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# Analgesic–antiinflammatory properties of *Proustia pyrifolia*

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**Abstract**

The antiinflammatory (per os and topic) and analgesic (per os) properties of the aerial part of *Proustia pyrifolia* a species in danger of extinction were investigated, and the major compounds of two of its active extracts were isolated. In addition, the evaluation of cytotoxicity in three tumoral cell lines and the acute toxicity of the crude methanol extract were also assayed, together with the antioxidant activity for the different extracts of this species. The results of the evaluation of the topic antiinflammatory activities induced by arachidonic acid, and phorbol 12-myristate 13-acetate of the different extracts showed that this species possesses active constituents that could diminish cyclo-oxygenase and lipoxygenases activities, the enzymes that allow the synthesis of proinflammatory endogenous substances as prostaglandin $E_2$ and leukotrienes, respectively. Our results corroborate the antiinflammatory and analgesic effects of *Proustia pyrifolia*, and could justify its use in folk medicine for the treatment of rheumatic and gout illnesses. From bio-active extracts β-sitosterol, quercetin and dihydroquercetin were obtained, and these compounds could explain in part the antiinflammatory, analgesic and antioxidant activities of this species. The crude methanol extract did not present acute toxicity or cytotoxic activity, however only this extract exhibited antioxidant activity.


*Keywords:* *Proustia pyrifolia*; Analgesic–antiinflammatory-toxicity; Steroids–flavonoids

*Abbreviations:* *A*, antinflammatory activity; AA, arachidonic acid; ADR, adriamicyn; AL, allopurinol; An, analgesic effect; A-549, human lung carcinoma; CC, column chromatography; $C_{control}$, median writhes reached in control animals which received only the vehicle; $CH_2C1_2$, dichloromethane; $C_{sample}$, median writhes reached in sample-treated animals; DCE, dichloromethane extract; $Et_2O$, ethyl ether; GME, crude methanol extract; HC1, chloride acid; HE, hexane extract; HT-29, human colon carcinoma; *I*, inhibition of xanthine oxidase; $I_c$, median inflammation reached in the control group; $IC_{50}$, inhibitory concentration; $I_s$, median inflammation in the sample-treated animals; IND, indomethacin; INF, aqueous extract; IR, infrared; $LD_{50}$, lethal dose; ME, methanol extract; NM, nimesulide; MeOH, methanol; *P*, mean pain; $PGE_2$, prostaglandin $E_2$; P-388, lymphoid neoplasm from DBA/2 mouse; S.E.M., standard error medium; SQF, Herbarium of the Escuela de Quimica y Farmacia; *T*, dermal antiinflammatory activity; TLC, thin layer chromatography; TPA, phorbol 12-myristate 13-acetate; $V_i$, initial paw volume; $V_f$, final paw volume; XO, xanthine oxidase; $\Delta W_c$, difference median values of the weights of the right and the left ear sections of the control animals; $\Delta W_s$, difference median values of the weights of the right and the left ear sections of the treated animals

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## 1. Introduction

Different species of *Proustia* genus have been frequently used as antiinflammatory and analgesic to treat gout and rheumatic illnesses, however, there is little information about their efficacy and acute toxicity (Muñoz et al., 1981). This genus accumulates sesquiterpene α-isocedrene derivatives that are typical for the subtribus Nassauviinae of the family Asteraceae (Zdero et al., 1986), and a guaianolide β-D-glucopyranoside has been previously isolated from *Proustia ilicifolia* (Bittner et al., 1989).

The objective of this research was to validate the use of a native shrub, *Proustia pyrifolia* DC. (Asteraceae) for folklore medicine, therefore, we explored its acute toxicity and its antiinflammatory and analgesic properties. For the in vivo assays oral administration was used, the same as in folklore medicine. The correlation among the different in vivo assays, allowed us to suggest the probable mechanism of action of the metabolites isolated from two bioactive extracts. We report the results obtained with crude methanol (GME), hexane (HE), dichloromethane (DCE), methanol (ME) and aqueous extracts (INF) in the biological assays. Antioxidant activity was also studied as it can be related with the antiinflammatory properties (Das and Maulik, 1994). To investigate other pharmacological activities not described by the folklore medicine, we evaluate GME cytotoxicity against three tumoral cell lines in search of potentially useful compounds that might help in cancer research.

## 2. Materials and methods

### 2.1. General experimental procedures

$^{1}H$ and $^{13}C$ NMR were recorded in $CDCl_3$ at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$; internal standard TMS. $^{1}D$ ($^{1}H$, $^{13}C$) and $^{2}D$ (COSY, HMQC, HMBC and ROESY) experiments were performed using the standard Bruker DISNMR pulse program.

Column chromatography (CC) was run using silica gel 60G (Merck 7734). TLC was performed on silica gel GF254 (Merck 5554); spots were detected under UV light, or after spraying Liebermann–Burchard reagent and then heating for about 5 min at 120°. IR spectra were made in KBr; melting point was determined on a Kofler hot stage microscope and is uncorrected.

### 2.2. Plant material

The aerial part of *Proustia pyrifolia* DC was collected at Cuesta La Dormida, Chacabuco, Chile, in January, and identified by Prof. Sebastian Teiller. A voucher specimen is kept at the Herbarium of the Escuela de Química y Farmacia (SQF 22143), Universidad de Chile.

### 2.3. Extraction and isolation

Air dried and powdered material (0.8 kg) of plant material was extracted with methanol at room temperature. After removing the solvent under vacuum this crude methanol extract (GME, 72.7 g) was used for additional pharmacological assays.

A separate portion of the powdered material (6.0 kg) was extracted successively at room temperature with *n*-hexane, $CH_2Cl_2$ and MeOH, yielding after removal of the solvents in vacuo, 131 g of HE, 73 g of DCE and 400 g of ME, respectively. Part of this last extract (20.0 g) was submitted to CC on silica gel with *n*-hexane/$Et_2O$ gradient (0, 10, 20, 50 and 100% $Et_2O$) yielding two fractions of increasing polarity (1–2). Fraction 1 was applied on Sephadex LH-20 with acetone/$CH_2Cl_2$ 1:1 yielding **1** (95.0 mg) and a rest fraction. The latter was rechromatographed on a chromatotron with *n*-hexane/acetone 5:4 yielding **2** (30 mg). Identification of these compounds was performed through comparison of the NMR data with those reported in the literature.

An amount of 100 g HE was subjected to repeated columns chromatography on silica gel and eluted with mixtures of *n*-hexane–$CH_2Cl_2$ (v/v), $CH_2Cl_2$–MeOH (v/v) and finally MeOH. Fractions of 500 mL were collected. Fractions eluted with *n*-hexane–$CH_2Cl_2$ (25:75 v/v) were joined and dried (25 g) and subjected to a second column chromatography over silica gel, and fractions of 200 mL were collected and monitored by TLC. From fractions (13–16) eluted with *n*-hexane–$CH_2Cl_2$ (50:50 v/v and 40:60 v/v) 281 mg of the compound **3** were obtained. Identification of this compound was performed by direct comparison of the melting point, chromatographic (TLC) and spectroscopic (IR) data with an authentic reference compound.

### 2.4. In vivo assays animals

Pirbright guinea pigs (220–300 g) of both sexes were used for the per os antiinflammatory study. CF-1 mice of either sex (20–25 g) were used to assess the analgesic and topic antiinflammatory effects, and acute toxicity. Animals under standard conditions from the Chilean Public Health Institute were fasted overnight before the day of the experiments.

### 2.5. Acute toxicity

For each dose, groups of 10 mice of both sexes were allowed free access to water. GME suspended in saline Arabic gum, 5%, were orally administered via a gastric catheter. They were weighed daily for a week to detect physiological alterations. In case of death of the animals, the $LD_{50}$ is determined by the Morgan Scoring method (Morgan, 1992).

### 2.6. Cytotoxicity assays

A screening procedure was used to assess the cytotoxicity of GME against the following cell lines: P-388 (lymphoid