ARTÍCULO ORIGINAL

Chemical properties and assessment of the antioxidant capacity of native species from the genus *Ugni*

Características químicas y evaluación de la capacidad antioxidante de especies del género *Ugni*

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ABSTRACT

Introduction: *Ugni molinae* Turcz., *Ugni candollei* Barn. (Berg) and *Ugni selkirkii* (Hook. et Arn.) Berg are Chilean species that share morphological characteristics and they are distributed in geographic locations with very diverse habitats. Its is considered important for the characterization of the Chilean flora to determine if there are similarities in the sort of chemical compounds among species with close morphological relations, growing in different habitats and their consequent biological activity.
Objective: to assess the chemical composition and the antioxidant capacity of leaf extracts from the Chilean species of the genus *Ugni, and* to compare with the *U. molinae* characteristics.
Methods: composition of chemical compounds was determined by chromatographic methods (HPLC-ESI-MS). The antioxidant capacity was assessed by DPPH, ABTS and by stabilization of the hydroxyl radical.
Results: as expected, given the great morphological similarity existing among the three species of *Ugni* that grow in Chile, similarities were found in their chemical composition. Nevertheless, it was also expectable to find variations among them. Thus, *U. candollei* and *U. selkirkii* are the species that present greater content and variety of phenolic and terpenic compounds. These species exert greater antioxidant capacity in comparison to*U. molinae*. *U. candollei* hightlights for its flavonoid content such as glycosides and quercetin derivatives, and the species *U. selkirkii*, is important in galotannins. *U. molinae* is characterized in ellagic acids derivatives.

Conclusion: these data and the morphological characteristics could become a useful toll in order to determine the closeness degree among these species.

Keywords: antioxidant capacity; chemical properties; genus *Ugni.*

RESUMEN

Introducción: *Ugni molinae* Turcz., *Ugni candollei* (Barn.) Berg y *Ugni selkirkii* (Hook. et Arn.) Berg son especies chilenas que comparten características morfológicas y se distribuyen en lugares geográficos con muy diversos hábitats. Se considera importante para la caracterización de la flora chilena determinar si hay similitudes en el tipo de compuestos químicos entre especies con relaciones morfológicas cercanas, que crecen en diferentes hábitats y su actividad biológica consecuente.
Objetivo: evaluar la composición química y la capacidad antioxidante de extractos de hojas de las especies chilenas del género *Ugni*, y compararlas con las características de la especie *U. molinae*.
Métodos: la composición química se determinó por métodos cromatográficos (HPLC-ESI-MS). La capacidad antioxidante se evaluó por los métodos DPPH, ABTS, y la estabilización del radical hidroxilo.
Resultados: como era de esperar, dada la gran similitud morfológica existente entre las tres especies de *Ugni* que crecen en Chile, se encontraron similitudes en su composición química. Sin embargo, también era esperable encontrar variaciones entre ellas. Por lo tanto, *U. candollei* y *U. selkirkii* son las especies que presentan mayor contenido y variedad de compuestos fenólicos y terpénicos. Estas especies ejercen una mayor capacidad antioxidante en comparación con *U. molinae*. *U. candollei* se caracteriza por su contenido en flavonoides como quercetina glucósidos y sus derivados, y la especie U. selkirkii, por la presencia de galotaninos. *U. molinae* se caracteriza por contener derivados del ácido elágico.
Conclusión: estos datos y las características morfológicas podrían convertirse en una herramienta útil para determinar el grado cercanía entre estas especies.

Palabras clave: capacidad antioxidante; género *Ugni;* propiedades químicas.

INTRODUCCIÓN

Genus *Ugni* Turczaninow belongs to the family *Myrtaceae* and comprises between 19 and 21 taxa located from Mexico, Central America, Venezuela, to Chile and Argentina.[1] The species of this genus can be shrubs or small trees, with perennial leafs and cultivated for both their ornamental importance and the attractiveness of their fruits. From all species, three grow in Chile: *Ugni molinae* Turcz, *Ugni candollei* (Barn.) Berg and *Ugni selkirkii* (Hook. et Arn.) Berg. These plants are shrubs distributed in Central Chile from the Maule Region to the Chiloe Island, including Juan Fernandez Archipelago.

Ugni molinae Turczaninow. This native plant is widely known by its local name "murtilla". It is distributed from the Maule Region to the Chiloe Island, including Juan Fernandez Archipelago and is also found in Río Negro and Neuquén, in Argentina. This species grows mainly near the coast, in both wet and dry environments, in the edge of forests or in rocky areas. *U. molinae* is an evergreen shrub, small in drought

conditions and it can reach 2 m in zones with high rainfall. Fruits of *U. molinae* are useful to relief circulatory disorders and for improving visual acuity, especially in the night. *U. molinae* leaves have been used by aboriginal people as stringent for treating diarrheas and dysenteries, as infusions.[2]

Ugni candollei (Barnéoud) Berg. This endemic species is known as "white murta". It is commonly distributes from Valdivia to Chiloe and it has also been described for Maule Region. It normally grows in the coastal zone. *U. candollei* is an evergreen shrub that can reach up to 2 m in height. No popular uses are known for this shrub.[2]

Ugni selkirkii (W. J. Hooker et Arnott) Berg. This species is endemic from Robinson Crusoe island (Masatierra Island), in Juan Fernandez Archipelago. It can reach 2 m height and it is pubescent in branches and young shoots[3]. No popular uses are known for this shrub. The current population trend is marked by a notorious declining, mainly due to the strong competition with expanding species that cover its habitat.[3-5]

Although no evolutionary studies on the group are known, given their morphological similarity it seems that these species are closely related. Because of such relation, they could share similar chemical compounds. There is only knowledge on the chemical composition of *U. molinae*, species assessed respect to its biological activity.[6-9] These authors have described the presence of phenolic substances such as phenolic acids and flavonoids, compounds with renowned antioxidant capacity, as well as compound of terpenic kind, such as pentacyclic triterpenic acids.

As *U. molinae* shares morphological characteristics with species of the genus that grow in Chile, *U. candollei* and *U. selkirkii,* probably share chemical properties and consequently develop similar biological activity. Therefore, the aim of this study is to assess the chemical composition and the antioxidant capacity of the leaf extracts of the Chilean species of the genus *Ugni, U. candollei* and *U. selkirkii,* and to compare with the *U. molinae* characteristics.[10]

Methods

1. Vegetal material

Biological material from the native species of the genus *Ugni* was collected in blooming season (November-March 2008-2009): *U candollei,* Los Ríos Region (Valdivia, Oncol Park, Oncol hill); *U. selkirkii*, Juan Fernandez Archipelago (Robinson Crusoe Island, Selkirk viewpoint, Portezuelo hill). Species were identified by the taxonomist Dr. Roberto Rodríguez, at the Faculty of Natural and Oceanographic Sciences, University of Concepción. A specimen from each species was recorded at the CONC herbarium (125465 and 162345, respectively).

2. Preparation of extracts

Biological material was dehydrated in the shade, at room temperature and the size was reduced in blade mill. For obtaining the extracts, 50 grams of the grinded sample in a Soxhlet apparatus were processed. The following solvents were successively used: hexane, chloroform, ethyl acetate and methanol, until exhaustion of vegetal material. The relation mass solvent was 1:6. Every extract was concentrated in evaporator and taken to dryness in liophilizer. Recognition reactions of secondary metabolites were carried out for every extract (Shinoda reaction for flavonoids recognition, $FeCl_3$ reaction for tannin recognition and foam test for saponin

recognition). Samples were stored in a dry place and protected from light until their utilization. For chemical and biological determinations, the extracts obtained with methanol were selected, due to their content of phenolic compounds and saponins.

3. **Chromatographic analysis**

The analyses of leaf extracts from the *Ugni* species were carried out by means of HPLC, according to Cho et al.[11]. For the analysis, a LC-MS system (Agilent Technologies Inc., Palo Alto, CA, USA) was used. This system is equipped with binary pumps, an online degasifier, automatic injector and a UV-VIS detector. UV traces were registered at 280 nm. Separation of phenolic compounds was carried out by means of a Zorbax Eclipse column XDB-C18 150 × 4.6 mm, 5 µm and 80 Å (Agilent Technologies Inc., CA-USA). Injection volume was 20 µL, with a flow of 1.0 mL/min. Solvent system was composed of the solvent A (double distilled water containing 0.1% formic acid v/v) and the solvent B (acetonitrile containing 0.1% formic acid). The gradient system was as follows: 0-5min, 5% B; 5-50 min, 100% B; 50-55 min, 100% B; 55-57.5 min, 100-5% B; and 57.5-60 min, 5% B. LC/MS detection was carried out immediately after the UV-VIS measurements. Analyses were carried out by means of Bruker Esquire 4000 (Bruker Daltonik, GmbH, Germany) ions trap ESI-IT mass spectrophotometer, operating under the following ion optics: capillary temperature, 225 ºC; capillary voltage, 5.7 kV; cone voltage, 35 V and voltage spray 2.8 kV. Nitrogen was used as nebulizer gas (pressure: 30 psi, temperature, 35 ºC) and drying gas (10 L/min). Products from mass spectra were recorded in a range of *m/x* 50-1500 in both positive and negative mode. Data were collected by means of the Esquire Control 5.2 software and processed by means of Data Analysis 3.2 software (Bruker Daltonics Esquire 5.2, Bruker Daltonik GmbH). Instrument parameters were optimized in a routine prior to the analysis of extracts.

4. **Antioxidant capacity**

Was carried out according to Joyeux et al.[12], stabilization of the DPPH radical; Ghiselli et al.[13], stabilization of the ABTS radical; Halliwell et al.[14], stabilization of the hydroxyl radical (OH). The antioxidant capacity was expressed as IC_{50}, which is defined as the final required concentration of the sample to reach 50% of the inhibition of the radical. As standard, gallic acid and Trolox® (Merck, Germany) were used. Three repetitions per extract were carried out.

5. Statistical analysis

All determinations were carried out in triplicate. Mean values and standard deviations (±SD) were calculated. Statistical tests were carried out in order to analyze correlations between values. Variance analysis (ANOVA) was used and differences were considered as significant at $P < 0.01$.

Results

U. candollei and *U. selkirkii* are species with high concentration of total polyphenols in the extracts obtained with methanol. *U. candollei* presents the greatest levels, including total flavonoids (0.070 ± 0.002 g EQ/g dry matter). With respect to the content of total saponins, no significant differences among species were observed, reaching a maximum of 0.015 g saponins/g dry matter in *U. selkirkii*.

Composition by chromatographic analysis

Tentative identification of the main phenolic and triterpenic compounds in the extracts of *U. candollei (Uc)* and *U. selkirkii (Us)* was carried out by means of HPLC-ESI-MS. The allocation of peaks was carried out by means of the fragmentation pattern analysis and its comparison with mass spectra from both standards and literature. Retention times and *m/z* in negative polarity are presented for the respective compounds in Table 1, and the chromatograms in Figure 1. Spectral properties were obtained from those signals considered more intense and pure. The positive mode was also used to confirm the identification of some compounds.

Methanolic extracts (table 1, Fig 1**).**

Table 1. HPLC-MS of methanolic extracts

Peak	t_R (min)	λmax (nm)	$[M-H]^-$	MS/MS Ions	Tentative identification	Species
1	2.4	295,272,240	343.2	190.9; 168.8	Galloylquinic acid	Uc[a], Us[b]
2	2.8		271.1	168.9; 124.9	Galloyl glucopiranose	Us
3	3.1	280	633.0	300.8; 274.9; 248.9	derivative Hexahydroxydiphenoi	Us
4	4.7	280	633.3		l galloyl glucose	Us
5	4.9		353.7	300.8; 275.0; 249.0	Hexahydroxydiphenoi l galloyl glucose	Uc
6	5.1	280	665.2		isomer	Uc
7	5.4		451.4	190.8	Caffeoilquinic acid	Us
8	5.5		706.5	352.9; 190.8	Caffeoliquinic acid	Uc
		326,295,240		405.0; 168.8	derivative	
9	12.3		887.3	352.9; 190.8	Gallate derivative	Us
		326,295,240			Caffeoliquinic acid	
10	12.8		635.3	442.9; 300.8;	derivative	Us
11	13.1	272	635.3	270.8; 248.9	Hexahydroxydiphenoi	Us
12	13.6	326,295,240	927.3	464.9; 312.9	l galloyl	Uc
				465.0; 482.9;	glucopiranóside	
13	13.9		943.5	313.2	derivative	Us
				462.9; 316.9;	Trigalloyl glucose	
14	14.0	280	615.4	298.9; 270.9	Trigalloyl glucose	Uc
15	14.1		871.5	470.9; 330.9;	isomer	Uc
16	14.3	280	943.6	270.8	Myricetin	Us
17	14.5	280	449.2	462.9; 300.9	Myricetin	Us
18	14.6	374,254	615.3	434.9; 303.0;	monomethylether	Uc
				285.0	derivative	
19	14.9	260	787.4	471.0; 331.0;	Quercetin hexose	Us
				270.9	galate	
20	15.1		927.4		Quercetin pentoside	Us
21	15.2		927.3	316.0	Myricetin	Uc
22	15.6	356,300,267	595.4	462.9; 300.9	monomethylether	Uc
23	15.9		595.5		derivative isomer	Uc
		355,255		643.9; 617.1;	Myricetin pentoside	
24	16.1	260	867.6	465.0; 447.9	Quercetin hexose	Uc
25	16.1		551.3	463.0; 316.0	galate isomer	Us
26	16.4		551.4	463.0; 301.0		Us
				432.9; 300.9	Tetragalloyl glucose	

27	16.6	374,254	895.4	432.8; 300.8;		Uc
28	16.9	356,300,267	898.8	178.9	Myricetin	Uc
29	17.9		521.2		deoxihexoside	Uc
30	20.7		301.1	433.0; 300.1	Quercetin hexoside	Uc
31	20.8	280	595.5	533.0; 507.1;	Quercetin	Us
32	23.2		329.3	312.9	hexosidopentóside	Uc
				532.9; 438.9;	Quercetin	
33	23.6		696.3	270.8	hexosidopentoside	Uc
34	25.9	260	1007.9		isomer	Us
35	27.1		813.7	447.0; 300.9;	Pentoside ellagic acid	Us
36	29.1	355,254	555.2	271.0	Methoxy flavone	Uc
37	29.1	355,254	975.3	450.9; 340.9;	derivative	Us
38	31.2		487.3	300.7	Metoxi flavona	Uc
39	31.6	355,254	679.6	330.9; 270.8;	derivative isómero	Us
40	33.8		649.9	210.9	Rhamnoside ellagic	Us
41	34.7		749.0	272.8; 178.8;	acid	Uc
42	35.2	367,256	701.6	150.8	Elagitannin	Uc
43	36.0		1268.5	330.8; 270.8;	Myricetin	Us
44	36.4	317,253	951.7	211.0	monomethyl ether	Us
45	40.9		748.9	293.0; 229.0;	Quercetin	Us
46	41.3	317,253	685.2	210.9; 171.0	Myricetin	Us
				649.3; 487.1;	monomethyl ether	
47	41.6		712.9	315.9	Decursin coumarin	Uc
48	41.8	367,256	685.2	503.2		Us
				633.3	Rhamnetin derivative	
		367,256		487.2	Madecassic acid	
		260		485.3; 439.2	Oleanolic glycoside	
				455.2	acid	
		356,280		633.3	Asiatic acid derivative	
				631.3; 605.7	Madecassoside	
		260		712.5	Trihydroxyursenoic	
		365		655.3; 633.4	acid	
				951.0; 633.7	Oleanoic acid	
				633.6	glycoside	
				712.5	Saponin derivative	
		354,254		639.3; 617.4	oleanolic acid	
		198			Oleanolic acid	
				550.4; 532.3	glycoside	
		194		639.2; 617.3	Oleanolic acid	
					glycoside	
		198			Prosaponin oleanane	
					Prosaponin oleanane	

		198			Oleanolic acid glycoside	
		198				
		194			Cumaroilic derivative of maslinic acid	
		194			Oleanolic acid glycoside	
		194			Cumaroil derivative of the maslinic acid isomer	
		198				
		194				
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[a]Uc: Methanolic Extract *U. candollei*.

[b]Us: Methanolic Extract *U. selkirkii*.

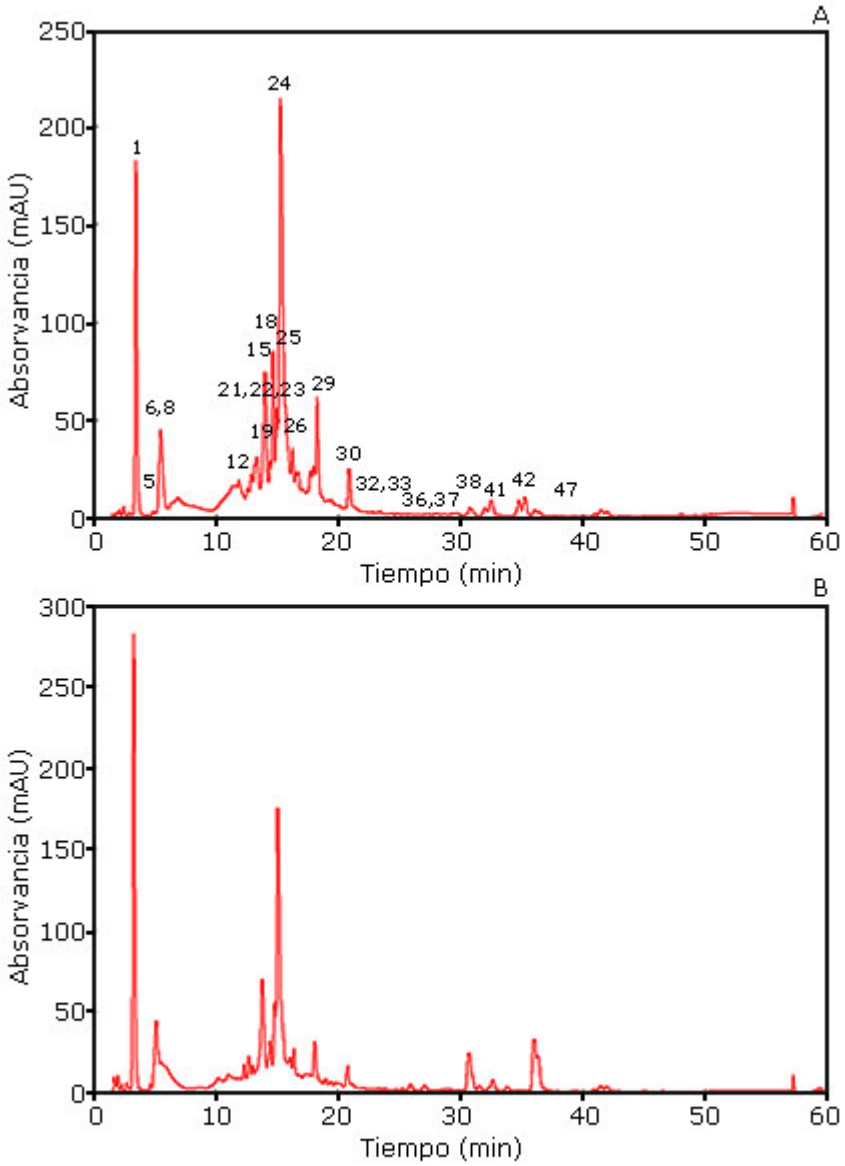

Fig. 1. HPLC-ESI-MS analysis of methanolic extracts. A) Chromatogram of the methanolic extract of *U. candollei*, B) Chromatogram of the methanolic extract of *U. selkirkii*.

Tannins: It must be taken into account that the most important compounds of certain species from the genus *Ugni* (*U. selkirkii* and *U. molinae*) derive from gallic and ellagic acids. Phenolic acids derived from quinic and caffeic acids are also reported for the species *U. candollei* and *U. selkirkii.*

Peak 1 (*Uc*, *Us*, t_R = 2.4 min), was tentatively identified as galloylquinic acid, because it presented $[M-H]^-$ de *m/z* 343.2 and its fragmentation provided ions of *m/z* 190.9 and 168.8, corresponding to quinic acid and gallic acid, respectively.

Peak 2 (*Us*, t_R = 2.8 min), was tentatively identified as a derivative from galloylglucopiranose, resulting a $[M-H]^-$ of *m/z* 271, a ionic fragment observed when a cross-ring fragmentation of the glucose ring is observed in the galloyl glucopiranose derivative. Ion fragments in MS2 in *m/z* 168.9 and 124.9 confirm the sequential loss and decarboxylation of the gallic acid.

Peaks 3 and **4** (*Us*, t_R = 3.1 min, 4.7 min), they have $[M-H]^-$ of *m/z* 633, which are dissociated in order to form *m/z* 302 hexahydroxidiphenoil (HHDP) galloyl glucose (ellagitannin), through the loss of 332 amu, which indicated the presence of a unit of galloyl glucose. This compound is known as sanguine H4 or sanguine H5, depending where the galloyl unit is attached.

Peak 5 (*Uc*, t_R = 4.9 min), has a $[M-H]^-$ of *m/z* 353.7, with a MS2 fragment of *m/z* 190.8. This suggests that the peak corresponds to 3-O-caffeoilquinic.

Peak 6 (*Uc*, t_R = 5.1 min) and **Peak 8** (*Uc*, t_R = 5.5 min), have ion fragments $[M-H]^-$ of *m/z* 353.7, with a MS2 fragment of *m/z* 190.8, suggesting that both peaks are other forms of caffeloil quinic acid derivatives.

Peak 7 (*Us*, t_R = 5.4 min), the presence of an MS2 ionic fragment of *m/z* 168.8 suggests that this compound is a galloyl derivative.

Peak 9 (*Us*, t_R = 12.3 min), it has an $[M-H]^-$ parental peak of *m/z* 887.3 and ionic fragments of *m/z* 300.8, suggesting that this compound is a derivative from HHDP-galloyl glucopiranose or an ellagitannin.

Peak 10 (*Us*, t_R = 12.8 min) and **Peak 11** (*Us*, t_R = 13.1 min), were identified as trigalloyl glucose derivatives, with [M-H] of *m/z* 635.3.

Peak 19 (*Us*, t_R = 14.9 min), has an $[M-H]^-$ of *m/z* 867.6 with MS2 ionic fragments of *m/z* 617.1, 643.9 and 465. They are exactly equal to those reported for tetragalloyl glucose.

Peak 24 (*Uc*, t_R = 16.1 min), exhibited a $[2M-H]^-$ of *m/z* 867.6. The pseudo molecular ion fragment was of *m/z* 300.1 (loss of a sugar of 132 units). This suggests that this compound is an ellagic acid pentoside.

Peak 27 (*Uc*, t_R = 16.6 min), exhibited a $[2M-H]^-$ of *m/z* 895.4, with a fragment of the pseudomolcular ion of *m/z* 450.9 (tertgalloic acid) and its deprotonized ion aglycon ion of *m/z* 300.9 (loss of one sugar of 146 units). This suggests that this compound is an ellagic acid rhamnoside.

Peak 28 (*Uc*, t_R = 16.9 min), exhibits a $[2M-H]^-$ of *m/z* 898.9 with a pseudo molecular ion of *m/z* 450.9 (tertgalloic acid) and its deprotonized aglycon ion of *m/z* 300.1 (ellagic acid). Consequently, it was speculated that this compound corresponded to a ellagitannin.

Flavonoids: In the extracts from the three species, the presence of several flavonoids derivatives of myricetin and quercetin were observed. This coincides with the previously reported for the species *U. molinae*. In our study it must be noted that quercetin derivatives were predominant in the continental species, whereas in the samples from *U. selkirkii* collected in the Island, myricetin glycosides predominate. Furthermore, by means of HPLC-ESI-MS, we were able to identify galloylated forms of quercetin and glycosylated myricetin. As seen in Table 1, flavonoids from the different species of the genus *Ugni* elute after 12 minutes.

Peak 12 (*Uc*, t_R = 13.6 min), had a $[2M-H]^-$ of *m/z* 927.3 and the pseudo molecular ion with a *m/z* 462.9. Its deprotonized aglycon ion of *m/z* 316.9 (loss of one sugar of 146 units) is consistent with a myricetin deoxyhexoside. Fragmentation pattern and retention time of myricetin rhamnoside (miricitrin) coincides with this compound.

Peak 13 (*Us*, t_R = 13.9) and **Peak 16** (*Us*, t_R = 14.3 min), have a $[2M-H]^-$ of *m/z* 943.5 and the pesudomolecular ion of *m/z* 470.9. The deprotonized aglycon of *m/z* 330.9 is consistent with a myricetin monomethyl ether derivative.
Peak 14 (*Uc*, t_R = 14.0 min) and **Peak 18** (*Uc*, t_R = 14.6) have a $[M-H]^-$ *m/z* of 615.3. MS2 ionic fragments of *m/z* 426.9 (loss of 152amu from a galloyl unit) and its deprotonized aglycon ion of *m/z* 300.9 (loss of one sugar of 162 units) are consistent with quercetin hexoside galate isomers.

Peak 15 (*Uc*, t_R = 14.1*)*, has a $[2M-H]^-$ of *m/z* 870 and the pseudo molecular ion of *m/z* 433. The deprotonized aglycon of *m/z* 301 (loss of one sugar of 132 units) is consistent with a quercetin pentoside.

Peak 17 (Us, t_R =14.5 min), exhibits a $[M-H]^-$ of m/z 449.2, with its deprotonized aglycon ion of *m/z* 316 (with a loss of a sugar of 132 units), suggesting it is a myricetin pentoside (arabinoside).

Peak 20 (*Us*, t_R =15.1 min), exhibits a $[2M-H]^-$ de *m/z* 927.4. Pseudo molecular ion was of *m/z* 463 with its deprotonized aglycon ion of *m/z* 316 (loss of one sugar of 146 units), suggesting that this compound is another myricetin deoxihexoside.
Peak 21 (*Uc*, t_R =15.2 min), exhibits a $[2M-H]^-$ of *m/z* 927.3. A pseudo molecular ion of *m/z* 463 and a deprotonized aglycon ion of *m/z* 301 (sugar loss of 162 units). This suggests that this compound is a quercetin hexoside.
Peak 22 (*Uc*, t_R = 15.6 min) and **Peak 23** (*Uc*, t_R = 15.9 min) have a pseudo molecular ion of *m/z* 595.4. In MS2, peaks produced ionic fragments of *m/z* 432.9 (loss of sugar of 162 units) and its deprotonized aglycon ion of *m/z* 302 (loss of sugar of 132 units). This suggests that these compounds are quercetin pentosides hexoside.

Peaks 25 and **26** (*Us*, t_R = 16.1 min, 16.4 min) exhibit a pseudo molecular ion $[M-H]^-$ of *m/z* 551.3. The ionic fragment of *m/z* 313 also suggests that these compounds are methoxyisoflavones isomers derivatives. The absence of other features does not allow a better allocation.

Peak 29 (*Uc*, t_R = 17.9 min) and **Peak 31** (*Us*, t_R = 20.8 min) produced ionic fragments of *m/z* 331, 271 and 211. These fragments are typical of a mono-galloyl-glycoside derivative. However, the loss of 190 amu from the pseudo molecular ion of *m/z* 521 in order to form the ionic fragment of *m/z* 331 could be assigned to the loss of methylglucuronic acid from monoethylether myricetin. The low intensity of the signals does not allow a better allocation.

Peak 30 (*Uc*, t_R = 20.7 min) produced a pseudo molecular ion of *m/z* 301.1 and MS2 fragments of *m/z* 272.8 (loss of 28 amu from a carbonyl group), 178.8 (retrocyclation after the fission over the bond 1 and 2) and 150.8 (a ring fragment via RDA) that coincide exactly with those produced by quercetin in negative mode.

Peak 33 (*Uc*, t_R = 23.6 min), produced a pseudo molecular ion of *m/z* 6963.3. MS2 fragments of *m/z* 649.3 (loss of a sugar of 162 units) and 315.9 (loss of 170 amu from water and gallic acid) only allow suggesting that this compound is a methyl quercetin (rhamnetin) derivative.
Other phenols

Peak 32 (*Uc*, t_R = 23.2 min) produced a pseudos molecular ion of *m/z* 329.3 $[M-H]^-$. MS2 ionic fragments of *m/z* 229 and 210.9 are consistent with coumarin decursin or decursinol angelate.

Glycoside triterpenic: In all studied extracts the presence of pentacyclic triterpenic derivatives was observed by means of HPLC-ESI-MS. Taking into account the main fragments, those compounds are derived from oleanoic, ursenoic acid, asiatic acid, madecassic acid and maslinic acid. The most abundant are those derived from oleanoic acid. Some of these compounds were previously reported in *U. molinae*. Different isomers and conjugated forms of these compounds elute between 27 and 42 min and in a zone corresponding to a complex mixture of saponins. For these substances, only in a few cases it was possible to assign an identity. In order to elucidate an exact structure, additional experiments (NMR) and the comparison to standards become necessary.

Peak 34 (*Us*, t_R = 25.9 min), produced a $[2M-H]^-$ of 1007.8 and a pseudo molecular ion of *m/z* 503.2, consistent with hydroxyasiatic acid (madecassic acid) or trihtdroxyoleanolic acid.

Peak 35 (*Us*, t_R = 27.1 min), contains the pseudo molecular ion $[M-H]^-$ of *m/z* 813.7, with an MS2 ionic fragment of *m/z* 633.3 (loss of one hexose and water). Other fragments observed in positive polarity suggest that this compound is a glycoside of oleanolic acid. **Peak 39** (*Us*, t_R = 31.6 min), **Peak 42** (*Uc*, t_R = 35.2) also presented the MS2 ionic fragment of *m/z* 633.3.

Peak 36 (*Uc*, t_R = 29.1 min), contains the pseudo molecular ion $[M-H]^-$ of *m/z* 555.2, with a MS2 ionic fragment of *m/z* 487.2, consistent with a derivative of the asiatic acid, arjunolic acid, trihydroxyoleanolic acid or trihydroxiursenoic acid.

Peak 37 (*Uc*, t_R = 29.1 min), contains the pseudo molecular ion $[M-H]^-$ of *m/z* 975.3, with a MS2 of *m/z* 485.3 and 439.2. These data and those observed in positive mode suggest that it corresponds to a madecasoside.

Peak 40 (*Us*, t_R = 33.8 min) also contain the fragment *m/z* 649.9, as well as the pseudo molecular ion of the ion fragments MS2 of *m/z* 605.7 and 631.3. By means of 1H and 13C NMR and hydrolysis, this structure could be unequivocally identified.

Peak 38 (*Uc*, t_R = 31.2 min), contains the pseudo molecular $[M-H]^-$ of *m/z* 487.3. All data were identical to those from trihydroxiursenoic acid.

Peak 41 (*Uc*, t_R = 34.7 min), **Peak 45** (*Us*, t_R = 40.9 min) and **Peak 47** (*Uc*, t_R = 41.6 min), produced some common fragments of *m/z* 712.9, 532.3 and 550. Ionic fragment of *m/z* 457 was observed in positive polarity. These compounds are glycosides from oleanolic acid. Their structure must be elucidated by means of NMR analysis.

Peak 43 (*Us*, t_R = 36.0 min) and **Peak 44** (*Us*, t_R = 36.4 min), have in common the ionic fragment of *m/z* 633. The fragment has been observed in several oleanane prosaponins. A loss of 176 amu (glucuronic acid) forms an ionic fragment of *m/z* 457. In fact, this fragment is observed in positive mode.
Peak 46 (*Us*, t_R = 41.3 min) and **Peak 48** (*Us*, t_R = 41.8 min) produced a pseudo molecular ion of *m/z 685.2* and MS2 fragments of *m/z* 639.3 ND 617.4, suggesting that they are cumaroilic derivatives of the maslinic or alphytolic acid.

Scavenging capacity of free radicals

The scavenging capacity of free radicals was investigated in the extracts obtained with methanol from the species. This study was carried out by means of several methods that study the stabilization of free radicals by donation of hydrogen atoms, or by electron transfer with later proton donation. table 2 indicates the scavenging capacity of free radicals. *U. candollei* capacity is more important, and it is related to

the polyphenols and total flavonoids contents (correlation coefficient equal to 0.881, 0.776 and 0.863 for the method DPPH, ABTS and OH, respectively $p<0.05$).

Table 2. Scavenging capacity of free radicals

Ugni species	Methanolic Extracts
	IC_{50} DPPH (µg/mL)
U. candollei	8.1 ± 0.2
U. selkirkii	9.2 ± 0.8
	IC_{50} ABTS (µg/mL)
U. candollei	11.0 ± 0.7*
U. selkirkii	11.9 ± 1.2*
	IC_{50} OH (µg/mL)
U. candollei	20.5 ± 1.3
U. selkirkii	25.4 ± 0.9

DISCUSSION

Hitherto, it is interesting to make a relation respect to the collection place of the samples. All species were collected over the sea level, in volcanic soils and close to the sea. *U. candollei* was collected in the Oncol hill, at 715 m above sea level. The hill belongs to the Oncol Park which is a private conservation park, located in the Municipality of Valdivia, in Los Ríos Region, distant 29 km from the city. This species concentrates the highest levels of both polyphenols and flavonoids. Factors that could contribute to this phenomenon are constituted by exposure to radiation, the high environmental humidity and the constant rainfall.[15,16] Authors[17,18] have described the participation of these factors on the biosynthesis of phenolic metabolites. Similarly, *U. selkirkii* was collected at the top of the Selkirk viewpoint, located in the Portezuelo hill, at 565 m above sea level, in Juan Fernandez Archipelago.

Chemical properties of *U. selkirkii*, collected in Juan Fernandez, are probably due to the weather characteristics, such as high humidity and the volcanic origin of the place[19]. The high content of iron and aluminum oxyhydroxides, with deficiency in the supply of nitrogen, phosphorous, potassium and sulfur favors the biosynthesis of both phenolic compounds and terpenoids, such as saponins.[19-21] What differentiates island and mainland soils, also volcanic, is that the latter are special at surface level and proximity to the craters. This would explain more important levels of certain nutrients, as well as the lack of other. Humidity, soil characteristics and radiation could influence on the genetic characteristics that determine the phenotypic characteristics of the species, because the adaptive response to natural habitat conditions makes the functions of these compounds become more important, as described by Vivanco[20], Davies & Schwinn[21] and Vermerris.[22] Finally, *U. molinae* was collected in valleys from the Biobío Region.[10]

These backgrounds, could explain chemical differences between species. Compounds identified from the extracts such as flavonoles, as well as gallic and ellagic derivatives can exert the scavenging capacity of free radicals through hydroxyl groups and their electronic stability.[23]
As expected, it was possible to determine similarities and variations in the chemical properties among the species of the genus *Ugni* that inhabit in Chile; *U. candollei* and *U. selkirkii* are the species that present greater content and variety of phenolic and terpenic compounds (pentacyclic triterpenic saponins derivatives from oleanoic acid, mostly). These species exert greater antioxidant capacity in comparison to *U. molinae*[10]. On the other hand, *U. candollei* exceeds because of its flavonoids content, such as glycosides and quercetin derivatives, whereas *U. selkirkii* is notorious in gallotannins. *U. molinae* is characterized in ellagic acids derivatives.[10] These data and the morphological characteristics could constitute as important evidences for evolutionary studies of these species.

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