Constituents and biological activities of *Schinus polygamus*

Silvia Erazo [a,*], Carla Delporte [a], Rosa Negrete [a], Rubén García [a], Mercedes Zaldívar [b], Gladys Iturra [a], Esther Caballero [c], José Luis López [c], Nadine Backhouse [a]

[a] *Department of Pharmacological and Toxicological Chemistry, School of Chemical and Pharmaceutical Sciences, University of Chile, P.O. Box 233, Santiago 1, Chile*
[b] *Department of Biochemistry and Molecular Biology, School of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile*
[c] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Salamanca, 37007 Salamanca, Spain*

Abstract

The folk medicine employs *Schinus polygamus* to treat arthritic pain and cleansing of wounds. As no reports of pharmacological studies supporting its anti-inflammatory and analgesic properties, extracts of increasing polarity were assayed on the base of fever, pain and inflammation, together with its antimicrobial activity. All the extracts showed pharmacological activities. From the most active extracts different metabolites were isolated that can in part explain the antipyretic, anti-inflammatory, and analgesic activity: β-sitosterol, shikimic acid together with quercetin, previously reported.

Also, the essential oil of leaves and fruits was obtained and compared with the oil obtained from *Schinus polygamus* collected in Argentine. Oils differed in composition and in antibacterial activity, where the Chilean species exhibited a wide spectrum of activity against Gram-positive and Gram-negative bacteria, and the most abundant compound found in leaves and fruits was β-pinene, meanwhile the Argentine species showed high activity against *Bacillus cereus*, and the main components resulted to be α-phellandrene and limonene.

Keywords: *Schinus polygamus*; Biological activities; Chemical constituents

1. Introduction

Schinus polygamus (Cav.) Cabr., Anacardiaceae, vernacular name "huingán", is a tree of about 1.2–2 m in height. In Chile, it grows from the 1st to the 9th Region and five species of the genus have been described (Navas, 1976). According to folk medicine infusion of the leaves of this plant has been used for cleansing of wounds, the bark decoction that produces a balsamic essence, is used to treat arthritic and feet pains. The latex that emanates from the bark is used as a plaster for muscles and tendons pains, dislocations, fractures and irritation of the skin. The resin is recommended in the chronic bronchitis (Muñoz et al., 1981). Also the aerial part was used for antifertility treatment (Montes and Wilkomirsky, 1987).

In , Mandich et al., described the presence of well-known flavonoids in *Schinus polygamus*, namely: kaempferol, quercetin and quercetin-3-*O*-galactoside.

In this study, we report for the first time the pharmacological studies to support the folk medicinal uses of this species such as the antipyretic, anti-inflammatory and analgesic activities of the aerial parts of the plant. These assays were made on the base that fever, pain and inflammation can be mechanistically linked. Also the antimicrobial activity of the extracts obtained from the leaves was assayed.

As the composition and antimicrobial activity of the essential oil obtained from *Schinus polygamus* leaves collected in Patagonia have been reported (González et al., 2004), the essential oil of leaves and fruits of the species growing in Chile was obtained to be compared.

2. Materials and methods

2.1. General experimental procedures

Solvents used for NMR were CD_3OD and DMSO-d_6. The measurements of the NMR spectra were carried out on a Bruker AMX - 300 (^{1}H NMR (300 MHz) ^{13}C NMR (75 MHz)(spectrometer.

* Corresponding author. Tel.: +56 2 6781638; fax: +56 2 2227900.
E-mail address: serazo@uchile.cl (S. Erazo).

Column chromatography (CC) was carried out using silica gel 60 G (Merck 7734). TLC were performed on silica gel GF 254 (Merck 5554), spots were detected by UV (254, 366 nm) and with Liebermann Burchard test and/or *p*-anisaldehyde and $AlCl_3$ 5% reagents.

GC/MS analysis was carried out on a Hewlett-Packard mod. 5890 series II gas chomatograph (GC) equipped with a 25 m × 0.2 mm i.d. HP U-2 column, with 0.33 μm thickness film. The initial oven temperature was held at 60 °C for 5 min, it was then increased at 3 °C/min up to 250 °C, at 10 psi with helium as carrier gas at a constant flow of 0.43 mL/min.

2.2. Plant material

The aerial part of *Schinus polygamus* (Cav.) Cabr., was collected in January in Cuesta La Dormida, Puente La Laja, Región Metropolitana, Chile and identified by Professor Carla Delporte. A voucher specimen is kept at the Herbario de Escuela de Química y Farmacia (SQF 21037), University of Chile.

2.3. Extraction and isolation

2.3.1. Essential oil

The essential oil was obtained separately by hydrodistillation from fresh collected fruits (250 g) and leaves (250 g) yielding 0.44% in leaves and 2.5% in fruits, which were assayed for the antimicrobial activity by means of bioautography agar overlay bioassay in order to determine the active compounds (Erazo et al., 1997, 2002a,b; Rahalison et al., 1991). Near to a 40% of the constituents were identified on the basis of their GC retention time (Rt) and mass spectra of each individually peak in the GC/MS coupled determination, which were compared with MS library (Wiley 130 K Database 1986) and literature. The results obtained were compared with those reported for essential oil from *Schinus polygamus* growing in the Patagonia.

2.3.2. Extracts

Ground dried aerial part (500 g) was extracted with methanol at room temperature, yielding 154.5 g. This methanol total extract (EMET) was used for the antipyretic, anti-inflammatory and analgesic assays.

Infusion was prepared from dried and ground material, adding boiling water to an exactly weighed amount, to obtain 20% (w/v) infusion.

Another quantity (2 kg) was sequentially extracted with *n*-hexane, dichloromethane and methanol yielding respectively and after THE removal of solvents in vacuo 55.3, 55.7 and 96 g, respectively. These extracts (EHEX, EDCM and EME-1) were used to carry out all the pharmacological assays.

The hexane extract (EHEX, 17 g) proved to be active and was submitted to CC on silica gel eluting with hexane–CH_2Cl_2 (8:2) from fractions 37–45 (50 mL volume each), the most abundant compound **1** (60 mg, 0.1%) was isolated, and a small amount (20 mg) was purified and identified according to their physical data and confirmed by comparison to a standard, previously isolated in our laboratory, as β-sitosterol (Backhouse et al., 2002).

The methanol extract (EME-1, 60 g) yielded compound **2** (186 mg, 0.02%) and **3** (150 mg, 0.01%) were eluted with CH_2Cl_2: MeOH (6:4) from fractions 69–78 (100 mL volume each) and CH_2Cl_2: MeOH (4:6) fractions 99–108 (100 mL volume each) respectively. Purification of **3** was carried out in Sephadex LH-20 column, eluted with hexane: CH_2Cl_2: MeOH (1:1.5:0.5) and followed by crystallization with CH_2Cl_2 and drops of MeOH**.** These compounds were identified by ^{1}H NMR and ^{13}C NMR and literature data.

Spectral data for compound **2** was compared and were according with those published for shikimic acid (Jackmann and Sternhell, 1969; Payne and Edmonds, 2005; Talapatra et al., 1989). Also, the MP 183 °C practically coincided with data published (184 °C).

Compound **3** was identified through comparison of the NMR data reported in the literature, as quercetin (Feresin et al., 2002; Mabry et al., 1970).

2.4. Antipyretic activity

Antipyretic activity was determined in rabbits using three animals for each dose (modified from USP XXII) and repeating each experiment three times at intervals not less than two weeks. Pyrexia was induced by i.v. injection of *Escherichia coli*, endotoxin (prepared in sterile saline) at a dose of 13 ng/kg. Rectal temperatures were recorded continuously for 180 min, with an Ellab Pyrogentester (model Z12DP) immediately after pyrogen injection. The areas under temperature versus time curves obtained for each pyrogen-treated animal with and without earlier oral administration of the samples were compared and the antipyretic effect (*E*) was calculated according to the following equation:

$$\%E = 1 - \frac{\text{area}_{\text{pyr+drug}}}{\text{area}_{\text{pyr}}} \times 100$$

where $\text{area}_{\text{pyr+drug}}$ represents the area under the curve obtained after plotting temperature versus time in min for drug-tested rabbits, and area_{pyr} is the corresponding area for animals treated only with pyrogen. These areas were calculated for the two time intervals: (0–90 min and 90–180 min), and the significance of the effect was estimated using the analysis of variance (ANOVA) test. The results are expressed as mean ± S.E.M.

2.5. Anti-inflammatory activity

The anti-inflammatory activity was evaluated in guinea pigs, in groups of 10–14 animals for each dose, using the carrageenan-induced paw edema (Winter et al., 1963). Paw volume was measured with an Ugo Basile plethysmometer (model 7150) initially, and 3 h after injecting 0.1 mL of sterile saline λ - carrageenan (1%). Anti-inflammatory activity (%A) was evaluated as:

$$\%A = \frac{(\%I_c - \%I_d)}{\%I_c} \times 100$$

where $\%\ I_c = 37.3 \pm 1.3$ is the mean inflammation reached in control guinea pigs which have received only the vehicle

(Backhouse et al., 2002), and I_d is the average inflammation in drug treated animals, expressed as $\%\ I_d = (V_f - V_i)/V_i \times 100$, where V_f and V_i are final and initial paw volumes, respectively, averaging % I over all the animals used in each test.

The significance of the drug-induced changes was estimated using Student's *t*-test, considering $p \leq 0.05$. The results are expressed as mean ± S.E.M.

In both assays, the samples were administered orally 1 h before the carrageenan or the endotoxin injection, by means of an intragastric catheter, suspended in propylene-glycol at a single dose of 600 mg/kg. Sodium naproxen (SN) (donated by Laboratorios Saval, Chile) was used as a positive control for both assays and was suspended in the same vehicle (Backhouse et al., 2002; Delporte et al., 1998).

λ-carrageenan was purchased from Sigma. *Escherichia coli* endotoxin was obtained at Instituto de Salud Pública, Chile.

2.6. Analgesic activity

The analgesic activity of the different extracts was evaluated in groups of 8 mice and 16 control receiving only the vehicle and 8 animals treated with sodium naproxen. All the animals received an intra-peritoneal injection of 0.5 mL of 0.6% acetic acid for inducing writhing. The analgesic effects were calculated by comparing the number of abdominal writhes of the treated and the control group, which only received the vehicle (Davies et al., 1997). The number of abdominal writhes of each mouse was counted for 30 min, beginning 5 min after acetic acid administration.

The following equation was used to calculate the mean pain percentage (%D):

$$\%D = \left[\frac{C_{\text{sample}}}{C_{\text{control}}}\right] \times 100$$

where C_{sample} is the mean writhes in sample-treated animals and C_{control} (41.6 ± 3.8) is the mean writhes in control animals which received only the vehicle. The analgesic effect (An) was calculated according to the following equation:

$$\%\text{An} = 100 - \%D$$

In analgesic assays, the extracts were orally administered by means of an intragastric catheter, suspended in saline arabic gum 1 h before the acetic acid, at doses of 600 mg/kg. 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. ($SD/\sqrt{n}$) values were calculated for the mean writhes.

Sodium naproxen (SN), obtained from Laboratorios Saval, Santiago, Chile, was used as a reference drug according to previous work (Delporte et al., 2002) and was suspended in the same vehicle.

2.7. Antimicrobial assays

The antimicrobial activity of the extracts was determined against *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (isolated from a patient), *Salmonella aviatum* (ATCC 2228), *Salmonella aeruginosa* (ATCC 14207), *Staphylococcus aureus* (ATCC 6538P), *Micrococcus flavus* (ATCC 10290), *Bacillus subtilis* (ATCC 6633), *Candida albicans* and *Saccharomyces cerevisiae* (isolated from a patient).

The extracts were dissolved in DMSO. Dilutions of 100 and 200 μg/mL were added to a fixed volume of Plate Count Agar (PCA). They were then superficially inoculated in overnight culture of the different microorganisms and incubated at 37 °C for 24 h for bacteria and 28 °C for 48 h for fungi and yeasts. Results were recorded as growth or growth inhibition at each extract concentration. (Erazo et al., 1997, 2002a,b).

The turbidimetric method (Balows et al., 1991) was used with serial dilutions of the extract in 4 mL of the Plate Count Broth or Tryptic Soy Broth. Both media were used to assay the MIC of the essential oil against *Staphylococcus aureus, Bacillus subtilis* and *Micrococcus flavus*.

3. Results and discussion

3.1. Extraction and isolation

Three compounds isolated and characterized from *Schinus polygamus* extracts were β-sitosterol (**1**) from EHEX, shikimic

Table 1
Antipyretic (E), anti-inflammatory (A) and analgesic (An) effects of *Schinus polygamus* extracts and sodium naproxen (SN)

Sample	Dose	% E		% A	% An
		0–90 min	90–180 min		
INF 20%	4 ml/kg	48.3* ± 5.3	21.5 ± 5.9		
	4 ml/kg			46.4* ± 3.3	
	0.4 ml/25 g				27.8* ± 2.8
EMEG	600 mg/kg	41.3* ± 8.1	0	45.9* ± 3.7	0
EHEX	600 mg/kg	13.3* ± 6.9	0	54.3* ± 3.2	55.8* ± 2.4
EDCM	600 mg/kg	0	42.2* ± 2.5	23.4* ± 2.6	36.4* ± 2.0
EME-1	600 mg/kg	33.4* ± 5.3	17.2 ± 4.8	49.2* ± 2.4	48.0* ± 2.2
SN	4 mg/kg			54.6* ± 0.8	
	12.5 mg/kg				70.0* ± 1.8
	25 mg/kg	51* ± 2.5	81* ± 3.2		

INF20%: infusion at 20% g/v; EMEG: global methanol extract; EHEX: hexane extract; EDCM: dichloromethane extract; EME-1: fraction methanol extract; SN: sodium naproxen.
* $p < 0.05$.

acid (**2**) and quercetin (**3**) from EME-1.The compounds 1 and 2 were not previously reported in this species.

3.2. Antipyretic, anti-inflammatory and analgesic activity

Table 1 shows the results for the pharmacological assays of the various extracts. The maximum effect of sodium naproxen was dose-dependent for the antipyretic, anti-inflammatory and analgesic activities (Backhouse et al., 1994; Delporte et al., 1998). Since all the extracts showed pharmacological activities, we assume that various active secondary metabolites are present. For the antipyretic activity the EMET showed the higher effect and EDCM was not active in the first interval of time, however, it was the only one with a significant effect in the second interval. All the extracts showed anti-inflammatory effect being EDCM the less active. Finally, for the analgesic assays, the EHEX showed the highest effect, followed by EME-1.

As mentioned above, β-sitosterol was isolated from the EHEX, as one of the major secondary metabolite justifying partially the anti-inflammatory and analgesic activity found for this extract (Gupta et al., 1980, Santos et al., 1995). Also, β-sitosterol has been informed to inhibit neutrophil migration into the inflamed tissue, as in acute topic inflammation it showed decreased myeloperoxidase activity (Gómez et al., 1999). These reports can explain its mechanism of action. On the other hand β-sitosterol has proved to increase the pain tolerance both in acetic acid induced writhing and in the hot plate methods, used to evaluate analgesic activity (Villaseñor et al., 2002).

Also the presence of flavonoids in the EME-1, as quercetin isolated from this extract, could be contributing to the analgesic and anti-inflammatory effects. The effect of flavonoids on arachidonic acid metabolism has been reported by various authors, indicating that the antioxidant properties (Havsteen, 2002) are important for lipoxygenase inhibition (Middleton et al., 2000).

Recently, several flavonoids including quercetin have been reported to inhibit nitric oxide production and iNOS enzyme activity. Nitric oxide is involved in different biological process

Table 2
Chemical composition of leaves and fruits of *Schinus polygamus* essential oil (GC-MS Analysis)

Rt (min) leaves–fruits		Compound	(%) Leaves–fruits	
9.60	9.58	α-Thujene	0.62	0.17
9.95	10.02	α-Pinene	4.06	6.55
–	10.62	Camphene	–	0.61
12.05	12.31	β-Pinene	8.91	19.07
12.66	–	β-Myrcene	0.26	–
14.39	14.42	*p*-Cymene	0.96	1.83
14.62	14.73	Limonene	2.36	5.75
16.79	16.98	1-Octanol	0.20	3.55
18.33	18.33	Linalool	1.37	0.79
19.08	19.08	Endo-fenchol	0.51	0.41
20.42	20.46	*trans*-pinocarveol	0.93	1.10
–	20.75	Camphor	–	2.46
21.82	21.87	Borneol	0.33	1.47
22.46	22.50	Terpinen-4-ol	5.98	3.96
23.18	23.44	α-Terpineol	6.83	12.11
23.41	23.58	Myrtenol	1.16	0.77
26.19	–	*trans*-Geraniol	0.55	–
27.14	27.04	1-Decanol	8.56	1.31
–	27.75	Endobornyl-acetate	–	0.32
–	30.74	α-Cubebene	–	0.11
32.76	–	β-Cubebene	0.42	–
–	32.01	α-Copaene	–	0.27
34.12	34.03	*trans*-Cariophyllene	2.96	0.10
35.61	35.55	α-Humulene	0.69	0.14
35.93	–	Aromadendrene	0.44	–
36.32	36.21	1-Dodecanol	5.57	0.34
37.30	37.25	Epibicyclosesquiphellandrene	0.58	0.16
37.57	37.56	α-Muurolene	0.56	1.54
38.19	38.18	γ-Cadinene	0.32	1.46
38.57	38.57	δ-Cadinene	3.46	3.37
39.66	39.62	Elemol	0.41	0.56
40.95	–	Spathulenol	0.81	–
–	41.51	Veridiflorol	–	0.47
43.10	43.06	Selina-3.11-dien-6-α-ol	0.68	0.29
43.90	–	Epoxy-alloaromadendrene	1.68	–
–	44.19	Muurolol	–	8.19
44.86	–	α-Cadinol	1.05	–
–	50.05	Longifolol	–	1.58

– not detected.

including inflammation and inmunoregulation, so the inhibition of iNOS may have a potential therapeutic value in the mentioned pathologies (Kee et al., 1999). Also, quercetin was investigated for anti-inflammatory activity on cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) catalyzed prostaglandin biosynthesis, showing a 40% of inhibition of COX-1 at 200 μm concentration (Rushdey et al., 2003). These important activities described for quercetin allow us to explain the effects found for the EME-1 extract of *Schinus polygamus*.

However, it must be considered that extracts are complex mixtures probably containing different active components with additive or synergistic activities.

3.3. Chemical constituents of essential oil

The essential oil of fresh fruits and leaves of *Schinus polygamus* contains a similar proportion of mono (48.4 and 46.8%, respectively) and sesquiterpene (41.9 and 46.8%) compounds. Near a 40% of constituents were identified and listed in Table 2, being β-pinene the main component of the Chilean essential oil, both in fruits (19.07%) and in leaves (8.91%).

Compounds found only in fruits are: camphene (0.61%), camphor (2.46%), endobornyl acetate (0.32%), veridiflorol (0.47%) and longifolol (1.58%). On the other hand, compounds found only in leaves are: β-myrcene (0.26%), transgeraniol (0.55%) β-cubebene (0.42%), aromadendrene (0.44%), spathulenol (0.81%), epoxy-alloaromadendrene (1.68%) and α-cadinol (1.05%).

The essential oil composition of the Chilean species is similar to the major constituents described for *Schinus longifolia* essential oil (Murray et al., 2005) and differs from Patagonia species witch are similar to *Schinus molle* (Marongiu et al., 2004) *Schinus fasciculata* and *Schinus areira* essential oils, being most of them species from Argentina; as these contains nearly 58.3% of monoterpenes, being the major constituents α-phellandrene (12.9%) and limonene (22.6%) and 20.4% of sesquiterpenes being α-cadinol and cubenol the main compounds (7.1% and 5.6%, respectively).

3.4. Antimicrobial activities

3.4.1. Antimicrobial activity of the essential oil

The essential oil of leaves of *Schinus polygamus* showed antibacterial activity on all the bacteria tested, at concentrations of 100 and 200 μg/mL; no activity against *Candida albicans* and *Sacharomyces cerevisiae* was observed.

Minimal inhibitory concentration (MIC) of essential oil of leaves against *Staphylococcus aureus, Bacillus subtilis* and *Micrococcus flavus* was 140, 10 and 13 μg/mL, respectively. MIC for the same strains for ampicillin as reference antibiotic was 5, 10 and 20 μg/mL, respectively.

Antimicrobial activities of essential oils are difficult to correlate to a specific compound due to their complexity and variability. In general, the antimicrobial activities have been mainly explained through C10 and C15 terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active site of target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils (Belletti et al., 2004)

3.4.2. Antimicrobial activity of the extracts

The EHEX and EME-1 extracts did not show antimicrobial activity. Only EDCM resulted to be active against *Staphylococcus aureus, Bacillus subtilis* and *Micrococcus flavus*.

4. Conclusions

The analgesic, anti-inflammatory and antimicrobial activities support scientifically the use of *Schinus polygamus* in popular medicine for the treatment of pains, inflammations and cleansing of wounds. The isolated compound β-sitosterol, one of the major secondary metabolite, together with the terpenoids α and β-pinene, major constituents of the essential oil, justify to some extent the anti-inflammatory and analgesic activity with pain tolerance. Also the presence of quercetin can explain partially the analgesic and anti-inflammatory effects by its inhibitory action over nitric oxide production and iNOS enzyme activity.

In our claim to compare the composition of the essential oil of species growing in different habitat more than their quantitative composition, as this study was carried out in fresh material, we found that the constituents of *Schinus* species clearly differs depending on its habitat. The essential oil composition of the Chilean species is similar to the major constituents described in *Schinus longifolia* essential oil and differs from Patagonia species which are similar to *Schinus molle*, *Schinus fasciculata* and *Schinus areira* essential oil, being most of them species from Argentina.

Even though the antimicrobial activity was observed only for the essential oil and EDCM extract against *Staphylococcus aureus, Bacillus subtilis* and *Micrococcus flavus*, these results indicate the presence of antimicrobial metabolites in *Schinus polygamus* that could justify its popular wound cleansing uses.

It is important to mention that shikimic acid was isolated for the first time from *Schinus polygamus*. Today this compound is considered of high value as a key starting material for the synthesis of neuramidase inhibitor developed under the name of "Tamiflu", for the treatment of antiviral infections (Krämer et al., 2003).

Acknowledgements

This research has been performed under the auspices of "Programa de Ciencias y Tecnología para el Desarrollo CYTED-Sub-programa X" and the "Secretaria Ejecutiva del Convenio Andrés Bello" (SECAB).

References

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

Backhouse, N., Delporte, C., Negrete, R., San Feliciano, S.A., López-Pérez, J.L., 2002. Bioactive phenolic derivatives from *Acaena splendens* methanol extract. Phytotherapy Research 16, 562–566.

Balows, A., Hausler Jr., W.J., Hermann, K.L., Isenberg, H.D., 1991. In: Shadomy, H.J. (Ed.), Manual of Clinical Microbiology, 5th ed. American Society for Microbiology, Washington DC, pp. 1105–1112.

Belletti, N., Ndagihimana, M., Sisto, C., Guerzoni, M.E., Lanciotti, R., Gardini, F., 2004. Evaluation of the antimicrobial activity of citrus essences on *Saccharomyces cerevisae*. Agricultural and Food Chemistry 52, 6932–6938.

Davies, N.M., Roseeth, A.G., Appleeyaard, C.B., Mcknight, W., Del Soldado, P., Calignano, A., Cirino, G., Wallace, J.L., 1997. No-naproxen vs, naproxen ulcerogenic, analgesic and anti-inflammatory effect. Alimentary Pharmacology Therapeutics 11, 69–79.

Delporte, C., Backhouse, N., Negrete, R., Salinas, P., Cassels, B.K., San Feliciano, A., 1998. Antipyretic, hypothermic and anti-inflammatory activities and metabolites from *Solanum ligustrinum* Lood. Phytotherapy Research 12, 1–5.

Delporte, C., Muñoz, O., Rojas, J., Ferrándiz, M., Payá, M., Erazo, S., Negrete, R., San Feliciano, A., Backhouse, N., 2002. Pharmaco-toxicological study of *Kageneckia oblonga* Rosaceae. Zeitschrift fur Naturforschung 57c, 100–107.

Erazo, S., González, V., Zaldívar, M., Negrete, R., 1997. Antimicrobial activity of *Psoralea glandulosa* L. International Journal of Pharmacognosy 35, 1–3.

Erazo, S., Negrete, R., Zaldívar, M., Backhouse, N., Delporte, C., Silva, I., Belmonte, E., López-Pérez, J.L., San Feliciano, A., 2002a. Methyl psilalate. A new antimicrobial metabolite from *Psila boliviensis*. Planta Medica 68, 66–67.

Erazo, S., Zaldívar, M., Delporte, C., Backhouse, N., Tapia, P., Belmonte, E., Delle Monache, F., Negrete, R., 2002b. Antibacterial diterpenoids from *Fabiana densa* var *ramulosa*. Planta Medica 68, 361–363.

Feresin, G., Tapia, A., Gutierrez, A., Delporte, C., Backhouse, N., Schmeda-Hirschmann, G., 2002. Free radical scavengers, anti-inflammatory and analgesic activity of *Acaena magellanica*. Journal of Pharmacy and Pharmacology 54, 835–844.

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

González, S., Guerra, P., Bottaro, H., Molares, S., Demo, M.S., Oliva, M.M., Zunino, M.P., Zygadlo, J.A., 2004. Aromatic plants from Patagonia Part I. Antimicrobial activity and chemical composition of *Schinus polygamus* (Cav.) Cabrera essential oil. Flavour and Fragrance Journal 19, 36–39.

Gupta, M.B., Nath, R., Srivastava, N., Shanker, K., Kishor, K., Bhargava, K.P., 1980. Anti-inflammatory and antipyretic activity of β-sitosterol. Planta Medica 39, 157–163.

Havsteen, H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacology and Therapeutics 96, 67–202.

Hollander, M., Wolfe, D.A., 1973. Non Parametric Statistical Methods. John Wiley and Sons, New York, pp. 68–70.

Jackmann, L.M., Sternhell, S., 1969. Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, vol. 5., 2nd ed. Pergamon Press, Oxford, England, p. 456.

Kee, K.H., Bong, S.Ch., Ha, K.Y., Yong, K.S., Pyo, K.H., 1999. Effects of naturally occuring flavonoids on nitric oxide production in the macrophage cell line Raw 264.7 and their structure activity-relationship. Biochemical Pharmacology 58, 759–765.

Krämer, M., Bongaerts, J., Bovenberg, R., Kremer, S., Müller, U., Orf, S., Wubbolts, M., Raeven, L., 2003. Metabolic engineering for microbial production of shikimic acid. Metabolic Engineering 5, 277–283.

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

Mandich, L., Bittner, M., Silva, M., Barros, C., 1984. Phytochemical screening of medical plants. Studies of flavonoids. Revista Latinoamericana de Química 15, 80–82.

Marongiu, B., Porcedda, A.P.S., Casu, R., Pierucci, P., 2004. Chemical composition of the oil and supercritical CO_2 extract of *Schinus molle* L. Flavour and Fragance Journal 19, 554–558.

Middleton Jr., E., Kandaswami, C., Theobarides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Pharmacological Reviews 52, 673–751.

Montes, M., Wilkomirsky, T., 1987. Medicina Tradicional Chilena. Editorial de la Universidad de Concepción, Santiago, Chile, p. 50.

Muñoz, M., Barrera, E., Meza, I., 1981. El uso medicinal y alimenticio de plantas nativas y naturalizadas de Chile, Edición ocasional N°33. Museo Nacional de Historia Natural, Santiago, Chile, p. 17.

Murray, A.P., Frontera, M.A., Tomas, M.A., Mulet, M.C., 2005. Gas chromatography-mass spectrometry study of the essential oil of *S. longifolia* (Lindl.) speg., *Schinus fasciculata* (Griseb.) I.M. Johnst. and *Schinus areira* L. Zeitschrift fur Naturforschung 60, 25–29.

Navas, L., 1976. Flora de la cuenca de Santiago de Chile Tomo II. Editorial de la Universidad de Chile, 278–280.

Payne, R., Edmonds, M., 2005. Isolation of shikimic acid from star aniseed. Journal of Chemical Education 82, 599–600.

Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M., Frenk, E., 1991. A bioautographic agar overlay method for detection of antifungal compounds from higher plants. Phytochemical Analysis 2, 199–203.

Rushdey, E.H., Ringbom, T., Torssell, K., Bohlin, L., 2003. Constituents of *Hypericum laricifolium* and their cyclooxygenase (COX) enzyme activities Chemical. Pharmaceutical Bulletin 51, 1439–1440.

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.

Talapatra, B., Das, A.K., Talapatra, S.K., 1989. Defuscin, a new phenolic ester from *Dendrobium fuscescens*: conformation of shikimic acid. Phytochemistry 28, 290–292.

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

Winter, Ch.A., Risley, E., Nuss, G., 1963. Anti-inflammatory and antipyretic activities of indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid. Journal of Therapy and Experimental Pharmacology 141, 369–373.