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; ΔW_c, difference median values of the weights of the right and the left ear sections of the control animals; ΔW_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

neoplasm from DBA/2 mouse, ATCC CCL-46), A-549 (human lung carcinoma, ATCC CCL-185) and HT-29 (human colon carcinoma, ATCC HTB-38). Cells were seeded into 16 mm wells (multi-dishes) (NUNC 42001) at concentrations of 1×104 cells/well (P-388), 2×104 cells/well (A-549) (HT-29), respectively, in 1 mL aliquots of MEM 10FCS medium containing the compound to be assessed at the concentrations assayed. In each case, a set of control wells was incubated in the absence of sample and counted daily. After 4 days at 37 °C, under a 10% CO_2, 98% humid atmosphere, P-388 cells were observed through an inverted microscope and the degree of inhibition was determined by comparison with the control, whereas A-549 and HT-29 were stained with crystal violet before examination (San Feliciano et al., 1993). Adriamicyn (ADR) as reference drug was used.

2.7. Xanthine oxidase activity

Both xanthine and xanthine oxidase (XO) from cow's milk were purchased from Sigma Co. and the standard inhibitor allopurinol (AL) was obtained from Laboratorios Saval, Chile; GME, HE, DCE and ME were evaluated at 50 mg/mL and those having an inhibition >50% were further tested for IC_{50} determination (Schmeda-Hirschmann et al., 1992). The inhibition of XO activity using xanthine as the substrate was spectrophotometrically measured in relation to the production of uric acid, which was determined at 290 nm using a UNICAM spectrophotometer. The assayed mixture consisted of 1.0 mL of test solution, 2.9 mL of phosphate buffer (Na_2HPO_4/KH_2PO_4; pH = 7.5), and 0.1 mL of enzyme solution. After preincubation of the mixture at 25 °C for 15 min, the reaction was initiated by adding 2.0 mL of substrate solution. The assayed mixture was incubated at 25 °C for 30 min. The reaction was stopped by adding 1.0 mL of 1 M HCl, and the absorbance was measured. The inhibition percent of xanthine oxidase activity (%I) was calculated as: $\%I = (A - B) - (C - D)/(A - B) \times 100$; where A is the activity of XO without test material (total uric acid), B the blank of A without XO, C the enzyme activity with test material (residual uric acid) and D the blank of C without the enzyme.

The IC_{50} determination of allopurinol was 0.035 mg/mL (0.267 mM). For XO activity, the significance of the drug-induced changes was estimated using the Wilcoxon test for independent data (Hollander and Wolfe, 1973).

2.8. Antiinflammatory activity per os

For each per os dose, the antiinflammatory activity was evaluated in groups of 10–15 guinea pigs and 16 control ones, using the λ-carrageenan-induced paw oedema (Backhouse et al., 1994). Paw volume was measured with an Ugo Basile plethysmometer (model 7150), and 3 h after injecting 0.1 mL of sterile saline (λ-carrageenan, 1%). Antiinflammatory activity (A) was evaluated as: $\%A = [\%I_c - \%I_s/\%I_c] \times 100$; where $\%I_c$ is the median inflammation reached in the control group receiving only the vehicle ($34.0 \pm 2.3\%$ paw volume increase), and $\%I_s$ corresponds to the median inflammation in the sample-treated animals, expressed as: $\%I = [V_f - V_i/V_i] \times 100$; where V_f and V_i are final and initial paw volumes, respectively (Backhouse et al., 1994).

2.9. Topic antiinflammatory activity

Eight mice were treated with the sample and after 5 min they received 2 mg of arachidonic acid (AA) or 2.5 μg of phorbol 12-myristate 13-acetate (TPA), dissolved in 20 μL acetone. Sixteen control subjects received only AA or TPA at the same concentration. Both the sample and the AA or TPA were applied to the inner (10 μL) and outer (10 μL) surfaces of the right ear. The left ear received only the acetone. Mice were sacrificed by cervical dislocation and a 6 mm diameter section of the right and left ears were cut and weighed (Lloret and Moreno, 1995). Dermal antiinflammatory activity (T) was evaluated according to the following equation: $\%T = [\Delta W_c - \Delta W_s/\Delta W_c] \times 100$; where ΔW is the difference median values of the weights of the right and the left ear sections of the control (ΔW_c) and the treated animals (ΔW_s), respectively (Delporte et al., 2002).

2.10. Analgesic activity per os

For each per os dose of the sample under study, the analgesic activity was evaluated in groups of eight mice and 16 control subjects, using a intraperitoneal injection of 0.5 mL of 0.6% acetic acid (Delporte et al., 2002). The analgesic effects were calculated by comparing the number of abdominal writhes of the treated and the control group, which only received the vehicle. The number of abdominal writhes of each mouse was counted for 30 min, beginning 5 min after acetic acid administration (Delporte et al., 2002).

The following equation was used to calculate the mean pain percentage: $\%P = [C_{sample}/C_{control}] \times 100$; where C_{sample} is the median writhes reached in sample-treated animals and $C_{control}$ (41.6 ± 3.79) is the median writhes reached in control animals which received only the vehicle (Delporte et al., 2002).

The analgesic effect, An, was calculated according to the following equation: $\%An = 100 - \%P$.

In antiinflammatory and analgesic assays, the dry extracts (GME, INF, HE, DCE and ME) were orally administered 1 h before λ-carrageenan, or acetic acid, respectively, by means of an intragastric catheter, suspended in saline Arabic gum. The dosages of extracts used in each assay were selected according to previous work on the same biological activity (Delporte et al., 1998).

For all in vivo pharmacological assays, the drug-induced changes were statistically estimated using the Wilcoxon test for independent data (Hollander and Wolfe, 1973). The effects were considered significant for $p \leq 0.05$. The S.E.M. values were calculated for the mean $\%I_c$ and $\%I_s$ values, for the mean writhes constriction and for the mean weight of ears in treated and untreated animals in each assay.

Sodium naproxen, obtained from Laboratorios Saval, Chile, was used as reference drug in per os analgesic and antiinflammatory assays and was suspended in the same vehicle; λ-carrageenan was obtained from Sigma. For the dermal antiinflammatory activity, nimesulide (AA-induced oedema) and indomethacin (TPA-induced oedema) from Laboratorio Chile and Laboratorio Madex, respectively, were use as reference drugs at the dose of 1 and 0.5 mg/20 μL/ear, respectively (Delporte et al., 2002).

3. Results and discussion

3.1. Phytochemical study

Two compounds were isolated and identified from ME, quercetin **1**, and dihydroquercetin **2**. These flavonoids were identified by their ^{1}HNMR and ^{13}C NMR data (Mabry et al., 1970; Agraval, 1989).

Repeated column chromatography of HE, one of the most active extracts, led to the isolation of compound **3**, that was identified as β-sitosterol. Identification of this compound was performed by direct comparison on TLC, melting point and spectroscopic (IR) data with authentic β-sitosterol (Nakanishi, 1962). The compounds **1**, **2** and **3** had not been described before for *Proustia pyrifolia*.

3.2. Acute toxicity and cytotoxicity activity

GME did not show acute toxicity per os up to the maxim dose of 2 g/kg and the weight of the mice had a normal variation after the 7 days of observation. Common side effects such as, mild diarrhea, loss of weight and depression were not recorded. It is important to carry out toxicological studies in other animal species in order to demonstrate its lack of toxicity. Table 1 shows the 50% inhibitory concentration (IC_{50}) of GME and adriamycin (ADR) against three tumour cell lines. GME did not present cytotoxicity against A-549 or HT-29, and only a weak cytotoxic activity against P-388.

3.3. Antioxidant activity

The antioxidant properties of GME, HE, DCE, and ME were evaluated as their abilities to inhibit XO. Table 2 shows the IC_{50} of the different extracts and reference drug (AL),

Table 1
Cytotoxic activities of GME of *Proustia pyrifolia* and reference drug

Cell lines	GME (IC_{50}, μg/mL)	ADR (IC_{50}, μg/mL)
P-388	10	0.017
A-549	20	0.053
HT-29	20	0.11

IC_{50}, inhibitory concentration; P-388, lymphoid neoplasm from DBA/2 mouse; A-549, human lung carcinoma; HT-29, human colon carcinoma; GME, crude methanol extract; ADR, adriamycin.

Table 2
Inhibitory effects on XO of different extracts of *Proustia pyrifolia* and reference drug

Sample	IC_{50} (mg/mL)
GME	46.5
DCE	>60
ME	>60
AL	0.035

IC_{50}, inhibitory concentration; GME, crude methanol extract; DCE, dichloromethane extract; ME, methanol extract; AL, allopurinol.

GME showed activity, but this activity is lower than the reference drug, however, both DCE and ME presented a weak effect and HE lack of effect. The low activity of ME could be explained by the weak inhibitory activity of the major compounds **1** and **2** against XO (Iio et al., 1986).

3.4. Antiinflammatory (per os and topic) and analgesic properties

Analgesic and antiinflammatory response to sodium naproxen (SN) is doses-related (Delporte et al., 2002). Table 3 shows the results for the pharmacological assays of the various extracts, together with the maximum effect of SN for the per os antiinflammatory and analgesic activities, and the antiinflammatory dermal maximum effect of nimesulide (NM) and indomethacin (IND) (Delporte et al., 2003).

In the assays carried out per os GME, HE and ME exhibited the strongest analgesic activities similar to the reference drug (SN). In relation to the results obtained in per os antiinflammatory studies, ME showed the strongest effect, and was similar to the reference drug (SN); HE did not present significant antiinflammatory activity.

It is important to point out the general correlation observed between (per os) antiinflammatory and analgesic activities found for the GME, DCE and ME. This could be explained in terms of the presence of compounds with a similar mechanism for both activities, as for example inhibition of the synthesis of prostaglandin E_2 (PGE_2). By the activation of the cyclo-oxygenase enzyme, the level of PGE_2 increases markedly, and its production provokes inflammation and pain (Dannhardt and Kiefer, 2001). Therefore, we assume that some active metabolites of these extracts could inhibit cyclooxygenase activity.

In the assays carried out topically, the AA-induced oedema response is rapid in onset with a short duration. In contrast, TPA produces a longer-lasting response with a delayed onset. The TPA model seems to be dependent mainly on leukotrienes, which are synthesized by the lipoxygenases enzymes, whereas the AA model is mainly related to PGE_2 (Lloret and Moreno, 1995).

For AA and TPA induced oedema, GME showed significant effect only against AA assay and its mechanism of action might be explained by cyclooxygenase inhibition.

Table 3
Antiinflammatory and analgesic activities of different extracts of *Proustia pyrifolia* and reference drugs

Sample	Dose	%*A* ± S.E.M.	%An ± S.E.M.	%TA-AA ± S.E.M.	%TA-TPA ± S.E.M.
INF	0.4 mL/25 g		53.2* ± 11.5	n.t.	n.t.
INF	4.0 mL/kg	33.3* ± 7.2			
GME	3.0 mg/ear			34.9* ± 3.5	24.5 ± 4.8
GME	600 mg/kg	33.6* ± 5.1	64.5* ± 9.7		
HE	3.0 mg/ear			26.2* ± 2.8	66.2* ± 12.6
HE	600 mg/kg	11.8 ± 3.0	60.5* ± 6.4		
DCE	3.0 mg/ear			55.8* ± 10.0	63.6* ± 10.1
DCE	600 mg/kg	38.1* ± 8.0	42.1* ± 10.5		
ME	3.0 mg/ear			8.7 ± 3.0	49.4* ± 8.9
ME	600 mg/kg	49.6* ± 7.0	64.8* ± 12.2		
SN	4.0 mg/kg	54.6* ± 0.8			
SN	12.5 mg/kg		↑70* ± 4.0	↑25.5* ± 4.0	
NM	1.0 mg/ear			↑48.8* ± 4.0	
IND	0.5 mg/ear				↑81.8* ± 20

Without asterisk $p > 0.05$; n.t.: non tested; *A*, antiinflammatory effect per os; An, analgesic effect; TA-AA and TP-TPA, topical antiinflamatory effects induced for AA and TPA, respectively; INF, aqueous extract; GME, crude methanol extract (or crude methanol extract); HE, hexane extract; DCE, dichloromethane extract; ME, methanol extract; SN, sodium naproxen (D-2-(6-methoxy-2-naphthyl) propionic acid); NM, nimesulide (4-nitro-2-phenoxymethanesulfonanilide); IND, indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-^{1}H-indole-3-acetic acid); ↑, maximum effect. Each group represents the median ± S.E.M. of eight animals pretreated with samples or reference drugs.

* $p \leq 0.05$.

On the contrary, HE and ME presented important activities only against TPA. Therefore, we can conclude that these extracts present active metabolites whose antiinflammatory effect might be explained by the inhibition of the synthesis of the leukotrienes.

DCE was active in both AA and TPA models, therefore, it must contain active compounds inhibitors of the synthesis of the leukotrienes and PGE_2.

In relation to the phytochemical study, our results indicate that the major component present for the HE was **3**, in a previous studies this steroid has been reported as antiinflammatory, analgesic and antipyretic when administered per os (Santos et al., 1995; Villaseñor et al., 2002). Also it has been demonstrated that **3** is an effective topic antiinflammatory agent mainly in acute inflammation induced by TPA; its effect on leukocyte migration to the inflamed site might be an important aspect of its mechanism of action (Gómez et al., 1999). Therefore, this steroid is one of the responsible compounds of the effects exhibited for HE against TPA-induced oedema and per os analgesic activity.

The phytochemical analysis showed for ME the presence of a high percentage of flavonoids, two of them were identified as **1** and **2**; these compounds have been previously reported to have antiinflammatory, antioxidant and antiallergic effects (Pathak et al., 1991; Pelzer et al., 1998). The ability of quercetin to inhibit nitric-oxide synthase, 5-lipoxygenase, phospholipase A_2 and C and cyclooxygenase-2, all proinflammatory enzymes has been reported (Lee et al., 1982; Rao et al., 1985; Chiesi and Schwaller, 1995; De Pascual-Teresa et al., 2004). Therefore, the per os and topical antiinflammatory activities of ME might be atributed in part to **1** and **2**.

In summary, **3** as well as **1** and **2** could contribute, at least in part, to the analgesic and antiinflammatory properties observed for HE and ME, respectively.

4. Conclusions

Our pharmaco-toxicological results corroborate that *Proustia pyrifolia* present analgesic and antiinflammatory effects and absence of acute toxicity. These results support scientifically the use of *Proustia pyrifolia* in popular medicine for the treatment of rheumatic and gout illnesses. Since all the extracts showed pharmacological activities, we assume that different active secondary metabolites are present in crude extracts and perhaps some of these compounds may operate in a synergistic manner.

The flavonoids quercetin and dihydroquercetin, and the steroid β-sitosterol were isolated, these compounds are responsible in part of the pharmacological effects observed.

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References

Agraval, P.K., 1989. Carbon-13 NMR of flavonoids. Studies in Organic Chemistry, vol. 39. Elsevier, p. 564.

Backhouse, N., Delporte, C., Negrete, R., Muñoz, O., Ruiz, R., 1994. Antiinflammatory and antipyretic activities of *Maytenus boaria* mol. International Journal of Pharmacognosy 32, 239–244.

Bittner, M., Jakupovic, J., Bohlmann, F., Silva, M., 1989. Coumarins and guaianolides from further Chilean representatives of the subtribe Nassauviinae. Phytochemistry 28, 2867–2868.

Chiesi, M., Schwaller, R., 1995. Inhibition of constitutive endothelial NO-synthase activity by tannin and quercetin. Biochemical Pharmacology 14, 495–501.

Dannhardt, G., Kiefer, W., 2001. Cyclooxygenase inhibitors—current status and future prospects. European Journal of Medicinal Chemistry 36, 109–126.

Das, D.K., Maulik, N., 1994. Antioxidant effectiveness in ischemia-reperfusion tissue injury. Methods in Enzymology 233, 601–610.

Delporte, C., Backhouse, N., Negrete, R., Salinas, P., Rivas, P., Cassels, B.K., San Feliciano, A., 1998. Antipyretic, hypothermic and anti-inflamammatory activities and metabolites from *Solanum ligustrinum*. Phytotherapy Research 12, 118–122.

Delporte, C., Backhouse, N., Salinas, P., San-Martín, A., Bohórquez, J., Loyola, A., 2003. Pharmaco-toxicological study of diterpenoids. Bioorganic and Medicinal Chemistry 11, 1187–1190.

Delporte, C., Muñoz, O., Rojas, J., Ferrándiz, M., Payá, M., Erazo, S., Negrete, R., Maldonado, S., Negrete, R., San Feliciano, A., Backhouse, N., 2002. Pharmaco-toxicological study of *Kageneckia oblonga*, Rosaceae. Zeitschrift für Naturforschung 57c, 100–108.

De Pascual-Teresa, S., Johnston, K.L., DuPont, M.S., O'Leary, K.A., Needs, P.W., Morgan, L.M., Clifford, M.N., Williamson, G., 2004. Quercetin metabolites downregulate cyclooxygenase. 2. Transcription in human lymphocytes ex vivo but not in vivo. Journal of Nutrition 134, 552–557.

Gómez, M., Saenz, M., García, M., Fernández, M., 1999. Study of topical antiinflammatory activity of *Achillea ageratum* on chronic and acute inflamation models. Zeitschrift für Naturforschung 54c, 937–941.

Hollander, M., Wolfe, D.A., 1973. Nonparametric Statistical Methods, 2nd ed. Wiley, New York, pp. 106–140.

Iio, M., Ono, Y., Kai, S., Fukumoto, M., 1986. Effects of flavonoids on xanthine oxidation as well as on cytochrome C reduction by milk xanthine oxidase. Journal of Nutritional Science and Vitaminology 32, 636–642.

Lee, T.P., Matteliano, M.L., Middleton, E., 1982. Effect of quercetin on human polymorphonuclear leukocyte lysosomal enzyme release and phospholipid metabolism. Life Science 31, 2765–2774.

Lloret, S., Moreno, J.J., 1995. Effects of an antiinflammatory peptide (antiflammin 2) on cell influx, eicosanoid biosynthesis and oedema formation by arachidonic acid and tetradecanoyl phorbol dermal application. Biochemical Pharmacology 50, 347–353.

Mabry, T.J., Markhan, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer-Verlag, New York, p. 294.

Morgan, B.J.T., 1992. Analysis of Quantal Response Data. Chapman & Hall, London, pp. 46–50.

Muñoz, M., Barrera, E., Meza, I., 1981. El uso medicinal y alimenticio de plantas nativas y naturalizadas en Chile. Museo Historia Natural, vol. 33. Publicación Ocasional, p. 32.

Nakanishi, K., 1962. Infrared Absorption Spectroscopy Practical. Holden-Day Inc./Nankodo Company Limited, San Francisco/Tokyo, p. 233.

Pathak, D., Pathak, K., Singla, A.K., 1991. Flavonoids as medicinal agents—recent advances. Fitoterapia 62, 371–385.

Pelzer, L.E., Guardia, T., Juárez, A.O., Guerreiro, E., 1998. Acute and chronic antiinflammatory effects of plant flavonoids. Il Farmaco 53, 421–424.

Rao, G.H., Radha, E., White, J.G., 1985. Irreversible platelet aggregation does not depend on lipooxigenase metabolites. Biochemical Biophysical Research Communication 131, 50–57.

San Feliciano, A., Gordaliza, M., Miguel del Corral, J.M., Castro, M.A., García-Grávalos, M.D., Ruíz-Lázaro, P., 1993. Antineoplastic and antiviral activities of some cyclolignans. Planta Medica 59, 246–249.

Santos, A.R.S., Niero, R., Cechinel Filho, V., Yunes, R.A., Pizzolatti, M.G., Delle Monache, F., Calixto, J.B., 1995. Antinociceptive properties of steroids isolated from *Phyllanthus corcovadensis* in mice. Planta Medica 61, 329–332.

Schmeda-Hirschmann, G., Loyola, J.L., Sierra, J., Retamal, R., Rodríguez, J., 1992. Phytotherapy Research 6, 184–188.

Villaseñor, I.M., Angelada, J., Canlas, A.P., Echegoyen, D., 2002. Bioactivity studies on beta-sitosterol and its glucoside. Phytotherapy Research 16, 417–421.

Zdero, C., Bohlmann, F., King, R.M., Robinson, H., 1986. α-Isocedrene derivatives, 5-methyl coumarins and other constituents from the subtribu Nassauviinae of the compositae. Phytochemistry 25, 2873–2882.