

PHYTOCHEMICAL CONSTITUENTS OF THE ROOT OF *BERBERIS MICROPHYLLA*MARÍA CRISTINA FURRIANCA¹, MARYSOL ALVEAR², TOMÁS ZAMBRANO³, VÍCTOR FAJARDO⁴, LUIS A SALAZAR^{3*}

¹Department of Nursing, Universidad de Magallanes, Chile. ²Department of Chemical Sciences and Natural Resources, Universidad de La Frontera, Temuco, Chile. ³Department of Basic Sciences, Center of Molecular Biology and Pharmacogenetics, Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, Temuco, Chile. ⁴Department of Science and Natural Resources, Universidad de Magallanes, Punta Arenas, Chile. Email: luis.salazar@ufrontera.cl

Received: 16 February 2017, Revised and Accepted: 20 March 2017

ABSTRACT

Objective: To objective of this work was to perform phytochemical qualitative and quantitative analyzes of the main secondary metabolites in the root of *Berberis microphylla*.

Methods: The extracts of *B. microphylla* root were tested through phytochemical screening and the quantification of the most important constituents was carried out using spectrophotometric and gravimetric techniques.

Results: Phytochemical screening of both extracts showed the presence of alkaloids, flavonoids, glycosides, cardiac glycosides, saponins, terpenes, and tannins, which are pharmacologically important. Quantification of the major phytochemicals groups showed that the ethanolic extract contains 3.9% alkaloids, 0.46% flavonoids, 9.53% tannins, and 3.60% saponins. Similarly, the methanolic extract contains 6.61% alkaloids, 0.41% flavonoids, 7.40% tannins, and 1.43% saponins.

Conclusion: This is the first time that the presence of tannins, flavonoids, and saponins in this plant has been reported. The medicinal properties of the root of *B. microphylla* may exist due to the presence secondary metabolites.

Keywords: Calafate, *Berberis microphylla*, Phytochemical constituents, Secondary metabolites, Patagonian species.

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INTRODUCTION

There are about 500 species of *Berberis* worldwide, located mainly in Eurasia with approximately 300 species and 200 species in South America [1]. In Southern Chile and Argentina, from the 60 species previously reported in 1961 [2], currently, 20 species are recognized [3]. This important reduction is explained by several species, such *Berberis heterophylla* and *Berberis buxifolia*, being grouped into one species, *Berberis microphylla* [4]. *Berberis* species are deciduous or evergreen shrubs with a height of up to 5 meters tall and thorny stems, which are native to temperate and subtropical regions of Europe, Africa, North and South America [5]. At present, a large number of species of *Berberis* have been reported with pharmacological and clinical use [6]. In homeopathy and ethnic medicine, these species have been used as medicine for their anti-inflammatory [7], antibacterial, antihypertensive, anticancer, antiarrhythmic [8], hypoglycemic [8,9], and hepatoprotective [10-12] properties. The major phytochemical studies have been conducted on Asian species, such as *Berberis jaeschkeana*, *Berberis asiatica*, *Berberis aristata*, and *Berberis lyceum*. These species showed a relatively high content of tannins, saponins, alkaloids, flavonoids, steroids, phenolic compounds, and carbohydrates [13-15].

In the Chilean-Argentina Patagonia, the *Berberis* genus is represented by 16 species of native shrubs [16], among which *B. microphylla* - whose common name is caulk - stands out as a symbol of Patagonia due to its widespread abundance in the region [17]. *B. microphylla* has also been medicinally used by the native peoples of Patagonia (Aónikenk, Selk'nam, Kawésqar) for its astringent, antipyretic, analgesic, antibacterial, and antiviral properties [18].

Although several articles on *B. microphylla* have reported the diversity of alkaloid compounds [19,20], there are currently no studies showing the presence of other secondary metabolites in this plant. For this reason, our research is important because it reports for the first

time the presence of the main secondary metabolites that have been described with biological activity. Therefore, the objective of this work was to perform phytochemical qualitative and quantitative analyzes of the main secondary metabolites in the root of *B. microphylla*.

METHODS

Sample collection

Roots of *B. microphylla* were collected in the settlement of Bahía Mansa (53° 36' 39, 38" S and 70° 55' 50, 56" O), about 54 km South of the city of Punta Arenas, Chile. The plants of *B. microphylla* were collected, and taxonomic authentication was carried out by Dr. Juan Marcos Henríquez, botanist, and taxonomist at the Institute of Patagonia, Universidad de Magallanes, Chile (Voucher No: 012837).

Methanolic and ethanolic extracts preparation of *B. microphylla* root

We prepared extracts of the root of *B. microphylla* in ethanol and methanol. For this purpose, 100 g of root was dried, ground and extracted with 1000 ml of alcohol (ethanol or methanol) for 72 hrs at room temperature. Then, the extract was filtered and concentrated in a rotary evaporator at 40°C. Finally, the extract was lyophilized and stored at 4°C until use.

Qualitative phytochemical analysis

We carried out qualitative assays on both ethanolic and methanolic extracts to identify the presence of secondary metabolites, such as alkaloids, flavonoids, glycosides, cardiac glycosides, saponins, resins, phenols, tannins, triterpenes, steroids, catechins, amines, and anthocyanins based on a protocol previously described [21].

Quantitative phytochemical analysis

We submitted both extracts to quantitative biochemical testing to determine the main secondary metabolites with described medicinal activity, such as alkaloids [22,23], flavonoids [24], saponins [25], and tannins [26].

Table 1: Phytochemical screening in alcoholic extracts of *B. microphylla* root

Constituents	Ethanol extract	Methanol extract
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Cardiac glycosides	+	+
Saponins	+	+
Resins	-	-
Phenols	+	+
Tannins	+	+
Terpenes	+	+
Triterpenes	-	-
Quinones	+	+
Catechins	-	-
Amines	-	-
Anthocyanins	-	-

-: Absence, +: Presence, *B. microphylla*: *Berberis microphylla*

Table 2: Phytochemical composition of *B. microphylla* root extract

Extract	Alkaloids		Flavonoids		Tannins		Saponins %
	mg equivalents berberine/g dry matter	%	mg equivalents quercetin/g dry matter	%	mg equivalents tannic acid/g dry matter	%	
Ethanol	1.16±0.05	3.90±0.15	0.13±0.11	0.46±0.37	2.85±0.12	9.53±0.41	3.60±0.53
Methanol	3.64±0.17	6.61±0.30*	0.22±0.21	0.41±0.37	4.09±0.36	7.40±0.65	1.43±0.27

Values expressed as mean±SD and correspond to mg equivalent g⁻¹ of dry weight and percentage. *Significant versus percentage of ethanol extract (p<0.05), SD: Standard deviation, *B. microphylla*: *Berberis microphylla*

Data analysis

Statistical analysis was completed using the R software version 3.2.2 GNU General Public License (GNU). All analyses were performed in triplicate. Continuous variables are presented as mean±SD. The comparison between both extracts was assessed by Student's t-test for independent samples. Two-tailed p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

Different reports highlight *B. microphylla* as an important source of alkaloid compounds [16,17]. However, there is little background information on other secondary metabolites of this plant. Thus, as far as we know, this work constitutes the first report describing the presence of other phytochemical constituents in the root of *B. microphylla*. Results of the qualitative phytochemical analysis are shown in Table 1. It is important to note that our results are consistent with those described for *B. asiatica* [15], *B. aristata* [14], *B. tinctoria* [27], and *B. lyceum* [28].

Quantitative analysis

We quantified four phytochemicals groups known to be important in natural medicine [29]. Interestingly, Tiwari *et al.* underlined the role that saponins and tannins perform in the control and management of hyperglycemia [30], which seems to be similar to alkaloids [28].

The total alkaloids contents were reported as mg equivalents of berberine. Their percentage in the methanolic extract was significantly higher compared to the percentage in the ethanolic extract. This may be due to the type of polarity of the solvent used. In the literature, there are various reports regarding the total alkaloid content of dry roots. For instance, *B. thunbergii* (3.47%) and *B. vulgaris* (2.22%) [31] show lower percentages than those found in *B. microphylla*, which may be due to the content of berberine, which is the main alkaloid, varying considerably with altitude and soil conditions for the root [31-33]. Total flavonoids were reported as mg equivalents of quercetin. No significant difference was found between the two extracts tested. In the literature, values of close to 2.8% of flavonoids in *B. aristata* are reported [34], a value higher than that found in *B. microphylla*. Total tannins contents were

reports as mg equivalents of tannic acid. The percentage of tannins is roughly similar in several species of *Berberis*; however, the root of *B. microphylla* contained a higher percentage of tannins than those described for *B. aristata* (0.6%), *B. asiatica* (1.7%), *B. chitria* (0.73%), and *B. lyceum* (0.96%) [35]. Moreover, the percentage of saponins is greater in *B. microphylla* than in *B. vulgaris* (0.3%) [36]. No significant differences were found in both extracts tested for these phytochemical groups. The phytochemical composition of both extracts was expressed in mg g⁻¹ of dry matter of the sample (±SD) and percentage (±SD), and the results are shown in Table 2.

CONCLUSION

The phytochemical study showed that the root extracts of *B. microphylla* contain important bioactive secondary metabolites, without qualitative differences between the ethanolic and methanolic extract of the plant. This would be the first time to report the presence of tannins, flavonoids, and saponins present in the root of *B. microphylla*. Quantitatively, the tannins would be the major group for both extracts tested. Therefore, their presence would support the traditional use of the plant in the treatment of various diseases. We are conducting further studies of the isolation of active components from the root of *B. microphylla* with cell lines to validate the medicinal use of this plant.

ACKNOWLEDGMENTS

The authors would like to thank Advanced Human Capital Formation Program, CONICYT-CHILE for their financial support of María Cristina Furriana Llana during her National Doctorate fellow (N°24121475).

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