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Research Article

Chemical Properties and Assessment of the Antioxidant Capacity of Leaf Extracts from Populations of *Ugni molinae* Growing in Continental Chile and in Juan Fernandez Archipelago.

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ABSTRACT

Ugni molinae Turcz is a Chilean native plant widely distributed in Central South Chile as well as in Juan Fernandez Archipelago. Its fruits are consumed fresh because of its organoleptic properties. Given the importance of the chemical compounds identified from *U. molinae*, it becomes important to know if there are variations in the chemical content and biological activity among populations growing geographically separated, as well as in different habitats, such as those populations that grow in Continental Chile and in Juan Fernandez Archipelago. The aim of this work was to assess the chemical composition and the antioxidant capacity of extracts from populations of *U. molinae* that grow in Continental Chile and in Juan Fernandez Archipelago. Composition of chemical compounds was determined by chromatographic methods (HPLC-ESI-MS). Antioxidant capacity was assessed by means of unspecific methods (DPPH and ABTS) and stabilization of the hydroxyl radical. Differences are observed in the chemical composition among continental and insular (Juan Fernandez Archipelago) populations of *U. molinae*. The latter presents greater content and variety of phenolic and triterpenic glycosides of oleanone type. These populations exert greater antioxidant capacity, probably due to the insular geoclimatic conditions.

INTRODUCTION

Ugni molinae Turcz, is a Chilean native plant commonly known with the local name of "Murtilla". The species is distributed between Maule Region and Chiloe Region, including Juan Fernandez Archipelago. The plant grows mainly in the coastal line, in both wet and dry environments, on forest borders and in rocky soils. *U. molinae* is an evergreen shrub, small in drought conditions, but it can reach 3 m height in zones with heavy rainfall. Fruits of *U. molinae* have been known by aboriginal people with the name "forest grapes", very useful to relieve circulatory disorders, as well as to enhance visual acuteness, especially during night. Murtilla fruits are consumed fresh, because of its organoleptic properties and it is also used for the preparation of jams, syrups, desserts and liquors among others[1]. *U. molinae* leaves have been used in infusions by aboriginal people as stringent, for the treatment of diarrheas and dysenteries.

Currently, there are records on the chemical composition of the leaves of *U. molinae* and its biological activity[2-10]. These authors have described the presence substances of phenolic kind, such as phenolic acids and flavonoids, compounds with a renowned antioxidant activity; and triterpenic compounds, such as pentacyclic triterpenic acids.

The biological activity conferred by these compounds to *U. molinae* turn this species into a beneficial one for the human being, different health areas.

The wide distribution of this species could affect the chemical composition of the populations from one region to another. There are geoclimatic backgrounds about how solar radiation, humidity and soil fertility can affect he synthesis of secondary metabolites[11], as well as how biotic factors affect[12]. As already mentioned, *U. molinae* inhabits Juan Fernandez Archipelago as an introduced species. This archipelago is more than 600 km off the Chilean coast and, whose peculiar climate and soil characteristics could be affecting the chemical composition of the populations that grow in these islands.

Given the importance of the compounds identified from *U. molinae*, it becomes important to determine if there are variations in the kind of the chemical compounds and the subsequent biological activity among populations geographically separated and in different habitats, such as the case in continental Chile and in Juan Fernandez Archipelago. For that reason, the aim of this study was to assess the chemical composition and the antioxidant capacity of extracts from populations of *U. molinae* that grow in both continental and insular Chile.

MATERIALS AND METHODS

Vegetal material: Biological material from populations of *U. molinae* was collected in blooming season (november – march, 2008 – 2009) in the Biobío Region and in Juan Fernandez Archipelago, Robinson Crusoe island. Species were identified by the taxonomist Dr. Roberto Rodríguez

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Table 1. HPLC-MS of methanolic extracts

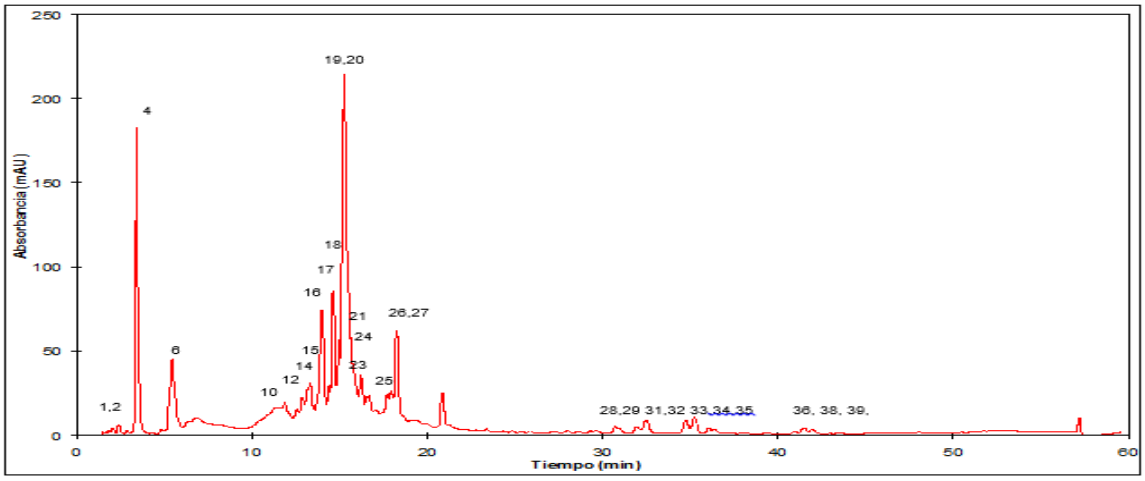
Peak	t_R (min)	λmax (nm)	$[M-H]^-$	Ions MS/MS	Tentative identification	Populations
1	2.4	271	331.1	168.8; 124.9	Galloil glucose	UmJF[a]
2	2.8	271	467.3	420.7; 328.9; 168.9	Digalloil glucose derivative	UmJF
3	3.1	280	633.0	300.8; 274.9; 248.9	Hexahydroxydiphenoil galloil glucose	UmBB[b]
4	3.3	280	331.2	168.8; 124.8	Galloil glucose isomer	UmJF
5	4.3	280	633.0	300.8; 274.9; 248.9	Hexahydroxydiphenoil galloil glucose isomer	UmBB
6	5.3	295,272,240	495.0	342.9; 190.9;168.8	Digalloilquinic acid	UmJF
7	8.9	280	782.2	768.1; 765.0; 300.8	Hexahydroxydiphenoil glucose -bis	UmBB
8	10.4	280	783.5	765.1; 300.7	Hexahydroxydiphenoil glucose -bis	UmBB
9	11.4	280	784.6	764.9; 300.8	Hexahydroxydiphenoilglucose -bis	UmBB
10	11.7	272	453.0	312.9; 168.8	Galloil derivative	UmJF
11	11.8	280	783.9	765.0; 300.8	Hexahydroxydiphenoil glucose -bis	UmBB,
12	12.4	356,300,267	615.3	462.9; 300.9; 254.9	Quercetin hexose gallate	UmJF
13	12.7	280	783.8	765.0; 300.8	Hexahydroxydiphenoil glucose -bis isomer	UmBB
14	13.1	280	635.3	482.9; 465.0; 313.2	Trigalloil glucose	UmJF
15	13.4	374,300255	631.5	479.0; 316.9; 298.9	Myricetin hexoside gallate	UmJF,UmBB
16	13.8	374,300,255	631.5	479.0; 316.9; 298.9	Myricetin hexoside gallate	UmJF
17	14.0	356,300,267	615.4	462.9; 300.9	Quercetin hexose gallate	UmJF,UmBB
18	14.3	374,300,255	631.5	479.0; 316.9; 298.9	Miricetin hexoside gallate isomer	UmJF
19	14.6	356,300,267	615.3	462.9; 300.9	Quercetin hexose gallate isomer	UmJF,UmBB
20	14.8	317,253	525.5	373.0; 359.0; 313.0; 211.0	Methoxy flavone derivative gallate Miricetin routinoside	UmJF UmJF
21	15.1	359,266	624.7	462.9; 315.9	Miricetin deoxyhexoside	UmBB
22	15.2	260	927.0	462.9; 315.9; 270.8	Quercetin pentoside hexoside	UmJF
23	16.1	355,254	599.4	462.9; 300.9	Quercetin pentoside hexoside isomer	UmJF
24	16.6	355,254	599.8	462.9; 300.9	Myricetin desoxyhexoside gallate	UmJF
25	17.5	374,300,260	615.4	462.9; 316.8	Myricetin desoxyhexoside gallate isomer	UmJF
26	17.8	374,300,260	615.5	462.9; 316.8		
27	18.1	374,254	317.7	298.8; 270.9; 230.9; 194.9	Myricetin	UmJF UmJF,UmBB
28	30.7	194	686.1	649.4	Oleanolic acid saponin derivative Asiatic acid	 UmJF
29	31.9	198	975.6	487.2	Oleanolic acid saponin derivative	UmBB
30	32.0	194	717.9	671.3; 649.3	Oleanolic acid saponin derivative isomer	UmJF,UmBB UmJF,UmBB
31	32.5	194	686.1	649.4	Prosaponin oleanane	UmJF,UmBB
32	34.7	194	670.0	633.3	Cumaroilic derivative of ursenoic acid	 UmJF
33	35.2	198	633.8	589.3; 513.2; 469.2	Prosaponin oleanane	UmJF
34	36.0	194	669.6	633.3	Prosaponin oleanane isomer	UmJF
35	36.4	194	669.6	633.3	Oleanolic acid glycoside	UmBB
36	40.9	194	748.9	712.5	Cumaroil derivative of maslinic acid	UmJF
37	41.3	194	685.2	639.3; 617.4	Oleanolic acid glycosideisomer	UmJF,UmBB
38	41.4	194	749.2	712.5; 532.4	Cumaroil derivative of maslinic acid isomer	
39	41.8	194	685.2	639.2; 617.3		

[a]*UmJF: Methanolic Extract U. molinae Juan Fernandez.*
[b]*UmBB: Methanolic Extract U. molinae R Biobío*

at the department of Botany, Faculty of natural and Oceanographic Sciences, University of Concepción. One sample of every species was recorded at the CONC herbarium (146511 and 116887, respectively).

Extracts obtaining: Biological material was dehydrated in the shade, at room temperature and the size was reduced by means of a blade mill. In order to obtain the extracts, 50 grams of the grinded samples were processed in a soxhlet apparatus. The following solvents were successively used: hexane, chloroform, ethyl acetate and methanol until exhaustion of vegetal material. Relation mass: solvent was 1:6. Every extract was concentrated in an evaporator and then taken to dryness in liophilizer. Recognition reactions for 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 the time for their utilization. For chemical and biological determinations, the extracts obtained with methanol were selected, due to their content of phenolic compounds and saponins.

Chromatographic analysis: Analyses of leaves extracts from populations of *U. molinae* (3 mg/mL) were carried out according to Cho et al. (2004)[13]. 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



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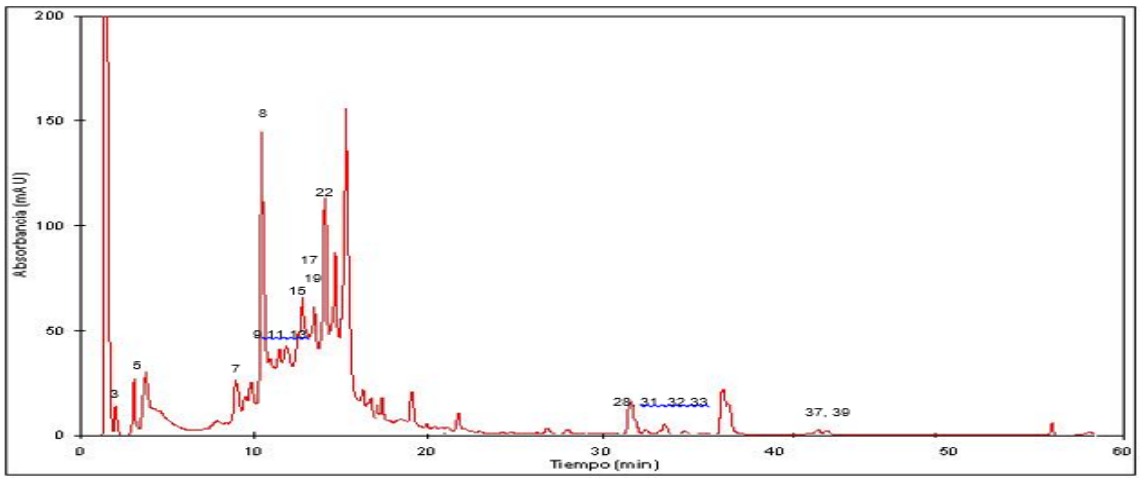

B

Fig 1: HPLC-ESI-MS analysis of methanolic extracts A: Chromatogram of the methanolic extract of U. molinae Juan Fernandez Archipelago, B: Chromatogram of the methanolic extract of U. molinae (R Biobío).

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.

Antioxidant capacity: Was carried out according to Joyeux et al. (1995)[14], stabilization of the DPPH radical; Ghiselli et al. (1998)[15], stabilization of the ABTS radical; Halliwell et al. (1987)[16], 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.

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 AND DISCUSSION

The population from Juan Fernandez Archipelago is the one with the highest concentration of total polyphenols in the extract obtained with methanol (0.068 ± 0.003 g EAG/g)[17]. Continental population instead, have lower levels of total polyphenols in comparison to the insular population. Nevertheless, these levels are considerable in the methanolic extract (0.054 ± 0.003 g EAG/g dry matter)[17]. The greatest content of total saponins (0.029 ±

0.003 saponin/g dry matter) was presented in the population from Juan Fernandez Archipelago[17].

It must be taken into account to establish a relation regarding the collection place of the populations. Samples from two populations were collected at the sea level, in volcanic soils and close to the sea. The difference between mainland and island soils, both of volcanic origin, is that the latter are special at surface level and close to the craters. This would explain more important levels of some nutrients and the lack of others[18-20]. The lack of nutrients in volcanic soils also contributes to the characteristic erosion of Juan Fernandez Island which is produced by both water and wind. Thus, their constant circulation would produce nutrient loss and metabolites that are stored in subterranean organs. This is the case of terpenic compounds[10].

Population from Juan Fernandez Archipelago is considered an invasive species, as well as a plague in the insular territory. One of the possible reasons for its success could be constituted by the important composition of secondary metabolites. The high humidity, the volcanic origin of the soils of the archipelago, the radiation and the erosion could favorably influence on the biosynthesis of terpenic nature compounds, such as saponins and phenolic compounds in an introduced species[10]. This would be justify the increment of both phenolic and terpenic compounds by increase in the activity of key enzymes, incrementing their precursors, recycling and distribution of nitrogen and carbon in enzymatic systems induced by a nutrient deficit in the soil, such as nitrogen and phosphorous, among other biosynthetic events[18-21-22].

The samples from the insular population were collected at the top of the Selkirk viewpoint, located in the Portezuelo Hill, at 565 ms bove sea level. On the other hand, sample from continental population were collected in valleys. As a general rule, in protective mechanisms, phenolic and terpenic compounds have a high participation, such as in allelopathic mechanisms, defense against both pathogens and predators[23-24]. These factors could be important for the adaptation of an introduced species into a new environment. Although it is true, there are no evidences on this subject for the insular population of *U. molinae*. These backgrounds, as a whole, could explain both differences between insular and mainland populations. This will be explained as follows.

Composition by chromatographic analysis: The tentative identification of the main phenolic and triterpenic compounds in the extracts from the populations of *U. molinae* of Juan Fernandez (*UmJF*), and Biobío region (*UmBB*) was carried out by means of HPLC-ESI-MS. The assignment of peaks was carried out by means of fragmentation pattern analysis and their comparison to the mass spectra from standards as well as described in the literature. Both retention time and *m/z* in positive polarity for the respective extracts are presented in Table 1, and the chromatograms, in Figure 1. Spectral characteristics were obtained from those signals considered intense and pure: positive mode was also used for the confirmation of some compounds.

Methanolic extracts (Table 1, Fig 1).: Tannins: It must be pointed out that the most important compounds from continental population are gallic acid and ellagic acid derivatives, whose tentative identification by means of HPLC-ESI-MS are reported in this study for the first time. As new data, phenolic acids derived from quinic acid are also reported for the insular population.

Peak 1 y 4 (*UmJF*, t_R = 2.4 min, 3.3 min), have a $[M-H]^-$ of *m/z* 331. MS2 fragmentation produced ionic fragments of *m/z* 168.8 after the loss of one unit of gallic acid and of *m/z* 124.9 after the decarboxylation of a unit of gallic acid [M-H-44]. Ion fragmentation intensity is different for each peak of galloil glucose, suggesting the existence of different isomeric forms.

Peak 2 (*UmJF*, t_R = 2.8 min), has a $[M-H]^-$ of *m/z* 467.3, corresponding to a derivative from digalloil glucose that has lost a molecule of water. Fragments of *m/z* 328.9 and 168.9 suggest the sequential loss of galloil units.

Peak 3 y 5 (*UmBB*, t_R = 3.1 min, 4.3 min), have a $[M-H]^-$ of *m/z* 633. These are dissociated to the form *m/z* 301 hexahydroxydiphenoil (HDDP), through the loss of 332 amu. This indicates the presence of a unit of galloil glucose (ellagitannin). This compound is also known as sanguiin H4, or sanguiin H5 depending on where it is bonded to the galloil unit[25].

Peak 6 (*UmJF*, t_R = 5.3 min), has a pseudo molecular $[M-H]^-$ of *m/z* 495, from which the MS2 fragmentation originated ionic products of *m/z* 342.9, 191 and 168.9, corresponding to the successive losses of two gallic acids and the gallic acid fragment, respectively. Data are consistent with a digalloil substitution of the quinic acid core.

Peaks 7, 8, 9, 11 and 13 (*UmBB*, t_R = 8.9 - 12.7 min) were identified as isomers of pedunculagin. All of them present a typical parental peak $[M-H]^-$ of *m/z* 783 and *m/z* 301 (loss of HDDP-glucose). This was assigned to bis-HDDP glucose (pedunculagin), previously found strawberry leaves and blackberry fruits[26].

Peak 10 (*UmJF*, t_R = 11.7 min). The presence of a MS2 ionic fragment of *m/z* 168.9 suggests that this compound is a galloil derivative.

Peak 14 (*UmJF*, t_R = 13.1 min), was identified as trigalloil glucose with a $[M-H]^-$ of *m/z* 635.3.

Flavonoids: In the extracts from the populations the presence of a number of myricetin and quercetin flavonols derivatives was observed. These coincide with the previously reported for the species *U. molinae*[5, 10]. It must be taken into account that in our study, the quercetin derivatives were predominant in the continental population, whereas in samples collected in Robinson Crusoe Island (Juan Fernandez Archipelago), myricetin glycosides predominate. Furthermore, for first time and by means of HPLC-ESI-MS we were able to identify galloilated forms of glycosilated quercetin and myricetin. In the insular population, a greater variety of these kind of compounds can be seen. As shown in Table 1, flavonoids from the different samples if *U. molinae* elute after 12 minutes.

Table 2. Scavenging capacity of free radicals from populations of *Ugni molinae*. Values are the average of de ± SD; n=3. * *P*<0.01.

Populations of *U. molinae*	Methanolic Extracts
Juan Fernandez	IC_{50} DPPH (µg/mL)
Biobío	9.5 ± 0.8
	13.5 ± 1.1
Juan Fernández	IC_{50} ABTS (µg/mL)
Bío-Bío	13.5 ± 0.3
	21.6 ± 0.5
Juan Fernández	IC_{50} OH (µg/mL)
Bío-Bío	18.2 ± 0.2*
	30.0 ± 0.8

Peak 12 (*UmJF*, t_R = 12.4 min), Peak 17 (*UmBB, UmJF*, t_R = 14.0 min) and Peak 19 (*UmBB, UmJF*, t_R = 14.6) Have a [M-H]$^-$ of m/z 615.3. MS2 ionic fragments of *m/z* 462.9 (loss of a sugar of 162 units) are consistent with quercetin hexoside gallate isomers.

Peak 15 (*UmBB, UmJF*, t_R =13.4 min), Peak 16 *(UmJF*, t_R =13.8 min) and Peak 18 (*UmJF*, t_R = 14.3 min), have a [M-H]$^-$ de *m/z* 631.5 and MS2 ionic fragments of *m/z* 479 (loss of 152 amu from a galloil unit) and a deprotonized aglycon of *m/z* 316.9 (loss of a sugar of 162 units) are consistent with quercetin hexoside gallate.

Peak 20 (*UmJF*, t_R = 14.8 min), exhibits a pseudo molecular ion of [M-H]$^-$ *m/z* 525.5. The loss of a galloil unit of 152 units and the formation of a fragment *m/z* 373 and the ionic fragment of *m/z* 313 suggest that this compound is a methoxy flavones gallate derivative[27]. The lack of other characteristics does not allow a better allocation.

Peak 21 (*UmJF*, t_R =15.1 min), exhibits a pseudo molecular ion [M-H]$^-$ of *m/z* 624.7. A MS2 ionic fragment of *m/z* 462.9 (loss of 162 units) and its deprotonized aglycon of *m/z* 315.9 (loss of a sugar of 146 units) suggest that this compound is myricetin hexose deoxyhexoside (routinoside).

Peak 22 (*UmBB*, t_R =15.2 min), exhibits a 2M-H]$^-$ of *m/z* 927.4. A pseudo molecular ion of *m/z* 463 and its (loss of a sugar of 146 units) deprotonized aglycon of *m/z* 316 (loss of a sugar of 146 units) suggest that this compound is another form of myricetin deoxihexoside.

Peak 23 (*UmJF*, t_R = 16.1 min), and Peak 24 (*UmJF*, t_R = 16.6 min) exhibit a pseudo molecular ion of *m/z* 599.4. In MS2 produced ionic fragments of *m/z* 462.9 (loss of a sugar of 132 units) and their deprotonized aglycon of *m/z* 300.9 loss of a sugar of 162 units. This suggests that these compounds are isomers of quercetin pentoside hexosides.

Peak 25 (*UmJF*, t_R = 17.5 min) and Peak 26 (*UmJF*, t_R = 17.8 min) have a pseudo molecular ion of *m/z* 462.9 (loss of one galloil unit of 152 units) and *m/z* 316.8 (loss of a sugar of 146 units) suggest that these compounds are isomer of myricetin desoxyhexoside gallate.

Peak 27 (*UmJF*, t_R = 18.1 min) has a pseudo molecular ion of *m/z* 317.7 and MS2 fragments, characteristic of myricetin in negative mode.

Triterpenic glycosides: In all studied extracts, the presence of pentacyclic triterpenic derivatives was observed by means of HPLC-ESI-MS. Regarding the main fragments, those derived from oleanolic, asiatic and maslinic acids must e noted. The most abundant compounds were those derived from oleanolic acid. Some of them have been previously reported for *U. molinae*[3]. However, the presence of pentacyclic triterpenic glycosides under the shape of saponins has not been yet reported. The different isomers and conjugated forms of these compounds elute between 30 and 42 minutes, 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) are needed, as well as the comparison to standards[28].

Peak 28 (*UmBB, UmJF*, t_R = 30.7 min), Peak 30 (*UmBB*, t_R = 32.0 min) and Peak 31 (*UmBB, UmJF*, t_R = 32.5 min) contain [M-H]$^-$ pseudo molecular ions of *m/z* 686.1 y *m/z* 717.9. A common MS2 ionic fragment of *m/z* 649.4 suggests that these compounds are saponins derived from the oleanolic acid, such as bayogenin hexose. By means of 1H and 13C NMR as well as hydrolysis, this structure could be unequivocally identified.

Peak 29 (*UmJF*, t_R = 31.9 min) contains a [2M-H]$^-$ of *m/z* 975.6, with a pseudo molecular ion *m/z* 487.2, consistent with asiatic acid. Arjunolic, trihydroxyoleanolic and trihydroxyursenoic acids also contain similar fragments.

Peak 32 (*UmBB, UmJF*, t_R = 34.7 min) and Peak 34, 35 (*UmJF*, t_R = 36.0 min, 36.4 min) have, in common, an ionic fragment of *m/z 633.* This fragment has been observed in a number of oleanone saponins.

Peak 33 (*UmBB, UmJF*, t_R = 35.2 min) contains a pseudo molecular ion [M-H]$^-$ of *m/z* 633.8, with ionic MS2 fragments of *m/z* 589.3, 513.2 and 469.2 (loss of one cumaroil unit). This suggests that this compound could be a derivative from the cumaroiloxydihydroxy ursenoic acid[29].

Peak 36 (*UmJF*, t_R = 40.9 min) and Peak 38 (*UmJF*, t_R = 41.4 min), have some fragments in common of *m/z* 749, 712.9 and 532.3. These compounds are oleanolic acid glycosides. Its structure must be elucidated with NMR analysis.

Peak 37 (*UmBB*, t_R = 41.3 min) and Peak 39 (*UmBB, UmJF*, t_R = 41.8 min) have a pseudo molecular ion of *m/z* 685.2 and MS2 fragments of *m/z* 639.3 and 617.4, suggesting that these compounds are cumaroilic derivatives from maslinic or alfitolic acids[29, 30].

Scavenging capacity of free radicals: The scavenging capacity of free radicals in the extracts obtained with methanol was investigated by means of a number of methods that study the stabilization of free radicals, by donating hydrogen atoms or by electronic session with subsequent donation of protons[31]. Table 2 shows that the population from Juan Fernandez Archipelago is the population with the greatest scavenging capacity of free radicals in the extract obtained with methanol. Which is related to the total content of phenols (correlation coefficient equal to 0.954 and 0.959 for the methods DPPH, ABTS and OH, respectively, *p<0.05*).

The antioxidant capacity of a compound to stabilize free radicals, such as DPPH, ABTS and the OH radical can be assessed by the proportion of free radicals and the number

of molecules that can stabilize them. This is directly determined by the chemical structure of the antioxidant molecule[31]. The transfer of hydrogen atoms or electrons followed by a transfer of protons depends on the probable antioxidant mechanism of the involved compounds and it is correlated with both content and variety[31]. Compounds identified in the studied extracts such as flavonoles, gallic and ellagic derivatives can exert their scavenging capacity of free radicals by means of hydroxyl groups and their electronic stability is limited by binding to sugars and the polymerization degree of the *in vitro* experiments. Differences in IC_{50} among methods could be associated to the sensibility and specificity of each one. To the total free radical scavenging capacity of the extracts with antioxidant capacity, pentacyclic triterpenic acids identified in such extracts would also contribute[32]. This is particularly important in the case of the hydroxyl radical that reacts mainly with *in vivo* lipids and the amphipathic nature of the triterpenic core could provide special protection to the lipid peroxidation[31].

Differences observed about the chemical properties between continental and insular populations of *U. molinae*. The latter population presents greater content and variety of phenolic glycoside triperpenic compounds of oleanone type. This exerts greater antioxidant capacity, probably due to the geoclimatic conditions of the archipelago.

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REFERENCES

1. Hoffmann A. Flora Silvestre de Chile, Zona Araucana, Fundación Claudio Gay, Chile, 1991, 160.
2. Avello M, Pastene E. Actividad antioxidante de infusos de *Ugni molinae* Turcz. (Murtilla). *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 2005; 4(2):33–39.
3. Aguirre MC, Delporte C, Backhouse N, Erazo S, Letelier ME, Cassels BK, Silva X, Alegría SM, Negrete R. Topical anti-inflammatory activity of 2 α-hydroxy pentacyclic triterpene acids from the leaves of *Ugni molinae*. *Bioorganic & Medicinal Chemistry* 2006; 14:5673-5677.
4. Suwalsky M, Orellana P, Avello M, Villena F, Sotomayor C. Human erythrocytes are affected *in vitro* by extracts of *Ugni molinae* leaves. *Food and Chemical Toxicology* 2006; 44:1393–1398.
5. Rubilar M, Pinelo M, Ihl M, Scheuermann E, Sineiro J, Nuñez MJ. Murta leaves (*Ugni molinae* Turcz.) as a source of antioxidant polyphenols. *Journal of Agricultural and Food Chemistry* 2006; 54:59-64.
6. Delporte C, Backhouse N, Inostroza V, Aguirre MC, Peredo N, Silva X, Negrete R, Miranda HF. Analgesic activity of *Ugni molinae* (murtilla) in mice models of acute pain. *Journal of Ethnopharmacology* 2007; 112:162-165.
7. Suwalsky M, Orellana P, Avello M, Villena F. Protective effect of *Ugni molinae* Turcz against oxidative damage of human erythrocytes. *Food and Chemical Toxicology* 2007; 45:130–135.
8. Avello M, Valdivia R, Mondaca MA, Ordóñez JL, Bittner M, Becerra J. Actividad de *Ugni molinae* Turcz. frente a microorganismos de importancia clínica. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 2009; 8(2):141-144a.
9. Avello M, Valdivia R, Sanzana R, Mondaca MA, Mennickent S, Aeschlimann V, Bittner M, Becerra J. Extractos a partir de berries nativos para su uso como preservantes naturales en productos cosméticos. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 2009; 8(6):479-486b.
10. Rubilar M, Jara C, Poo Y, Acevedo F, Gutierrez C, Sineiro J, Shene C. Extracts of maqui (*Aristotelia chilensis*) and murta (*Ugni molinae* Turcz): Sources of antioxidant compounds and α-glucosidase/α-amylase inhibitors. *Journal of Agricultural and Food Chemistry* 2011; 59:1630-1637.
11. Oh MM, Trick H, Rajashekar C. Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. *Journal of Plant Physiology* 2009; 166:180-191.
12. Charudattan R, Dinoor A. Biological control of weeds using plant pathogens: Accomplishments and limitations. *Crop Protection* 2000; 19:691-695.
13. Cho MJ, Howard LR, Prior RL, Clark JR. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture* 2004; 84 (13):1771-1782.
14. Joyeux M, Mobstein A, Anton R, Mortier F. Comparative antilipoperoxidant antinecrotic and scavenging properties of terpenes and biflavones from Ginkgo and some flavonoids. *Planta Medica* 1995; 61:126-129.
15. Ghiselli A, Nardini M, Baldi A. Antioxidant activity of different phenolics fractions separated from Italian wines. *Journal of Agricultural and Food Chemistry* 1998; 46(2):363.
16. Halliwell B, Gutteridge JMc, Aruoma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hidroxyl radical. *Analytical Biochemistry* 1987; 165:215-219.
17. Avello M, Bittner M, Becerra J. Efectos antibacterianos de extractos de especies del género *Ugni* que crecen en Chile. *Revista Cubana de Plantas Medicinales* 2013; 18(2):247-257a.
18. Kovacik J, Klejdus B, Backor M, Repcak M. Phenylalanine ammonia-lyase activity and phenolic compounds accumulation in nitrogen-deficient *Matricaria chamomilla* leaf rosettes. *Plant Science* 2007; 172:393-399.

19. Giorgi A, Mingozzi M, Madeo M, Speranza G, Cocucci M. Effect of nitrogen starvation on the phenolic metabolism and antioxidant properties of yarrow (*Achillea collina* Becker ex Rchb.). *Food Chemistry* 2009; 114:204-211.
20. Lattanzio V, Cardinali A, Ruta C, Morone I, Linsalata V, Cicco N. Relationship of secondary metabolism to growth in oregano (*Origanum vulgare* L.) shoot cultures under nutritional stress. *Environmental and Experimental Botany* 2009; 65:54-62.
21. Treutter D. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biology* 2005; 7:581-591.
22. Harbaum-Piayda B, Walter B, Bengtsson G, Hubbermann EM, Bilger W, Schwarz K. Influence of pre-harvest UV-B irradiation and normal or controlled atmosphere storage on flavonoid and hydroxycinnamic acid contents of pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*). *Postharvest Biology and Technology* 2010; 56:202-208.
23. Waniska RD. in Proceedings of International Consultation. Structure, Phenolic Compounds and Antifungal Proteins of *Sorghum* Caryotipes, Chandrashekar, A., Bandyopadhyay, R., Hall, A.J., eds., ICRISTAT, India, 2000, 72.
24. Vivanco J, Cosio E, Loyola V, Flores H. Mecanismos químicos de defensa en las plantas. *Investigación y Ciencia* 2005; 341:68-75.
25. Quindeau S, Feldman KS. Ellagitannin chemistry. *Chemical Reviews* 1996; 96:475-503.
26. Hager T, Howard L, Liyanage Lay JO, R Prior. Ellagitannin composution of blackberry as determined by HPLC-ESI-MS and MALDI-TOF-MS. *Journal of Agricultural and Food Chemistry* 2008; 56:661-669.
27. Wang ZT. Characterization of fifty-one flavonoids in a Chinese herbal prescription Longdan Xiegan decoction by high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and photodiode array detection. *Rapid Communications in Mass Spectrometry* 2008; 22:1767-1778.
28. Augustin J, Kuzina V, Andersen S, Bak S. Molecular activities, biosynthesis and evolution of triterpenoid saponins. *Phytochemistry* 2011; 72:435-457.
29. Lee SM, Park JG, Lee YH, Lee CG, Min BS, Kim JH, Lee HK. Anti-complementary activity of triterpenoids from frutis of *Zyzyphus jujuba*. *Biological & Pharmaceutical Bulletin* 2004; 27:1883-1886.
30. Plastina P, Bonofiglio D, Vizza D, Fazio A, Rovito D, Giordano C, Barone I, Catalano S, Gabriele B. Identification of bioactive constituents of *Ziziphus* jujube fruits extracts exerting antiproliferative and apoptotic effects in human breast cancer cells. *Journal of Ethnopharmacoly* 2012; 140:325-332.
31. Niki E. Assessment of antioxidant capacity *in vitro* and *in vivo*". *Free Radical Biology Medicine* 2010; 49:503-515.
32. Wang ZT. Characterization of fifty-one flavonoids in a Chinese herbal prescription Longdan Xiegan decoction by high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and photodiode array detection. *Rapid Communications in Mass Spectrometry* 2008; 22:1767-1778.