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Phenolic composition and antioxidant capacity of *Ugni molinae* Turcz. leaves of different genotypes

Marcelo Peña-Cerda [a], Jorge Arancibia-Radich [a], Paula Valenzuela-Bustamante [a], Rodrigo Pérez-Arancibia [a], Andrés Barriga [b], Ivette Seguel [c], Lorena García [d], Carla Delporte [a,*]

[a] Laboratorio de Productos Naturales, Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, PO Box 8380492, Santos Dumont 964, Santiago, Chile
[b] Unidad de Espectrometría de Masa, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, PO Box 8380492, Sergio Livingstone 1007, Santiago, Chile
[c] Instituto Nacional de Investigaciones Agropecuarias (INIA, Carillanca, Chile), PO Box 58-D, Temuco, Chile
[d] Advanced Center for Chronic Diseases (ACCDis), Departamento de Bioquímica y Biología Molécular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, PO Box 8380492, Sergio Livingstone 1007, Santiago, Chile

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ABSTRACT

Ugni molinae Turcz. is a native shrub of Chile, known for its edible berries and its leaves, which have been the focus of recent attention, as a good source of phenolic compounds to be used in cosmetics and food products. The aim of this study was to assess the differences in the phenolic composition and antioxidant capacity of the ethanolic extracts from the leaves of 10 genotypes of *U. molinae*, that were cultivated under the same soil, climate and agronomical management. Antioxidant activity was assessed by complementary methods (ORAC-Fl, FRAP and DPPH assay), phenolic composition of each extract was analyzed by LC–MS. Phenolic and flavonoid total contents were determined by Folin-Ciocalteu and $AlCl_3$ methods. Significative differences were found by these methods, and ellagitannins, gallic acid derivatives and flavonols were identified as responsible for these differences, showing the influence of the genotype on the phenolic composition of *U. molinae* leaves.

1. Introduction

Ugni molinae Turcz. (Myrtaceae), commonly known as "murtilla", "murta", or "Chilean guava", is a small evergreen shrub that growns in the downhills of the Coastal and Andes Mountain Ranges in the south-central regions of Chile (Schreckinger, Lotton, Lila, & de Mejía, 2010). Murtilla fruits have gained a lot of attention from Chilean farmers due to a rising consumption of the berries in countries around the world. Moreover, the leaves have been traditionally consumed as infusions and liquors, since the Chilean indigenous people incorporated these preparations into their diet (Schreckinger et al., 2010), and also used them to treat infections and several types of pains (Montenegro, 2000).

The chemical composition of *U. molinae* leaves and fruits presents a rich variety of phenolic compounds (Junqueira-Gonçalves et al., 2015; Rubilar et al., 2006) and triterpenoids (Aguirre et al., 2006; Arancibia-Radich et al., 2016). It is known that the leaves specially possess very high levels of these compounds (Quilaqueo, Echeverría, Ihl, Bifani, & Mauri, 2012), and several studies have demonstrated the presence of different phenolic compounds, such as myricetin, quercetin and some of their glycosides; epicatechin; gallic acid derivatives and ellagitannins in the leaves ethanolic extracts (Avello et al., 2014; Rubilar et al., 2006).

These phenolic compounds are an essential part of the human diet and the focus of considerable interest due to their antioxidant properties. Naturally occurring antioxidants have the ability to prevent free radical tissue damage; by precluding formation of free radicals, scavenging them and promoting their decomposition (López-Alarcón & Denicola, 2013). Recent studies have also shown that these compounds exert their biological activity through the modulation of many cellular pathways (for example, NF-kB, AP-1 or Nrf-2) involved in metabolism, survival, proliferation and antioxidant defences (Forbes-Hernandez et al., 2016).

Free radicals are originated from endogenous or exogenous sources and their excessive production leads to oxidative stress,

* Corresponding author at: Laboratory of Natural Products, Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santos Dumont 964, PO Box 8380492, Santiago, Chile.

E-mail addresses: mpenac@ciq.uchile.cl (M. Peña-Cerda), jarancib@ciq.uchile.cl (J. Arancibia-Radich), p.valenzuelabustamante@gmail.com (P. Valenzuela-Bustamante), rpereza@postqyf.uchile.cl (R. Pérez-Arancibia), anbarr@gmail.com (A. Barriga), iseguel@inia.cl (I. Seguel), logarcia@ciq.uchile.cl (L. García), cdelpor@uchile.cl (C. Delporte).

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which is suggested to be an important factor in the pathophysiology of cardiovascular problems, atherosclerosis, neurodegenerative diseases, cancer, chronic renal failure, diabetes mellitus and other diseases (Young & Woodside, 2001).

Ethanolic extracts from *U. molinae* leaves have demonstrated *in vitro* antioxidant capacity (Rubilar et al., 2006, 2011; Shene et al., 2012). Due to this, they are being incorporated in cosmetics and as edible films in food products to increase their shelf life and durability (Gómez-Guillen et al., 2007; Hauser, Peñaloza, Rodríguez, Guarda, & Galotto, 2014; Quilaqueo et al., 2012). Also, regular intake of murtilla leaves infusions has proven to increase the ORAC value of human plasma (Avello & Pastene, 2005), and also to diminish lipid peroxidation and TBARS formation (Avello, Pastene, Bustos, Bittner, & Becerra, 2012).

Nevertheless, the phenolic composition and the closely related antioxidant capacity may vary under many circumstances, one of them being the genotype (Cardeñosa, Girones-Vilaplana, Muriel, Moreno, & Moreno-Rojas, 2016; Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005). In *murtilla*, a comparative study performed with fruits from three genotypes proved that total phenolic content (TPC) and antioxidant capacity changes significantly due to the influence of genotype and harvest year (Alfaro et al., 2013). Yet, none of these studies have been developed using the leaves, which according to literature, contain a higher content of phenolic compounds.

In 1996, the *Instituto Nacional de Investigaciones Agropecuarias* (INIA), started a domestication plan to introduce the berries of this native shrub in the markets, by investigating and improving their cultivation and production. INIA collected 100 accessions from 36 different sites, located between VII and X Regions of Chile. The genetic variability among the samples was confirmed by Random Amplified Polymorphic DNA (RAPD) and microsatellite detection, discriminating 45 different accessions (Ramos, Ravest, Méndez, & Hinrichsen, 2012). The berries are collected every year from this domesticated crops in the months of April–May, after which, the leaves are pruned and discarded, wasting a valuable source of phenolic compounds.

Based on this, the aim of this work was to comparatively investigate *in vitro* antioxidant activity and phenolic composition of the ethanolic extracts (ETEs) of *Ugni molinae* leaves from 10 genotypes obtained from INIA, cultivated under the same soil, climate and agronomical management, in order to correlate phenolic composition with antioxidant activity and to identify the most active antioxidant genotypes; therefore, those with greater projections in the food and cosmetic industries. Also, it is possible to provide an added value to the crops, since now both berries and leaves could be economically exploited, with no product losses in the process.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu reagent, aluminum chloride, iron chloride, sodium carbonate, sodium acetate, hexane, dichloromethane, ethyl acetate, ethanol, methanol, and the reference compounds quercetin and gallic acid were purchased from Merck S.A (Darmstadt, Germany). The reagents: 2,4,6-Tris(2-pyridil-)-s-triazine (TPTZ), 2, 2′-azobis(2-methylpropionadimine) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were purchased from Sigma-Aldrich Chemical (St. Louis, MO). In LC–MS analyses, Milli-Q Water was used for the mobile phase in all measurements, and Hibar Purospher Star RP-18 column was purchased from Merck S.A.

2.2. Plant material

Ten genotypes of *U. molinae* leaves grown under the same climate and soil conditions were obtained from the *Instituto Nacional de Investigaciones Agropecuarias* (INIA, Carillanca, Chile) in the month of May 2013. Normally, after fruits are harvested, leaves are pruned and discarded, so this study will also provide added value to the murtilla's cultivar. The leaves were preserved under the same agronomical management and recollected from different plants with a same height, in order to diminish the probability that the differences found among them were not due to the genotype alone.

The selected genotypes were those that presented a better relative agronomic fruit quality according to previous INIA studies. A voucher sample of each genotype was kept in the herbarium of the Faculty of Chemical and Pharmaceutical Sciences of University of Chile. The number of the selected accessions corresponds to 14-4 (SQF-22549), 19-1 (SQF-22554), 19-1^{HA} (SQF-22553), 8-2 (SQF-22571), ZF-18 (SQF-22550), 22-1 (SQF-22552), 23-2 (SQF-22556), 27-1 (SQF-22555), 31-1 (SQF-22551) and 19-2 (SQF-22557).

2.3. Preparation of ethanolic extracts (ETEs) from Ugni molinae leaves

Dried and grinded leaves (2.0 kg) were successively extracted by maceration at room temperature with hexane, dichloromethane, ethyl acetate and ethanol, to obtain HE, DCE, EAE and ETE's fractions respectively. We used ETEs in accordance to one study that compared the TPC of ethanol, methanol and water extracts from a sample of wild murtilla leaves, which showed that, under the same conditions, ethanol extracts have a greater TPC than aqueous extracts (Rubilar et al., 2006). So, ETEs of the 10 genotypes were selected for this study as a source of phenolic compounds, because it is well known that they posses a major phenolic composition and antioxidant capacity in comparison to others fractions.

2.4. Determination of total phenolic and flavonoid content

Total phenolic content (TPC) of the ETEs was determined by modifying the Folin-Ciocalteu method described by Cicco, Lanorte, Paraggio, Viggiano, and Lattanzio (2009) to perform it in a 96-well plate Thermo Scientific Multiskan GO Spectrophotometer. The ETEs were dissolved in a water/methanol (8:2) solution at a concentration of 0.5 mg/mL. The temperature of the spectrophotometer was set at 40 °C and 30 μL of sample was mixed in a well with 30 μL of Folin-Ciocalteu reagent (1:10 in water). The mixture was allowed to equilibrate for 2 min and then 240 μL of 5.0% (w/v) sodium carbonate was added. The absorbance of the coloured mixture was measured at 765 nm after a 20 min reaction. A calibration curve was prepared with gallic acid (2.0–10.0 μg/mL) as a standard compound ($y = 0.062x + 0.053$, $R^2 = 0.998$ and $F_{calc} = 0.30 < F_{tab} = 3.71$). Total phenolic content was expressed as mg gallic acid equivalent (GAE) per g of dry extract (DE).

Total flavonoid content (TFC) was determined using $AlCl_3$ complexation method described by Chang, Yang, Wen, and Chern (2002), with some modifications. With the spectrophotometer at 25 °C, 30 μL of a methanolic solution of the ETEs (3.0 mg/mL) were mixed with 10 μL of 1 M sodium acetate and 240 μL of distilled water. The absorbance of the mixture at 415 nm was measured after 30 min of reaction. Total flavonoid content was calculated using a calibration curve elaborated with quercetin (4.0–14.0 μg/mL) as standard ($y = 0.049x - 0.003$, $R^2 = 0.999$ and $F_{calc} = 0.06 < F_{tab} = 3.49$), and expressed as mg quercetin equivalent (QE) per g of dry extract (DE).