A091 Antioxidant compounds from *Hypericum triquetrifolium* Turra

F. Conforti [a], G. A. Statti [a], R. Tundis [a], F. Menichini [a] and P. Houghton [b]
[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi della Calabria, 87036 Rende (CS), Italy. [b] Department of Pharmacy, King's College London, 150 Stamford Street, London SE1 WA, United Kindom.

The aim of the present study was to investigate the antioxidant activity of the total extract and of specific compounds of *Hypericum triquetrifolium* Turra. The *in vitro* antioxidant activity tests were carried out using the lipid peroxidation of liposomes assay where the thiobarbituric acid test (TBA test) has been applied to asses the efficacy of the plant extract to protect liposomes from lipid peroxidation (1). Reactive oxygen species constitute a key mechanism of tissue injury and they are of significant relevance in the risk of cardiovascular disease (2) and in the pathology of arteriosclerosis, malaria and rheumatoid arthritis and could play a role in neurodegenerative disease and ageing processes (3).

The ground leaves of *Hypericum triquetrifolium* Turra were extracted with methanol. The ethyl acetate soluble part of this extract fractioned by column chromatographyed afforded five compounds: hypericin, I3-II8-biapigenin, quercetin-3-O-galactoside, kaempferol-3-O-glycoside and (-)-epicatechin. The flavonoids were isolated for the first time from this plant. Their structures were confirmed on the basis of NMR spectroscopic analysis. The antioxidant activity shown by *Hypericum triquetrifolium* Turra methanolic extract (IC_{50} 0.18 mg/ml) is related to the presence of phenolic compounds (4): quercetin-3-O-galactoside (IC_{50} 0.25 mg/ml), kaempferol-3-O-glycoside (IC_{50} 0.51 mg/ml) and particularly I3,II8-biapigenin and (-)-epicatechin, that showed the most antioxidant activity (IC_{50} 0.016 mg/ml and 0.036 mg/ml, respectively), whilst the xanthone hypericin shown low antioxidant activity (IC_{50} 1 mg/ml).

Our observations demonstrated the varying inhibitory effects of the five structurally-related natural compounds from *Hypericum triquetrifolium* Turra on oxidation of liposomes. It appears that I3,II8-biapigenin and (-)-epicatechin are effective antioxidants against liposomes.

References: 1. Fernàndez, J. et al. (1997), Food Chemistry 59: 345-353. **2.** Hertog, M.G.L. et al (1993), Lancet 342: 1007-1011. **3.** Moure, A. Et al. (2001), Food Chemistry 72: 145-171. **4.** Chen, Z.Y et al. (1996), Chemistry and Phisics of Lipidis 79: 157-163.

A092 Evaluation of mediators involved in immunocytotoxicity induced by isocoumarins from *Paepalanthus bromelioides*

Karina Ferrazzoli Devienne [a], Maria Stella Gonçalves Raddi [b], Iracilda Zeppone Carlos [b] and Wagner Vilegas [a]
[a] Instituto de Química de Araraquara-UNESP, CP 355,14801-970, Araraquara, SP, Brazil. [b] Faculdade de Ciências Farmacêuticas de Araraquara- UNESP, CP 502,14801-902, Araraquara, SP, Brazil.

Isocoumarins are isolated in great variety of microorganisms, plants and insects, and showed to have considerable biological activity. The isocoumarin 9,10-dihydroxy-5,7-dimethoxy-1H-naphtho (2,3c)pyran-one (paepalantine) and 8,8'-paepalantine dimer (paepalantine dimer), isolated from *Paepalantuhs bromelioides*, showed cytotoxicity on murine macrophages and others cells lines (1,2). Actually, *in vitro* studies on macrophages have been applied to immunocytotoxicity testing which have included the measurements of both cytotoxic and immune responses directly on the culture cells. The effects and influence of the cellular mediators have been used as analytical probes or as indicator in cytotoxicity techiques on macrophages (3). The hydrogen peroxide (H_2O_2), nitric oxide (NO) and tumoral necrosis factor (TNF-α) are effector molecules for the microbicidal and cytotoxic response of macrophages after a stimuli. Considering the possibilities of these molecules involvement in cytotoxicity induced by paepalantines, we investigate whether stimulation with these isocoumarins could be lead to NO, H_2O_2 and TNF-α production by macrophages (4,5,6). The results demonstrated that were not detected H_2O_2 and NO in supernatants of these cells when treated with paepalantines and their toxic effects in this cells system are not mediated by these mediators production. The TNF-α production by paepalantine at 20 µg/ml was not statistically different when compared to untreated cells, however, the macrophages treated with the same concentration of paepalantine dimer significantly increased this cytokine production (67±32 U/ml). The results suggest that paepalantine dimer has effect on macrophages secretory and cellular activities, showing a differential effect on production of cytokines and others cytotoxic molecules. The amount of this cytokine found in the supernatants appears is not responsible by viability decreased of macrophages and the mechanisms of cytotoxicity-induced paepalantines to remain unknown.

Acknowledgements: FAPESP.

References: 1. Devienne K.F. et al. (2002) Z. Naturforsch., 57c: 85-8. **2.** Coelho R.G. et al. (2000) Fitoterapia, 71: 497-500. **3.** Barile F.A. (1994) Introduction to *in vitro* cytotoxicology. CRC Press. Boca Raton. **4.** Pick E. and Mizel D. (1981) J. Immunol. Methods. 46: 211-26. **5.** Green L.C. et al. (1982) Anal. Biochem., 126: 131-8. **6.** Kirikae T et al. (1996) Biochem. Biophys. Res. Commun., 227: 227-35.