A015 Bioassay-guided fractionation of extracts of basidiomycetes for inhibitory activity on collagenase and elastase

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In a previous study we reported the ability of dichloromethane extracts of basidiomycetes to inhibit the activity of the metalloendopeptidase *Clostridium histolyticum* collagenase (EC 3.4.24.3) and the serine proteinase human neutrophil elastase (EC 3.4.21.37) (1). The bioassay-guided fractionation led to the isolation of free fatty acids (stearic acid, palmitic acid, linoleic acid).

We extracted the fruit bodies of *Heterobasidion annosum* (Fr.) Bref. and *Lactarius deterrimus* Grög. with dichloromethane. The separation of the active fraction was performed by column chromatography on silica gel for several times. The final separation and the identification of the free fatty acids was performed by GC-MS. For the GC determination, the fatty acids were esterified to their methyl esters. The identification was carried out comparing the retention times of reference substances and their mass spectra. The quantification of the fatty acids was performed using an internal standard.

We characterized the pharmacological activity by enzyme assays. We estimated the activity of the collagenase by degradation of resorufin-labeled casein fluorimetrically (2). The determination of the elastase activity was performed by a spectrophotometrical method using a 4-nitroanilide peptide substrate (3).

The results of the quantification of the free fatty acids correlated with the results of the collagenase assay. The results of the determination of the free fatty acids and the results of the elastase assay differed. Thus, we can assume that there are additional elastase-active compounds in the extracts.

References: 1. Rennert B. and Melzig M.F. (2002) Phytother. Res. 16 (S1): S81-83. **2.** Twining S.S. (1984) Anal. Biochem. 143: 30-34. **3.** Melzig M.F., Löser B. et al. (1999) Pharmazie 54: 712.

A016 Effects of propolis on hypoxanthine-xanthine oxidase-induced toxicity in cultivated endothelial cells and on the inhibition of neutrophil elastase activity

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Propolis is used by bees (*Apis mellifera)* as a glue to seal the hives and to protect the beehive against outside invaders and enemies. It is collected from different plant sources and contains, depending on the region of collection, different polyphenolic compounds such as flavonoids, phenolic acids and its esters, fatty acids, diterpenic acids and other compounds (1). Propolis is appreciated for its antibacterial, antifungal, anti-inflammatory and immuno-stimulating activities.

We evaluated the free radical scavenger activity and the inhibition of neutrophil elastase activity of different ethanolic and water extracts of propolis.

We tested the decline of endothelial cells (ECV-304 cell-line) after oxidant injury with hypoxanthine-xanthine oxidase and determined protection provided by propolis and its constituents chrysin, caffeic acid and its phenethyl ester. In these experiments the cells were incubated with both hypoxanthine-xanthine oxidase and test substance for 1 h.

The 70% ethanolic extract of propolis demonstrated the strongest beneficial effect. It showed a 50% restitution of cells at a concentration of 1.1 µg/ml. The scavenging efficiency of a purchased 85% ethanolic extract of propolis was weaker (EC_{50} = 2.8 µg/ml).

Both extracts showed a significant inhibition of human neutrophil elastase activity (IC_{50} = 3.4 µg/ml and 2.4 µg/ml respectively).

The inhibition of neutrophil elastase activity and protection of endothelial cells against oxygen radicals contribute to an anti-inflammatory effect of propolis which was shown for both chronic and acute inflammations in rat adjuvant arthritis (2).

References: 1. Velikova, M., Bankova, V. et. al. (2000) Z. Naturforsch. 55 c, 790- 793. **2.** Park, E. H., Kahng, J. H. (1999) Arch. Pharm. Res. 22, 554-558.