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Phytochemical and biological study of Radal *Lomatia hirsuta* (Proteaceae)

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Abstract

The anti-inflammatory property of *Lomatia hirsuta* (Lam.) Diels ex Macbr. (Proteaceae), leaves (radal), a plant used in Chilean traditional medicine for bronchial troubles and asthma, was evaluated. The biological assays showed infusion of *L. hirsuta* leaves inhibits the inflammation induced by λ-carrageenan corresponding to a 29.2% anti-inflammatory effect, and to 53.5% of the maximum effect observed with sodium naproxen (4 mg/kg) in the same experimental conditions. The coumarins, umbelliferone and scopoletin, were the major compounds isolated, along with quercetine, rhamnetin and iso-rhamnetin, with minor quantities or quercitrine and no presence of toxic naphthoquinone derivates. These results supported the folk use of *L. hirsuta*.

Keywords: Lomatia hirsuta; Flavonoids and coumarin; Anti-inflammatory activity

1. Introduction

Lomatia hirsuta (Lam.) Diels ex Macbr. (Proteaceae), is a wild tree growing in Chile from Coquimbo to Chiloé (IV–X Regions), from the sea to submountain zones through 700–1200 m of altitude, beside *L. ferruginea* (Cav.) R.Br. and *L. dentata* (R. et P.) R. Br. It is also present in Argentina, Ecuador and Perú (Rodríguez et al., 1982). The aqueous extract of stem and leaves of this tree, commonly known as 'radal', is used in traditional medicine of Chile for cough treatment, bronchial troubles and asthma (San Martín, 1983).

Naphthoquinones, such as lomatiol, juglone and naphthazasin, have been found to be the main metabolites in earlier chemical studies on various *Lomatia* species (Moir and Thompson, 1973), including some native plants, such as *L. ferruginea*, *L. dentata*, but not in *L. hirsuta* leaves. Also, flavonoids, such as 3-methylether of quercetin and kaempferol, and rhamnetin, iso-rhamnetin and quercetrin have been determined

rhamnetin and quercetrin have been determined in some *Lomatia* species (Mehendale and Thomson, 1975).

The aim of this study was to investigate the main chemical composition of *L. hirsuta* leaves and determine the anti-inflammatory property of an infusion of its leaves, to corroborate its popular use, since airway inflammation is recognized as one of the principal factors that triggers the asthmatic process (Weinberger, 1993).

2. Material and methods

2.1. Plant material

The plant material was collected at Vilches, near Talca city (Maule Region) in a mountain site. A voucher specimen (SQF No. 17184) identified by Prof. R. Peña has been deposited at the Herbarium of Escuela de Química y Farmacia, Universidad de Chile.

The air-dried ground leaves (5 kg) were extracted sequentially at room temperature with *n*-hexane, chloroform, ethyl acetate and methanol, yielding after removal the solvents in vacuo (% w/w of extracts), respectively: hexane, chloroform, ethylacetate and methanol (1.14, 1.62, 2.32, and 4.30).

The separation, isolation and purification of substances were performed by flash column and thin layer chromatography (TLC) techniques using silica gel (Merck) as adsorbent. The spots on TLC were detected using a UV lamp (254 and 365 nm), intensifying the fluorescence by chromogenic reactives.

The characterization of the chemical structures of purified metabolites was made by the determination of the physical constants and the use of different spectral techniques (UV, IR, NMR and Mass) together with comparison with authentic samples.

The aqueous extract for the biological assay was prepared from dried and ground material, adding boiling water to obtain 20% (w/v) extract.

2.2. Anti-inflammatory activity

The anti-inflammatory activity was evaluated in Pirbright guinea pigs (200–300 g weight) of either sex in groups of 10 animals for each dose, using the carrageenan-induced paw edema method (Winter et al., 1963). Infusions were administered orally at the dose of 800 mg/kg 1 h before the injection of 0.1 ml of sterile saline λ-carrageenan (1%) suspended in saline *Acacia* gum (5%). The paw volume was measured with a plethysmometer (Ugo Basile, model 7150) immediately (V_i), and 4 h after (V_f) the samples. Results were calculated as percent inhibition (%A) compared with a control group (Backhouse et al., 1994). The significance of the drug-induced changes was estimated using Student's t-test (Bowman and Rand, 1980). Results were compared with a standard anti-inflammatory agent, sodium naproxen (Laboratorio Saval-Syntex, Chile) which showed a maximum effect at 4 mg/kg.

Anti-inflammatory effect was calculated by the following formula:

$$\%I_d = [(V_f - V_i)/V_i] \times 100$$

where V_f is the final volume and V_i is the initial volume of the hind paw.

$$\%I_d = (\sum \%I/n) \pm Sm$$

where n is the number of animals considered in each assay

$$\%A = [(\%I_c - \%I_d)/\%I_c] \times 100$$

Were $\%I_c$ is the mean control inflammation and I_d is the average inflammation in drug-treated animals reached in control guinea pigs which have received only vehicle ($34.0 \pm 2.32\%$ paw volume increase for a group of 20 animals), over all the animals used in each test.

3. Results

Sodium naproxen administered orally and suspended in saline arabic gum showed a dose-related anti-inflammatory effect, where the maximum was reached at a dose of 4.0 mg/kg, with an inflammation of $17.1\% \pm 0.8$ that compared with the control of $37.7\% \pm 1.26$. This corresponds to a 54.6% anti-inflammatory effect.

The infusion of *L. hirsuta* (800 mg/kg) inhibits the inflammation with 29.2% of anti-inflammatory effect. This corresponds to the 53.5% of maximum effect observed with sodium naproxen (4 mg/kg) under the same experimental conditions.

The coumarins, umbelliferone and scopoletin (0.012 and 0.001% of dried weight), are the major compounds found in the *L. hirsuta* chloroform and ethyl acetate extracts, besides quercetin, rhamnetin and iso-rhamnetin flavonoid compounds. Quercetrine was found to be a minor flavonoid glycoside in the methanolic extract. According to the technique of Moir and Thompson (1973), no naphthoquinone derivatives were found in the diverse extracts analysed of *L. hirsuta* leaves.

4. Discussion and conclusions

The phytochemical analysis of the different extracts showed two coumarins, umbelliferone and scopoletin, to be the main metabolites, accompanied by a few flavonoid compounds (quercetin, quercetrine, rhamnetin and iso-rhamnetin), but not toxic naphthoquinone derivatives.

The infusion of *L. hirsuta* leaves has a mild anti-inflammatory activity (29.2%) on the carrageenan-induced paw edema method compared with sodium naproxen (53.5%). In a search for the active metabolites responsible of the anti-inflammatory effect, it was found that benzopyrones are useful for treatment of edemas in animals (Casley-Smith, 1984). The major compound, umbelliferone, showed anti-inflammatory properties evaluated by the carrageenan-induced paw edema (Hardt and Ritschel, 1983). On the other hand, scopoletin also was reported as an anti-inflammatory and analgesic compound (Zhu and Huang, 1989), besides having antirheumatic (Ye et al., 1981) and antibronchoconstrictor and anti-arrhythmic properties (Ojewole, 1983).

Although no toxic naphtoquinone derivatives were found in the diverse extracts of *L. hirsuta* (Moir and Thompson, 1973), more toxicological and clinical studies are needed to assess the safety of folk use.

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References

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

Bowman, W.C. and Rand, M.J., 1980. Texbook of Pharmacology, 2nd. ed. Black, Oxford, pp. 18–22.

Casley-Smith, J., 1984. Benzopyrones for treatment of high protein edemas in animals (PCT. Int. Appl. Wo. 85:865). Chemical Abstracts 105, 30049b.

Hardt, T., Ritschel, W., 1983. Investigation of dose-response relationship upon intraperitoneal administration of coumarin and 7-hydroxicoumarin on the carrageenan induced edema of the rats. Arzneimittel-Forschung/Drug Research 33, 1662–1666.

Mehendale, A., Thomson, H., 1975. Binaphthoquinones in *Lomatia ferruginea*. Phytochemistry 14, 801–802.

Moir, M., Thompson, H., 1973. Naphthoquinones in *Lomatia* species. Phytochemistry 12, 1351–1353.

Ojewole, J., 1983. Antibronchoconstrictor an antiarhythmic effects of chemical compounds from Nigerian medicinal plants. Fitoterapia 54, 153–161.

Rodríguez, R., Matthei, O., Quezada, M., 1982. Flora Arbórea de Chile. Editorial de la Universidad de Concepción, p. 200.

San Martín, J., 1983. Medicinal plants in central Chile. Economic Botany 37, 216–227.

Weinberger, S., 1993. Medical Progress. Recent Advances in Pulmonary Medicine. The New England Journal of Medicine 328, 1389–1397.

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

Ye, H.Z., Fan, Y.H., Liu, C.W., Chin, Y.S., 1981. Study of the antirheumatic active principle of ding gont-teng (*Erycibe obtusifolia* Benth.) (Chung Ts'ao Yao 12:5-7). Chemical Abstracts 105, 30019b.

Zhu, H., Huang, J., 1989. Antiinflammatory effect of scopoletin (Zhongcaoyao, 15:462-465). Chemical Abstracts 102, 72550b.