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Biomass, resin and essential oil content and their variability in natural populations of the Chilean crude drug "bailahuén" (*Haplopappus* spp.)

[Biomasa, contenido de resina y aceite esencial y su variabilidad en poblaciones naturales de las especies de droga cruda chilena "bailahuén" (*Haplopappus* spp.)]

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Abstract
Bailahuén (*Haplopappus rigidus, Haplopappus baylahuen, Haplopappus multifolius* and *Haplopappus taeda*; Asteraceae) are medicinal shrubs native to the Andes Mountains of Chile widely used to treat hepatic ailments. At present, exploitation of bailahuén is based on wild collections, affecting the abundance of natural populations. Variability of biomass production and concentration of active compounds in different wild populations of the four *Haplopappus* species was studied in order to select the best plant material for cultivation. Resins were extracted with dichloromethane and essential oils by distillation. Biomass production of the populations was highest for *H. baylahuen* and *H. rigidus* compared with the other species, reaching between 0.56 and 1.61 kg and 1.11-1.48 kg per plant, respectively. No differences were found among populations of the same species. In some *H. rigidus* populations, the resin content was about a third of the dry weight, whereas plants of *H. multifolius* had mean values of about 8%. In *H. baylahuen* (11.3-27.7%) the resin content of leaves differed significantly among populations, whereas the essential oil ranged from 0.02-0.38 mL*100g DM^{-1} in *H. baylahuen,* 0.03-0.5 mL*100 g DM^{-1} for *H. rigidus*, and 0.08-0.35 mL*100 g DM^{-1} for *H. taeda*. Resin content in stems differed only among *H. baylahuen* (6.3-15.5 %) and *H. rigidus* (10.7-21.2%) populations. No significant differences in essential oil content among species could be detected because of the large amount of variation observed among populations. The variation between plants of the same population may indicate favorable selection potential for future breeding programs.

Keywords: *Haplopappus baylahuen, Haplopappus multifolius, Haplopappus rigidus, Haplopappus taeda,* resin, essential oils.

Resumen
Bailahuén (*Haplopappus rigidus, Haplopappus baylahuen, Haplopappus multifolius* y *Haplopappus taeda*; Asteraceae) son arbustos medicinales de la Cordillera de los Andes Chilena ampliamente usados para tratar malestares hepáticos. Actualmente, la explotación del bailahuén se basa en la recolección silvestre, afectando su abundancia. La variabilidad en diferentes poblaciones silvestres de las cuatro especies de *Haplopappus* fue usada en orden a determinar la potencialidad para seleccionar el mejor material vegetal para cultivo en producción de biomasa y contenido de principios activos. La resina se extrajo por inmersión en diclorometano y el aceite esencial por destilación. Los mayores rendimientos de biomasa se observaron en *H. rigidus* y *H. baylahuen*, con valores poblacionales que fluctuaron entre 1,11-1,48 y 0,56-1,61 kg $planta^{-1}$, respectivamente, sin presentar diferencias entre poblaciones de la misma especie. El contenido de resinas alcanzó en algunas poblaciones de *H. rigidus* hasta un tercio del peso seco, mientras que en *H. multifolius* fue de aproximadamente 8%. En hojas, sólo se observaron diferencias significativas en el contenido de resinas entre poblaciones de *H. baylahuen* (11,3-27,7%). El contenido de aceites esenciales en hojas permitió diferenciar poblaciones de *H. baylahuen* (0,02-0,38 mL*100g MS^{-1}), *H. rigidus* (0,03-0,50 mL*100g MS^{-1}) y *H. taeda* (0,08-0,35 mL*100g MS^{-1}). En tallos, el contenido de resina difirió significativamente entre poblaciones de *H. baylahuen* (6,3-15,6%) y *H. rigidus* (10,7-21,2%). La alta variación del contenido de aceite esencial entre poblaciones no permitió establecer diferencias entre especies. La variación entre poblaciones de la misma especie sugiere un buen pronóstico para futuras selecciones y mejoramiento genético.

Palabras Claves: *Haplopappus baylahuen, Haplopappus multifolius, Haplopappus rigidus, Haplopappus taeda*, resina, aceites esenciales.

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INTRODUCTION

"Bailahuén" is the common name of resinous *Haplopappus* species native to Chile (*Haplopappus baylahuen* Remy, *Haplopappus rigidus* Phil., *Haplopappus multifolius* Philippi ex Reiche, *Haplopappus taeda* Reiche; Asteraceae), where they are widely used to treat hepatic ailments (Mellado *et al*., 1996). The herb, including leaves and stems, is used for its choleretic, colagogic and enemagogic properties (Schrickel and Bittner, 2001). Secondary metabolites from different *Haplopappus* species have been described by Torres *et al*., 2004, Torres *et al*., 2006, Urzua *et al*., 2004, Demirci *et al*., 2006, Faini *et al.,* 2007.

Studies of the national market in Chile estimated an annual consumption of 23 tons of bailahuén. About 80% of the plant material sold in the main cities of Central Chile (Coquimbo to Biobio Regions) was identified as *H. multifolius* Philippi ex Reiche and only 10% corresponds to the official medicinal plant, *H. baylahuen* (Res. N°548, Diario Oficial reference, 2009), whereas plants of *H. taeda* Reiche seems to be the most exported species (Vogel *et al*., 2007). Although these species are used for the same therapeutic purposes, the chromatographic patterns of the resins are characteristic and distinct for each and are not affected by environmental variables (Vogel *et al*., 2005).

At present, exploitation of bailahuén is based on wild crafting, affecting the abundance and spatial disposition of natural populations. At the same time, bailahuén species grow in habitats characterized by high stress conditions, including high radiation levels, drought, low soil fertility and grazing, which limit their natural regeneration (San Martín *et al*., 2005, Vogel *et al*., 2007).

The secure supply of high quality raw material is possible growing a selected plant material with high resin content, essential oil and biomass production. Also the bailahuén crop could help to reduce the anthropogenic pressure and the loss of genetic variability on wild populations.

For define the cash crop potential of these Haplopappus species, their resin contents are compared with Grindelia species ones. Grindelia and Haplopappus are genders closed that share the capacity to produce resin. In the 80's, *Grindelia camporum* Greene is described like an attractive crop for arid zones for biofuel production. Economic studies showed that Grindelia crop are feasible when the resin contents achieve to 15% (Hoffmann and Mc Laughlin, 1986).

For future selection and plant breeding programs, inter- and intraspecific variability of the main *Haplopappus* species marketed as bailahuén was studied by determining the aerial biomass production including leaves and stems. The resin and essential oil content were quantified as determinant factors in the quality of the medicinal plant material.

MATERIALS AND METHODS

Collection sites

H. baylahuen and *H. rigidus* were collected in the Andes Mountains of the Region de Atacama; *H. multifolius* in the Region de Valparaiso and Metropolitana and *H. taeda* in the Region del Libertador Bernardo O'Higgins and del Maule (Table 1, figure 1).

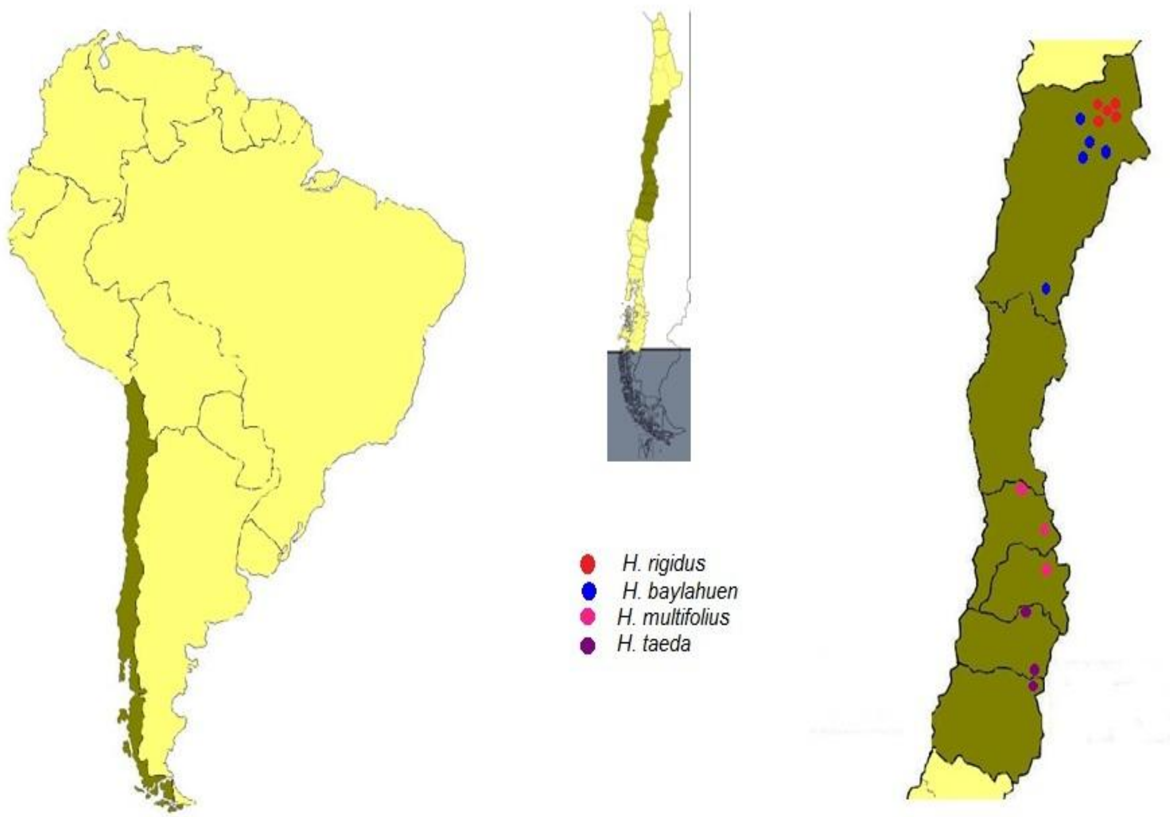
All species grow in high elevated areas with an average temperature of about 10° C (Table 1). However, northern and southern populations are exposed to precipitation and humidity gradients. *H. baylahuen* and *H. rigidus* commonly inhabit areas with precipitation of about 44 mm per year and a relative humidity of about 27%, whereas *H. multifolius* grows in areas with precipitation between 287 and 508 mm/year and relative humidity from 50 to 60%. The precipitation in the natural habitat of *H. taeda* oscillates between 1,000 and 1,800 mm per year (Di Castri and Hajek, 1976; Amigo and Ramírez, 1998; Mena, 2009).

Based on soil analyses, all species grow in well drained soils with low levels of nutrients and salinity. However, the optimal pH is different for each species. While *H. baylahuen* and *H. rigidus* inhabit moderate to highly alkaline soils, *H. multifolius* and *H. taeda* are growing in neutral and moderately acid soils, respectively.

Plant material

Leaf and stem portions of *H. baylahuen*; *H. rigidus; H. multifolius* and *H. taeda* were collected from natural habitats (Table 1, Figure 1) during the flowering time (January-March) in 2005 and 2006. The species were identified *in situ* using specific keys (Reiche, 1902; Tortosa and Bartoli, 2002) and were compared with herbarium specimens at the Universidad de Concepción (CONC 50597, 19832, 27652, 58465) and the Museo de Historia Natural, Santiago de Chile. Specimens were deposited in Herbarium of Universidad de Talca, Chile (AJIM 2429, 2485, 2488, 3359). Each population was characterized, and the taxonomic identity of each plant determined by chromatographic patterns of the chemical compounds in resins. Samples were taken from five populations of *H. baylahuen*, five of *H. rigidus*, two of *H. multifolius* and three of *H. taeda* with five replicates (individuals) per population. The populations were selected using historical information and collection data from specimens of the Herbarium of the Universidad de Concepción. Additional areas were located with the help of wild collectors and local people.

Figure 1



Collection sites of the Chilean Haplopappus species studied

Biomass determination

The plant material was dried at room temperature under shade for two weeks. Once dried, the leaves and stems were separated and the biomass production of the five individuals from each collection site and species were estimated based on the dry weight measurements.

Resin and essential oil content

Resins were extracted using dichloromethane (DCM) as solvent. Dry, crushed plant material (25 g) was placed in an Erlenmeyer flask with 150 mL of DCM for 30 minutes. The mixture was then filtered and the DCM soluble mixture evaporated under reduced pressure to obtain the crude resins. Resins were characterized by thin layer chromatography on silica gel F254, with DCM:MeOH 98:2 as the mobile phase. The chromatograms were evaluated under UV light (254 and 365 nm) to detect the presence of flavonoids and coumarins, respectively (Vogel, 2005).

Essential oils were isolated from 150 g of dry material by distillation using a Clevenger type distillation apparatus for one hour. The essential oil volume was measured with a graduated burette.

Table 1
Characterization of collection sites of Chilean *Haplopappus* species

Population	Latitude/ Longitude (south /west)	Altitude (m.a.s.l.)	Bioclimatic belt[1]	Ombrotype[1]	pH soil[2]	Nitrogen level[2] (ppm)	Electrical conductivity (dS/m)
H. baylahuen							
Cerro Vicuña	26º 37'/ 69° 37'	2,888	Mesomediterranean	Perarid	-	-	-
Rincón del Toril	28º 16'/ 69° 44'	2,592	Mesomediterranean	Perarid	7.4	1	0.109
Las Vizcachas	27º 45'/69° 34'	2,870	Mesomediterranean	Perarid	-	-	-
Los Castaños	27º 44'/69° 42'	2,550	Mesomediterranean	Perarid	-	-	-
San Félix	29º 20'/ 70° 17'	2,343	Mesomediterranean	Perarid	7,4	1	0.153
H. rigidus							
Salar de Pedernales[A]	26º 25'/ 69° 16'	3,588	Mesomediterranean	Perarid	6.5	19	0.107
Salar de Pedernales[B]	26º 27'/69° 18'	3,014	Mesomediterranean	Perarid	-	-	-
Acerillos	26º 32'/69° 17'	3,648	Mesomediterranean	Perarid	-	-	-
Cerro Blanco	26º 30'/69°16'	3,778	Mesomediterranean	Perarid	6.6	3	0.112
Tinogasta	26º 53'/69° 17'	3,522	Mesomediterranean	Perarid	7.9	1	0.442
H. multifolius							
Los Libertadores	32º 51'/ 70° 10'	2,085	Supramediterranean	Dry	-	-	-
La Disputada	33º 15'/ 70° 20'	1,814	Supramediterranean	Subhumid	6.9	1	0.074
H. taeda							
Las Vegas	35º 55'/ 70° 29'	1,534	Supramediterranean	Subhumid	6.2	15	0.510
Pichoante	35º 05'/70° 29'	1,480	Supramediterranean	Subhumid	6.5	3	0.064
Altos de Cantillana	33° 57'/70° 49'	1,896	Supramediterranean	Subhumid	5.8	31	0.156

[A] Km 107
[B] Km 90
[1] Classification was according to Amigo and Ramirez (1998) using the weather station closest to the collection area.
[2] Soil analyses.

Data analysis
Biomass, resins and essential oil content were evaluated using both parametric (T-Student and Tukey) and non-parametric tests (Kruskal-Wallis) using SPSS V11 software.

RESULTS
Biomass
H. rigidus aerial biomass exceeds a kilogram of dry material per plant in all populations studied, similar to some populations of *H. multifolius* y *H. taeda*. *H. taeda*, which gave intermediate biomass yields among its populations, had the highest percentage of leaves in comparison to other species, reaching up to 90%. Among *H. taeda* populations the lowest leaf percentage was from material from Las Vegas and the lowest biomass production from Altos de Cantillana (Table 2).

Table 2
Aerial parts dry biomass, resin and essential oil content in wild-growing Chilean populations of *H. baylahuen, H. rigidus, H. multifolius* and *H. taeda*

Population	Biomass (kg DM* plant^{-1})	Leaves (%)	Resin content (%)			Essential oil (mL*100g DM^{-1})		
			Leaves	Stems	Difference among both	Leaves	Stems	Difference among both
H. baylahuen	1	1	1	2	3	2	2	3
Cerro Vicuña	1,61 ±0,69 a	50 ±12 a	27,7 ± 4,1 a	15,6 ± 3,6 a	*	0,07 ±0,03 c	0,04 ±0,01 b	*
Rincón del Toril	0,56 ±0,36 a	46 ± 9 a	20,7 ±3,7 ab	9,5 ± 6,8 ab	*	0,05 ±0,05 c	0,05 ±0,03 b	ns
Las Vizcachas	1,38 ±0,31 a	45 ±10 a	16,9 ±7,1 ab	11,6 ±1,7 ab	*	0,14 ±0,09 b	0,12 ±0,03 a	ns
Los Castaños	0,76 ±0,68 a	62 ±10 a	14,5 ± 5,1 b	9,8 ± 2,4 ab	ns	0,38 ±0,24 a	0,16 ±0,02 a	ns
San Félix	0,61 ±0,42 a	39 ±19 a	11,3 ± 1,8 b	6,3 ± 2,4 b	*	0,02 ±0,01 c	0,03 ±0,03 b	ns
H. rigidus	1	1	1	2	3	2	2	3
Salar km107	1,11 ±0,75 a	55 ±14 a	30,8 ± 3,0 a	17,1 ± 3,8 a	*	0,05 ±0,03 c	0,05 ±0,04 b	ns
Salar km 90	1,48 ±0,44 a	40 ± 4 a	29,9 ± 3,6 a	10,7 ± 2,1 b	*	0,07±0,06bc	0,02 ±0,01 b	*
Acerillos	1,21 ±0,48 a	50 ± 9 a	25,2 ± 8,6 a	13,6 ±3,0 ab	*	0,50 ±0,04 a	0,04 ±0,01 b	*
Cerro Blanco	1,14 ±0,51 a	48 ±10 a	31,3 ±3,0 a	21,2 ±8,6 a	*	0,29±0,24ab	0,12 ±0,11 a	ns
Tinogasta	1,15 ±0,51 a	56 ±10 a	33,6 ±1,2 a	11,5 ±0,5 ab	*	0,03 ±0,01 c	0,01 ±0,01 b	*
H. multifolius	3	3	3	3	3	3	3	3
Los Libertadores	0,31 ±0,20 a	42 ± 20a	7,5 ± 5,7 a	4,3 ± 2,4 a	*	0,14 ±0,05 a	0,05±0,01a	*
La Disputada	0,25 ±0,10 a	50 ± 3 a	8,1 ± 2,2 a	4,5 ± 0,8 a	*	0,11 ±0,04 a	0,06±0,03 a	*
H. taeda	2	2	1	1	3	2	1	3
Las Vegas	1,09 ±0,43 a	74 ± 3 b	15,4 ±5,7 a	17,9 ±3,4 a	ns	0,08 ±0,05 b	0,03 ±0,01 a	*
Pichoante	0,75 ±0,20 a	81 ± 1 a	13,9 ±4,0 a	13,9 ±4,0 a	ns	0,30±0,13ab	0,06 ±0,05 a	*
A. Cantillana	0,14 ±0,08 b	90 ±10 a	18,3 ±1,7 a	11,7 ±2,6 a	*	0,35 ±0,18 a	0,07 ±0,05 a	ns

Values for each species in columns followed by different letters indicate significant difference, by [1]Tukey, [2]Kruskal- Wallis y [3] T-Student tests, P ≤ 0.05. * Significant difference P ≤ 0.05; ns: not significant

Resin content

Resin content in leaves and stems for Chilean *Haplopappus* species are reported in Table 2. *H. rigidus* showed the highest resin content in leaves, making up about a third of the dry material in some plants. The lowest resin content in both, leaves and stems, was found in *H. multifolius.*

Although each species has a limited distribution and the topography contributes to geographic isolation of the natural populations, only among *H. baylahuen* populations were significant differences in resin content for leaves (Table 2). The most distant populations, Cerro Vicuña and San Félix, differ significantly in resin content of leaves and stems. Stems of *H. rigidus* from Cerro Blanco had twice as much resin as those from Salar de Pedernales Km 90. *H. multifolius* and *H. taeda* showed homogeneous contents for each species and part of the plant.

Standard deviations reveal high intra-population variation in both leaves and stems. In all species populations could be found where the resin content of some plants doubled that of other individuals.

Essential oil

In general, essential oil content is low, not exceeding population averages of 0.5 mL 100 gDM^{-1} in leaves. At the same time, the high inter and intra-population variation doesn´t show significant differences among species in leaves and stems.

Results for each species suggest significant differences among populations, except in leaves and stems of *H. multifolius* and *H. taeda* stems.

Plants of *H. baylahuen* collected in Los Castaños were distinguished by their high essential oil content (Table 1). Differences among leaves and stems were significant only in Cerro Vicuña, in which the stems had the largest essential oil content. Leaves of *H. taeda* from Las Vegas exhibited essential oil content significantly less than those of Altos de Cantillana.

DISCUSSION

Aerial biomass is the main factor that determines biomass yield. The reported values for wild plants don´t consider sustainable harvest, which is based on annual growth of a plant. Thus, the highest biomass values of *H. baylahuen* and *H. rigidus* don't necessarily indicate a major potential yield. According to Vogel (2007) only 7% of the biomass of *H. baylahuen* was annual growth, while biomass production for *H. multifolius* and *H. taeda* reached 24% and 40%, respectively. In fact, Gonzalez (2003) found in young cultivated plants of *H. taeda* and *H. multifolius*, have greater biomass production rates than *H. baylahuen.*

Of the four species studied only *H. taeda* showed differences in biomass among the populations studied, which could be due to the presence of younger plants in Altos de Cantillana than in Las Vegas and Pichoante.

Several studies indicate that the surface resin production protects xerophytic plants against herbivory, UV radiation (Johnson, 1983), and water loss by transpiration (Johnson and Brain 1985; Zabala and Ravetta, 2001). In fact, the four species are highly resinous when grown in geographical areas where they are exposed to stress factors such as radiation, drought, and major temperature fluctuations. One would expect that cultivated plants have lower concentrations of these protecting compounds. However, resin content was similar in wild and cultivated plants of *H. baylahuen* and *H. taeda*, but in *H. multifolius* it was even higher in cultivated plants (15%). Other environmental factors formerly studied that could affect the resin contents are radiation and variation from year to the other. However no significant differences between plants cultivated in sun and shade could be detected. In the same population of *H. taeda* great yearly variation has been observed. For example, samples collected in 2002 had resin content of 37g*100g DM^{-1} (González, 2003), twice the value that was observed in 2005.

Resin productions of the four Haplopappus species were compared with the *Grindelia camporum* E. Greene ones, in order to define their cash crop potential. Resin content of *Grindelia camporum* in native stands is similar to *H. multifolius* (8%), being among the studied species the less resinous. However, an economic feasibility study indicates that *Grindelia camporum* plants needs to produce 15% of resin for to be a profitable crop (Hoffmann and Mc Laughlin, 1986). In order to improve the resin production and the cold adaptation of *G. camporum,* studies in *G. chiloensis* (Cornel) Cabrera were performed to look for a genetic variability source. Ravetta *et al*. (1996) informed about significant differences in resin content among populations, with values lower than *H. rigidus* and similar to *H. baylahuen* and *H. taeda*. This fact indicates the excellent potential of the Chilean bailahuén species for resin production.

Resin chromatographic patterns are identical for each *Haplopappus* species, even for populations of different provenances (González, 2003; Vogel *et al*., 2005, Vogel *et al*., 2007). Analysis of individuals indicates that each of the populations studied consists of a single species. Atypical chemotypes were not encountered.

In nature, essential oils evaporate from plants surfaces under high environmental temperatures, increasing resin viscosity and causing diminished water loss by limiting transpiration (Muñoz, 1987). All *Haplopappus* populations evaluated had low essential oil content with average as high as 0.50 mL * 100 gDM^{-1}.

The high intra-population variability in secondary metabolite content has also been shown in wild populations of other Chilean medicinal plants, such as *Buddleja globosa* (Vogel *et al.*, 2010; Vogel *et al.*, 2011) and *Drimys winteri* (Muñoz-Concha *et al.*, 2004). This fact is attributed mainly to genetic differences among plants as they are exposed to the same environmental conditions. The age of the plant is another factor that influences the concentration of secondary metabolite content.

The present research provides preliminary information for the selection of plant material with high biomass, resin and essential oil production. The *H. rigidus* population with the highest resin and essential oil content can be found in Cerro Blanco. In the species *H. baylahuen* populations situated at Cerro Vicuña and Los Castaños are appropriated for selection of individuals with high resin and essential oil content, whereas in *H. taeda*, a population from Pichoante is the most suitable for both compounds. In *H. multifolius* both of the studied populations showed similar characteristics.

CONCLUSIONS

Both, biomass production and resin content, vary significantly among species, *H. rigidus* is the most resinous species. Resin content in leaves varied significantly only among different *H. baylahuen* populations. Essential oil content is not suitable for identification of species because of the large amount of variation among populations.

In most populations, resin and essential oil content are higher in leaves than stems.

Biomass production and leaf percentages per plant are similar among populations of the same species, except for *H. taeda.*

The high variation observed among individuals from the same populations indicates the potential for selection of plants with desirable characteristics in future breeding projects of the *Haplopappus* species studied.

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