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Murta Leaves (*Ugni molinae* Turcz) as a Source of Antioxidant Polyphenols

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Extracts from Murta leaves are used by Chilean natives for their benefits on health and cosmetic properties, which are mainly due to the presence of polyphenolic compounds. Extraction of such compounds is strongly influenced by several variables, the effects of which are studied in this work; the antioxidant power of the resulting extracts was measured by two different methods [2,2-diphenyl-1-picrylhydrazyl (DPPH) and thiobarbituric acid reactive substances (TBARS)]. On the whole, maximum values of polyphenolic yields and antiradical power (DPPH method) were attained at 50 °C (from 25 to 50 °C) and a solvent-to-solid ratio (v/w) of 15:1 (15:1−25:1). The solvents assayed were ethanol, methanol, and water. The highest polyphenolic yield values (2.6% expressed as gallic acid) were reached with methanol, whereas maximum EC_{50} was attained by the ethanol extract (0.121 mol gallic acid/mol DPPH). Contact time was shown to have only a slight influence in alcoholic extraction, while in water a remarkable effect of increasing contact times (30−90 min)was observed. Just water was the solvent that offered the best result when the antioxidant power was measured by the TBARS method. High-performance liquid chromatography−mass spectrometry analysis revealed the presence of polyphenols, basically flavonols and flavanols, sometimes glycosilated; myricetin and quercetin glycosides were detected in all extracts, whereas epicatechin was present in alcoholic extracts and gallic acid was only present in water.

KEYWORDS: Murta (*Ugni molinae* Turcz.); polyphenols; DPPH; TBARS; HPLC-MS

INTRODUCTION

Murta is a wild shrub growing in the south of Chile, especially in the Coast Mountains and in part of the pre-Andean mountains. Although it was originally named "Myrtus ugni" (after the vernacular name "Uñi"), nowadays, it is scientifically known as *Ugni molinae*, from Juan Ignacio Molina (1737−1829). It was identified and classified by Western botanics for the first time in 1844. Despite the fact that very scarce scientific literature can be found about the characterization and identification of its components, Chilean natives have transmitted and tapped the cosmetic and health-beneficial properties of its extracts for centuries. Alcoholic beverages and infusions made from leaves are commonly used to lessen urinary tract pain, and they also act as astringents, stimulants, and phytoestrogenic substances (*1*). Furthermore, a great number of cosmetic products containing extracts of murta leaves have appeared in the Chilean market in the past several years. The antioxidant and phytoestrogenic properties of such extracts could prevent the oxidative processes responsible for the premature aging of skin tissues.

The properties of extracts obtained from murta are in part due to the presence of different phenolic compounds (*2*), and their ability to scavenge free radicals has been widely reported in recent scientific works. These free radicals play a main role in the oxidative processes taking place in the human body and in lipid-containing foods. By inhibiting the generation of these nonstable species, polyphenolic compounds can prevent the progression of a great number of diseases such as arteriosclerosis and cancer (for apoptosis induction) and also behave as antiviral agents against some diseases such as diarrhea, arthritis, influenza, and poliomyelitis (*3*, *4*). Besides, several studies showed that these species can be successfully employed to delay fish oil degradation and cholesterol oxidation in commercial meat products such as pork sausage, raw and roast ham, bacon, and hamburgers (*5*, *6*).

Although the latter considerations strengthen the viability of polyphenolic extraction from murta leaves, the economical feasibility of an eventual industrial process must be supported by an efficient extraction procedure. Previous findings have reported the influence of some variables (e.g., temperature, contact time, solvent-to-solid ratio, etc.) on the yields of phenolics capable of being extracted from diverse natural matrixes such as almond hulls, pine sawdust, or grape byprod-

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Table 1. Extraction Conditions of the Experimental Design[a]

experiment	t	T	L/S	t	T	L/S
1	30	25	25	–1	–1	+1
2	30	50	25	–1	+1	+1
3	30	25	15	–1	–1	–1
4	30	50	15	–1	+1	–1
5	90	25	25	+1	–1	+1
6	90	50	25	+1	+1	+1
7	90	25	15	+1	–1	–1
8	90	50	15	+1	+1	–1
9	60	37.5	20	0	0	0
10	60	37.5	20	0	0	0
11	60	37.5	20	0	0	0
12	60	37.5	20	0	0	0

[a] Not coded/coded variables.

ucts (*7*, *8*). The positive or negative role of each factor in mass transfer phenomena involved in the process is not always obvious; chemical characteristics of the solvent and the diverse structure and composition of the natural products ensure that each material−solvent system shows different behaviors, which cannot be predicted.

For this reason, a study about the effect of both the above-mentioned variables and the solvents employed on the total soluble solids, phenolics yield, and antioxidant capacity of extracts is undertaken in this work. Conditions maximizing antioxidant activity of extracts will be considered as the optimal ones. Finally, because very few scientific studies on murta can be found and practically none on identification can be found, a characterization of the polyphenolic compounds responsible for the properties of Murta extracts will be carried out by high-performance liquid chromatography−mass spectrometry (HPLC-MS) analysis.

MATERIALS AND METHODS

Raw Material. Samples of murta leaves (*Ugni molinae* Turcz.) were supplied by "Estación Experimental INIA Carillanca-Chile" and were not subjected to any pretreatment before extraction. They were stored at room temperature until use. The solvents used to achieve extraction were analytical grade methanol, 96° ethanol (Sudelab-Chile), and distilled water.

Experimental Design. A full factorial 2^3 experimental design was developed to evaluate the influence of the temperature (T), time of contact (t), and solvent-to-solid ratio (L/S) on the extraction process to which Murta leaves were subjected (*9*). Temperature values varied between 25 and 50 °C, the contact time varied between 30 and 90 min, and the solvent-to-solid ratio varied between 15:1 and 25:1 (v/w). Variables were coded in the way that their value ranged between +1 and −1, taking, as a central point, the zero value. So

$$t = (t - 60)/30 \quad (1)$$

$$T = (T - 37.5)/12.5 \quad (2)$$

$$\mathrm{L/S} = (\mathrm{L/S} - 20)/5 \quad (3)$$

Table 1 shows the factorial design matrix, with variables in both coded/noncoded form, for better comprehension. Experiments 9−12 are relevant to the central point of the experimental design, which was repeated four times. Numbers 1−12 corresponded with those in **Tables 2**−**4**.

Data were adjusted to a response surface R:

$$R = a_0 + a_1 t + a_2 T + a_3 \mathrm{L/S} + a_{12} tT + a_{13} t\mathrm{L/S} + a_{23} T\mathrm{L/S} + a_{123} tT\mathrm{L/S} \quad (4)$$

where a_0 is the value of the objective function in the central point conditions, a_1, a_2, and a_3 represent the principal effects associated to each variable, and the other ones represent the crossed effects among variables.

Extraction Processes. *Characterization in Extractable Compounds.* The maximum weight of total extracted substances was assessed after extraction in Soxhlet, employing boiling ethanol as a solvent during 8 h. After the solvent was evaporated in an Arquimed Rotavapor RV05-ST, values were determined by increasing the weight of the flasks. All of the results were expressed as the percentage of the initial amount of employed sample in a dry basis. The humidity (8.9 ± 0.98%) was assessed by maintaining the murta leaves in a stove at 105 °C until constant weight.

Determination of Soluble Solids after Batch Extraction. Samples and solvent were disposed in capped flasks (solvent-to-solid ratios of 15:1, 20:1, and 25:1) and maintained at 140 rpm in a GFL-3032 orbital incubator shaker. At the time fixed for each experiment, a filtration was realized, and the solvent was evaporated of the liquid phase, after determining the solids gravimetrically.

Determination of Phenolic Compounds. The total phenolics were assayed colorimetrically by means of the Folin−Ciocalteu method, as modified by Singleton and Rossi (*10*). A 2.5 mL amount of 10-fold diluted Folin−Ciocalteu reagent, 2 mL of 7.5% sodium carbonate, and 0.5 mL of phenolic extract were mixed well. The absorbance was measured at 765 nm after 15 min of heating at 45 °C. A mixture of water and reagents was used as a blank. The content of phenolics was expressed as gallic acid equivalents, and the yield was referred to the initial sample, in a dry basis.

Antioxidant Activity. The antioxidant power will be assessed by using two different methods. In the first, the ability of phenolic compounds to scavenge free radicals will be expressed as a function of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition percentage, or by EC_{50}. Because different tests usually lead to different results, the antioxidant power was also determined by thiobarbituric acid reactive substances (TBARS) assay, which measures the capacity of polyphenols to inhibit the oxidation processes on a lipidic substrate.

DPPH Radical Scavenging Capacity. The hydrogen-donating ability of the crude extract was determined by the method described by Von Gadow et al. (*11*). A volume of 1.85 mL of 6.1×10^{-5} M DPPH methanol solution was used. The reaction was started by the addition of 150 μL of sample. The bleaching of DPPH was followed at 515 nm (Spectronic Génesis 5) for 16 min at 25 °C. The inhibition percentage (IP) of the DPPH radical was calculated as follows:

$$\mathrm{IP} = \frac{(\mathrm{absorbance}_{t=0\mathrm{min}} - \mathrm{absorbance}_{t=16\mathrm{min}})}{\mathrm{absorbance}_{t=0\mathrm{min}}} \times 100 \quad (5)$$

The values of DPPH radical scavenging capacity were expressed as an inhibition percentage in order to compare the efficiency of the different experimental design conditions and to select the optimal ones. However, when these values were higher than 90%, the incomplete exhaustion of the antioxidant (once reacted with all DPPH free radical) probably took place. In this case, the dilution of extracts and the assessment of the inhibition percentage of diluted samples were advisable (*12*); the resultant antioxidant capacity was expressed as the quantity of active compound needed to decrease the initial DPPH concentration by 50% and was named EC_{50}, which was expressed as mol gallic acid/mol DPPH. This parameter was used to compare the antioxidant capacity of optimal extracts obtained with the three solvents employed.

TBARS Assay. Freeze-dried samples obtained by using the optimal extraction conditions of the experimental design were analyzed by using the TBARS assay (*13*) in order to measure the ability of extracts to inhibit lipid peroxidation at pH 7.4. A liposome system from egg lecithin, as described by Miyake et al. (*14*), was used. The experiments were conducted in a physiological saline buffer PBS (pH 7.4) made of 3.4 mM Na_2HPO_4−NaH_2PO_4 and 0.15 M NaCl. Together with the buffer, the corresponding extract to measure, a 0.5 mg/mL solution of phospolipid liposomes and 100 μM of $FeCl_3$ was added. The reaction was started by the addition of 100 μM ascorbate, and the reactive mixture was incubated at 37 °C for 60 min, after adding 0.1 mL of 2% (w/v) butylated hydroxytoluene (BHT), 1 mL of 1% (w/v) thiobarbituric acid (TBA), and 2.8% (w/v) trichloroacetic acid. The resulting solutions

Table 2. Percentage of Total Soluble Solids from Murta Leaves Subjected to the Extraction Conditions of the Experimental Design[a]

experiment	solvent used for extraction		
(t, T, L/S)	ethanol	methanol	water
1 (–, –, +)	18.69 ± 1.52	16.36 ± 0.66	5.84 ± 0.15
2 (–, +, +)	30.96 ± 0.82	31.54 ± 0.32	11.68 ± 0.82
3 (–, –, –)	14.37 ± 0.39	22.43 ± 1.96	8.41 ± 0.09
4 (–, +, –)	21.73 ± 0.84	24.18 ± 1.47	10.51 ± 0.14
5 (+, –, +)	26.87 ± 1.29	30.96 ± 0.82	11.68 ± 0.23
6 (+, +, +)	**36.80 ± 0.70**	**39.72 ± 0.18**	**20.44 ± 3.80**
7 (+, –, –)	17.87 ± 0.49	29.09 ± 0.49	8.76 ± 2.45
8 (+, +, –)	30.84 ± 0.19	30.49 ± 1.47	19.03 ± 0.98
9 (0, 0, 0)	13.08	20.56	8.22
10 (0, 0, 0)	13.08	24.30	7.97
11 (0, 0, 0)	14.95	22.43	8.40
12 (0, 0, 0)	14.02	20.56	8.11

[a] The highest values are in bold.

Table 3. Percentage of Total Phenolic Compounds from Murta Leaves Subjected to the Extraction Conditions of the Experimental Design[a]

experiment	solvent used for extraction		
(t, T, L/S)	ethanol	methanol	water
1 (–, –, +)	0.19 ± 0.01	0.53 ± 0.12	0.03 ± 0.00
2 (–, +, +)	0.79 ± 0.11	1.29 ± 0.04	0.06 ± 0.01
3 (–, –, –)	0.23 ± 0.03	0.59 ± 0.06	0.06 ± 0.00
4 (–, +, –)	0.82 ± 0.03	1.47 ± 0.07	0.15 ± 0.06
5 (+, –, +)	0.55 ± 0.02	1.17 ± 0.14	0.10 ± 0.00
6 (+, +, +)	1.55 ± 0.07	**2.64 ± 0.16**	**0.25 ± 0.02**
7 (+, –, –)	0.62 ± 0.01	1.42 ± 0.06	0.07 ± 0.03
8 (+, +, –)	**1.79 ± 0.11**	2.53 ± 0.00	0.15 ± 0.04
9 (0, 0, 0)	0.87	1.94	0.07
10 (0, 0, 0)	0.97	2.28	0.11
11 (0, 0, 0)	0.94	2.52	0.06
12 (0, 0, 0)	1.00	2.38	0.06

[a] Highest values are in bold.

were heated in a water bath at 80 °C for 20 min to promote the formation of a pink pigment resulting from the reaction with malondyaldehyde [$(MDA)_2$−TBA]. The chromogen was extracted into 2 mL of butan-1-ol, and the extent of peroxidation was measured at 532 nm in the organic layer. Results were expressed as EC_{50}, g/L extract.

HPLC-MS Analysis. Filtered crude extracts (20 μL) were directly injected into the HPLC system. The reverse-phase HPLC apparatus with a pump PU-980 connected to a quaternary gradient unit LG-1580-04, a JASCO UV-1575 UV−vis detector, and a Rheodyne model 7725 loading sample injector with a 20 μL sample loop were used to determine the phenolic composition of the different fractions. The column (250 mm × 4.6 mm) was a C_{18} Hypersil ODS (5 μm particle size) (Supelco).

The two solvents used to make the gradient were (A) 0.5% acetic acid Milli-Q water solution and (B) methanol. The solvent gradient in volumetric ratios of solvents A and B 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 carried out using 280 nm as a preferred wavelength. The flow rate was set to 0.7 mL/min. Three determinations were made on each extract obtained.

The equipment used for electrospray mass spectrometry in positive ion mode was a HP-Serie1100-MSD, working with nitrogen as the drying gas at 13 L/min and 350 °C, nebulizer pressure at 40 psig, and fragmentor voltage at 60 V. Murta extracts were dissolved in ethanol, methanol, and water, filtered through a 0.45 μm nylon filter, and then injected at 10 μL volume.

Statistical Analysis. The results reported in this work are the average of at least three measurements, and the coefficients of variation, expressed as the percentage ratio between standard derivations (SD) and the mean values, were found to be ≤10 in all cases. Significant variables were calculated, subjecting results to a linear regression, using SPSS statistical program version 11 (SPSS Inc., Chicago, IL). Only variables with a confidence level superior to 95% ($p < 0.05$) were considered as significant.

RESULTS AND DISCUSSION

Results from characterization in extractable compounds of murta leaves (Soxhlet) indicate that the highest yield in extractable compounds (44%) corresponded to methanol, followed closely by ethanol (40%) and water (34%). The same tendency was found when samples were subjected to the extraction conditions of the experimental design (**Table 2**). In this case, higher values of soluble solids were reached at the conditions of experiment 6. Slight differences were found between these values and those obtained by using Soxhlet (∼3−5%) when alcohols were used, indicating the practically complete exhaustion of soluble solids and the high efficiency of the batch extraction under certain experimental conditions. For water, however, the differences between Soxhlet and batch

Table 4. Inhibition Percentage (DPPH Assay) from Murta Leaves Subjected to the Extraction Conditions of the Experimental Design[a]

experiment	solvent used for extraction		
(t, T, L/S)	ethanol	methanol	water
1 (–, –, +)	22.75 ± 0.86	47.24 ± 1.47	2.50 ± 0.54
2 (–, +, +)	78.20 ± 3.56	94.66 ± 0.32	7.00 ± 1.77
3 (–, –, –)	45.84 ± 5.88	85.48 ± 5.56	8.31 ± 2.05
4 (–, +, –)	**95.09 ± 0.43**	**95.26 ± 0.11**	35.47 ± 0.50
5 (+, –, +)	65.24 ± 0.80	94.07 ± 1.40	9.78 ± 0.98
6 (+, +, +)	94.18 ± 0.54	94.68 ± 0.09	21.86 ± 0.43
7 (+, –, –)	93.75 ± 1.21	94.08 ± 0.11	16.41 ± 0.09
8 (+, +, –)	94.32 ± 0.97	94.25 ± 0.04	**93.48 ± 0.07**
9 (0, 0, 0)	94.16	95.01	10.6
10 (0,0, 0)	95.60	95.00	13.91
11 (0, 0,0)	95.18	95.29	7.47
12 (0, 0, 0)	95.31	94.72	7.34

[a] The highest are values in bold.

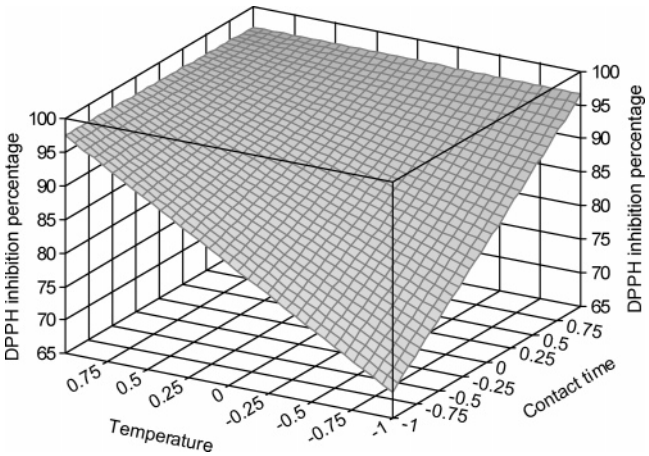

Figure 1. Response surface for DPPH inhibition percentage of Murta leaves extracts in methanol.

extraction were higher, attaining ∼15%. The results from **Table 2** show that increasing temperatures, once fixed other variables, favored extraction; this fact was expected, because it is recognized that high temperature enhances both the solubility of solute and the diffusion coefficient. Despite this, temperature cannot be increased indefinitely, because the stability of phenolic compounds can decrease and the denaturation of membranes can happen at temperatures higher than 50 °C (*15*). The solvent-to-solid ratio had also a positive effect on yields; in fact, the higher the solvent-to-solid ratio was, the higher the total amount

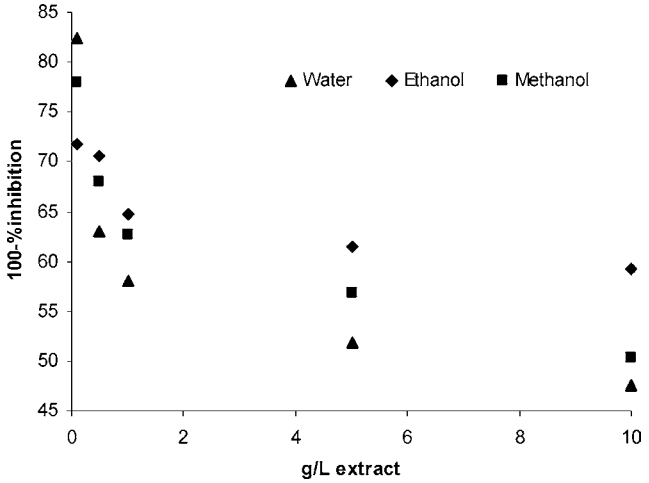

Figure 2. Values of peroxidation inhibition percentage for different phenolic concentrations obtained by TBARS assay. Extracts were obtained at the conditions at which the inhibition percentage was maximum (experiment 4 for alcohols and experiment 8 for water).

of solids extracted, despite the solvent used. This is consistent with mass transfer principles; the driving force during mass transfer is the concentration gradient between the solid and the bulk of the liquid, which is greater when a higher solvent-to-solid ratio is used. Similar results about the effect of temperature and solvent-to-solid ratio on the extraction of soluble solids were previously reported for milled berries by Cacace et al. (*15*), who found a linear relationship of temperature and L/S ratio with solids yield.

The yields of phenolic compounds obtained under the conditions of the experimental design are showed in **Table 3**. Also in this case, the highest values for phenolics were obtained with methanol (experiment 6). A value of 2.64% was attained for this solvent, being the ethanol top value 1.79%, at experiment 8. Values for water were noteworthily lower (0.25% in the best case, experiment 6). From comparing values of **Tables 2** and **3**, it can be deduced that methanol was also the most selective solvent for polyphenolic compounds, since the polyphenols: extractables ratio in this solvent was about two and six times higher than in ethanol and water extracts, respectively. In general, values of phenolic compounds yields were between 0.03 and 2.64 g/100 g residue, which were similar to those detected for other agricultural materials. As an example, oat hulls and apple byproducts contain 0.056 g/100 g solid and 0.11 g/100 g, respectively (*16*, *17*). Likewise, Pastrana-Bonilla et al. (*18*) reported values of 0.169 and 0.195 g total phenols/100 g residue for extracts of bronze (Early Fry) and purple (Paulk) Muscandine grapes, respectively. Even so, abundant literature supports the fact that the total phenols capable of being extracted with polar solvents (water, methanol, and ethanol) can vary largely as a function of the employed material, from values of 1.03×10^{-3} g/100 g solid for *G. avellana* hulls to 3.9 g/100 g solid found in buckwheat extracts (*19*, *20*). Response surfaces obtained (only

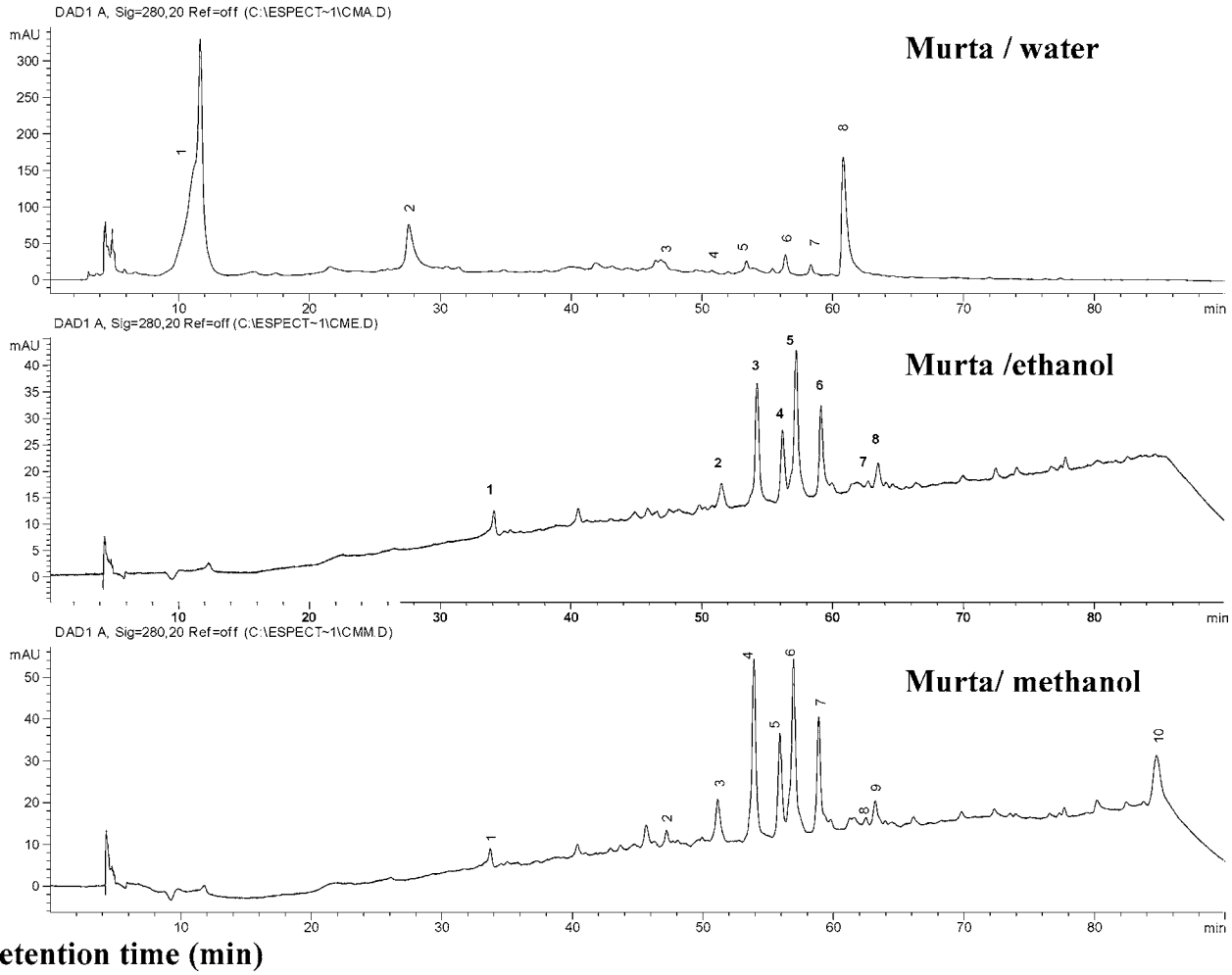

Figure 3. Chromatograms corresponding to aqueous, ethanol, and methanol extracts obtained in conditions at which the total phenol content was maximum (experiment 8 for ethanol and experiment 6 for water and methanol).

Table 5. Identification of Phenolic Species Contained in a Methanol Extract of Murta Leaves

peak no.	retention time (min)	λ_{max} (nm)	(*m*/*z*)	positive ion (*m*/*z*)	identification
1	33.7	280	291	(290 + 1)	epicatechin
2	47.2	260.3	319	(318 + 1)	myricetin
3	51.1	266.3	319	(318 + 146 + 146 + 23) 633	myricetin dirhamnoside
4	53.9	258.4	319	(318 + 162 + 23) 503	myricetin glucoside
5	55.9	258.4	303	(302 + 146 + 146 + 23) 617	quercetin dirhamnoside
6	56.9	260.3	319	(318 + 146 + 23) 487	myricetin rhamnoside
7	58.9	256.4	303	(302 + 162 + 23) 487	quercetin glucoside
8	62.4	264.3	287	(286 + 162 + 23) 471	kaempferol glucoside
9	63.2	258.3	303	(302 + 146 + 23) 471	quercetin rhamnoside
10	84.7	314			NI[a]

[a] Nonidentified.

Table 6. Identification of Phenolic Species Contained in an Ethanol Extract of Murta Leaves

peak no.	retention time (min)	λ_{max} (nm)	(*m*/*z*)	positive ion (*m*/*z*)	identification
1	34.1	280	291	(290 + 1)	epicatechin
2	51.1	266.3	319	(318 + 146 + 146 + 23) 633	myricetin dirhamnoside
3	53.9	258.4	319	(318 + 162 + 23) 503	myricetin glucoside
4	55.9	258.4	303	(302 + 146 + 146 + 23) 617	quercetin dirhamnoside
5	56.9	260.3	319	(318 + 146 + 23) 487	myricetin rhamnoside
6	58.9	256.4	303	(302 + 162 + 23) 487	quercetin glucoside
7	62.4	264.3	287	(286 + 162 + 23) 471	kaempferol glucoside
8	63.2	258.3	303	(302 + 146 + 23) 471	quercetin rhamnoside

Table 7. Identification of Phenolic Species Contained in an Aqueous Extract of Murta Leaves

peak no.	retention time (min)	λ_{max} (nm)	(*m*/*z*)	positive ion (*m*/*z*)	identification
1	11.7	271	171	(171 + 484 + 23) 678	gallic acid derivate
2	27.6	265	155.950		NI[a]
3	53.9	258.4	319	(318 + 132 + 23) 473	myricetin xyloside
4	51.1	266.3	319	(318 + 146 + 146 + 23) 633	myricetin dirhamnoside
5	53.9	258.4	319	(318 + 162 + 23) 503	myricetin glucoside
6	56.9	260.3	319	(318 + 146 + 23) 487	myricetin rhamnoside
7	58.9	256.4	303	(302 + 162 + 23) 487	quercetin glucoside
8	58.9	254.4	303	(302 + 132 + 23) 457	quercetin xyloside

[a] Nonidentified.

significant variables are indicated) for polyphenolics yields were

$$\%\ \text{polyphenols}_{\text{ethanol}} = 0.860 + 0.309t + 0.419T + 0.124tT \quad (6)$$

where $F_{\text{mod}} = 70.207$ and $p = 0.000$.

$$\%\ \text{polyphenols}_{\text{methanol}} = 1.730 + 0.484t + 0.530T \quad (7)$$

where $F_{\text{mod}} = 8.602$ and $p < 0.008$.

$$\%\ \text{polyphenols}_{\text{water}} \times 10^2 = 9.773 + 3.353t + 4.538T + 2.948t\text{L/S} \quad (8)$$

where $F_{\text{mod}} = 10.483$ and $p < 0.004$.

As can be observed, temperature and contact time were significant in all cases but no effect of solvent-to-solid ratio as single variable was found. Ethanol and aqueous models include mixing effects, time−temperature in ethanol equation and time-solvent-to-solid ratio in the aqueous one.

In **Table 4**, the DPPH inhibition percentage values for the Murta leaves extracts are shown. The highest values corresponded to experiment 4 for alcohols, while experiment 8 was the optimal when water was used as a solvent (in this case, variability was considerable). The only difference between conditions of both experiments is the extraction time, keeping the same values of temperature (50 °C) and solvent-to-solid ratio (15:1). Alcohols therefore do not deserve further than 30 min extraction times, while increasing times from 30 to 90 min favored the achievement of aqueous extracts with enhancing antioxidant activity. Despite the differences in the nature of the plant matrix, the same effect of contact time was observed in previous studies; no differences in inhibition percentage of methanol and ethanol extracts from almond hulls (*Prunus amygdalus*) were observed with further extraction times. However, significant increases were found when water was used as a solvent (*8*). For either alcohols or water, higher values of temperature favored the increase of the DPPH inhibition percentage. The influence of each variable on the inhibition percentage of extracts was expressed in the following equations:

$$\%\ \text{inh}_{\text{ethanol}} = 80.80 + 13.20t + 16.78T \quad (9)$$

where $F_{\text{mod}} = 5.24$ and $p < 0.027$.

$$\%\ \text{inh}_{\text{methanol}} = 89.98 + 6.81t + 7.25T - 7.05tT \quad (10)$$

where $F_{\text{mod}} = 7.23$ and $p < 0.037$.

$$\%\ \text{inh}_{\text{water}} = 19.51 + 15.10T - 14.07\text{L/S} \quad (11)$$

where $F_{\text{mod}} = 6.54$ and $p < 0.020$.

In **Figure 1**, the response surface for the extracts obtained using methanol at L/S = 15 is plotted as an example. As can be observed, the influence of temperature on the DPPH

inhibition percentage of the extracts decreases concomitantly with increasing values of contact time and vice versa. Because inhibition percentage values for alcohols were similar and higher than 90%, successive dilutions of extracts obtained in the optimal conditions for the three solvents were made in order to assess the value of EC_{50} (see Materials and Methods). Little differences were found between EC_{50} values of alcoholic extracts (0.121 and 0.140 mol gallic acid/mol DPPH for ethanol and methanol, respectively), while a lower antioxidant activity was detected in the aqueous one (0.211). Values of EC_{50} for optimal extracts in each solvent were also determined by means of the TBARS method, and from **Figure 2**, an opposite tendency to that from DPPH assay can be deduced. As can be observed, ethanol did not reach a 50% inhibition, whereas the aqueous extract was the most efficient, $EC_{50} = 7.0$ g/L (for methanol, $EC_{50} = 10$ g/L). Once more, the polar paradox is observed when alcoholic and water extracts are subjected to TBARS assay. The fact that polar antioxidants are more effective in lipidic systems was previously observed for other plant extracts (*21*). It is explained in basis of the protective effect exerted by the air−lipidic interface created between the lipid system and the hydrophilic extract against the air contact oxidation. By contrast, in absence of this protection, the oxidation processes are more active (*22*, *23*).

HPLC-MS analysis showed the differences in polyphenolic species extracted by using the different solvents. In **Figure 3** and **Tables 5**−**7**, the chromatograms and the corresponding data about polyphenolic compounds detected for water, ethanol, and methanol, respectively, are shown. Differences can be noted in two aspects, phenolic composition and polymerization degree of the detected compounds. In the former aspect, gallic acid was only detected in water extract, while epicatechin and kaempferol derivatives were extracted exclusively with alcohols as solvents. Myricetin rhamnoside, myricetin dirhamnoside, myricetin glucoside, and quercetin glucoside were detected in all solvents, although in water in a low percentage. Many of these compounds identified in Murta extracts are similar to those found in other similar agricultural materials (*8*, *17*, *20*).

Referred to the aspect of polymerization, an ascendant trend from 50 min is observed for alcohols. Often, these "ascending wide peaks" are explained in basis of the presence of complex polymeric compounds, where polyphenols are joined among them or with other compounds such as sugars, proteins, etc. (*24*). So, besides the different phenolic composition, differences in antioxidant activity between alcoholic and water extracts could be supported by the presence of these complex polymeric compounds.

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