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ORIGINAL ARTICLE

In vitro and *in vivo* anti-inflammatory effect of extracts from four species of the genus *Baccharis*

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Keywords:
anti-inflammatory; Baccharis; edema; extracts; phospholipase A2 inhibitors; lipoxygenase (source: MeSH-NLM).

ABSTRACT

Objective. To evaluate the *in vitro* and *in vivo* anti-inflammatory effect of extracts from four species of the genus *Baccharis*. **Methods.** This was an experimental study with a quantitative approach. Leaves of *Baccharis* were collected from the provinces of Ayopaya and Chapare in the Department of Cochabamba. The species *B. pentlandii*, *B. perulata*, *B. genistelloides*, and *B. dracunculifolia* were identified at the "Martín Cárdenas" National Forestry Herbarium. Aqueous and ethanolic extracts (10%) were prepared from each species. The *in vitro* anti-inflammatory effect was assessed using Cayman's Lipoxygenase and Phospholipase A2 Inhibitor Screening Assay Kits, while the *in vivo* effect was evaluated through the carrageenan-induced paw edema model in 50 male Wistar rats. Descriptive analyses were conducted, with data normality assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests, and group comparisons performed through the Kruskal-Wallis test and one-way ANOVA. **Results.** The aqueous extract of *B. perulata* inhibited lipoxygenase activity by 91.9 %, while the aqueous extract of *B. pentlandii* inhibited phospholipase A2 activity by 81.2 %. In contrast, the ethanolic extract of *B. dracunculifolia* exhibited the greatest anti-inflammatory effect on paw edema volume in rats (90.7 %), with statistically significant differences after the first hour ($p < 0.05$). **Conclusions.** Extracts of *Baccharis* (*B. perulata*, *B. pentlandii*, and *B. dracunculifolia*) exhibited *in vitro* and *in vivo* anti-inflammatory effects superior to indomethacin, supporting their potential as natural alternatives for inflammation treatment.

Efecto antiinflamatorio *in vitro* e *in vivo* de extractos de cuatro especies del género *Baccharis*

Palabras clave:
antiinflamatorio; Baccharis; edema; extractos; inhibidores de fosfolipasa A2; lipooxigenasa (fuente: DeCs-BIREME).

RESUMEN

Objetivo. Evaluar el efecto antiinflamatorio *in vitro* e *in vivo* de extractos de cuatro especies del género *Baccharis*. **Métodos.** El estudio fue experimental con enfoque cuantitativo. Las hojas del género *Baccharis* se recolectaron de las provincias Ayopaya y Chapare del Departamento de Cochabamba. Las especies *B. pentlandii*, *B. perulata*, *B. genistelloides* y *B. dracunculifolia* se identificaron en el Herbario Nacional Forestal "Martín Cárdenas". De cada especie se elaboraron extractos acuosos y etanólicos al 10 %. El efecto antiinflamatorio *in vitro* se determinó con el Kit de ensayo de detección de inhibidores de lipooxigenasa y fosfolipasa A2 de Cayman; in vivo con el modelo de edema plantar inducido por carragenina en 50 ratas macho Wistar. Se realizó un análisis descriptivo. Para determinar la normalidad de datos se utilizó la prueba de Shapiro-Wilk y Kolmogorov-Smirnov; para comparar los grupos analizados se empleó la prueba de Kruskal-Wallis y ANOVA de una vía. **Resultados.** El extracto acuoso de *B. perulata* inhibió la lipooxigenasa un 91,9 %, mientras que el extracto acuoso de *B. pentlandii* inhibió la fosfolipasa A2 un 81,2 %. Por otro lado, el extracto etanólico de *B. dracunculifolia* presentó mayor efecto antiinflamatorio del volumen de edema plantar de ratas de un 90,7 %, con diferencias estadísticamente significativas después de la primera hora ($p < 0,05$). **Conclusiones.** Los extractos de *Baccharis*: *B. perulata*, *B. pentlandii* y *B. dracunculifolia* tienen efecto antiinflamatorio *in vitro* e *in vivo* superior a la indometacina, lo que respalda su uso como alternativa natural para el tratamiento de la inflamación.

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INTRODUCTION

Worldwide, inflammation represents a major public health problem [1], as it is associated with a large number of autoimmune diseases [2-5] that deteriorate the population's quality of life and generate a high socioeconomic burden [6]. To manage the inflammatory response, non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids are commonly used [7,8]. However, high doses or prolonged use can lead to gastrointestinal, renal, cardiovascular, metabolic, endocrine, immune, and neurological adverse effects [9,10]. Therefore, identifying natural alternative therapies with anti-inflammatory potential and minimal or no side effects is essential.

Plants of the *Baccharis* genus, comprising more than 350 species distributed mainly in Latin America, are widely recognized in pharmacognosy due to their secondary metabolites, such as diterpenes, triterpenes, and flavonoids [11]. International studies conducted on extracts of *Baccharis trimera* (carqueja) have demonstrated anti-inflammatory and immunomodulatory effects in rat pleurisy models and inhibition of inflammatory mediators *in vitro* [12], whereas extracts of *Baccharis dracunculifolia* (chilca blanca) have shown anti-inflammatory activity in ulcerative colitis models in rats [13].

Bolivia's vast cultural and biological diversity is reflected in an extensive range of traditional medicine practices, the most prominent being the use of medicinal plants [14]. Within this context, the *Baccharis* genus—represented by nearly 60 species in Bolivia—is traditionally used for bruises, sprains, and dislocations due to its anti-inflammatory effects [15]. Pharmacological studies on extracts of these species have identified flavonoids such as 5,4-dihydroxy-6,7,8,3-tetramethoxyflavone, 8-methoxycirsilineol, 5,4-dihydroxy-6,7,8-trimethoxyflavone, and sideritoflavone, all of which exhibited significant anti-inflammatory effects against cyclooxygenases (COX-1, COX-2) and tumor necrosis factor-alpha (TNF-α) [16,17].

Based on this background, the development of this study is justified, as no research evaluating the anti-inflammatory effects of *Baccharis* using combined *in vitro* and *in vivo* models has been identified. Furthermore, considering Bolivia's long history of traditional use and extensive biodiversity, validating the therapeutic effects attributed to these species is necessary to promote the development of accessible alternative therapies. Therefore, the objective of this study was to evaluate the *in vitro* and *in vivo* anti-inflammatory effects of extracts from four *Baccharis* species.

METHODS

Study type and area

This study followed a quantitative, experimental approach with a cross-sectional *in vitro* design (lipoxygenase and phospholipase A2 inhibition assays were performed once) and a longitudinal *in vivo* design (inflamed volume measured over time). It was randomized, controlled, and conducted in parallel groups. The study was conducted at the Center for Pharmaceuticals, Food, and Cosmetics (CEFAC, by its Spanish acronym), affiliated with the Faculty of Pharmaceutical and Biochemical Sciences of the Universidad Mayor de San Simón (UMSS), in Cochabamba, Bolivia, during 2023-2024.

Population and sample

Although the study population could not be established, the plant and animal samples met the following criteria:

a) Plant sample: Plants from four species of the *Baccharis* genus were included, with healthy leaves collected from municipalities in the Department of Cochabamba. Excluded were *Baccharis* species previously studied at CEFAC for anti-inflammatory effects, as well as those with damaged or contaminated leaves.

b) Animal sample: Fifty male Wistar rats (*Rattus norvegicus albinus*), of the same species, aged 3-4 months, weighing 200-300 grams, and certified by a biological testing unit were included. Rats previously used in other experiments or presenting any pathology were excluded.

Variables and data collection instruments

The study variables were:

a) Lipoxygenase (LO) inhibition: Determined using the LO inhibitor screening assay kit (item 760700, Cayman) [18]. Absorbance values were read using a Stat Fax 303 Plus.

b) Phospholipase A2 (PLA2) inhibition: Determined using the PLA2 inhibitor screening assay kit (item 10004883, Cayman) [19]. Absorbances were also read using a Stat Fax 303 Plus.

c) *In vivo* anti-inflammatory effect: Assessed using the carrageenan-induced paw edema model

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described by Winter et al. [20]. The inflammation volume at 0, 1, 3, 5, and 7 hours was measured using a manual plethysmometer.

Data for "absorbance," "inflammation volume," and "percentage of anti-inflammatory effect" were recorded using an observation guide instrument.

Data collection techniques and procedures

According to Valenzuela [21], *Baccharis* species grow year-round, with greater leaf production during wet months. Therefore, in February, *Baccharis dracunculifolia* (chilca blanca) was collected from the municipality of Independencia, Ayopaya Province. Meanwhile, *Baccharis pentlandii* (chilca clara), *Baccharis perulata* (Yurak chilca), and *Baccharis genistelloides* (carqueja amarga) were collected in the municipality of Colomi, Chapare Province, Cochabamba Department. Stems were cut using pruning shears and placed in labeled plastic bags. Following Arnelas et al. [22], the *Baccharis* species were identified at the "Martín Cárdenas" National Forestry Herbarium, belonging to the Faculty of Science and Technology at UMSS.

The leaves of each *Baccharis* species were washed thoroughly with water and disinfected with 80-ppm sodium hypochlorite. They were then dried in a drying chamber at 40°C for 72 hours and later ground in a porcelain mortar. Aqueous and ethanolic extracts at 10 % were prepared for each species; ethanolic extracts were macerated for seven days, and aqueous extracts for two days. All extracts were filtered and concentrated using a rotary evaporator until three-quarters of the solvent was removed. Each extract was then labeled and refrigerated at 2-8°C.

The detection of LO inhibitors was conducted following the manufacturer's instructions, using a microplate in triplicate as follows:

a) Blank: 100 µl of buffer were pipetted into the first three wells.

b) Standard: 90 µl of LO + 10 µl of buffer.

c) 100% initial activity (IA): 90 µl of LO + 10 µl of methanol.

d) Inhibitors: 90 µl of LO + 10 µl of inhibitor (indomethacin or extracts).

The plate was incubated for five minutes at room temperature. The reaction was initiated by adding 10 µl of substrate (arachidonic acid) to each well. The plate was then placed on a shaker for ten minutes. Enzymatic catalysis was stopped by adding 100 µl of chromogen to each well. The plate was covered and shaken for five minutes. Finally, the cover was removed and absorbances were measured between 490 and 500 nm using a Stat Fax 303 Plus.

PLA2 inhibitors were detected in a microplate according to the following procedure:

a) Blank: 10 µl of buffer + 10 µl of methanol were pipetted into the first three wells.

b) Standard: 10 µl of PLA2 + 10 µl of thioetheramide-PC.

c) 100% initial activity (IA): 10 µl of PLA2 + 10 µl of methanol.

d) Inhibitors: 10 µl of PLA2 + 10 µl of inhibitors (indomethacin or extracts).

Then, 200 µl of substrate (phosphatidylcholine analog) were added to all wells, followed by 10 µl of DTNB (5,5'-dithiobis-2-nitrobenzoic acid) to initiate the reaction. The plate was shaken for 10 seconds to mix the contents, covered, and incubated for 15 minutes at 25°C. Finally, the cover was removed and absorbances were read between 405 and 420 nm on a Stat Fax 303 Plus.

Concentrations and LO/PLA2 inhibitor groups:

G1: Indomethacin 100 µg/ml

G2: Aqueous extract of *B. perulata* (Yurak chilca) 100 µg/ml

G3: Ethanolic extract of *B. perulata* (Yurak chilca) 100 µg/ml

G4: Aqueous extract of *B. pentlandii* (chilca clara) 100 µg/ml

G5: Ethanolic extract of *B. pentlandii* (chilca clara) 100 µg/ml

G6: Aqueous extract of *B. genistelloides* (carqueja amarga) 100 µg/ml

G7: Ethanolic extract of *B. genistelloides* (carqueja amarga) 100 µg/ml

G8: Aqueous extract of *B. dracunculifolia* (chilca blanca) 100 µg/ml

G9: Ethanolic extract of *B. dracunculifolia* (chilca blanca) 100 µg/ml

Calculations: First, the mean absorbance of the blank, 100% IA, and inhibitor groups was determined. Then, the blank mean was subtracted from the means of 100% IA and the inhibitors. The percentage of LO and PLA2 inhibition was calculated using the following formula:

$$\% \ inhibition = \left[\frac{AI - Inhibitor}{AI}\right] * 100$$

To evaluate the *in vivo* anti-inflammatory effect, the rats were acquired from the Biological Testing Unit of the Faculty of Pharmaceutical and Biochemical Sciences, Universidad Mayor de San Andrés (UMSA), La Paz (Bolivia). The rats were transported in boxes provided by the Biological Testing Unit and placed on the floor of a private vehicle; neither air conditioning nor heating was used during transport, as it was not necessary. Subsequently, they were acclimatized at CEFAC for 7 days in groups of five, housed in metal cages under controlled conditions (temperature 22 ± 3 °C, 12-hour light–dark cycles, wood-chip bedding changed every 48 hours, pellet diet, and *ad libitum* access to water). The rats were fasted for 12 hours prior to the experiment, with free access to water. Afterward, an independent staff member randomly assigned five rats to each of the following groups:

G1: Negative control.

G2: Indomethacin 10 mg/kg body weight

G3: Aqueous extract of *B. perulata* (Yurak chilca) 400 mg/kg

G4: Ethanolic extract of *B. perulata* (Yurak chilca) 400 mg/kg

G5: Aqueous extract of *B. pentlandii* (chilca clara) 400 mg/kg

G6: Ethanolic extract of *B. pentlandii* (chilca clara) 400 mg/kg

G7: Aqueous extract of *B. genistelloides* (carqueja amarga) 400 mg/kg

G8: Ethanolic extract of *B. genistelloides* (carqueja amarga) 400 mg/kg

G9: Aqueous extract of *B. dracunculifolia* (chilca blanca) 400 mg/kg

G10: Ethanolic extract of *B. dracunculifolia* (chilca blanca) 400 mg/kg

First, the analysis personnel measured the baseline volume of the right hind paw of each rat using a manual plethysmometer, according to previous studies [23–25]. Then, an independent staff member administered 1 ml of the corresponding treatment intraperitoneally. Thirty minutes later, edema was induced by subcutaneous injection of 0.1 ml of 1% carrageenan into the plantar aponeurosis of the right hind paw of each rat, following Amado et al. [26]. Subsequently, paw volume was measured at 1, 3, 5, and 7 hours. The percentage of inhibition was calculated using the mean volume ($\bar{x}$) of the negative control and the mean volume of each treatment, using the following formula:

$$\% \ inhibition = \frac{\bar{X}(V_d - V_o)\ negative\ control - \bar{X}(V_d - V_o)\ treatments}{\bar{X}\ (V_d - V_o)\ negative\ control} \times 100$$

where:

Vd = volume after carrageenan injection

V0 = volume before carrageenan injection

Data analysis

Data were tabulated in Microsoft Excel 2016 and subsequently imported into the SPSS 25 statistical package, where absolute and relative frequencies of the variables were calculated. Normality was assessed using the Shapiro–Wilk test ($n < 50$) and the Kolmogorov–Smirnov test ($n \geq 50$). Group comparisons were performed using the Kruskal–Wallis test and one-way ANOVA, with a 95% confidence level and statistical significance set at $p < 0.05$.

Ethical considerations

This study was part of the research project *"Biotechnological application in the valorization of plant products for productive and medicinal purposes"*, whose protocol was approved by the Bioethics Committee of the Faculty of Medicine, UMSS, Cochabamba (Bolivia), under registration code CE-25, issued on January 13, 2023. All ethical principles established by the World Medical Association (WMA) for the use of animals in biomedical research, reaffirmed at the 203rd Meeting in Buenos Aires, Argentina, in April 2016, were observed [27].

RESULTS

The *in vitro* inhibition of LO by *Baccharis* extracts at a concentration of 100 µg/ml showed that the aqueous extract of *B. perulata* (Yurak chilca) exhibited the highest inhibition (91.9 %), compared with 85.6 % inhibition produced by indomethacin, 79.6 % by the aqueous extract of *B. pentlandii* (chilca clara), 75.9 % by the ethanolic extract of *B. pentlandii*, 72.2 % by

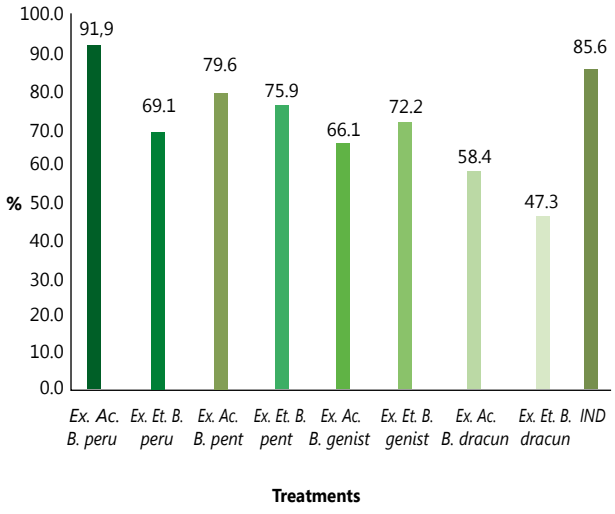

Figure 1. Percentage of lipoxygenase inhibition

** B: Baccharis, Ex: extract, Aq: aqueous, Et: ethanolic, peru: perulata, pent: pentlandii, genist: genistelloides, dracun: dracunculifolia, IND: indomethacin.*

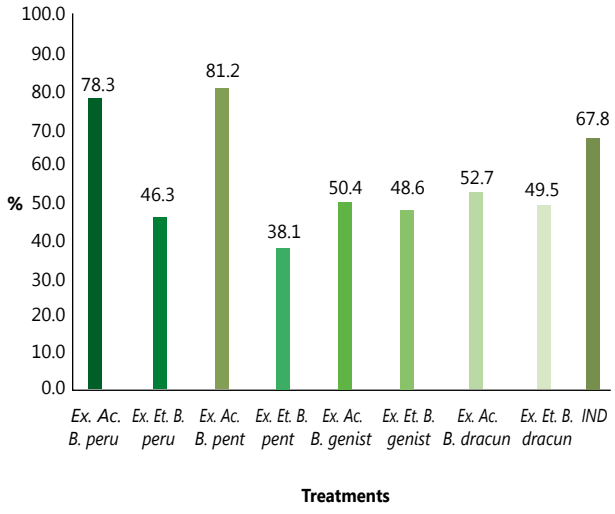

Figure 2. Percentage of phospholipase A2 inhibition

** B: Baccharis, Ex: extract, Aq: aqueous, Et: ethanolic, peru: perulata, pent: pentlandii, genist: genistelloides, dracun: dracunculifolia, IND: indomethacin.*

the ethanolic extract of *B. genistelloides* (carqueja amarga), 69.1 % by the ethanolic extract of *B. perulata*, 66.1 % by the aqueous extract of *B. genistelloides*, and 58.4 % by the aqueous extract of *B. dracunculifolia* (chilca blanca) (see Figure 1).

Regarding the *in vitro* inhibition of PLA2, the aqueous extract of *B. pentlandii* (chilca clara) showed 81.2 % inhibition, higher than the 78.3 % of the aqueous extract of *B. perulata* (Yurak chilca), the 67.8 % inhibition produced by indomethacin, and the values obtained for the remaining *Baccharis* extracts, which exhibited lower percentages (see Figure 2).

Regarding the percentage inhibition of inflammation volume at 1, 3, 5, and 7 hours after carrageenan administration, the ethanolic extract of *B. dracunculifolia* (chilca blanca) exhibited 90.7 % inhibition at 7 hours, followed by the ethanolic extract of *B. pentlandii* (chilca clara) with 85.3 % and the aqueous extract of *B. perulata* (Yurak chilca) with 82.9 %. These values were higher than those of the other *Baccharis* extracts and indomethacin (see Table 1).

The LO inhibition absorbance values and the inflammation volume at the fifth hour followed a normal distribution ($p > 0.05$), whereas the PLA2 absorbance values and inflammation volume at 0, 1, 3, and 7 hours did not follow a normal distribution ($p < 0.05$) according to the Shapiro–Wilk and Kolmogorov–Smirnov tests (see Table 2).

Table 1. Initial volume and percentage inhibition of inflammation volume over time

Treatment	Initial volume	% inhibition			
		1 h	3 h	5 h	7 h
Aqueous extract *B. perulata*	1.13	31.3	42.5	66.7	82.9
Ethanolic extract *B. perulata*	1.14	10.3	25.4	33.3	47.1
Aqueous extract *B. pentlandii*	1.15	17.2	32.6	40.7	56.5
Ethanolic extract *B. pentlandii*	1.15	33.8	41.7	68.5	85.3
Aqueous extract *B. genistelloides*	1.14	31.7	38.5	45.9	79.3
Ethanolic extract *B. genistelloides*	1.15	26.8	31.3	40.5	72.4
Aqueous extract *B. dracunculifolia*	1.14	22.0	29.8	35.1	62.1
Ethanolic extract *B. dracunculifolia*	1.16	34.1	44.2	62.2	90.7
Indomethacin	1.14	27.6	46.9	63.7	79.3

Table 2. Normality test for study variables

Variables	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	*p-value*	Statistic	df	*p-value*
Phospholipase A2				0.888	27	0.007
Lipoxygenase				0.975	27	0.733
Volume 0 h	0.171	50	0.001			
Volume 1 h	0.161	50	0.002			
Volume 3 h	0.149	50	0.007			
Volume 5 h	0.108	50	0.200			
Volume 7 h	0.191	50	0.000			

The comparison of independent groups using treatments as a factor for normally distributed data indicated statistically significant differences in LO inhibition absorbances and inflammation volume at the fifth hour in at least two groups, according to the one-way ANOVA ($p < 0.05$) (see Table 3).

The comparison of independent groups for non-normally distributed data showed no statistically significant differences in inflammation volume before carrageenan administration; however, there were significant differences in PLA2 inhibition absorbances and inflammation volumes at 1, 3, and 7 hours in at least two groups, according to the Kruskal-Wallis test ($p < 0.05$) (see Table 4).

DISCUSSION

In this study, the anti-inflammatory effects of extracts from four *Baccharis* species were evaluated using *in vitro* assays, such as LO inhibition, using the method by Sircar et al. [28] modified by Evans et al. [29], which employs arachidonic acid (AA) as a substrate, while PLA2 inhibition was assessed using the method by Reynolds et al. [30]. Thus, *in vitro* LO and PLA2 inhibition are considered effective models for identifying plants with anti-inflammatory activity, as plant lipoxygenase is similar in several aspects to the enzyme that hydrolyzes AA in animals [31]. Moreover, several natural secondary metabolites are known to inhibit these enzymes [32]. The *in vivo* anti-inflammatory effect was evaluated using carrageenan-induced paw edema in rats, a widely used biological model in preclinical studies for assessing anti-inflammatory drugs [20].

Regarding *in vitro* LO inhibition, the aqueous extract of *B. perulata* (Yurak chilca) demonstrated higher inhibition than indomethacin. Comparing these results with those of Abad et al. [33], several extracts, particularly dichloromethane and hexane extracts of *B. pentlandii*, *B. obtusifolia*, *B. subulata*, and *B. latifolia*, achieved 100% inhibition. These differences may be related to the solvent used and the *Baccharis* species analyzed. In the study by Torres et al. [34], ethanolic extracts of *B. boliviensis* (chijua) and *B. tola* (tola)

Table 3. One-way ANOVA for independent groups

Variables	One-way ANOVA		
	SS	df	*p-value*
Lipoxygenase			
Between groups	0.107	8	
Within groups	0.004	18	0.000
Total	0.111	26	
Volume 5 hours			
Between groups	0.527	9	
Within groups	0.216	40	0.000
Total	0.743	49	

Table 4. Kruskal–Wallis test for independent groups

Variable	Kruskal-Wallis		
	Test statistic	df	*p-value*
Phospholipase A2	23.273	8	0.003
Volume 0 h	11.927	9	0.217
Volume 1 h	31.557	9	0.000
Volume 3 h	24.456	9	0.004
Volume 7 h	29.798	9	0.000

at 200 µg/ml showed lower LO inhibition than the extracts analyzed in this work, likely due to lower concentrations of secondary metabolites capable of inhibiting this enzyme.

The PLA2 inhibition percentages of the aqueous extracts of *B. pentlandii* and *B. perulata* were similar to those obtained from the mixture of oleanolic and ursolic acids isolated from the ethanolic extract of *B. uncinella* (vassoura) in the study by Zalewski et al. [35]. Nevertheless, they were higher than the percentages reported by Torres et al. [34] for ethanolic extracts of *B. boliviensis* and *B. tola*—perhaps due to poor solubility of the compounds present in these species.

The inflammation-inhibition percentages at 3, 5, and 7 hours were highest for the ethanolic extracts of *B. dracunculifolia* (chilca blanca) and *B. pentlandii* (chilca clara) at 400 mg/kg. These results are consistent with those of Dos Santos et al. [36] at 3 hours using hydroalcoholic extract of *B. dracunculifolia*, Pérez et al. [37] using extract of *B. incarum* (tola de río), and González et al. [38] using *B. latifolia* extract at 5 hours. However, they were lower than the 62 % inhibition observed at 2 hours in Rivera's study [39] with ethanolic extract of *B. buxifolia* (tayanca), which even surpassed diclofenac and dexamethasone. This difference may be due to the distinct *Baccharis* species used and the induction of edema with 1% albumin.

This study showed statistically significant differences in *in vitro* LO and PLA2 inhibition in at least two groups, contrary to the findings of Torres et al. [34], who reported no significant differences.

Statistically significant differences were also observed in inflammation inhibition using treatments as a factor, consistent with the findings of Dos Santos et al. [36], Pérez et al. [37], González et al. [38], and Rivera-Vicuña [39].

One strength of this study was the use of both *in vitro* and *in vivo* models, allowing identification of plants with anti-inflammatory activity and evaluation of anti-inflammatory drugs. Another strength was the inclusion of multiple *Baccharis* species, facilitating ethnopharmacological comparisons.

A limitation was the lack of identification and quantification of the secondary metabolites responsible for the anti-inflammatory effect, as well as the absence of toxicity evaluation for potential therapeutic applications.

Future studies should identify and quantify the secondary metabolites responsible for the anti-inflammatory effect, conduct chronic inflammation models, and assess extract toxicity.

Conclusions

The aqueous and ethanolic extracts of *Baccharis* species demonstrated significant *in vitro* and *in vivo* anti-inflammatory activity. The aqueous extracts of *B. perulata* (Yurak chilca) and *B. pentlandii* (chilca clara) showed greater *in vitro* inhibition of LO and PLA2 than indomethacin. Meanwhile, the ethanolic extract of *B. dracunculifolia* (chilca blanca) exhibited the strongest anti-inflammatory effect against carrageenan-induced edema at 1, 3, 5, and 7 hours.

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Authorship contribution

JVN: methodology, formal analysis, investigation, funding acquisition, supervision, writing and final review of the manuscript.

ZBG: conceptualization, methodology, and final review of the manuscript.

JPD: project administration, validation, and final review of the manuscript.

SZV: project administration, validation, and final review of the manuscript.

SVF: investigation, data curation, resources, and final review of the manuscript.

BAFM: resources, investigation, and final review of the manuscript.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.