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In memoriam Professor Luis Astudillo, Universidad de Talca, Chile

Identification of isoquinoline alkaloids from *Berberis microphylla* by HPLC ESI-MS/MS

[Identificación de alcaloides isoquinolínicos en *Berberis microphylla* G. Forst mediante CLAE IES-MS/MS]Loreto MANOSALVA¹, Ana MUTIS², Juan DÍAZ³, Alejandro URZÚA⁴,
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Abstract: *Berberis microphylla* (G. Forst) is a native plant growing in Patagonia. In recent years Patagonia *Berberis* are becoming important due to their interesting biological properties related to their alkaloids content. The aim of this study was determine the distribution and proportion of isoquinoline alkaloids in leaves, stems and roots of *B. microphylla* collected in two different climatic zones from Chilean Patagonia. Using by HPLC ESI-MS/MS isocorydine, jatrorrhizine, palmatine, reticuline, scoulerine, tetrahydroberberine and thalifendine were detected for the first time in this specie, and the presence of allocryptopine, berberine, calafatine and protopine, previously isolated in *B. microphylla* was corroborated. The alkaloids profile showed differences of compounds in samples collected in two climatic zones, where more compounds were detected in plants from Lago Deseado than Cerro Sombrero. Furthermore, a greater number of alkaloids were found in stem and root extracts and berberine and thalifendine were detected in higher proportion in these structures.

Keywords: *Berberis microphylla*, Patagonia, isoquinoline alkaloids, HPLC ESI-MS/MS

Resumen: *Berberis microphylla* (G. Forst) es un arbusto nativo que crece en la Patagonia. Actualmente, esta planta ha sido foco de estudio dada las propiedades biológicas que presenta, atribuidas principalmente al contenido de alcaloides. El objetivo de este estudio fue determinar la distribución y proporción de alcaloides isoquinolínicos en hojas, tallos y raíces de *B. microphylla* colectadas en dos zonas climáticas de la Patagonia chilena. Mediante CLAE IES-MS/MS se informa por primera vez la presencia de isocoridina jatrorrhizina, palmatina, reticulina, escoulerina, tetrahydroberberina y talifendina en esta especie y se confirma la presencia de allocriptopina, berberina, calafatina y protopina, identificados previamente en *B. microphylla*. El perfil de alcaloides mostró diferencias en la presencia de compuestos en las muestras colectas en las dos zonas climáticas, observándose un mayor número de compuestos en plantas provenientes de Lago Deseado. Además, un mayor número de compuestos se identificó en extractos de tallos y raíces donde berberina y talifendina fueron detectados en mayor proporción.

Palabras clave: *Berberis microphylla*, Patagonia, alcaloides isoquinolínicos, CLAE IES-MS/MS

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INTRODUCTION

Berberis microphylla G. Forst, (*Berberidaceae*) locally called “calafate” or “michay” is a native shrub that grows wild in southern cone of South America (Chilean and Argentinean Patagonia) (Moore, 1983; Orsi, 1984). The plants had been used in traditional medicine as a treatment of different diseases such as, fevers, inflammations and diarrhea (Muñoz *et al.*, 2004). In recent years, Patagonia *Berberis* has also gained prominence importance due to their interesting pharmacological (Morales *et al.*, 1993; Martínez *et al.*, 1997; Alarcon *et al.*, 2014), antifungal (Enriz & Freile, 2006; Freile *et al.*, 2006; Pitta-Alvarez *et al.*, 2008) and antibacterial properties (Freile *et al.*, 2003) based on the presence of alkaloids.

To date, few chemical studies in *B. microphylla* (known also *B. buxifolia*) in relation to the presence of isoquinolinoline alkaloids have been reported (Fajardo *et al.*, 1979; Leet *et al.*, 1983). Alkaloids are naturally secondary metabolites and its biosynthesis in some plants family is influenced for abiotic factors in order to tolerate and survive under harsh weather conditions (Bustamante *et al.*, 2006; Cuadra and Fajardo, 2008). *B. microphylla* grown in Patagonia is exposed to stressful habitats such as strong wind, cloudy and cold days and arid soils (Garredau *et al.*, 2013). Moreover, the altitude, soil nutrients and phenological stage of the plant affects the alkaloids content in *Berberis* species (Chandra & Purohit, 1980; Andola *et al.*, 2010a; Echeverria & Niemeyer, 2012; Niemeyer, 2014). The literature indicate that the presence of berberine (major alkaloid in *Berberis* plant) could be restricted to a specific organ or distributed in different organs of plants; including leaves, stem and roots (Khamidov *et al.*, 2003; Končić *et al.*, 2010). However, in *B. microphylla* only has been studied the presence of alkaloids but its distribution in the plant is unknown.

In the last decades, several methods have been report for the determination of alkaloids in plant, included colorimetry (Shamsa *et al.*, 2008), thin layer chromatography (TLC) (Patel *et al.*, 2012), capillary zone electrophoresis (CZE) (Gong *et al.*, 2003) and nuclear magnetic resonance (H-NMR, C-NMR) (Quevedo *et al.*, 2008; Fajardo *et al.*, 2009). In some cases, the traditional methods mentioned above require sample extractions and long time for completing the whole analysis (Chan *et al.*, 2007). In the recent years, high performance liquid chromatography-electrospray tandem mass spectrometry (HPLCESI-MS/MS) has demonstrated

to have an advantage over other methods for the high sensitivity, specific and rapid analysis for the identification of components in complex alkaloid mixtures (Chen *et al.*, 2000; Fabre *et al.*, 2000; Luo *et al.*, 2005; Ding *et al.*, 2007).

This work reported a qualitative and semi-quantitative study of the isoquinoline alkaloids found in leaves, stems and roots of *B. microphylla* collected in two different climatic zones from the Patagonia determined by HPLC ESI-MS/MS. Furthermore, the results have been discussed with the purpose to find some relation between the variation of alkaloids in the plant and the habitat that they grow.

MATERIALS AND METHODS

Plant material

Representative samples of leaves, stems and roots of *B. microphylla* were collected during flowering season at Cerro Sombrero (52°48'26.7"S; 68°52'50.1"W) and Lago Deseado (54°22'12.4"S; 68°45'45.0"W) in November and December 2011. Sample collections were performed in the Province of Tierra del Fuego in two climatic zones corresponding to cold steppe (Cerro Sombrero) and submontane (Lago Deseado). In cold steppe the climatic data indicate that the rainfall gradient is low (250-350 mm), mean annual temperature is 6.8 °C, soils present a pH 6-7 and erode readily for strong winds. Further south, submontane zone is characterized by more acid soil (pH 4 - 5.2), rainfall increases (338-852 mm) and the temperature decreases to 2.7 °C (annual mean) (Moore, 1983). Voucher specimens (178056-Cerro Sombrero; 178057-Lago Deseado) were deposited in the Herbarium of the Universidad de Concepción, Concepción, Chile.

Alkaloids extraction

Extraction was carried out according to the methodology described by Cabezas *et al.* (2009) with some modifications. Oven dried and powdered leaves (100 g), stems (300 g) and roots (300 g) of *B. microphylla* were sequentially extracted (24, 48 and 72 h) with methanol at room temperature. Methanolic extracts were evaporated *in vacuo* at 40 °C, and the residue was reconstituted with 200 mL 10% HCl for 1 h under agitation (orbital shaker, MS-NOR, Taiwan), and allowed to stand for 12 h at 10° C and then filtered. The filtrate was washed with CHCl₃ (5 x 100 mL). The aqueous phase was adjusted to pH 10 with NH₄OH and extracted with CHCl₃ (5 x 100 mL). The solvent was evaporated for obtaining the extract containing alkaloids.

Alkaloid standards

Berberine (purity, > 90%), palmatine (purity, > 97%) and jatrorrhizine (purity >95%) were purchased from Sigma Aldrich (St. Louis, USA) and all solvent used for extraction were analytical grade. Calafatine was obtained from Laboratorio de Productos Naturales, Universidad de Magallanes (Punta Arenas, Chile). HPLC grade acetonitrile, methanol water and formic acid were purchased from Merck (Darmstadt, Germany).

HPLC ESI-MS/MS

The chromatographic separation was carried out using a RP-C18 BioSuite column (2.1 x 150 mm, 3 μ m), injecting 10 μ L at 0.2 mL/min and 35 °C. 0.01 g of standards and sample extracts were dissolved in 10 mL of methanol and submitted to LC-MS/MS. The chromatographic separation was performed using a linear gradient solvent system consisting of 0.1% formic acid (A) and acetonitrile (B). The linear gradient was composed of 0–3 min 10% B, 3–35 min 10–70% B, 35–40 min 70% B, 40–50 min 70–10% B, then again, under the initial conditions (10% B) for 10 min. Each standard was injected with an electro spray ionization (ESI) source into the mass spectrometer (LC-MS MS Shimadzu Prominence coupled at mass spectrometer Applied Biosystems/MDS Sciex3200 Qtrap, Massachusetts, USA). The ion source temperature was set to 400° C, and the capillary voltage was 5.5 kv. For alkaloids determination, data were collected as positive-ion spectra by means of Enhanced Mass Scan (EMS) over a m/z 100–1000 Da range at 1000 Da/s and Enhanced Product Ion (EPI) over a m/z 50–1000 Da range at 4000 Da/s. The CUR gas was 20 psi, GS1 30 psi and GS2 60 psi.

In addition, the content of alkaloids was performed calculating the relative amounts of the individual alkaloids present in the plant extracts. The ion intensities were extracted at the m/z values of the molecular (M^+) or pseudo-molecular ($M+H^+$) ions of the corresponding detected compounds. The relative ion peak area of each compound from the sample was compared to the relative ion peak area of the total alkaloids.

RESULTS

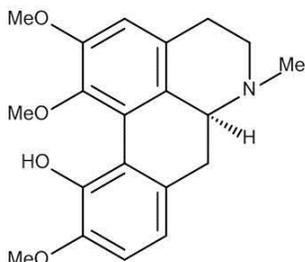
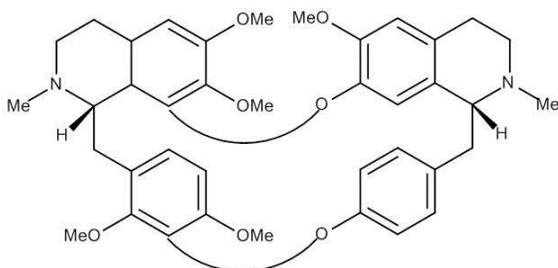
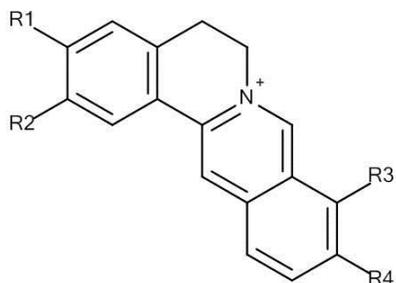
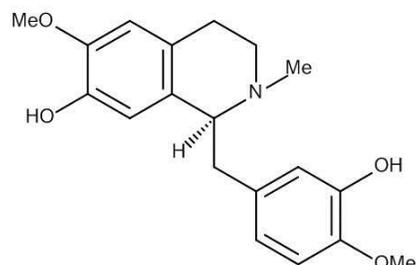
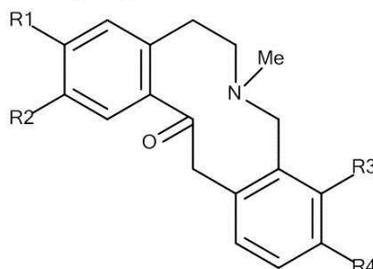
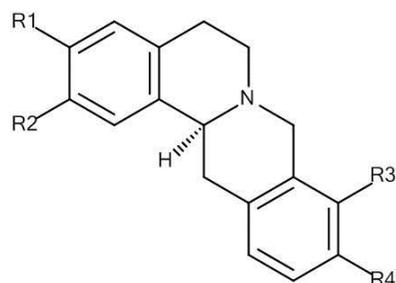
Identification of alkaloids by HPLC ESI-MS/MS

The identification of isoquinoline alkaloids in samples of *B. microphylla* were analysis using HPLC

ESI-MS/MS. The ESI-MS spectra recorded in the positive ionization mode exhibited the $[M+H]^+$ ion for the tertiary bases, and $[M]^+$ ion for the quaternary salts that allowed the determination of molecular weight. All compounds were identified by comparison retention time and MS spectra with those authentic standard or referring the literature and base data. Table 1 shows the spectral data and retention time of each alkaloid identified in *B. microphylla* collected in Patagonia. Different structural type of isoquinoline alkaloids identified in the samples of *B. microphylla* can be classified as: a) aporphine: isocorydine; b) benzylisoquinoline: reticuline; c) bisbenzylisoquinoline: calafatine; d) protopine: allocryptopine and protopine; e) protoberberine: thalifendine, jatrorrhizine, palmatine and berberine; f) tetrahydroprotoberberine: scoulerine and tetrahydroberberine (Figure 1).

Compounds were characterized by MS spectra showing their expected parent ion and MS/MS spectra and characteristic fragment ions. In the case of aporphine-type alkaloids the MS spectra of $[M+H]^+$ ion m/z 342 was tentatively identified as isocorydine (Jeong *et al.*, 2012). The rupture of $[M+H]^+$ for isocorydine produce a characteristic fragment ion at m/z 311, this could be formed by the loss of CH_3NH_2 . The ion m/z 311 could lead to the formation of the m/z 279 fragment because due to loss of CH_3OH , and by lose of OCH_3 and CH_3 from the ion at m/z 279, the ion at m/z 248 and m/z 264 are generated respectively. A benzylisoquinoline-type alkaloid eluting at 13.1 min was assigned as reticuline because the rupture $[M+H]^+$ at m/z 330 producing the characteristic fragments ions at m/z 192 and m/z 137. The ion m/z 192 could lead to the formation of the m/z 177 fragment due to loss of CH_3 (Schmidt *et al.*, 2005). Calafatine, a bisbenzylisoquinoline-type alkaloids was identified comparing the MS spectra with authentic standard. The $[M+H]^+$ at m/z 653 yielded ions product m/z 610 and m/z 622 corresponding to losses of CH_2NCH_3 and OCH_3 respectively. Four protoberberine-type alkaloids thalifendine, jatrorrhizine, palmatine and berberine eluting at 18.1, 18.7, 19.6 and 20.0 min respectively, were identified in base of their MS/MS spectral with authentic standard and previous report (Jeong *et al.*, 2012). The fragmentation of $[M]^+$ at m/z 322 corresponding to thalifendine yielded the characteristic ion at m/z 307 by the loss of CH_3 .

Figure 1
Structure of type of isoquinoline alkaloids identified in *B. microphylla*

Aporphine type**Isocorydine***m/z* 342**Bisbenzylisoquinoline type****Calafatine***m/z* 653**Protoberberine type****Thalifendine***m/z* 322 ($R_1=R_2=-OCH_2O-$, $R_3=OCH_3$, $R_4=OH$)**Jatrorrhizine***m/z* 338 ($R_1=R_3=R_4=OCH_3$, $R_2=OH$)**Palmatine***m/z* 352 ($R_1=R_2=R_3=R_4=OCH_3$)**Berberine***m/z* 336 ($R_1=R_2=-OCH_2O-$, $R_3=R_4=OCH_3$)**Benzylisoquinoline type****Reticuline***m/z* 330**Protopine type****Allocryptopine***m/z* 370 ($R_1=R_2=-OCH_2O-$, $R_3=R_4=OCH_3$)**Protopine***m/z* 354 ($R_1=R_2=-OCH_2O-$, $R_3=R_4=-OCH_2O-$)**Tetrahydroprotoberberine type****Scoulerine***m/z* 328 ($R_1=R_4=OCH_3$, $R_2=R_3=OH$)**Tetrahydroberberine***m/z* 340 ($R_1=R_2=-OCH_2O-$, $R_3=R_4=OCH_3$)

Then the ion fragments *m/z* 307 loss CO producing the fragments ion at *m/z* 279 (Jeong *et al.*, 2012). The MS data of jatrorrhizine was compared with authentic standard. $[M]^+$ *m/z* 338 was fragmented to *m/z* 322

indicating a loss of CH_4 from molecular ion. Further fragmentation yielded *m/z* 308 and *m/z* 294 ions by losses of $2CH_3$ and C_2H_4O respectively. The ion *m/z* 308 could lead to the formation of the *m/z* 280

fragment due to loss of CO (Wu *et al.*, 2005). The alkaloid palmatine, $[M]^+$ m/z 352, was identified due to losses of CH_4 , $2CH_3$ and C_2H_4O producing the fragments m/z 336, 322 and 308 respectively. The ion m/z 322 could lead to the formation of the m/z 294 fragment due to loss of CO (Wu *et al.*, 2005).

The presence of $[M]^+$ at m/z 336 in accordance with authentic standard corresponded to berberine. The $[M]^+$ produced the characteristic fragments ions at m/z 320, 306, 292, 278 by the losses of CH_4 , $2CH_3$, C_2H_4O and $2CH_3-CO$ respectively (Wu *et al.*, 2005).

Table 1
Mass Spectral data and retention times (tR) of isoquinoline alkaloids identified from *B. microphylla* by HPLC ESI-MS/MS

Compounds	tR (min)	$[M+H]^+$	$[M]^+$	m/z fragment ion (% base peak)
Aporphine-type				
Isocorydine ^b	13.08	342		190(54), 207(36), 248(18), 222(27), 264(27), 265(72), 279(100), 296(36), 311(27)
Benzylisoquinoline-type				
Reticuline ^{a,b}	13.1	330		137(100), 192(92), 177(28), 207(14)
Bizbenzylisoquinoline-type				
Calafatine ^c	16.6	653		610(62), 622(100)
Protopine-type				
Allocryptopine ^b	17.4	370		188(100), 206(28), 290(28)
Protopine ^{a,b}	14.2	354		149(3), 177(12), 189(100), 206(18), 247(18), 275(37)
Protoberberine-type				
Thalifendine ^b	18.1		322	251(6), 279(44), 292(6), 307(100)
Jatrorrhizine ^c	18.4		338	280(54), 294(100), 307(72), 308(36), 322(100)
Palmatine ^c	19.6		352	279(2), 294(18), 308(57), 322(47), 336(100)
Berberine ^c	20.0		336	205(1), 234(2), 263(9), 275(11), 278(62), 292(78), 320(100), 306(59)
Tetrahydroprotoberberine-type				
Scoulerine ^b	13.8	328		151(11), 178(100)
Tetrahydroberberine ^b	18.5	340		149(21), 176(100), 324(2)

tR = Retention time

Compounds identified using a MS database (<http://spectra.psc.riken.jp/>); b MS data of literature; c authentic standard

Two tetrahydroprotoberberine-type alkaloids scoulerine (13.8 min) and tetrahydroberberine (18.5 min) showed characteristic tandem MS spectra as reported in the literature (Jeong *et al.*, 2012; Tang *et al.*, 2013). The $[M+H]^+$ at m/z 328 was identified as

scoulerine and the rupture of this ion produced a characteristic fragments ions at m/z 178 and m/z 151 formed by RDA (retro Diels-Alder) or B-ring cleavage (Jeong *et al.*, 2012). The alkaloid tetrahydroberberine displayed $[M+H]^+$ ion at m/z 340

and the losses of $C_{10}H_{12}O_2$ and $CH_3-C_{10}H_{10}O_2N$ assigned to the fragments ions m/z 176 and m/z 149 respectively (Tang *et al.*, 2013). Protopine and allocryptopine eluting at 14.2 and 17.4 min respectively, are protopine-type alkaloids identified in samples of *B. microphylla*. The $[M+H]^+$ ion at m/z 354 and $[M+H]^+$ was tentatively identified as protopine by comparing with literature data (Tang *et al.*, 2013). The $[M+H]^+$ ion at m/z 354 produced a fragment ion at m/z 275 that could result from the loss of $CH_3-NH_2-CH_2(OH)_2$. The rupture type RDA from $[M+2H]^+$ at m/z 355 produced two fragments ions at m/z 149 and m/z 206. The ion m/z 206 could lead to the formation of the m/z 189 fragment due to loss of OH (Tang *et al.*, 2013). Other protopine-type alkaloid was putatively identified as allocryptopine on the basis of previous report (Tang *et al.*, 2013). The $[M+H]^+$ ion at m/z 370 produced a several fragments ions at m/z 290, 206 and 188 by the losses of $2CH_3O-OH$, $C_{10}H_{12}O_2$ and $C_{10}H_{12}O_2-H_2O$ respectively (Tang *et al.*, 2013).

Analysis of alkaloids in plant extracts by HPLC ESI-MS/MS

Table 2 shows the proportion of isoquinoline alkaloids in leaves, stems and roots extracts of *B. microphylla* collected in Cerro Sombrero and Lago Deseado analyzed by HPLC ESI-MS/MS. The results indicate the presence of seven alkaloids (isocorydine, jatrorrhizine, palmatine, reticuline, scoulerine, tetrahydroberberine and thalifendine) not reported before in *B. microphylla* and confirmed the presence of four alkaloids (allocryptopine, berberine, calafatine, protopine) previously reported for this specie (Podesta *et al.*, 1987). Furthermore, a greater number of compounds were observed in stem and root extracts, stand out berberine and thalifendine in stems and roots, and tetrahydroberberine in leaves. Also, there were found differences in alkaloid content between samples collected in two climatic zones differences Protopine only was identified in samples collected in Cerro Sombrero whereas, isocorydine, tetrahydroberberine, scoulerine and reticuline only were identified in sample from Lago Deseado.

Table 2
Identification and proportion of isoquinoline alkaloids in *B. microphylla* from Patagonia

Compounds	Cerro Sombrero			Lago Deseado		
	Leaves (%) ^a	Stems (%) ^a	Roots (%) ^a	Leaves (%) ^a	Stems (%) ^a	Roots (%) ^a
Allocryptopine	-	0.32	1.29	-	1.36	1.58
Berberine	-	78.50	76.43	24.60	85.49	79.82
Calafatine	-	-	0.23	-	-	1.10
Isocorydine	-	-	-	-	1.19	-
Jatrorrhizine	-	-	0.16	-	2.55	2.68
Palmatine	-	-	6.27	-	-	3.05
Protopine	-	2.18	3.05	-	-	-
Reticuline	-	-	-	-	-	1.22
Scoulerine	-	-	-	-	1.87	-
Tetrahydroberberine	-	-	-	75.36	-	-
Thalifendine	-	18.93	12.55	-	4.49	10.51

-: not detected

a: Proportion (%) of each compound with a total alkaloids identified in the samples

DISCUSSION

The identification of major and minor compounds in herbs is of great importance to understand its quality and biological properties (Liang *et al.*, 2004; Deevanhxay *et al.*, 2009). Phytochemical analyses of *B. microphylla* showed the presence of different structural type of isoquinoline alkaloids distributed in different organs of the plant. *Berberis* species contains alkaloids with antimicrobial, antiviral, insecticide and pharmacological activities (Moreno-Murillo *et al.*, 1995; Ivanovska and Philipov, 1996; Küpeli *et al.*, 2002; Yeşilada and Küpeli, 2002; Singh *et al.*, 2007; Quevedo *et al.*, 2007; Shahid *et al.*, 2009; Maliwichi-Nyirenda *et al.*, 2011; Potdar *et al.*, 2012).

The alkaloid profiles in *B. microphylla* showed the presence of isocorydine, jatrorrhizine, palmatine, reticuline, scoulerine, tetrahydroberberine and thalifendine not reported before in *B. microphylla*. These compounds have been isolated from different organs in other species of *Berberis*. Khamidov *et al.* (1997a; 1997b) described the presence of isocorydine as minor alkaloid in leaves and stems of *B. thumbergii* and leaves of *B. densiflora*, whereas Yosupov *et al.* (1993) found this compound in young shoots and leaves of *B. heteropoda*. Jatrorrhizine and palmatine were identified in aerial part, seed and root-barks of *B. julianae*, *B. thumbergii* and *B. vulgaris*, respectively (Brázdovičová *et al.*, 1975; Brázdovičová *et al.*, 1980; Suau *et al.*, 1998). Reticuline has been isolated from *B. heteropoda* in young shoots and leaves extract from *B. integerrima* (Karimov *et al.*, 1993).

Furthermore, tetrahydroberberine and thalifendine also was identified in *Berberis* plants (Karimov, 1993). Other alkaloids present in the extracts as allocryptopine, berberine, calafatine and protopine, have been previously reported in *B. microphylla* (Podesta *et al.*, 1987). Even more, berberine is present in all species of *Berberis* and it can be found in leaves, fruit, stem and roots (Hussaini and Shoeb, 1985; Weber *et al.*, 1989; Karimov *et al.*, 1993; Khamidov *et al.*, 1997a; Andola *et al.*, 2010b), and it has been reported together with the compounds more active biologically (Bhardwaj & Kaushik, 2012).

Pharmacological studies indicate that berberine have extensive properties, such as antimicrobial, antiinflammatory, antioxidant, hypoglycemic and hypolipidemic effects, and effects against cancer and the cardiovascular system (Serafim *et al.*, 2008). In this regard, Bandyopadhyay *et al.* (2013)

reported antibacterial activity of berberine against drug resistant *Escherichia coli* strain, probably due to its intercalation with nucleic acid inhibiting the multiplication of cells. This mechanism together with apoptosis induction in cells could be explaining the anticancer activity reported for berberine (Meeran *et al.*, 2008; Abd El-Wahab *et al.*, 2013). Moreover, antiinflammatory activity has been associated to content of protoberberine alkaloids, palmatine, berberine and jatrorrhizine in herb extracts, probably by inhibition of pro-inflammatory cytokine production (Chao *et al.*, 2009; Kim *et al.*, 2009). These compounds also exhibited antifungal effect. Volleková *et al.* (2003) in a study using *in vitro* dilution agar plate method against human pathogens showed that jatrorrhizine was the most effective agent against fungal species tested with MIC ranges from 62.5 to 125 µg/mL. In addition, antibacterial activity of jatrorrhizine was reported by Ali *et al.* (2013) as the most active antimicrobial compound against resistant *Staphylococcus aureus* strain.

Alkaloids reported in this study showed an uneven distribution in plant organs, where the great number of alkaloids was found in stem and roots. Facchini & De Luca (1995) studied the relationships between plant development and alkaloid biosynthesis in specific organs in *Papaver somniferum*, finding accumulation of morphine in aerial organs and roots, whereas sanguinarine only was accumulated in roots, even more, and the biosynthesis organ-specific is regulated by tyrosine/dopa decarboxylase gene family.

Other factors involved in the distribution and accumulation of alkaloids in organs and tissue of plant could be related with environmental conditions, such as light and temperature. The organs of plant that normally grow in absent of light, such as roots and rhizomes produce alkaloids in largest amount (Cromwell, 1933; Tomè & Colombo, 1995). In addition, in different ecological niches, plants behave differently in terms of biochemical aspects in order to better adapt to their environmental. The altitude, temperature, UV radiation and soils nutrition affect the presence and content of alkaloids (Katoch *et al.*, 2011; Andola *et al.*, 2011; Ghanavi *et al.*, 2013). Variation in the presence of alkaloids in *B. microphylla* could be influenced by the climatic zones where plants were collected. In Lago Deseado was found a greater number of alkaloids in *B. microphylla*. This zone is characterized for low temperature (2.7 °C annual mean), acid soils (pH 4 - 5.2), a lower potassium concentration (K) (66

mg/Kg) and phosphorus (P) (2 mg/Kg) of the soil in relation to samples collected in Cerro Sombrero (K, 346 mg/Kg; P, 13 mg/Kg); factors that could cause a stress on the plant and thus to influence the synthesis of compounds. Gremigni *et al.* (2001) reported the relation about the content of potassium and the production of alkaloids in *Lupinus angustifolius*, finding an increase of the concentration of quinolizidine alkaloids by deficiency of potassium. Similarly, Yaber Grass *et al.* (2009) reported an increase of pyrrolizidine alkaloids content in *Senecio grisebachii* plants in response to P-deficient treatments, in greenhouse assays.

The content of alkaloids also dependent of the variety, development stage and organ of plant (Furuya *et al.*, 1972; Maknickiene & Asakavičiūtė, 2008). In *B. microphylla* was observed differences in the proportions of alkaloids among the organs; berberine was detected in higher proportion in stems and roots. Similar results in *Berberis* species indicated that berberine is the alkaloid more abundant in stem and root extracts (Andola *et al.*, 2010a). Other reports in *Berberis* indicate that the occurrence of alkaloids is restricted to a specific organ of the plant, whereas other compounds are distributed in the whole plant (Chandra & Purohit 1980; Gorval & Grishkoves, 1999; Khamidov *et al.*, 2003).

The results obtained in our study increase the knowledge about the alkaloid composition of *B. microphylla*. The compounds identified are known to have different bioactivities. This evidence suggests that this plant can be used in future studies of their biological activity.

CONCLUSIONS

An HPLC ESI-MS/MS method was applied to investigate the presence of isoquinoline alkaloids in different organs of *B. microphylla* collected in two locations of the Patagonia. For the first time, seven alkaloids not reported for this specie were identified and also was confirmed the presence of four compounds previously isolated from *B. microphylla*. The presence of alkaloids was associated with organ/plant and could be affected for environmental conditions. Our methodology, carried out in this study, provides sensitivity and specificity for characterization of the alkaloids in *B. microphylla* and other related species.

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