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11

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Abstracts

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PI456 Effect of natural and semisynthetic flavonoids on the expression of heme oxygenase-1

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The natural flavonoid quercetin is known to activate the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) which regulates the expression of antioxidant and phase II xenobiotic metabolism enzymes such as heme oxygenase-1, superoxide dismutases and glutathione *S*-transferases. This study examined whether the expression of heme oxygenase-1 could also be activated either by natural derivatives of quercetin, isoquercitrin (quercetin-3-*O*-glucoside) and taxifolin (dihydroquercetin), or by new semisynthetic galloylated derivatives, 3-*O*-galloylquercetin and 7-*O*-galloyltaxifolin. In murine macrophage RAW264.7 cells, 7-*O*-galloyltaxifolin at the concentrations from 25 μM significantly induced the expression of *Hmox1* gene encoding heme oxygenase-1 and increased the protein levels of the enzyme as well. In contrast, the other tested compounds had negligible effects on the expression of heme oxygenase-1. The induction of *Hmox1* gene expression by 7-*O*-galloyltaxifolin was accompanied by nuclear accumulation of Nrf2 and by downregulation of Keap1 (Kelch-like ECH-associated protein 1), a negative regulator of the Nrf2 activity. The increase in Hmox1 mRNA levels by 7-*O*-galloyltaxifolin was, at least partially, suppressed by SB203580 and PD98059, pharmacologic inhibitors of p38 mitogen-activated protein kinases (p38 MAPKs) and p44/42 MAPKs, respectively. We conclude that 7-*O*-galloyltaxifolin induces heme oxygenase-1 via activation of the MAPK/Nrf2 signaling pathway. This work was supported by grants GACR P301/11/0767 and LF_2012_10.

PI457 Isolation and characterization of an arabinose-specific lectin from the ascomycete *Xylaria hypoxylon*

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Lectins are proteins that have the ability to bind specific sugars. For years some commercially available lectins have been used as biochemical tools for affinity chromatography, microarray or fluorescence microscopy experiments. In search of a lectin that binds specifically to arabinoxylans from *Plantago ovata* we found a lectin that exhibits haemagglutination activity with 4% rabbit red blood cell suspension. This was isolated from fresh mushroom bodies of *Xylaria hypoxylon*, ("Stag's horn fungus") grown in North Rhine Westphalia, Germany. The isolation procedure[1] involved aqueous extraction, protein precipitation with 80% saturated ammonium sulfate, dialysis against double distilled water, anion exchange chromatography on DEAE-cellulose and finally gel filtration on Biogel P-100. The native molecular mass was found to be ~50 kDa by gel filtration. However in SDS-PAGE, the protein dissociated into smaller subunits of molecular mass ~ 16 kDa. ESI LC-MS results also suggested small subunit nature of the lectin. Surprisingly, besides D-galactose and lactose, L-arabinose was able to inhibit haemagglutinating activity up to a concentration of just 0.49mM. Biochemical characterization of this lectin is in progress. [1] Liu, Q., Wang, H. & Ng, T. B. First report of a xylose-specific lectin with potent hemagglutinating, antiproliferative and anti-mitogenic activities from a wild ascomycete mushroom. *Biochim. Biophys. Acta* 1760, 1914 – 1919 (2006)

PI458 Anti-diabetic effects of the silkworm (*Bombyx mori.*) extracts in the db/db mice

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The anti-diabetes mechanism of silkworm powder and extracts turned out to have the inhibitory activity of α-glycosidase. The major functional component of silkworm powder was 1-deoxynojirimycin(1-DNJ), and it exerts blood glucose-lowering effect. This study compared with polyhydroxylated alkaloid contents such as 1-deoxynojirimycin(DNJ), Fagomine, and 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) according to three silkworm varieties. Changes of food and water intakes, body weight and blood glucose with db/db mice were investigated. In addition, the oral glucose tolerance test carried out by maltose in ICR mice. The contents of 1-DNJ was very similar among the three varieties, but the contents of polyhydroxylated alkaloid were the highest in Yeonnokjam. The 1-DNJ contents of the YR70 group were more than those of other groups that used other extract methods. The anti-diabetic effects of the extracts and powder of Yeonnokjam are tested on the db/db mice. The blood glucose level decreased significantly in YR70 group, but food and water intake and body weight do not changed considerably. Based on these results, the silkworm extracts can be developed as a new natural drug.

PI459 Potential therapeutic activity of some lichen extracts from Usnea aurantiaco-atra on human cancer cell lines

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Lichens have demonstrated cytotoxic activity against many human cancer lines. In this work extracts of the Antarctic lichen, Usnea Aurantiaco-Atra, isolated with n-hexane, diethyl ether and methanol using a Soxhlet process and purified with solid phase extraction, were evaluated in vitro using two different human cancer lines (HeLa: human cervical cancer and HT-29: human colon adenocarcinoma). The MTT assay revealed significant cytotoxicity in all the fractions after purification and elution with acetonitrile. Since Usnea Auriantiaco-Atra grows in the Antarctic region, a highly UV-exposed area, antioxidant activity has been also evaluated for its potential therapeutic utilization. Antioxidant activities (AA), reducing powers (RP) and total phenolic contents (TPC) have been also determined.

PI460 Schistosomicidal potential of endophytic fungi associated with *Vochysia divergens* Pohl

Pedroso RCN[1], Pimenta LP[1], Lima WC[2], Soares MA[3], Magalhães LG[1], Crotti AEM[1], Silva MLA[1], Cunha WR[1], Pauletti PM[1], Januário AH[1]
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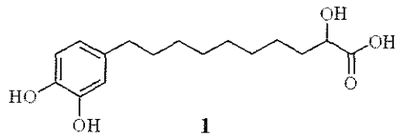
Schistosomiasis, caused by trematode flatworms of the genus *Schistosoma*, is one of the most significant, neglected tropical diseases in the world. *Vochysia divergens* (Vochysiaceae), popularly known as "Cambará", is a typical species of the Mato Grosso Pantanal. In this work, ethyl acetate extracts of endophytic fungi 43W and 53W strains associated with *V. divergens* roots were chemically investigated and also evaluated *in vitro* against *Schistosoma mansoni* adult worms for viability and motor activity. The compound 2-hydroxy-10-(3,4-dihydroxyphenyl) decanoic acid (1) was identified as the major constituent from the strain 53W. The structural elucidation, established by NMR spectroscopic and mass spectrometric analysis of 1 as well as the biological results will be presented.



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PI461 Antimicrobial activity of *Cladonia incrassata* acetone extract

Dieu A[1], Millot M[1], Champavier Y[2], Chulia JA[1], Vergnaud J[1], Chaleix V[1], Bressollier P[1], Sol V[1], Gloaguen V[1]
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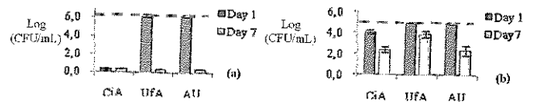
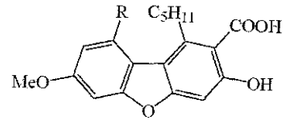
Lichens of the genera *Cladonia* and *Usnea* biosynthesize usnic acid, a widely spread dibenzofuran derivative endowed with antimicrobial activity. Usnic acid contents of *Cladonia incrassata* and *Usnea florida* acetone extracts were assessed by HPLC. Evaluation of antimicrobial activities against *Staphylococcus aureus* and *Candida albicans* showed that *C. incrassata* extract is more effective than usnic acid. Phytochemical study of this extract was initiated using a bioautographic protocol for tracking down active compounds. Two dibenzofurans isolated by preparative TLC and semi-preparative HPLC were further identified by NMR and MS as didymic acid and condidymic acid respectively. The strong antimicrobial activity of *C. incrassata* extract can be attributed to these two molecules, whose potential use as preservatives is currently under study.



Activity of 0.1% solutions of C. incrassata acetone extract (CiA), U. florida acetone extract (UfA) and usnic acid (AU) against S. aureus **(a)** and C. albicans **(b)**



R = C3H7: didymic acid; R = C5H11: condidymic acid

PI462 Unusual sesterterpenes from the lichen *Leprocaulon microscopicum*

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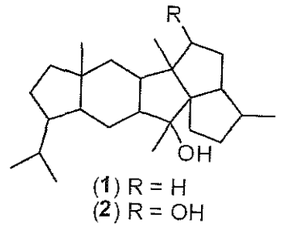
Leprocaulon microscopicum is a lichen belonging to anamorphic Lecanorales, growing in various countries and widely spread in humide areas of Limousin, France. Its chemical composition is partially described in the literature and publications still mention some unknown substances[1]. A phytochemical study of acetonic and hydro-methanolic extracts led to the isolation of (-)-usnic acid, dibenzofuran derivatives and terpenes. Among terpenoids, the common triterpene zeorin as well as two new sesterterpenoids (1) and (2) have been characterized in *L. microscopicum*. Structures were established on the basis of mass spectrometry and 2D NMR experiments. With molecular formulae $C_{25}H_{42}O$ and $C_{25}H_{42}O_2$, the new compounds featured a rare pentacyclic skeleton, closely related to retigeranic acid, the only sesterterpene isolated from lichens[2]. Thus, the present work notably extends the knowledge of the genus *Leprocaulon* and lichen chemistry. References: 1. Lamb and Ward (1974) Journ. Hattori Bot. Lab. 38: 499 – 553. 2. Kaneda et al. (1972) Tet. Let. 13: 4609 – 4611.



(**1**) R = H
(**2**) R = OH

PI463 Docking studies to evaluate mushrooms low molecular weight compounds as inhibitors of the anti-apoptotic protein BCL-2

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Several reports indicate that mushrooms have the ability to promote apoptosis in tumour cell lines, but the mechanism of action is not quite well understood. Inhibition of the interaction between Bcl-2 (anti-apoptotic protein) and pro-apoptotic proteins could be an important step that leads to apoptosis. Therefore, the discovery of compounds with the capacity to inhibit Bcl-2 is an ongoing research topic on cancer therapy. Herein, Autodock4 virtual screening was applied to a dataset of 40 low molecular weight compounds present in mushrooms, using 3D Bcl-2 protein structure (PDB:2XA0) as target. Results suggested that steroids mainly ergosta-4,6,8(14),22-tetraen-3-one, lucidenic lactone, cerevisterol, ganoderic acid w and ganoderic acid x, with a binding energy lower than -10 kcal/mol, had the ability to interact with Bcl-2. Acknowledgements: FCT and COMPETE/QREN/EU- project PTDC/AGR-ALI/110062/2009, PEst-OE/AGR/UI0690/2011 (CIMO) and grant BPD/4609/2008 (L. Barros).

PI464 Effects of THC, THC acid and CBD on MPP^+ or glutamate affected dissociated mesencephalic cultures of mice

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Phytocannabinoids become of interest for studies on neuroprotection. Two major events leading to neuronal degeneration are oxidative stress and excitotoxicity. In cell culture systems, these events can be induced by the use of either the complex I inhibitor MPP^+ or high doses of glutamate. In our study, we investigated the effects of tetrahydrocannabinol (THC), THC acid (THCA) and cannabidiol (CBD) on MPP^+ or glutamate affected dissociated mesencephalic cultures of mice. On the 8th day *in vitro*, cannabinoids (0.001 to 10 μM) were administered alone or concomitantly with MPP^+ (10 μM) or glutamate (30 μM) for 48 h. Using tyrosine hydroxylase immunocytochemistry, dopaminergic neurons were stained and counted. While 10 μM of CBD decreases the dopaminergic cell number, THCA has no effect and THC increases the number of surviving neurons at a concentration of 1 and 10 μM. MPP^+ treatment results in a degeneration of about a half of the dopaminergic cells. Against this cell degeneration, all chosen phytocannabinoids display neuroprotective effect at 10 μM. Administration of glutamate for 48 h leads to a reduction of dopaminergic cell count by about 30%. Phytocannabinoids support the cell survival in glutamate treated cultures significantly already at low concentrations. Cannabinoids might be candidates for neuroprotective agents in disorders in which excitotoxicity and oxidative stress occur.