## ORIGINAL PAPER

# Phenolic Constituents of the Chilean Herbal Tea *Fabiana imbricata* R. et P.

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Abstract "Pichi" or "pichi romero" (Fabiana imbricata R. et. P., Solanaceae) is a Chilean plant used as a tea in the Andean regions of Chile and Argentina. A very simple and direct method was developed for the qualitative analysis of polyphenols in the tea by high-performance liquid chromatography (HPLC) with diode array detection and electrospray ionization tandem mass spectrometry. The phenolic constituents identified in the teas were chlorogenic acid (3-O-caffeoylquinic acid), p-hydroxyacetophenone, scopoletin and quercetin derivatives. The glycosides were mainly glucosides from *p*-hydroxyacetophenone and scopoletin while di- and tri-glycosides from quercetin were the main flavonoids. The content of the main phenolic compounds in the teas (g/100 g lyophilized infusion) was 0.8-1.9 % for scopoletin, 0.4-6.2 % for p-hydroxyacetophenone and 2.1-4.3 % for rutin, respectively. The health-promoting properties reported for this herbal tea can be associated with the presence of several phenolics with known antioxidant, diuretic and antiinflammatory activity.

**Keywords** Chilean herbal tea · *Fabiana imbricata* · HPLC-DAD-MS · Chlorogenic acid · Quercetin derivatives

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# Abbreviations

HPLC-DAD	High-performance liquid chromatography
	with diode array detection
HPLC-MS	High-performance liquid chromatography
	mass spectrometry
HPLC-DAD-	High-performance liquid chromatography
ESI-MS	with diode array detection electrospray
	ionization mass spectrometry
CID	Collision induced dissociation
LC/MS	Liquid chromatography – mass
	spectrometry

## Introduction

Herbal teas are an important group of products with nutraceutical properties widely used all over the world. Teas include the widespread infusions of Camellia sinensis [1] but also infusions from plants with a more restricted distribution such as Lycium barbarum from China [2], the endemic Lamiaceae Ballota rotundifolia and Teucrium chamaedrys from Turkey [3], and Phlomis lychnitis from Spain [4]. The consumption of herbal teas is widespread in Latin American countries, where the tradition of use include pre-hispanic herbs and European plants introduced by the early Spanish and Portuguese settlers. From the native species used as teas in Chile, the best known are the leaves of "boldo" (Peumus boldus Mol., Monimiaceae), the Solanaceae Fabiana imbricata R. et P., known under the common names of "pichi" or "pichi romero" and the Haplopappus spp. (Asteraceae) described under the common name of "baylahuen" [5]. Boldo is widely used as medicinal and functional tea in Chile and worldwide. The shrub F. imbricata is common in the Andean slopes in central Chile and Argentina. The infusion (tea) of the aerial parts is consumed in both countries. Supplementation of human diet with herbal teas that contain high amounts of antioxidant compounds may have potential benefits. At the same time,

increasing consumption of herbal teas is a worldwide trend. with a population paying more attention to healthy foods and beverages. Fabiana imbricata contains among its secondary metabolites the flavonoid rutin, the coumarin scopoletin, oleanolic acid and several sesquiterpenes [6-8] as well as Dmanno-heptulose, perseitol and D-glycero-D-manno-octulose [9]. The main terpenoid of the aerial parts of *F. imbricata* is the triterpene oleanolic acid. However, the tea of the plant has not been properly investigated looking for a fast characterization of the constituents. Phenolic compounds such as flavonoids, coumarins, phenolic acids and tannins are widespread in plants, often occurring in high amounts, present several relevant biological activities and can be used as chemotaxonomic markers [10]. Liquid chromatography with diode array detection hyphenated with tandem mass spectrometry has been successfully applied to provide tentative structures of phenolic compounds in extracts from natural sources. Indeed, metabolome analysis based on HPLC-DAD-ESI-MS fingerprinting technique is a powerful tool in phytochemistry [11], plant taxonomy and fast characterization of phenolic compounds in medicinal herbs [12], crop plants and edible fruits [13, 14]. The aim of the present study was to evaluate the antioxidant activity, total phenolics, total flavonoids content and to identify the main phenolic compounds in F. imbricata tea.

#### **Materials and Methods**

#### Samples

Aerial parts of F. imbricata were collected at the Andean slopes near Las Trancas, comuna de Pinto, VIII Region on December, 2007. Additional samples were collected at the same place in December 2010 and February 2011 as well as at the Universidad de Talca on November, 2010 and at Vilches Alto, VII Region, on January 2011. Voucher herbarium specimens have been deposited at the Herbario de la Universidad de Talca. All herbal samples were dried at room temperature (20-30 °C) for two weeks in dark conditions and powdered in a waring blender (Somela Frutty Mix BL1501, Somela S.A., Santiago de Chile). The tea was prepared adding 50 ml of boiling water to 2.5 g of the powdered herb and the infusion was allowed to cool throughout the extraction process to mimic tea brewing. After filtering on Whatman Nº 1 filter paper, the infusion was kept at -20 °C and lyophilized using a Labconco Freeze Dry System Model 77520, Labconco Corporation, Kansas City, Missouri, USA to afford a light brown powder. Dried extracts were kept in a freezer for futher analysis. For HPLC analysis, 5 mg of the lyophilized extract was dissolved in 1 ml MeOH: $H_2O$  (1:1 v/v).

#### Chemicals

Folin–Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), NaNO<sub>2</sub>, NaOH, gallic acid, quercetin and chlorogenic acid (3-*O*-caffeoylquinic acid) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). HPLC-grade acetonitrile, methanol (MeOH), and HPLC-grade water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Analytical grade ethyl acetate, chloroform, acetic acid, formic acid, sodium carbonate, 2-aminoethyldiphenyl borate were obtained from Merck (Darmstadt, Germany). The purity of the chemicals used was as follows: NaOH reagent grade,  $\geq$ 98 %, NaNO<sub>2</sub> ACS reagent  $\geq$ 97.0 %, gallic acid (purity $\geq$ 99 %), quercetin (purity $\geq$ 97 %), chlorogenic acid (purity $\geq$ 95 %), Folin–Ciocalteu phenol reagent (2 M, respect to acid, Sigma), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, 95 % purity) (Sigma-Aldrich).

#### HPLC-DAD Analysis

The HPLC system used for DAD analysis of extracts was Merck-Hitachi (LaChrom, Tokyo, Japan) equipment consisting of a L-7100 pump, a L-7455 UV diode array detector, and a D-7000 chromatointegrator. A 250 mm×4.60 mm i.d., 5 µm Kromasil 100-5 C18 column (Eka Chemicals, Brewster, NY, USA) maintained at 25 °C was used. Approximately 5 mg of each extract obtained as explained above was dissolved in 1 ml MeOH:H<sub>2</sub>O (1:1 v/v), filtered through a 0.45  $\mu$ m PTFE filter (Alltech Associates Inc., Deerfield, Illinois, USA) and submitted to HPLC-DAD and HPLC-MS analysis. The compounds were monitored at 250 nm, and UV spectra from 200 to 600 nm were recorded for peak characterization. The HPLC analyses were performed using a linear gradient solvent system consisting of 1 % formic acid (A) and MeOH (B) as follows: 90 to 75 % A over 20 min; followed by 75 to 40 % A from 20 to 45 min; 40 to 25 % A from 45 to 50 min; 25 to 40 % A from 50 to 55 min; 40 to 75 % A from 55 to 60 min; 75 to 90 % A from 60 to 75 min. The flow rate was 1 ml/min and the injected volume was 20 µl.

#### ESI-MS/MS Analysis

pt?>Mass spectra were recorded using an Agilent 1100 (USA) liquid chromatography system connected through a split to an Esquire 4000 Ion Trap LC/MS system (Bruker Daltoniks, Germany) or a Merck-Hitachi 6200 Intelligent Pump and L 4000 UV detector coupled to a EBE trisector VG Autospec Micromass spectrometer (Micromass-Water Autospec, U.K.) operating at 70 eV. Full scan mass spectra were measured between m/z 150 and 2000 u in positive ion mode. For the Ion Trap LC/MS system, nitrogen was used as nebulizer gas at 27.5 psi, 350 °C and at a flow rate of 8 l/min. The mass spectrometric conditions for positive ion mode

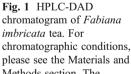
were: electrospray needle, 4000 V; end plate offset, -500 V; skimmer 1, 56.0 V; skimmer 2, 6.0 V; capillary exit offset, 84.6 V; capillary exit, 140.6 V. Collision induced dissociation (CID) spectra were obtained with a fragmentation amplitude of 1.00 V (MS/MS) using helium as collision gas.

#### Main Phenolics in F. imbricata Tea

The standards of scopoletin and *p*-hydroxyacetophenone used for quantification were isolated and purified from F. imbricata. Rutin was from Sigma-Aldrich. HPLC-DAD analysis suggested that the purities of all compounds used as markers were >98 % and their chemical structures were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS [15]. The main phenolics were quantified using calibration curves. The concentration range used was between 50 and 400 mg/l for rutin, 8-24 mg/l for scopoletin, and 12-50 mg/l for phydroxyacetophenone. The stocks solutions were mixed and diluted with methanol to appropriate concentration ranges for the establishment of calibration curves. Calibration curves were plotted after linear regression of the peak areas versus the concentrations. All calibration curves showed good linear regression within tested ranges with  $r^2$ values of 0.9986, 0.9999 and 0.9998 for scopoletin, phydroxyacetophenone and rutin, respectively. The data are expressed as percentage in the lyophilized infusion.

Total Phenolic Content

The total phenolic content (TPs) was determined by the Folin– Ciocalteu method as previously described [13, 14]. All samples and gallic acid were dissolved in 50 % (v/v) aqueous methanol. Samples (50  $\mu$ l) were placed into test tubes and 250  $\mu$ l Folin–Ciocalteu was added. The mixture was left to



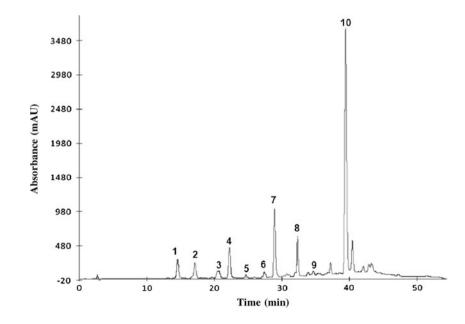
Methods section. The identification of compounds is summarized in Table 1. Compound 1: p-Hydroxyacetophenone derivative; 2: p-Hydroxyacetophenone glucoside; 3: Scopoletin glucoside; 4: Chlorogenic acid (3-O-caffeoylquinic acid); 5: Quercetin-O-rhamnose-dihexoside; 6: p-Hydroxyacetophenone; 7: Scopoletin; 8: Quercetin-dirhamnoside hexoside: 9: Unknown; 10: Rutin stand for 5 min, and 750  $\mu$ l of 20 % sodium carbonate solution and 5 ml distilled water were added. After 30 min of incubation at room temperature (20 °C) the resulting absorbance was measured at 765 nm. The calibration curve was performed with gallic acid (concentrations ranging from 31.3 to 500  $\mu$ g/ml) and the results were expressed as mg of gallic acid equivalents per 100 g of dry plant material.

#### Scavenging of DPPH Radicals

The scavenging of DPPH radicals was assayed as previously reported [12, 13]. All extracts were dissolved in 50 % (v/v) aqueous methanol to prepare stock solutions of 1 mg/ml. These stock solutions were serially diluted with methanol, mixed with an equal volume of DPPH solution (60  $\mu$ M) and shaken vigorously. The mixture was incubated at room temperature for 30 min before the absorbance was read at 517 nm. Solutions of quercetin were used as a positive control. The scavenging activity was determined by comparing the absorbance with that of the blank (100 %) containing only DPPH and solvent. Antiradical DPPH<sup>-</sup> bleaching activity is expressed as SC<sub>50</sub> (scavenging concentration of 50 %) of the lyophilized infusions in µg/ml which denoted the concentration of sample required to scavenge 50 % of DPPH free radicals. Values lower than 50 µg/ml are considered high, while values between 50 and 100 µg/ml are considered moderate.

# **Results and Discussion**

The phenolic constituents of the herbal tea from *Fabiana imbricata* were analyzed for the first time by HPLC-DAD-ESI-MS. Approximately 10 compounds were detected in the tea and 9 from them were characterized on the basis of the



Peak	Rt (min)	$\lambda_{\max}$ (nm)	MW	$\left[M{+}H\right]^{+}$ and ions	Tentative identificaction
1	14.47	264	618	619, 321, 137	p-Hydroxyacetophenone derivative
2	16.86	264	298	299, 137	p-Hydroxyacetophenone glucoside
3	20.65	336, 287, 234	354	355, 193	Scopoletin glucoside
4	22.33	327, 300 sh, 245	354	355, 309, 163	Chlorogenic acid <sup>a</sup>
5	24.40	355, 255	772	773, 627, 465, 303	Quercetin-O-rhamnose-dihexoside
6	27.59	273	136	137	<i>p</i> -Hydroxyacetophenone <sup>a</sup>
7	28.93	341, 296, 250, 235	192	193	Scopoletin <sup>a</sup>
8	32.33	327, 300 sh, 245	608	609, 347, 318, 201, 186	Unknown
9	34.68	355, 253	756	757, 611, 465, 303	Quercetin-dirhamnoside hexoside
10	39.46	355, 296 sh, 262 sh, 255	610	611, 465, 303	Rutin <sup>a</sup>

<sup>a</sup> Identity confirmed by coinjection of standards. sh: shoulder

Compounds. 1: *p*-Hydroxyacetophenone derivative; 2: *p*-Hydroxyacetophenone glucoside; 3: Scopoletin glucoside; 4: Chlorogenic acid (3-*O*-caffeoylquinic acid); 5: Quercetin-*O*-rhamnose-dihexoside; 6: *p*-Hydroxyacetophenone; 7: Scopoletin; 8: Quercetin-dirhamnoside hexoside; 9: Unknown; 10: Rutin

UV spectra and MS fragmentation patterns in comparison with literature or with reference substances. The compounds comprise p-hydroxyacetophenone derivatives, scopoletin and its glucoside, chlorogenic acid and three glycosides of quercetin. A representative chromatogram of the tea constituents is presented in Figure 1 and the identification of compounds is shown in Table 1, in accordance with literature [8, 12, 15]. The results (Table 2) suggested that three compounds account for most of the phenolics in the infusion, namely rutin (2.1-4.3 %), p-hydroxyacetophenone (0.4-6.2 %) and scopoletin (0.8-1.9 %). Total phenolics in the lyophilized teas ranged from 7.2 to 19.2 %. The highest TPs were found in a sample collected on February 2011, during summer time in the southern hemisphere. On a dry herbal tea weight basis, the single constituent content in infusion ranged between 0.062-0.187 %, 0.051-0.401 % and 0.103-0.428 % for scopoletin, p-hydroxy acetophenone and rutin, respectively (Table 2). In previous studies on F. imbricata,

Razmilic et al. [8] reported values ranging from 0.24 to 2.40 g/100 g crude drug for scopoletin and 0.195–1.95 g/100 g crude drug for rutin, respectively. The extracts, however, were methanol extracts from the aerial parts. In a further study, rutin in the aerial parts varied between 0.99 and 3.35 % while the scopoletin content varies between 0.09 and 0.64 % in wild samples collected in years 2000 and 2001 [15].

The free radical scavenging effect of the teas, measured by the DPPH bleaching assay should be considered moderate, with SC<sub>50</sub> values between 59.2 and >100 µg/ml. Under the same experimental conditions, the SC<sub>50</sub> of quercetin was 29.3 µg/ml. Quercetin is a well known free radical scavenger and antioxidant compound [16] and the genuine of the main flavonol glycosides from the *Fabiana* tea. The best free radical scavenging effect of the infusions was found for samples collected in spring and summer time and shows a trend associated with larger amounts of the marker compounds in infusions.

Table 2 Total phenolics (TPs), scavenging of DPPH radical and % (w/w) yield of extraction of *Fabiana imbricata* teas on the basis of dry starting material and main phenolic compounds: scopoletin, *p*-hydroxyacetophenone and rutin in the lyophilized infusion from *Fabiana imbricata* aerial parts

Collection place, month and year	$TP^1$	DPPH <sup>-1</sup>	Yield of extraction (%) $\pm$ SD	Compound (% in lyophilized infusions)		
				Scopoletin	<i>p</i> -Hydroxyacetophenone	Rutin
Las Trancas, Dec. 2007	10.4±0.3	83.7±5.8	6.39±0.15	1.3±0.2	3.9±0.2	2.1±0.1
Univ. Talca, Nov. 2010	$7.2 \pm 0.1$	>100 (39.3)	13.35±0.21	$0.8 {\pm} 0.1$	$0.4{\pm}0.1$	$3.0{\pm}0.1$
Las Trancas, Dec. 2010	$19.2 {\pm} 0.3$	$61.6 \pm 3.1$	9.85±0.16	$1.9 {\pm} 0.2$	$4.1 \pm 0.1$	$4.3{\pm}0.2$
Las Trancas, Febr. 2011	$12.1 \pm 0.1$	$59.2 \pm 2.9$	4.99±0.11	$1.2 \pm 0.2$	$6.2 \pm 0.2$	$2.1{\pm}0.2$
Vilches Altos, Jan. 2011	$9.8 {\pm} 0.1$	>100 (41.4)	$10.45 \pm 0.24$	$1.4 {\pm} 0.1$	$2.2{\pm}0.1$	$2.9{\pm}0.2$
Quercetin		29.3±2.3				

<sup>1</sup> All measurements are expressed as mean $\pm$ S.D. (*n*=3). TPs and DPPH calculated on the basis of the lyophilized infusions. TPs expressed as mg of gallic acid equivalents/100 g of lyophilized infusions. Antiradical DPPH<sup>-</sup> decoloration activity is expressed as SC<sub>50</sub> in µg dry lyophilized infusion/ml and if higher than 100 µg/ml, as percent inhibition at 100 µg/ml. Yield of extraction is expressed as g of lyophilized infusion per 100 g dry crude drug weight

#### Conclusions

The evaluation of the antioxidant status of different teas will promote research on the identification and quantification of active components of these teas that may help protect consumers against free radical damage and oxidative stress-related diseases. The main constituents identified in this herbal tea or its aglycones are known bioactive compounds and the tea can be considered a good source of antioxidants. Scopoletin presents antioxidant and hepatoprotective effect [17, 18], while chlorogenic acid (3-*O*-caffeoylquinic acid) and quercetinbased flavonol glycosides are known by their antioxidant effect [16]. Recent findings suggest that flavonoids including quercetin and caffeic acid derivatives such as chlorogenic acid can be regarded as anti-obesity natural compound [19].

When compared with other herbal teas, *F. imbricata* infusions present a phenolic-rich pattern characterized by the occurrence of *p*-hydroxyacetophenone, scopoletin and quercetin glycosides as main constituents. According to literature, the compounds described for *F. imbricata* comprises liposoluble terpenes that occur in the plant exudate, but also alkaloids and the anthraquinones physcion and erythroglaucin have been reported [20, 21]. The large variation in the constituent content, compared with previous studies, can be explained by different extraction methods (boiling water for tea, methanol for studies on the crude drug) and environmental factors affecting secondary metabolite production. The method presented provides a fast and reliable quality control for the crude drug and for comparisons with other *Fabiana* species used as medicinal plants in other Andean countries.

Herbal teas provide a dietary source of biologically active compounds, including polyphenols, which help to prevent a wide variety of diseases. The antioxidant activity of polyphenols is mainly due to their ability to scavenge free radicals and to increased activity of some detoxifying enzymes [16, 22]. Thus, herbal teas should be considered a source of antioxidant agents available in everyday life.

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