

A033 In vivo investigation of the anti-inflammatory activity of proanthocyanidins from *Ribes nigrum* leaves

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Ribes nigrum (Grossulariaceae) leaves are used in European traditional medicine for treating disorders of inflammatory nature. Previous experiments (1, 2) have shown that the most active fractions contained proanthocyanidins (PAC).

The mechanism of action of the PAC was evaluated on two rat models: the oedemas induced by different irritants in paw and the pleurisy model induced by carrageenan. Leaves were extracted with acetone (70% v/v in water). Isolation of an enriched PAC fraction in the resulting extract was carried out by MPLC on RP8 with water-acetone (9:1).

Oedemas in the rat paw: carrageenan, zymosan, concanavalin-A, nystatin and histamin were injected in rat paws. PAC solutions (10 and 30 mg/kg, *i.p.*), indomethacin (10 mg/kg, *i.p.*), mepyramine maleate (2 mg/kg, *i.p.*) or saline solution were tested. The PAC fraction (30 mg/kg) showed the highest inhibitory activity on carrageenan (55±7%) and on concanavalin-A (66±2%) induced oedemas versus respectively 57±9% and 21±5 % with indomethacin.

Rat pleurisy induced by carrageenan: PAC solutions (30 mg/kg, *i.p.*), indomethacin, nimesulid (10 mg/kg, *i.p.*) or saline solution were tested. The exsudate and the lungs were collected from the thoracic cavities for assessment of exsudate volume, leukocyte accumulation and histological modification of the tissues. Exsudate volume was reduced by 51±5 % with PAC fraction and respectively by 87±9 % and 71±6 % with indomethacin and nimesulid. However, the amount of leukocyte found in the exsudate (in million/ml) was lower with PAC fraction (25) than with reference drugs (28 and 35, respectively). Histological sections examination of lungs and cytokines assays in exsudate are currently underway.

These results indicate that PAC are potential anti-inflammatory agents, working partially by reducing the leukocyte extravasation.

References: 1. Tits, M. et al. (1991) *Planta Med.* 57(suppl 2): A134. 2. Garbacki, N. et al. (2002) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 365: 434-441.

A034 Tagitinin C from *Tithonia diversifolia*: anti-inflammatory activity and high performance liquid chromatographic quantification

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Tithonia diversifolia (Asteraceae) aerial parts are traditionally used in a decoction for treatment of disorders of malaria in São Tomé e Príncipe. Its main antimalarial activity has been attributed to a sesquiterpene lactone, tagitinin C (1). As this class of compounds contains potential anti-inflammatory drugs, we have investigated the activity of crude ether extract from *T. diversifolia* (TDE) as well as isolated tagitinin C (TGC) on an animal model, the croton oil induced-oedema in mice ear skin.

Croton oil-induced oedema in mice ear skin: TDE (100, 200, 400 and 800 µg/ear), TGC (100, 200 and 400 µg/ear) and indomethacin (250 µg/ear) as reference drug were applied topically. The inhibition of inflammatory response occurred in a dose-dependent way. TDE (800 µg/ear) reduced the oedema by 59±5 % and TGC (400 µg/ear) by 79±10%. In comparison, indomethacin inhibited the oedema by 71±12%. We investigated then with a plasmid reporter gene assay if TGC was able to inhibit the NF-κB transcription factor as did many anti-inflammatory drugs (2). TGC, at a concentration of 3 µM, inhibited the induction of NF-κB by TNFα at 49±7%.

A HPLC method was developed to quantify the amount variation of TGC in the different studied samples we obtained from São Tomé e Príncipe. The system, consisting of a RP-8 select B column with diode array detector and an isocratic elution with acetonitrile:acetate buffer pH 4.8 (45:55), allowed the quantification of TGC in TDE. Calibration curves were constructed in the concentration range 0.01-0.4 mg/ml. The amount of TGC in the samples was determined from the peak area by applying the linear equation obtained from the calibration curve.

These results indicate that TGC is a potential agent for therapy of inflammatory diseases. Moreover, the HPLC method can be readily used to compare TGC concentrations of *T. diversifolia* aerial parts.

References: 1. Goffin, E. et al. (2002) *Planta Med.* (in press). 2. Baldwin A.S. (1996) *Annu. Rev. Immunol.* 14: 649-81.