Antioxidant properties and TLC characterization of four Chilean *Haplopappus*-species known as bailahuén

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Abstract

"Bailahuén" is the common name of a medicinal shrub native to Chile where this resinous herb is widely used for its liver stimulating properties. Although the official species is *Haplopappus baylahuen* Remy (Asteraceae), other species of the same genus are also used in different regions as "bailahuén". A thin layer chromatography (TLC) method for rapid identification of different species and detection of adulterations is described for four of the species: *Haplopappus baylahuen*, *Haplopappus taeda* Reiche, *Haplopappus multifolius* Phil., and *Haplopappus remyanus* Wedd. To confirm efficiency in all species antioxidant properties were screened in resins, infusions, and methanolic extracts by tests of lipid peroxidation in erythrocytes and free radical scavenging activity by DPPH. In both studies *Haplopappus baylahuen* showed the lowest antioxidant capacity. In DPPH analyses, infusion and resins of *Haplopappus baylahuen* also showed the lowest and *Haplopappus remyanus* the major inhibiting activity of free radicals, while *Haplopappus multifolius* proved to have the highest result when the methanolic extracts were used. The chemical characterization of the studied species showed important levels of flavonoids and coumarins, with flavonoids predominating in *Haplopappus taeda*, coumarins in *Haplopappus multifolius* and both of them in *Haplopappus baylahuen* and *Haplopappus remyanus*.

Keywords: *Haplopappus* spp.; TLC characterization; Antioxidant activity; Coumarins; Flavonoids

1. Introduction

Bailahuén (*Haplopappus baylahuen* Remy; Asteraceae) is a medicinal plant native to Chile with known choleretic and cholagogue properties (Montes and Wilkomirsky, 1985). It is traditionally used by the local population, but it is also exported in small amounts as a digestive in tea bags. The natural habitat of this species is limited to the mountain areas of Chile between latitudes 26 and 28°S. Nevertheless, in other regions of Chile other endemic *Haplopappus* species, such as *Haplopappus multifolius* Phil. (32–34°S), *Haplopappus remyanus* Reiche (29–32°S), and *Haplopappus taeda* Wedd. (34–35°S), are collected, sold, and exported. We suppose that the confusion may even extend to some scientific publications, as plant material of some investiga-

Abbreviations: TLC, thin layer chromatography; DCM, dichloromethane; MeOH, methanol; LB, Liebermann reagent

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tions (Núñez-Alarcón et al., 1993) reported as "*Haplopappus baylahuen*" was collected in areas where only *Haplopappus taeda* is present. All of the above-mentioned species are known in the rural areas as highly effective medicinal plants, this being the reason why they were studied in the present investigation comparing their chemical composition and antioxidant activity. Several biological activities related with antioxidant properties of different plant extracts and their compounds including hepatoprotective (Sohn et al., 2003), gastroprotective (Kahraman et al., 2003), cytoprotective (Potapovich and Kostyuk, 2003), and immunomodulatory (Pandima Devi et al., 2003) have been recently reported.

All bailahuén production comes from the wild collection and although only the aerial parts are used the plants are mostly eradicated. The actual over-exploitation has strongly limited the natural populations of bailahuén and its former, very common use as a popular medicinal plant in Chile.

2. Materials and methods

2.1. Plant material

Species were collected in their natural habitat at the moment of flowering and identified taxonomically by Dr. José San Martín, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, using specific keys (Reiche, 1896–1911; Tortosa and Bartoli, 2002). Samples were compared with those of the Herbarium of the Universidad de Concepción. Voucher specimens were deposited in the Herbarium of Universidad de Talca referring AJIM 2489 to *Haplopappus baylahuen*, AJIM 2486–2487 to *Haplopappus remyanus*, AJIM 2485 to *Haplopappus multifolius*, and AJIM 2488 to *Haplopappus taeda*. Duplicates were deposited in the Herbarium of the Museo Nacional de Historia Natural (MNHN), Santiago de Chile, and in the Herbarium of the Universidad de Concepción. Samples used for the experiments were dried at room temperature in a well-ventilated drying room.

2.2. Extracts

For the following procedures only dried leaves were employed, with three repetitions for each species and extract. Dry plant material (100 g) was immersed in 250 ml dichloromethane (DCM) for 30 s to obtain resins with yields of 17.9% for *Haplopappus baylahuen*, 13.4% for *Haplopappus remyanus*, 14.6% for *Haplopappus multifolius*, and 20.6% for *Haplopappus taeda*. For methanolic extracts other 100 g were macerated in 500 ml methanol (MeOH) for 72 h. Organic solvents were removed to obtain dry extracts. For the infusion 5 g of dried leaves were treated with 50 ml bidistilled boiling water for 5 min. All extracts were lyophilized for further analyses.

2.3. Studies of antioxidant activities

Antioxidant activity of the different extracts was studied in two procedures: by lipid peroxidation of erythrocyte membranes and by free radical scavenging by DPPH, both procedures as reported by Schmeda-Hirschmann et al. (2003). Catechin was used as the standard compound in the lipid peroxidation and Trolox in the DPPH assay. Each experiment was repeated three times to analyze results statistically. Lipid peroxidation of the erythrocytes was only studied in infusions and methanolic extracts because of the low solubility of resins.

2.4. TLC characterization

Resins were submitted to the characterization by thin layer chromatography (TLC; silica gel 60 F254, Merck; mobile phase, DCM:MeOH 98:2). Chromatograms were evaluated under UV light at 254 and 365 nm to detect the presence of flavonoids and coumarins, respectively. Finally, chromatograms were developed with the Liebermann reagent (LB) with terpenes appearing. To confirm the presence of flavonoids, TLC was additionally sprayed with a diphenylborinic acid ethanolamine complex. Prenyletin was identified by co-chromatography with the pure compound.

3. Results

3.1. Antioxidant effects

In both, methanolic extracts and infusions of the different *Haplopappus* species lowest activity in lipid peroxidation of erythrocytes was observed in *Haplopappus baylahuen*, the official bailahuén species, showing inhibition percentages of about 50% at a concentration of 200 μg/ml whereas the other species reached the same levels at only 50 μg/ml. Differences in activities between the concentrations of the same species were highly significant (Table 1). The lipid peroxidation was inhibited by the standard compound catechin with an IC_{50} value of 73.8 μg/ml.

In the DPPH study, also all *Haplopappus baylahuen* extracts, i.e. infusion, methanolic extract, and resins showed lowest activities when compared with other *Haplopappus* species (Table 2). Best antioxidant activity was obtained from the methanolic extract of *Haplopappus multifolius*, from the resins of *Haplopappus remyanus*, and from the infusions of *Haplopappus remyanus* and *Haplopappus taeda*. Infusion is the traditional form of application of bailahuén, commonly used as an herbal tea.

3.2. Chemical compounds

The resins of the four *Haplopappus*-species studied showed characteristic TLC chromatograms for each species (Fig. 1). These TLC profiles remain characteristic even

Table 1
Inhibition of the aqueous and methanolic extracts of different bailahuén-species (*Haplopappus* spp.) on the lipid peroxidation of erythrocytes at different concentrations

Species	Inhibition (%) of the aqueous extract at different concentrations (μg/ml)				Inhibition (%) of the methanolic extract at different concentrations (μg/ml)			
	500	200	100	50	500	200	100	50
Haplopappus baylahuen	77.5 b	57.5 c	32.2 c	24.3 b	96.0 a	51.3 c	38.6 c	27.3 b
Haplopappus remyanus	91.8 a	80.2 b	77.8 b	58.5 a	95.7 a	94.2 a	84.0 a	56.5 a
Haplopappus multifolius	92.2 a	88.8 a	83.6 a	59.7 a	86.9 b	74.3 b	70.3 b	60.2 a
Haplopappus taeda	94.3 a	86.2 a	81.5 a	56.2 a	97.8 a	94.8 a	82.1 a	62.5 a

Values in columns followed by different letters show significant differences between species (Duncan; $\alpha \leq 0.05$). Differences of inhibition between concentrations of the same species are all highly significant ($\alpha \leq 0.01$).

Table 2
Antioxidant properties of different bailahuén-species (*Haplopappus* spp.) determined by DPPH and expressed as micro equivalents Trolox (TRE)

Species	Infusion	Methanolic extract	Resins
Haplopappus baylahuen	661 c	1,985 c	682 c
Haplopappus remyanus	3,784 a	3,536 b	3,971 a
Haplopappus multifolius	2,771 b	5,170 a	2,730 b
Haplopappus taeda	3,433 a	2,957 b	2,481 b

Values in columns followed by different letters indicate significant differences between species (Duncan; $\alpha \leq 0.05$).

when samples are taken from wild plants, cultivated plants or from individuals grown under different light conditions. *Haplopappus baylahuen* presents flavonoids (detected by the yellow and orange spots) and few coumarins at low concentrations (detected by grey spots with blue fluorescence when observed under UV light at 365 nm and marked in Fig. 1 with pencil). High concentrations of terpenes, which turned brown when the LB reagent was applied, appeared at Rf 0.62–0.77. No prenyletin could be detected for this species. *Haplopappus multifolius* showed principally the presence of coumarins, some of them described before (Chiang et al., 1982; Núñez-Alarcón and Quiñónez, 1995; Torres et al., 2004) with prenyletin (Rf 0.83) being the most abundant. The main compounds of *Haplopappus taeda* resin are flavonoids. *Haplopappus remyanus* presents both, flavonoids and coumarins with characteristic spots at 0.63 and 0.83, which are not present in *Haplopappus taeda*.

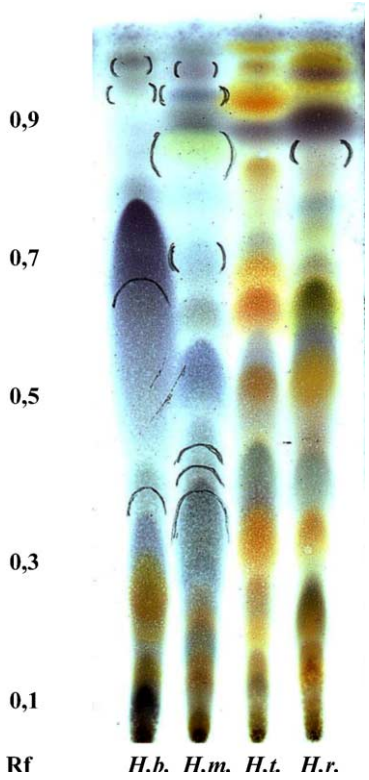

Fig. 1. Identification of the bailahuén-species *Haplopappus baylahuen* (H.b.), *Haplopappus multifolius* (H.m.), *Haplopappus taeda* (H.t.), and *Haplopappus remyanus* (H.r.) by TLC (silica gel F254; DCM/MeOH, 98:2; sprayed with LB).

4. Discussion

As the four species studied are all used in popular medicine for the same purpose and as the same medicinal plant, we supposed that chemical compounds would be very similar. The present study shows that this is not the case. Besides the botanical and morphological differences between the species they can also be distinguished clearly by their flavonoid- or coumarin-content that show characteristic marks in the TLC of external resins, which are common for the four species.

The main compounds of *Haplopappus baylahuen* were found to be terpenes, some flavonoids and coumarins other than prenyletin. As no prenyletin could be detected for this species, as reported before (Schwenker et al., 1967), we suppose that the plant material of that study may correspond to *Haplopappus multifolius*, the species with the very characteristic presence of prenyletin in high concentrations or *Haplopappus remyanus*, which also produces this compound, but only in traces. The presence of coumarins in the latter species has not been previously described, whereas labdanes, monoterpene esters, and flavanones have been reported (Zdero et al., 1991). Flavonoids proved to be the main compounds of *Haplopappus taeda*. Eight of these compounds have been identified in this species before (Marambio and Silva, 1989). Our assumption, that a former study which reports the presence of flavonoids and powerful hepatoprotective properties of *Haplopappus baylahuen* (Núñez-Alarcón

et al., 1993) may have used *Haplopappus taeda*, would fit with our results.

The antioxidant properties of aqueous *Haplopappus baylahuen* extract reached similar values as catechin and was three times superior to the inhibition of lipid peroxidation of aqueous boldo (*Peumus boldus*) extract (Schmeda-Hirschmann et al., 2003). Nevertheless, the other *Haplopappus* species studied showed even significantly higher antioxidant effects than the official bailahuén-species. Considering the relationship between the antioxidant activity and hepatoprotective effects (Sohn et al., 2003), this study does confirm the effectiveness reached by the popular application of *Haplopappus multifolius* in the region of Santiago, of *Haplopappus taeda* southward and *Haplopappus remyanus* as bailahuén, by showing even higher, up to five-fold antioxidant activity than *Haplopappus baylahuen* in spite of the differences in chemical composition.

5. Conclusions

The locally used bailahuén-species *Haplopappus remyanus*, *Haplopappus multifolius*, and *Haplopappus taeda* showed higher antioxidant effects in DPPH assay and lipid peroxidation in erythrocytes than the official one, *Haplopappus baylahuen*, although species differ in their respective main compounds with flavonoids predominating in *Haplopappus taeda*, coumarins in *Haplopappus multifolius*, and both of them in *Haplopappus remyanus* and *Haplopappus baylahuen*. The species can be identified easily by TLC of the resins.

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