A029 Anti-inflammatory natural products with inhibitory activity against the transcription factor NF-κB

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NF-κB (nuclear factor-kappa B) is a ubiquitous transcription factor found in mammalian cells and plays a pivotal role in the induction pathways of inflammatory stimuli (e.g. TNF-α and IL-1). The identification of small molecule inhibitors of this pathway is currently under investigation by a team of eight international laboratories funded by the EU (1). The approach is to screen ethnobotanically used plants in inflammation and identify active extracts by employing a series of targeted-molecular based assays. A total of 129 plant species and 533 extracts have been screened thus far. Among these extracts, 5 have shown potent NF–κB inhibitory activity and a further 64 are of interest.

Two plants that yielded extracts with inhibitory activity were *Ochna macrocalyx* and *Helichrysum stoechas*. Assays included HeLa cells incorporating the luciferase firefly gene (controlled by the IL-6 promoter), EMSA (electrophoretic mobility shift assay). *Ochna macrocalyx* Oliv. (Ochnaceae) is a medicinal tree used by the Washambaa, indigenous people inhabiting the Western Usambara mountains in Tanzania. An ethyl acetate fraction (200 µg/ml) showed good inhibitory activity in EMSA. Three compounds were isolated from the active ethyl acetate extract and characterised but none showed inhibitory activity (50 µg/ml) in the luciferase firefly assay. A second example, *Helichrysum stoechas* (L.) Moench. (Compositae), was collected from Southern Spain and again an ethyl acetate extract displayed potent inhibitory activity in the luciferase firefly assay. In this case an acetophenone was isolated following bioassay-guided fractionation and was found to be an active inhibitor of NF-κB in the luciferase assay. Recently, this compound has also been shown to have effective anti-inflammatory activity using an *in vivo* model (2).

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References: 1. Bremner, P. and Heinrich, M (2002) J. Pharmacy and Pharmacology 54: 453-472. **2.** Sala, A. et al. (2001) J. Nat. Prod. 64: 1360-1362.

A030 Inhibitory effect of *Eucaliptus globulus Labill.* and *Thymus vulgaris* L. on nitric oxide production

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The physiological actions of nitric oxide (NO) are mediated by small NO concentrations acting as a labile intracellular messenger molecule. However, when NO is synthesized in high amounts by inflammatory cells, it possesses cytotoxic properties and be involved in the pathogenesis of acute and chronic inflammatory conditions. Thus, inhibition of NO accumulation induced by inflammatory stimuli could be a good therapeutic strategy to use in inflammatory diseases.

In this work, in order to determinate the anti-inflammatory properties of the *Eucaliptus globulus* Labill. (E.g.) and *Thymus vulgaris* L. (T.v.) extracts (obtained in powder form and dissolved in water), the J774A.1 murine macrophage cell line was treated with these extracts at different doses and then evaluated cell vitality, NO production and scavenging activity.

J774A.1 cell line is maintened continuously in DMEM plus 10% FBS at 37ºC under 5% CO_2 humidified air. Cells were incubated 5 h with E.g. and T.v. extracts at diverse doses (8.5, 16.8 and 50.4 µg) and then administered LPS (lipopolysaccharide, 1µg/ml) plus Interferon-γ (IFN-γ, 15 ng/ml) for 24 h. Nitrite (NO) concentrations were measured according to the Griess reaction. Cell viability was assessed by the MTT reduction method, and the scavenging effect was carried out using PAPA NONOATE as NO donor.

Both, E.g. and T.v. extracts induced a significant dose-dependent inhibition of NO ($p<0.001$ vs. LPS+IFN-γ treated cells). Cell vitality in plant-extracts treated cells did not show significant differences versus non-treated cells. The scavenging effect of E.g. and T.v. extracts was observed at 16.8 and 50.4 µg of concentration ($p<0.01$ vs. control group).

Our results suggest that E.g. and T.v. extracts poduce inhibition of nitric oxide production by the scavenging effect, although we can not discard an effect on iNOS gene transcription.

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