone, U.K.). Filtrate was concentrated in a rotary evaporator at 35 °C (Büchi R3000, Germany) and lyophilized for 24 h (Uniequip Unicryo MC4L-60, Germany). In the experiments carried out for testing the antioxidant activity and enzyme inhibition, the solid was dissolved in the same solvent that was previously used for extraction.

Fractionation of Crude Extracts. Fractionation was performed using the method described by Rubilar et al.[21] Briefly, the lyophilized crude extract (LCE) (1 g) was defatted with petroleum ether (3 × 10 mL) and the solvent evapored in a rotary evaporator. Then, defatted LCE was suspended in 10 mL of distilled water. After the addition of acetic acid (50 μL), the monomeric and oligomeric components were extracted with ethyl acetate (5 × 2.5 mL) from the acidified aqueous mixture, to obtain an aqueous fraction called fraction A. After separation of these phases, fraction A was lyophilized. Solvent from the organic fraction was evaporated under vacuum and the residue suspended in water. The resulting suspension was then filtered through a sintered glass filter. The residue was washed with deionized water, and the filtrates were pooled, centrifuged, and then lyophilized to yield an organic-aqueous fraction (fraction B), which contained those species soluble in both ethyl acetate and water.

Determination of Total Polyphenols. Total phenolic content was determined through reaction with Folin−Ciocalteu (FC) reagent (Fluka, Japan), following the method reported by Velioglu et al.[22] Briefly, solutions of LCE and fractions A and B in deionized water were prepared to give a final concentration in the range of 25−400 mg/L. Each solution (200 μL) was mixed with 1 mL of FC reagent (which had previously been diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 800 μL of Na_2CO_3 (60 g/L) (Sigma-Aldrich, Germany) solution was added to the mixture. After the tubes had been heated for 15 min at 45 °C in a water bath, they were allowed to cool in the dark for 30 min, after which the absorbance was measured at 765 nm (Genesys 6, Thermo Scientific, USA). The results were expressed as gallic acid equivalents per gram of dry mass (mg GAE/g dm).

DPPH Method. The radical scavenging activity was determined by using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma-Aldrich, Germany).[23] Solutions of LCE and fractions A and B in deionized water were prepared to give a final concentration in the range of 5−400 mg/L. An aliquot of each solution (3.2 mL) was mixed with 800 μL of 400 μM DPPH in ethanol. After 30 min of incubation in the dark, the absorbance was measured at 520 nm. The radical scavenging activity was obtained from interpolation into a calibration curve performed with Trolox (Calbiochem, Germany) solutions as standards (0−250 μg of Trolox/mL). The antioxidant activity was expressed as IC_{50}, which was defined as the final concentration of the tested sample required for the inhibition of DPPH radical by 50%.

Inhibition of AAPH Induced by Linoleic Acid Oxidation. Inhibition of lipid peroxidation was determined according to the method of Hamauzu and Iijima.[24] Solutions of LCE and of fractions A and B in deionized water were prepared to give a final concentration in the range of 25−100 mg/L. The assay was prepared by mixing 4 mL of mixture reagent (75 mL of linoleic acid in 100 mL of 0.1 M SDS in 10 mM phosphate buffer, pH 7.4), 0.02 mL of 2% 2,2′-azobis(2-methylpropionamidine)dihydrochloride (AAPH) (Sigma-Aldrich, Germany), and 0.3 mL of each solution of LCE and fractions. The rate of oxidation was monitored by recording the increase in absorption at 233 nm (Genesys 6, Thermo Scientific, USA) caused by conjugated diene hydroperoxide formation at 50 °C for 90 min. Data were interpolated in a calibration curve using Trolox solutions as standards. Antioxidant activity was expressed as mg of Trolox equivalents per gram of dry mass of plant material (mg TE/g dm) used for extraction. Measurements were run with the buffer as blank and compared to a separate AAPH-free control to check for spontaneous oxidation. AAPH shows a relatively high absorbance below 260 nm; therefore, its absorbance was subtracted from each experimental value.

Determination of Antihemolytic Activity. The preventive action of plant extracts against the hemolytic effect of H_2O_2 solutions on washed human red blood cells was examined by the in vitro test of Naim et al.[25] and Yuan et al.,[26] with slight modifications. A human blood sample (4.5 mL) was obtained, erythrocytes were separated from the plasma, and the buffy coat was separated by centrifugation (Z300 Hermle Labortechnik, Germany) and washed three times by centrifugation in 10 volumes of 10 mM saline or isotonic sodium phosphate buffer (pH 7.4).[25] The supernatant and buffy coat of white cells were removed with each wash. During the last washing, the erythrocytes were obtained by centrifugation and resuspended in the same buffer to give a 4% v/v suspension of erythrocytes. Solutions of LCE and of fractions A and B in deionized water were prepared to give a final concentration in the range of 25−500 mg/L. Each solution (0.6 mL) was mixed with 1.0 mL of the erythrocyte suspension, and then 0.4 mL of 0.5% H_2O_2 was added to induce the oxidative degradation of the membrane. The mixture was incubated for 120 min at 37 °C. After centrifuging (2500 rpm, 10 min), the extent of hemolysis (hemoglobin liberation) was determined by measuring the absorbance at 540 nm. The results were expressed as the sample concentration (mg/L) required to achieve 50% of hemolytic activity inhibition (IC_{50}).

α-Amylase Inhibition Assay. The α-amylase inhibition assay was performed using the chromogenic method adopted from Sigma-Aldrich. Briefly, porcine pancreatic α-amylase (EC 3.2.1.1, type VI, Sigma-Aldrich, Germany) was dissolved in ice-cold distilled water to a

concentration of 4 U/mL. Potato starch (0.5%, w/v) in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride was used as substrate. Solutions of LCE in deionized water were prepared to give a final concentration in the range of 25−100 mg/L. The concentration range of fractions A and B varied between 25 and 750 mg/L. An aliquot of each solution (200 μL) and 400 μL of starch solution were mixed and preincubated for 5 min. The reaction was started by adding 200 μL of the enzyme solution; the reaction mixture was then incubated at 37 °C for 3 min. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M NaOH). The test tubes were incubated in a boiling water bath for 15 min and then cooled to room temperature. After the addition of 3.6 mL of deionized water, absorbance was measured at 540 nm. The same reaction mixture using Acarbose (Glucobay Bayer, Chile), without LCE or fraction solution, was used as a control. Preliminary experiments were carried out to establish optimal conditions using 0.25% (w/v) starch, 1 U/mL of α-amylase, and 1 mg/mL of inhibitor concentration. The α-amylase inhibition was calculated as

$$\%\ \text{inhibition} = \frac{A_C - A_E}{A_C} \times 100$$

where A_E represents the sample absorbance and A_C the control sample absorbance. The results were expressed as the sample concentration (mg/L) required to inhibit 50% of the enzyme activity (IC_{50}).

α-Glucosidase Inhibition Assay. The α-glucosidase inhibitory assay was performed according to the chromogenic method described by Kim et al.[27] with slight modifications, using α-glucosidase from baker's yeast (G-5003, Sigma-Aldrich, Germany). Solutions of LCE in deionized water were prepared to give a final concentration in the range of 25−100 mg/L. The concentration of fractions A and B was established between 25 and 500 mg/L. Fifty microliters of α-glucosidase (0.6 U/mL) was premixed with each LCE or fraction solution (50 μL) at various concentrations prepared in 67 mM sodium phosphate buffer containing 100 mM NaCl. The mixture was preincubated for 10 min at 37 °C and pH 6.8. The enzymatic reaction was initiated by adding 125 μL of 10 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) (N-1377, Sigma-Aldrich, Germany); the mixture was then incubated at 37 °C for 30 min. The reaction was stopped by adding 5.9 mL of 100 mM Na_2CO_3 solution. The activity of α-glucosidase was determined by measuring the absorbance of *p*-nitrophenol released at 400 nm. The same reaction mixture using Acarbose (Glucobay Bayer, Chile) (without LCE or fraction solution) was used as a control. The α-glucosidase inhibitory activity was calculated using a relationship similar to the one used for α-amylase inhibition.

Inhibition Type of Maqui Leaf Extract. To examine the inhibition mode of maqui leaf crude extract, α-amylase and α-glucosidase activities were measured with increasing substrate concentrations in the absence and presence of maqui leaf extract, which was added at several concentration values. α-Amylase activity was quantified by measuring the maltose equivalents released from starch at 540 nm. α-Glucosidase activity was quantified by measuring the *p*-nitrophenol equivalents released from pNPG at 400 nm. The Michaelis−Menten constant (K_m), maximum enzyme reaction rate (V_{max}), and inhibition mode of maqui leaf crude extract on the α-amylase-catalyzed hydrolysis of starch and on the α-glucosidase-catalyzed reaction of pNPG were obtained from Lineweaver−Burk linearization of data.

Chromatographic Analysis. As a preliminary assay, thin layer chromatography (TLC) of plant crude extracts, fractions A and B, was carried out according to the method of Jerez et al.[28] Samples were applied to silica gel 60 F254 plates (20 cm × 20 cm, Merck, Darmstadt, Germany), and plates were developed with toluene−acetone−formic acid (3:6:1 v/v/v). Separated components were first viewed under UV light at 365 nm and then by spraying with 5% vanillin dissolved in ethanol, which was acidified with 10% HCl, followed by heating with hot air. The flavans and procyanidins were revealed as orange to reddish spots.

Table 1. Total Polyphenols, Radical Scavenging Activity, Inhibition of Linoleic Acid Oxidation, and Antihemolytic Activity of Crude Extracts from Murta and Maqui Leaves, Fruits, and Stems and Their Aqueous Fraction (Fraction A) and Organic-Aqueous Fraction (Fraction B)

		crude extract	fraction A	fraction B
		total polyphenol (mg GAE/g dm)		
murta	leaves	32.5 ± 3.1	29.1 ± 1.8	2.4 ± 0.3
	fruits	10.1 ± 1.6	7.7 ± 1.1	1.7 ± 0.3
	stems	15.8 ± 0.2	11.9 ± 0.5	*a*
maqui	leaves	69.0 ± 0.9	15.9 ± 0.7	5.6 ± 0.7
	fruits	45.7 ± 1.1	11.0 ± 0.8	*a*
	stems	25.8 ± 0.3	1.1 ± 0.2	2.0 ± 0.5
		IC_{50} DPPH (mg of extract/L)		
murta	leaves	21.6 ± 0.2	18.0 ± 0.8	13.8 ± 0.4
	fruits	82.9 ± 1.9	>400	25.2 ± 0.4
	stems	42.3 ± 1.2	40.2 ± 0.5	*a*
maqui	leaves	8.0 ± 0.1	26.9 ± 0.8	29.4 ± 1.2
	fruits	399.8 ± 17.5	>400	*a*
	stems	43.1 ± 1.7	17.7 ± 0.5	29.5 ± 0.0
		inhibition of linoleic acid oxidation (mg TE/g dm)		
murta	leaves	7.6 ± 0.3	10.9 ± 1.1	0.5 ± 0.1
	fruits	6.9 ± 0.4	0.3 ± 0.2	1.0 ± 0.2
	stems	3.0 ± 0.7	4.8 ± 1.8	*a*
maqui	leaves	5.1 ± 0.6	4.1 ± 0.6	0.8 ± 0.1
	fruits	4.6 ± 0.8	31.2 ± 7.2	*a*
	stems	1.7 ± 0.3	0.7 ± 0.1	0.1 ± 0.0
		IC_{50} antihemolytic activity (mg of extract/L)		
murta	leaves	233.4 ± 2.7	>500	>500
	fruits	127.8 ± 6.4	>500	>500
	stems	364.3 ± 17.6	479.6 ± 45.7	*a*
maqui	leaves	35.8 ± 2.5	409.8 ± 33.3	92.3 ± 3.2
	fruits	>500	280.0 ± 40.4	*a*
	stems	>500	>500	160.2 ± 43.2

[a] Insufficient amount of lyophilized sample for analysis.

Then, crude extracts were analyzed by HPLC-MS as described by Rubilar et al.[21] Samples were filtered through a 0.45 μm nylon filter and injected (20 μL) into an HPLC-DAD system (Jasco UV-1575, Japan). A C18 Hypersil ODS column (250 mm × 4.6 mm, 5 μm particle size, Supelco) was used. Flow rate was set to 0.7 mL/min. The solvents used were 0.5% acetic acid−water solution (A) and methanol (B). The elution gradient was as follows: 0−10 min, 95A/5B; 10−60 min, 50A/50B; 60−80 min, 30A/70B; and 80−90 min, 95A/5B. Detection was performed at 280 nm. Three determinations were made on each sample. The equipment used for electrospray mass spectrometry in the positive ion mode was a HP 1100-MSD series. The conditions were as follows: nitrogen was used as the drying gas at 13 L/min and 350 °C with nebulizer pressure at 40 psig and fragmentor voltage at 60 V.

Statistical Analysis. All determinations were performed at least in triplicate; average values and standard deviations (±SD) were calculated. Statistical tests were carried out to analyze correlations between two variables, and the Student *t* test was applied to determine the

significance of variable association. The level of significance adopted in the statistical test was 95.0% ($p < 0.05$).

RESULTS AND DISCUSSION

Total Polyphenol Content. As shown in Table 1, all crude extracts from murta and maqui showed a higher total polyphenols content than their derived fractions. Among the murta crude extracts, the stem crude extract had a higher polyphenolic content than the crude extract of fruit. The opposite was found for maqui crude extracts, total polyphenolic content being higher in the fruit than in the stems. The crude extract of maqui leaves (69.0 ± 0.9 mg GAE/g dm) was richer in polyphenols than that of murta leaves (32.5 ± 3.1 mg GAE/g dm). The same observation was obtained with the crude extract of maqui stems, which contained a total polyphenol concentration of 25.8 ± 0.3 mg GAE/g dm, higher than the 15.8 ± 0.2 mg GAE/g dm measured in the crude extract from murta stems. Additionally, when the yields in polyphenols from fruit extracts were compared, the total polyphenolic content in crude extracts from maqui fruit (45.7 ± 1.1 mg GAE/g dm) was also much higher than that of crude extracts from murta fruit (10.1 ± 1.6 mg GAE/g dm).

In the analysis of the total content of polyphenols in fractions from murta and maqui leaf extracts, fractions A showed a higher content than fractions B, as shown in Table 1. The total content of polyphenols in fraction A from murta leaves (29.1 ± 1.8 mg GAE/g dm) was very close to that of crude extracts from murta leaves, thus indicating that the polyphenolic compounds had a high affinity for the aqueous phase. It is worth noting that when both fractionations were carried out with murta stems or maqui fruit crude extracts, the amount of the lyophilized nonaqueous fraction (fraction B) was not enough for polyphenol content determination, showing a negligible yield.

Radical Scavenging Activity. The results regarding free radical scavenging capacity of maqui and murta crude extracts indicated that maqui leaf crude extracts were those more active, showing an IC_{50} value of 8.0 ± 0.1 mg/L (Table 1). The concentration of murta leaf crude extract required for inhibiting 50% of the initial DPPH radical concentration was 2- and 4-fold lower than stem and fruit crude extracts concentration, respectively. With regard to maqui extracts, the required leaf crude extract concentration to inhibit 50% of DPPH was 5-fold lower than that required for stem and 50-fold lower than for fruit crude extracts. Fraction B, obtained from murta leaf crude extracts, showed the highest radical scavenging activity, with an IC_{50} of 13.8 ± 0.4 mg/L; however, this value was not better than that of crude extract of maqui leaves (Table 1). Fraction A obtained from murta and maqui fruits was not able to reach 50% inhibition of DPPH in the concentration range tested.

The polyphenol content and radical scavenging activity of the crude extract of maqui leaves and fraction B of murta leaves presented correlation coefficients (r) equal to 0.996 and 0.989, respectively. This positive and high linear relationship cause/effect means that as the polyphenol content in the extract increased, the radical scavenging activity also increases. By transforming the r values to Student t values are obtained t_{exptl} of 29.17 and 16.73, respectively, higher than t_{theor} of 2.447. Therefore, the association between these variables was significant ($p < 0.05$).

In general, for all murta and maqui crude extracts and their fractions, the polyphenol content and radical scavenging activity presented a significant correlation ($p < 0.05$) except fraction A of maqui fruit and stem.

Table 2. Effect of Crude Extracts of Murta and Maqui Leaves, Fruits, and Stems and Their Aqueous Fraction (Fraction A) and Organic-Aqueous Fraction (Fraction B) on α-Amylase and α-Glucosidase Inhibition

		crude extract	fraction A	fraction B
		IC_{50} α-amylase inhibition (mg of extract/L)		
murta	leaves	79.5 ± 3.1	110.1 ± 1.9	165.0 ± 30.2
	fruits	>100	>750	>750
	stems	56.6 ± 1.2	>750	*a*
maqui	leaves	>100	314.2 ± 2.9	521.5 ± 7.6
	fruits	41.5 ± 3.6	>750	*a*
	stems	>100	>750	>750
		IC_{50} α-glucosidase inhibition (mg of extract/L)		
murta	leaves	12.5 ± 1.8	153.0 ± 3.8	215.7 ± 6.3
	fruits	69.2 ± 5.0	457.8 ± 9.1	61.3 ± 7.0
	stems	39.1 ± 5.2	108.5 ± 1.1	*a*
maqui	leaves	6.1 ± 0.9	139.1 ± 4.7	2.4 ± 0.3
	fruits	47.9 ± 2.7	197.3 ± 4.2	*a*
	stems	1.1 ± 0.1	112. 3 ±16.5	189.4 ± 27.7

[a] Insufficient amount of lyophilized sample for analysis.

Inhibition of AAPH Induced by Linoleic Acid Oxidation. The analysis of crude extracts of murta and maqui showed that leaf crude extracts had the highest antioxidant activity, with values of 7.6 ± 0.3 mg TE/g dm for murta and of 5.1 ± 0.6 mg TE/g dm for maqui (Table 1).

Higher activities were obtained in the fractions A from murta leaves and maqui fruit, showing values of 10.9 ± 1.1 and 31.2 ± 7.2 mg TE/g dm, respectively. Therefore, the fractionation of crude extracts allowed for increasing inhibitory activity of lipid oxidation for these raw materials. Previous studies showed that antioxidant compounds of a hydrophilic nature obtained from murta leaves were more effective in lipid systems,[10] in agreement with the results presented in this study.

Polyphenol content in fractions A of murta leaves and maqui fruit and antioxidant activity were linearly correlated ($p < 0.05$), showing correlation coefficients of 0.763 and 0.853, respectively. The coefficient of determination indicates that about 58% of antioxidant activity in aqueous fractions of murta leaves and 72% of antioxidant activity in aqueous fractions of maqui fruit are due to the polyphenol content in the extracts.

Determination of Antihemolytic Activity. The maqui leaf crude extract showed the highest antihemolytic activity with an IC_{50} of 35.8 ± 2.5 mg/L (Table 1). In contrast, the antihemolytic activity of murta leaf crude extract was low, showing a IC_{50} value of 233.4 ± 2.7 mg/L. Maqui leaf crude extracts were more active because one-sixth of the concentration of murta leaf crude extract was required to achieve 50% inhibition of hemolytic activity induced on erythrocytes.

It is concluded that crude extracts from maqui leaves were more active than those obtained from murta. With regard to the polyphenols−erythrocyte interaction that may explain this activity, Chaudhuri et al.[29] suggested that several polyphenols contained in plant extracts may interact with the erythrocyte cell membrane, reducing the flow and dissemination of free radicals in it, thus preventing deterioration.

As shown in Table 1, some crude extracts (maqui fruit and stem) were not able to inhibit 50% of the hemolytic activity in the

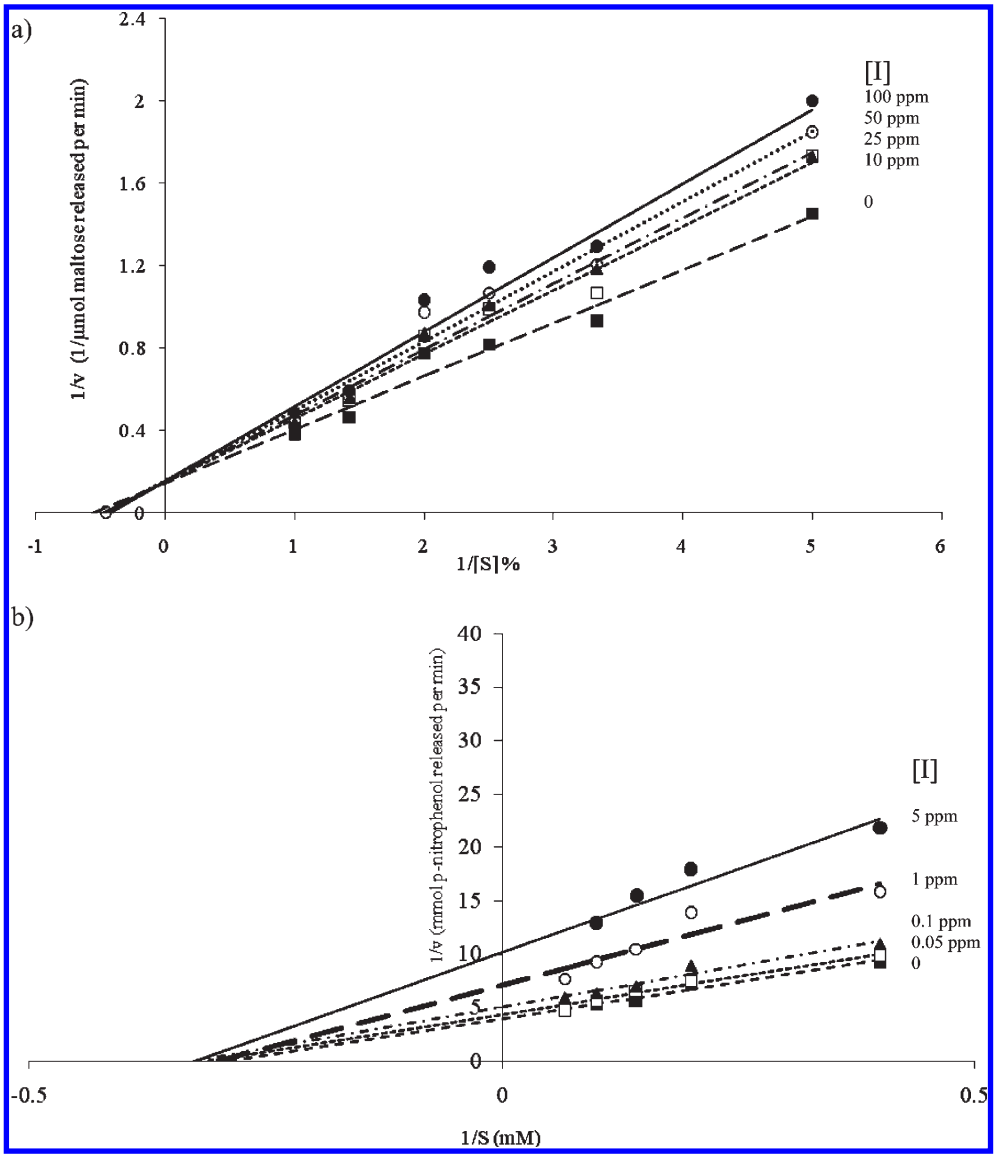

Figure 1. (a) Double-reciprocal plot of the initial velocity v of the hydrolysis reactions catalyzed by α-amylase at various substrate concentrations [S] in the absence and presence of several maqui leaf extract concentrations [I]. The maqui leaf extract concentration was (■) 0 ppm, (□) 10 ppm, (▲) 25 ppm, (○) 50 ppm, or (●) 100 ppm. (b) Double-reciprocal plot of the initial velocity v of the hydrolysis reactions catalyzed by α-glucosidase at various pNPG concentrations [S] in the absence and presence of several maqui leaf crude extract concentrations [I]. The maqui leaf crude extract concentration was (■) 0 ppm, (□) 0.05 ppm, (▲) 0.1 ppm, (○) 1 ppm, or (●) 5 ppm.

tested concentration range, demonstrating further the importance of fractionation in those cases.

The polyphenol content of maqui leaf crude extracts and antihemolytic activity present a linear correlation (correlation coefficient equal to 0.750) and a significant correlation ($p < 0.05$) between variables.

α-Amylase Inhibition. α-Amylase inhibiting activities are shown in Table 2. The crude extract of maqui fruit was the most active in inhibiting α-amylase because it showed the lowest IC_{50} value (41.5 ± 3.6 mg/L).

Also remarkable was the capacity of crude extract of murta stem to inhibit α-amylase, an IC_{50} value of 56.6 ± 1.2 mg/L being obtained. In contrast with these results, the majority of the extracts were not able to inhibit 50% of α-amylase activity in the tested concentration range (Table 2).

A positive control was analyzed together with plant extracts and fractions, using acarbose as specific inhibitor; its effectiveness was observed when obtaining an IC_{50} value of 3.4 mg/L. In general, the trend of plant extracts tested was a weak inhibition of α-amylase compared to acarbose.

The polyphenolic content of crude extract of maqui fruit and the inhibition of α-amylase showed a strong linear correlation (correlation coefficient of 0.854), and the inhibiting activity of the crude extracts from maqui fruit may be explained by their polyphenolic content of 73%; however, their correlation could be not significant ($p > 0.05$) because t values were at the limit of acceptance ($t_{exptl} = 4.028 < t_{theor} = 4303$).

α-Amylase is one of the main enzymes in humans responsible for the hydrolysis of α-1,4-glycosidic internal links in starch, producing simple sugars such as glucose, maltose, and dextrins. The inhibitors of this enzyme can delay carbohydrate digestion and reduce the rate of glucose absorption. Consequently, these α-amylase inhibitors can decrease postprandial plasma glucose levels

Table 3. Kinetic Properties of Maqui Leaf Crude Extract on α-Amylase and α-Glucosidase

α-amylase			α-glucosidase		
maqui leaf extract (ppm)	K_m (%)	V_{max} (μM/min)	maqui leaf extract (ppm)	K_m(mM)	V_{max} (mM/min)
0	1.8	6.81	0	3.5	0.25
10	2.1	6.75	0.05	3.2	0.22
25	2.1	6.48	0.1	3.1	0.20
50	2.2	6.41	1	3.3	0.14
100	2.3	6.33	5	3.1	0.10
mode of inhibition	mixed type		mode of inhibition	noncompetitive type	

and improve glucose tolerance in diabetes patients. Several side effects related with the intake of synthetic α-amylase inhibitors have been reported including abdominal distension, flatulence, bloating, and possible diarrhea. Studies carried out by Apostolidis et al.[19] have shown that a possible cause of these effects might be the excessive inhibition of α-amylase, which gave as a result an abnormal colon bacteria fermentation of undigested carbohydrates; this effect is claimed to be one of the disadvantages of using acarbose. For this reason, a current trend is the search for natural alternatives that could complement the action of specific inhibitors. Future studies will be focused on evaluating the possible side effects of natural inhibitors through in vivo assays of crude extracts from maqui and murta.

α-Glucosidase Inhibition. The effect of crude extracts and their respective fractions on α-glucosidase inhibition is shown in Table 2. The results are expressed as IC_{50}. The most active inhibitor of α-glucosidase was crude extract of maqui stems with an IC_{50} of 1.1 ± 0.1 mg/L, followed by crude extract of maqui leaves with an IC_{50} of 6.1 ± 0.9 mg/L. Crude extracts of murta and maqui fruit showed IC_{50} values of 69.2 ± 5.0 and 47.9 ± 2.7 mg/L, respectively. In contrast, acarbose showed a IC_{50} value of 247.4 mg/L, indicating that it is unable to block the active center.

α-Glucosidase is a key enzyme for metabolizing nonabsorbable oligosaccharides into absorbable monosaccharides in the small intestine. Inhibition of this enzyme could delay the digestion of oligosaccharides and disaccharides to monosaccharides, diminishing glucose absorption and consequently reducing prostprandial hyperglycemia.

Therefore, natural and strong α-glucosidase inhibitors from native plant sources such as maqui and murta could be an attractive strategy for controlling hyperglycemia and the development of functional food for diabetes patients.

Inhibition Type by Maqui Leaf Crude Extract. We found that polyphenols from maqui leaves showed an inhibitory effect on α-glucosidase and α-amylase activities, strong and weak, respectively. Because polyphenols from maqui leaves were more active in most of the activities tested, the next step was obtaining more information about this plant matrix and the inhibition mode of maqui leaf crude extract against both enzymes. To accomplish this objective, the initial hydrolysis reaction rate of α-amylase was determined for several substrate concentrations [S] (0.2−1% starch) in the absence and presence of maqui leaf extract concentrations [I] (10−100 ppm), as indicated in Figure 1a. The Michaelis−Menten constant (K_m) for α-amylase was found to be 1.8% starch, with a V_{max} value of 6.8 μM min^{-1} (Table 3). The double-reciprocal plots showed that straight lines were obtained with starch substrates for α-amylase. The results indicated that maqui leaf crude extracts act as mixed-type inhibitors, binding to either α-amylase (E) or enzyme−substrate (ES) complex, resulting in a decrease in the apparent affinity of α-amylase for starch (increase in K_m) and a decrease in the apparent maximum enzyme reaction rate (V_{max}). A similar type of acarbose inhibition and for the two acarbose analogues for porcine pancreatic α-amylases was reported by Yoon and Robyt.[30] Similar behavior is reported in the literature for finger millet seed coat phenolics, which behave as noncompetitive inhibitors on pancreatic α-amylase, the K_m value for this enzyme being about 1% starch.[31]

The initial reaction rate of α-glucosidase was measured for substrate concentrations [S] in the range of 2.5−15 mM pNPG, either in the absence or in the presence of maqui leaf extract concentrations [I] (0.05−5 ppm), as indicated in Figure 1b. The Michaelis−Menten constant (K_m) for α-glucosidase was found to be 3.5 mM starch, with a V_{max} value of 0.25 mM min^{-1} (Table 3). The results of this study indicate that binding of phenolic compounds to enzyme affected the velocity of the α-glucosidase reaction rate, proportionally to the concentration of the phenolic compounds in the reaction mixture, not modifying the K_m value. Thus, a noncompetitive inhibition by maqui leaf crude extracts upon α-glucosidase-catalyzed pNPG hydrolysis was found in this study. Reversible noncompetitive inhibition of α-glucosidase is reported in the literature for aqueous extracts from the gall of *Rhus chinensis* (AEGRC).[32] α-Glucosidase inhibition by several flavonoids has been reported as mixed-type and almost noncompetitive.[33]

Polyphenolic Compounds by Chromatographic Analysis. In this study, a tentative identification by thin-layer chromatography (TLC) and HPLC-MS of the polyphenolic compounds from murta and maqui extracts was carried out.

The identification of the peaks was made by HPLC-MS on the basis of the chromatographic data and by comparison with standards and the literature. Retention times, spectral characteristics (λ_{max}), and m/z in positive ions are presented for the respective compounds in the Supporting Information. Spectral characteristics (λ_{max}) were obtained from the samples for which the signals of the respective compounds were most intense and pure; m/z values with masses in parentheses refer to the assumed structural units that rendered the experimentally found fragments. Sugar moieties linked to a phenol moiety found in both plant matrices were the following: glucose or galactose (162 mass units), rhamnose (146), xylose or arabinose (132), all of them naturally occurring in plants.

Crude extracts and fractions B from maqui leaves and crude extract of maqui stems and maqui fruit were analyzed. In relation to murta species, crude extract of leaves and its fraction B were analyzed.

High and low molecular weight procyanidins were separated from the most active crude extracts and fractions by means of TLC. Procyanidins with high molecular weight appeared under UV light as dark spots. The results of the TLC showed that the extracts contain procyanidins of high molecular weight such as flavan-3-ol polymers. In contrast, procyanidins of low molecular weight appeared as reddish spots with vanillin in acid medium. TLC results showed that only fraction B of murta leaves contained flavan-3-ols in their monomeric form (catechin or epicatechin) and their derivatives. In the assay, vanillin joins carbons 6 and 8 of the A benzene ring of tannin. A higher content of monomers (catechin and epicatechin) provides a more intense color, whereas color is less intense in the presence of flavan-3-ol dimers or polymers (procyanidins).

Crude extracts of maqui leaves and fraction B of murta leaves showed the presence of flavan-3-ol polymers and flavonols such as glycosylated quercetin and glycosylated kaempferol, according to HPLC-MS identification (Supporting Information).

Phenolic compounds, such as hydroxybenzoic acids, flavan-3-ol monomers, and polymers were found in the crude extract of murta leaves (Supporting Information); a monomer (epi)catechin with a mass of m/z 291 was also identified. The identification of phenolic compounds in these two crude extracts through HPLC-MS (Supporting Information) showed the presence of procyanidins, glycosylated quercetin, and quercitrin. It is worth noting that quercitrin was not found in the other crude extracts.

Identification of phenolic compounds through HPLC-MS (Supporting Information) showed the same composition for crude extracts of murta stem and maqui fruit: mainly flavan-3-ol polymers and the flavonols quercetin and myricetin. TLC assays revealed that these extracts contained high molecular weight hydrophilic compounds that remained on the baseline of the plate. In addition, the crude extract of maqui fruit was free of monomer flavan-3-ols and their derivatives, whereas the crude extract of murta leaves revealed reddish spots.

Chromatographic profiles obtained through HPLC-MS (Supporting Information) of crude extract of maqui leaves, fraction B of maqui leaves, and crude extract of maqui stems showed that these extracts contained proanthocyanidins and quercetin. The results of TLC assay indicated that the three extracts contained high molecular weight compounds and flavan-3-ol polymers (dark spots). However, only the crude extract of maqui stems contained low molecular weight compounds.

Furthermore, the evaluation of antioxidant activity, digestive enzyme inhibition, and the phenolic compound profiling in some extracts and their fractions represent a preliminary approach to the those potential biological properties of murta and maqui.

Although quantification, bioactivity, and contribution of each polyphenol in these native plants have not been evaluated yet, our findings support the use of native Chilean shrubs as sources of biological compounds for future applications. Considering that, to date, there have not been reported studies about nonantioxidant activities of murta and maqui; these results might be an important contribution to the study of the in vitro biological activities of these plants matrices, which are used in traditional medicine.

Further research is required and it continues in our laboratory to evaluate the role of the compounds identified in murta and maqui, which are responsible for the biological activities reported in this study.

ABBREVIATIONS USED

GAE/g dm,gallic acid equivalents per gram of dry matter; DPPH,2,-2-diphenyl-1-picrylhydrazyl; A,aqueous; OA,organic-aqueous; HPLC-MS,high-performance chromatography−mass spectrometry; TLC,thin layer chromtography; AAPH,2,2′-azobis(2-methylpropionamidine)dihydrochloride; mg TE/g dm,mg of Trolox equivalents per gram of dry mass; IC_{50},concentration of extract required to inhibit 50% of enzyme activity.

ASSOCIATED CONTENT

Supporting Information. Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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