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Histological leaf characterization of four Bailahuen species (*Haplopappus* spp.) for taxonomic identification for quality control of the drug

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ABSTRACT

Leaf morphology of four *Haplopappus* species (*Haplopappus baylahuen*, *H. rigidus*, *H. multifolius* and *H. taeda*) are histologically described in order to identify and differentiate the Chilean *Haplopappus*-species sold as "Bailahuen" for quality control of the drug. In leaves of five plants of each identified species transverse sections of 25 μm were taken from the middle of the leaf. These were stained with saphranine and fast green before being studied under an optical microscope together with the surface imprint of the cuticle. The design of the cuticle lines is very characteristic for each *Haplopappus* species. They also can be identified by the thickness of the leaves, together with presence and density of filamentous trichomes, fibers, and density of stomata.

1. Introduction

Haplopappus baylahuen Remy, Asteraceae, a medicinal plant native to the hills of the Atacama Desert, between latitudes 26–28° S, Chile (Vogel et al., 2005), has been traditionally used for indigestion and as a natural choleretic, cholagogue (Montes and Wilkomirsky, 1987) and emmenagogue (Hoffmann et al., 1992). Due to the very arid conditions of its natural habitat the shrubs are covered with a thick layer of aromatic resins. The wild collection of this species is not sustainable because of its low growing rates and difficulties to regrow after harvest (Vogel et al., 2007).

Because of the scare availability of this raw material, *H. baylahuen* is commonly substituted by other resinous species of the same genus, mainly by *H. multifolius* Phil. ex Reiche, a species growing in the mountainous regions between 32 and 34° S or the southern *H. taeda* Reiche, distributed in the Andean mountains between latitudes 34–35° S (Vogel et al., 2005). The confusion reached even the German Homoeopathic Pharmacopeia HAB 2000, where the description of *H. baylahuen* fits the characteristics of *H. multifolius*. Moreover, 80% of the "bailahuen" samples found in the Chilean market correspond to *H. multifolius*, the species that grows in the Andean mountains close to the capital Santiago de Chile (Hoffmann et al., 1992; Gonzalez et al., 2012). Vogel et al. (2007) estimated the Chilean demand for this drug of about 23 tons per year, 93% of them being identified as *H. multifolius* (80%), *H. baylahuen* (10%), and *H. taeda* (3%). They also describe the three above mentioned *Haplopappus* species botanically, illustrating the study with morphological drawings. For quality control of dried leaves the species can also be identified by the TLC-fingerprint of their resins (Vogel et al., 2005).

To complement the tools that would permit taxonomic identification of bailahuén drug, the present study describes the micromorphological characteristics of *H. baylahuen, H. multifolius, H. taeda*, and *H. rigidus* Phil. leaves. The latter species is also mentioned by Hoffmann et al. (1992) for its medicinal use, and was considered in the study because it shares the natural habitat with *H. baylahuen*.

2. Materials and Methods

The principal *Haplopappus* species sold in the Chilean market as "bailahuen" (*Haplopappus baylahuen, H. multifolius, H.taeda*) were included in the present study, together with *H. rigidus* as a potential

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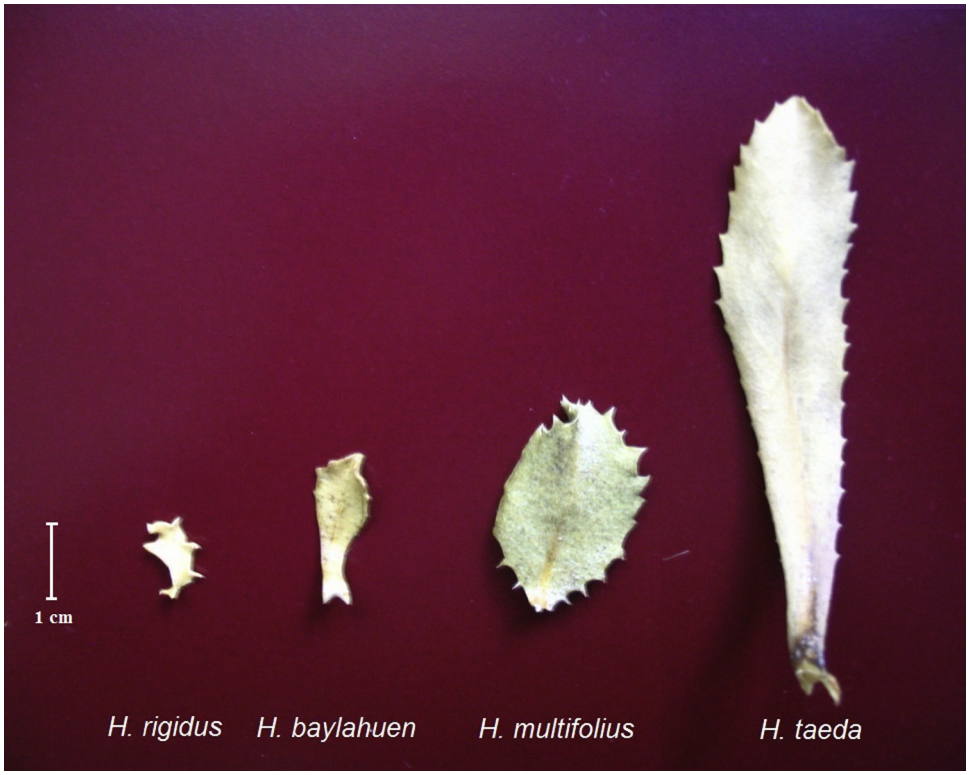

Fig. 1. Leaf shape of the four *Haplopappus* species used as "bailahuen".

falsification because of its neighborhood to the *H. baylahuen* habitat. From each species five individuals were collected in the Andean Mountains. However *H. baylahuen* and *H. rigidus* were from the Atacama Desert in Northern Chile, while those of *H. multifolius* and *H. taeda* were from Central Chile. Details about geographical location, climate conditions and soil properties of the collection sites are described by Gonzalez et al. (2012). All *Haplopappus* samples were taxonomically identified by Dr. J. San Martín by their flower morphology according to Reiche (1902) and Tortosa and Bartoli (2002), together with a comparative study of the plant specimens in the Chilean Herbaria (CONC, Departamento de Botánica, Universidad de Concepción, and SGO, Museo de Historia Natural, Santiago de Chile). The identities were also confirmed by the species-specific TLC fingerprints of the resins (Vogel et al., 2005). Voucher specimens of *H. baylahuen* (N°2429), *H. multifolius* (N°2488), *H. taeda* (N°3359) and, *H. rigidus* (N°2485) were deposited in the Herbarium of Universidad de Talca, Chile.

Fully expanded leaves from the five wild-growing plants of each species were dried in the laboratory at room temperature and the middle third of them prepared for microscopical studies according to World Health Organization (1998).

From each species five recently collected and dried leaves were painted with transparent nail varnish on the upper and lower sides. The varnish was peeled off and put on a slide to observe it with an optical microscope (Leitz). Samples were examined for the stomatal size (μm), their distribution and density (number per mm^2), the presence and type of trichomes and secretory resin glands, along with the pattern of the surface lines. From each species 20 replicates were taken, with 10 samples from the upper (adaxial) and lower (abaxial) surface of the leaf, respectively.

Small sections cut out from the middle part of a mature leaves (0.5 × 0.5 cm) were fixed in FAA (5% formaldehyde, 5% glacial acetic acid, 90% ethanol) for 10 days, and then dehydrated in ethanol (70, 80, and 95%) for one hour each. Then samples were embedded in a methacrylate medium using Technovit 7100 kit (Heraeus Kulzer GMBH, Wehrheim, Germany). Cross-sections (25 μm) were made in a microtome MICRON HR400 and stained with saphranine and fast green according to D'Ambrogio (1986). At least 10 selected sections per leaf were observed in a light microscopy (Leitz) at different magnifications.

Micromorphological qualitative characters, such as epidermis, trichomes, secretory glands, fibers, bundle sheath and type of the vascular bundle were described.

The thickness of the leaf and cuticle were measured in 10 sections from five leaves per species.

The length of the leaf was measured in three mature leaves from five individuals per species.

Data were statistically analyzed by the Kruskal-Wallis- test (SPSS V 11.0).

3. Results and Discussion

The leaves of the different species show differences in overall thickness with the highest values for *H. baylahuen*, followed by *H. rigidus* and *H. taeda,* while the thinnest leaves were observed in *H. multifolius* (Table 1). *H. taeda*, the species with the largest leaves (7.4 cm), has the thinnest cuticle (Fig. 1) with 14.5 μm compared with 23–27 μm found in the other species (Table 1).

H. baylahuen and *H. rigidus* have higher secretory gland density than *H. mutifolius* and *H. taeda*. The stomatal size is similar for all studied species, with mean values between 36 and 40 μm, but their density on the upper surface of the leaves is higher in *H. baylahuen* than in *H. taeda* and *H. rigidus*, whereas on the lower surface of the leaves, stomata distribution is denser in *H. rigidus* than in *H. taeda* (Table 1).

The quantitative traits recorded may be affected by the environmental conditions of the natural habitat (Li et al., 2015). The very arid climate conditions of the Atacama Desert, where *H. baylahuen* and *H. rigidus* are growing (Gonzalez et al., 2012), may have influenced leaf size and thickness, with very small but thick leaves,

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