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Abstracts

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PF56 Plant species from the Peruvian Amazon rainforest (Peru) and their antimicrobial activity

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The plant species reported here are traditionally used by Indigenous and Mestizo populations from the Iquitenian surroundings (Peruvian Amazon) for microbial infections. Inhabitants of various ethnic origins were interviewed and selected plants extracts were evaluated for their antimicrobial properties against 36 sensitive and multi-resistant bacteria or fungi. Of the 39 plants analyzed (50 methanolic extracts), 9 species showed MIC ≤0.3 mg/ml for one or several microorganisms and only 6 extracts were inactive. This study supports the traditional use of these plants. It may help to discover new chemical classes of antibiotics that could serve as selective agents against multi-resistant bacteria.

PF57 *Cistus ladanifer* as a source of phenolic compounds with antifungal activity

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A screening of the antifungal potential of phenolic extract of *Cistus ladanifer* from Northeast Portugal, against *Candida* species was performed. The extract was characterized by HPLC-DAD-ESI/MS. Phenolic acids and derivatives, ellagic acid derivatives and flavonoids, such as catechins, flavonols and flavones, were found in the sample. The most abundant group was ellagic acid derivatives in which punicalagin gallate, a derivative of punicalagin attached to gallic acid, was found in highest amount. These compounds could be related to the strong inhibition of *C. albicans*, *C. glabrata* and *C. parapsilosis* growth. Moreover, the best antifungal activity was against *C. glabrata*, where the studied extract was able to cause at least 3 Log of reduction at concentrations below 50 µg/mL and a total growth inhibition at concentrations above 625 µg/mL.

PF58 Effects of Ocimum sanctum Linn (OS) leaf extract on stress, memory and attention in healthy humans

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Anti-stress effects and memory enhancing effects of OS have been documented in animal models nut no human studies available. Double blinded RCT on healthy human adults.300 mg capsules of ethanolic leaf extract of OS or placebo were administered to 30 volunteers for 30 days. Recordings were taken on day 0,15 and 30. Parameters assessed: a) STAI questionnaire b) Sternberg memory task c) Stroop task d) Heart rate (HR) & e) GSR. **Results:** showed significant improvement in reaction time $p = 0.043$ in Sternberg task, and improved % of correct responses in facilitation task of Stroop ($p = 0.01$). STAI, HR, GSR and P300 latency showed no significant change.P300 amplitude showed a significant increase ($p = 0.02$).All changes were significant only at day 30. OS possesses memory enhancing effects at dose of 300 mg/day od by 30 days. There is also significant improvement in attention as assessed by P300 amplitude. However no significant reductions in Stress parameters were seen at this dose/duration of OS. Further trials have to been conducted in more subjects and for longer duration for the effects to be validated

PF59 Phytochemical investigation of *Rauvolfia nukuhivensis*, a Marquesan traditional medicinal plant

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Rauvolfia nukuhivensis (Apocynaceae) is an endemic species of the Marquesas archipelago where it is used as a traditional gynecological antiseptic. Over-exploited because of the frequent use of the bark (macerate), the plant is now classsified as an endangered species ("Critical Rare" UICN status). Data regarding pharmacological principles and their chemical identity were not available until now. The phytochemical investigation of the main constituents of this popular medicinal plant resulted in the isolation and identification of several alkaloids belonging to the sandwicine and ajmaline type, among them also formerly unknown derivatives. In order to test the efficacy against human pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) bioassays were carried out, showing moderate antifungal activities of some compounds.

PF60 Analysis of leaf epidermal characters of medicinal and poisonous Brazilian menispermaceae

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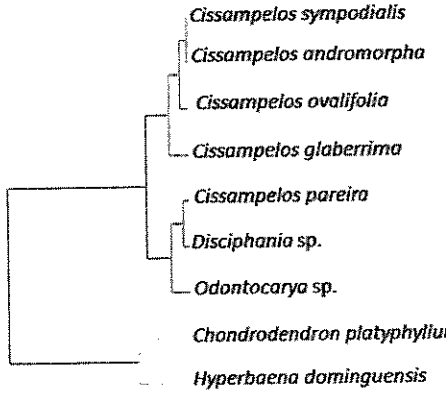
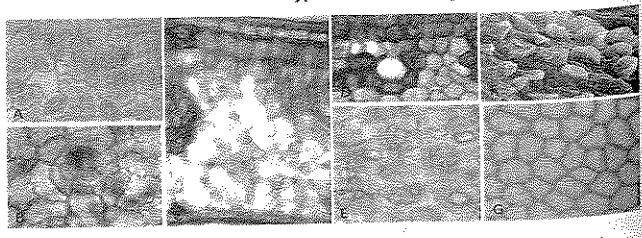
We carried out a multivariate analysis of leaf anatomy of five genera and nine species of Menispermaceae known to be toxic or used as medicinal in Brazil. The dendrogram obtained by Ward's method showed two groups (A and B), with cophenetic correlation coefficient of 0.9178. The group A with six species and two subgroups: the subgroup A1 brought together four species of *Cissampelos* with anomocytic stomata (Fig 1A), without inclusions of calcium oxalate (Fig.1E) and sclereids; the subgroup A2 with *Cissampelos pareira*, *Odontocarya* and *Disciphania*. The group B consists of *Chondrodendron platyphyllum* with anticlinal walls cells straight (Fig 1G) and *Hyperbaena dominguensis* with cyclocytic stomata (Fig.1B), braciform cells in the spongy parenchyma (Fig 1C), and secretory canals (Fig 1D). *Cissampelos sympodialis* and *Chondrodendron platyphyllum* were the species with lower similarity in the dendrogram. Financial support: CNPq.





A. anomocytic stomata; B. cyclocytic stomata; C. braciform cells in the spongy parenchyma; D. secretory canals; E: inclusions of calcium oxalate; F. Papillae; G. Anticlinal walls cells straight.

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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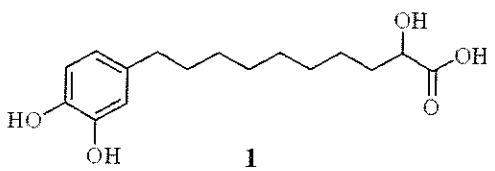
Schistosomiasis, caused by trematode flatworms of the genus *Schistosoma*, is one of the most significant, neglected tropical diseases in the world. *Vochysia divergens* (Vochysiaceae), popularly known as "Cambará", is a typical species of the Mato Grosso Pantanal. In this work, ethyl acetate extracts 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.



1

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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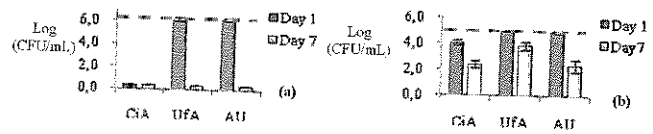
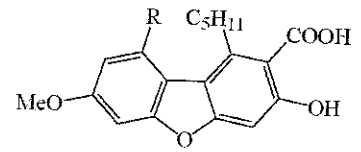
Lichens of the genera *Cladonia* and *Usnea* biosynthesize usnic acid, a widely spread dibenzofuran derivative endowed with antimicrobial activity. Usnic acid contents of *Cladonia incrassata* and *Usnea florida* acetone extracts were assessed by HPLC. Evaluation of antimicrobial activities against *Staphylococcus 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.

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

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CIMO-ESA, Polytechnic Institute of Bragança, Portugal

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.

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ple HPLC method for detecting adulteration nkgo extracts with flavonol aglycones

uth H[1], Savage K[1], Dowell A[1], Mouatt P[1]
ern Cross Plant Science, Southern Cross University,
e 2480, Australia

more than 45 flavone glycosides, most of which are
nes quercetin, kaempferol and isorhamnetin [1].
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e determination of absolute configurations -hydroxy acids by the expansion of Marfey's od

K[1], Lim C[2], Kim S[2], Oh DC[1]
ral Products Research Institute, College of Pharmacy,
National University, Seoul 151 – 742; [2]College of
acy, Seoul National University, Seoul 151 – 742,
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taneously. We believe that our approach may be
r natural product chemists.

trace level voltammetric determination of mercury and toxic metals in tea matrices

elli C[1], Melucci D[1], Locatelli M[2]
rtimento di Chimica "G. Ciamician", Università degli

(II) by square wave anodic stripping voltammetry (SWASV) in matrices involved in food chain as tea leaves is proposed. The digestion of each matrix was carried out using a concentrated HCl-HNO_3-H_2SO_4 acidic attack mixture. 0.01 mol/L EDTA-Na_2 + 0.06 mol/L NaCl + 2.0 mol/L $HClO_4$ was employed as the supporting electrolyte. The voltammetric measurements were carried out using a conventional three electrode cell, employing, as working electrodes, a gold electrode (GE) and a stationary hanging mercury drop electrode (HMDE). The analytical procedure has been verified on the standard reference materials Spinach Leaves NIST-SRM 1570a, Tomato Leaves NIST-SRM 1573a and Apple Leaves NIST-SRM 1515. For all the elements, the precision as repeatability, expressed as relative standard deviation (s_r) was of the order of 3 – 5%, while the accuracy, expressed as relative error (e) was of the order of 3 – 7%. Once set up on the standard reference materials, the analytical procedure was applied to commercial tea leaves samples. A critical comparison with spectroscopic measurements is also discussed.

PJ35 Mutual interference problems in the simultaneous voltammetric determination of ultra-trace total mercury(ii) and toxic metals in medicinal herbs matrices

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The work describes the the voltammetric determination of mercury (II), copper (II), lead (II), cadmium (II), zinc (II) by square wave anodic stripping voltammetry (SWASV) in medicinal herbs. The digestion of each matrix was carried out using a concentrated HCl-HNO_3-H_2SO_4 acidic attack mixture. 0.01 mol/L EDTA-Na_2 + 0.06 mol/L NaCl + 2.0 mol/L $HClO_4$ was employed as the supporting electrolyte. The voltammetric measurements were carried out using a conventional three electrode cell, employing, as working electrodes, a gold electrode (GE) and a stationary hanging mercury drop electrode (HMDE). The analytical procedure has been verified on the standard reference materials Spinach Leaves NIST-SRM 1570a, Tomato Leaves NIST-SRM 1573a and Apple Leaves NIST-SRM 1515. For all the elements, the precision as repeatability, expressed as relative standard deviation (s_r) was of the order of 3 – 6%, while the accuracy, expressed as relative error (e) was of the order of 3 – 7%. Once set up on the standard reference materials, the analytical procedure was applied to commercial medicinal herbs samples. A critical comparison with spectroscopic measurements is also discussed.

PJ36 Analysis of phenolic compounds in flowers from wild medicinal plants from northeastern Portugal

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This study aimed to analyse phenolic compounds in wild medicinal flowers of *Crataegus monogyna*, *Cytisus multiflorus*, *Malva sylvestris* and *Sambucus nigra*, by HPLC-DAD-ESI/MS. Flavonols and flavones were the main groups in almost all the studied samples. *C. multiflorus* sample gave the highest levels of flavonoids, being a chrysin derivative the most abundant flavone. *C. monogyna* revealed the highest concentration in phenolic acids that were not found in *C. multiflorus*; 5-*O*-caffeoylquinic acid was the most abundant phenolic acid found in the first species, being a procyanidin trimer also found. Kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside were the main flavonols present in *M. sylvestris* and *S. nigra*, respectively. The studied flowers could be selected for processing extracts with health-promoting properties or to be incorporated into functional beverages or products with bioactive properties related to oxidative stress. Acknowledgements: PEst-OE/AGR/UI0690/2011, SFRH/BPD/4609/2008 (L. Barros), Ramón y Cajal (M. Dueñas).

peripheral neuropathy (DPN) as well as diabetes mellitus. We carry on the study on bioactive fraction and compounds of Radix Astragali for DN and DPN. The bioactive fraction was identified by LC/MS^3 on the basis of separated active compounds, besides astragaloside IV (ASI) was focus on in our long term study. 16 peaks in the HPLC spectrum were determined by analysis their ESI-MS^3 spectra and retention time with isolated compounds and referring to literatures. Astragalosides and flavonoides are the main constituents in bioactive fractions.

PI32 A simple HPLC method for detecting adulteration of ginkgo extracts with flavonol aglycones

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Ginkgo leaf contains more than 45 flavone glycosides, most of which are based on the aglycones quercetin, kaempferol and isorhamnetin [1]. Adulteration with rutin, quercetin and other plant extracts has been reported [2,3]. USP and EP/BP monographs for ginkgo extract stipulate 22 – 27% flavonoids, but pharmacopoeial methods cannot effectively detect adulteration with aglycones, because calculation of glycoside content is based on the aglycone content after acid hydrolysis. We developed a modification to the pharmacopoeial methods that enables quantification of both glycosides and free aglycones and applied it to 5 leaf samples and 8 retail ginkgo products. Free flavonol aglycones were not present in leaf samples or in two products. Most products largely met their label claim for flavonol glycoside content and relative aglycone content by USP (Identification Test B), but our method revealed high levels of free quercetin and kaempferol in three products, suggestive of adulteration. The presence of free aglycones in these products meant that the pharmacopoeial methods for calculating flavonol glycosides overestimated the glycoside content by up to 40%. We suggest the USP and EP/BP monographs for ginkgo extract be modified to increase their ability to detect adulteration with flavonol aglycones. References: 1. Lin, L.-Z. et al. (2008) *J Food Agric Chem* 56:6671 – 9. 2. Franz, C. et al. (2011) *Food Funct* 2:720 – 30. 3. Liu, C. et al. (2005) *Analyst* 130:325 – 9.

PI33 Facile determination of absolute configurations of α -hydroxy acids by the expansion of Marfey's method

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α-Hydroxy acids are often biosynthetically incorporated in natural small molecules such as depsipeptides from nonribosomal peptide synthetase pathways. While the absolute configurations of α-amino acids are conveniently determined by the LC/MS-based analysis of Marfey's derivatives of amino acids, the current methods to determine the absolute configurations of corresponding α-hydroxy acids require more complicated steps. So we expanded Marfey's method and developed a facile procedure determining the absolute configurations of α-hydroxy acids. The method was evaluated with the LC/MS analysis of the reaction products of various L-D-α-hydroxy acids coupled with Marfey's reagent (L-FDAA). This new method is operationally simple and applicable at a submilligram scale without any purification of the reaction mixture. We applied this facile procedure to a natural depsipeptide, zygosporamide, which bears L-phenylalanine, L-D-leucine, and L-leucic acid, and successfully determined the absolute configurations of its α-amino acids and α-hydroxy acid simultaneously. We believe that our approach may be practically useful for natural product chemists.

PI34 Ultratrace level voltammetric determination of total mercury and toxic metals in tea matrices

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An analytical procedure regarding the voltammetric determination of mercury (II) and copper (II), and copper (II), lead (II), cadmium (II), zinc (II) by square wave anodic stripping voltammetry (SWASV) in matrices involved in food chain as tea leaves is proposed. The digestion of each matrix was carried out using a concentrated HCl-HNO_3-H_2SO_4 acidic attack mixture. 0.01 mol/L EDTA-Na_2 + 0.06 mol/L NaCl + 2.0 mol/L $HClO_4$ was employed as the supporting electrolyte. The voltammetric measurements were carried out using a conventional three electrode cell, employing, as working electrodes, a gold electrode (GE) and a stationary hanging mercury drop electrode (HMDE). The analytical procedure has been verified on the standard reference materials Spinach Leaves NIST-SRM 1570a, Tomato Leaves NIST-SRM 1573a and Apple Leaves NIST-SRM 1515. For all the elements, the precision as repeatability, expressed as relative standard deviation (s_r) was of the order of 3 – 5%, while the accuracy, expressed as relative error (e) was of the order of 3 – 7%. Once set up on the standard reference materials, the analytical procedure was applied to commercial tea leaves samples. A critical comparison with spectroscopic measurements is also discussed.

PI35 Mutual interference problems in the simultaneous voltammetric determination of ultra-trace total mercury(ii) and toxic metals in medicinal herbs matrices

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The work describes the the voltammetric determination of mercury (II), copper (II), lead (II), cadmium (II), zinc (II) by square wave anodic stripping voltammetry (SWASV) in medicinal herbs. The digestion of each matrix was carried out using a concentrated HCl-HNO_3-H_2SO_4 acidic attack mixture. 0.01 mol/L EDTA-Na_2 + 0.06 mol/L NaCl + 2.0 mol/L $HClO_4$ was employed as the supporting electrolyte. The voltammetric measurements were carried out using a conventional three electrode cell, employing, as working electrodes, a gold electrode (GE) and a stationary hanging mercury drop electrode (HMDE). The analytical procedure has been verified on the standard reference materials Spinach Leaves NIST-SRM 1570a, Tomato Leaves NIST-SRM 1573a and Apple Leaves NIST-SRM 1515. For all the elements, the precision as repeatability, expressed as relative standard deviation (s_r) was of the order of 3 – 6%, while the accuracy, expressed as relative error (e) was of the order of 3 – 7%. Once set up on the standard reference materials, the analytical procedure was applied to commercial medicinal herbs samples. A critical comparison with spectroscopic measurements is also discussed.

PI36 Analysis of phenolic compounds in flowers from wild medicinal plants from northeastern Portugal

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This study aimed to analyse phenolic compounds in wild medicinal flowers of *Crataegus monogyna*, *Cytisus multiflorus*, *Malva sylvestris* and *Sambucus nigra*, by HPLC-DAD-ESI/MS. Flavonols and flavones were the main groups in almost all the studied samples. *C. multiflorus* sample gave the highest levels of flavonoids, being a chrysin derivative the most abundant flavone. *C. monogyna* revealed the highest concentration in phenolic acids that were not found in *C. multiflorus*; 5-*O*-caffeoylquinic acid was the most abundant phenolic acid found in the first species, being a procyanidin trimer also found. Kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside were the main flavonols present in *M. sylvestris* and *S. nigra*, respectively. The studied flowers could be selected for processing extracts with health-promoting properties or to be incorporated into functional beverages or products with bioactive properties related to oxidative stress. Acknowledgements: PEst-OE/AGR/UI0690/2011, SFRH/BPD/4609/2008 (L. Barros), Ramón y Cajal (M. Dueñas).

PI37 Qualitative and quantitative analysis of flavonoids in Saba Senegalensis P. leaves by HPLC-DAD-ESI-MS/MS and HPTLC-UV

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Saba senegalensis P. is a tendrilled liana widespread throughout Tropical West Africa, from Senegal to Nigeria. Leaves are essentially used in traditional medicine of several countries as an antiseptic and wound healing agent. To provide major information about the chemical content of the leaves, we performed analysis of secondary metabolites by HPTLC-UV. On our samples collected in Mali, the polyphenolic profile is mainly represented by two flavonoids. Liquid chromatography (LC) coupled to electrospray ionsation (ESI) and tandem mass spectrometry (MS/MS) was used for the identification of these two compounds. Comparison of retention time, UV and MS spectral data of standard compounds allowed us to characterize unambiguously: quercitrin and myricitrin. Quantification was achieved by HPLC-DAD and myricitrin was the main component (average 80%) regardless the date of harvest. Moreover, we optimized a rapid quantitative analysis of quercitrin and myricitrin by thin-layer chromatography with densitometric detection. The results obtained were compared to those of HPLC-DAD ones. This present study described for the first time a qualitative and quantitative fingerprint of *Saba senegalensis* P. and could be helpful in the chemotaxonomic study of this genus and for medicinal purposes.

PI38 Fractal dimension in mass spectra from herbal extracts: Hypothesis for a new method of phytocomplex characterization

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In order to optimize the quality control of phytochemical products, we propose a non-conventional method of analysis of complex systems, called *fractal analysis*, applied to ESI (*Electrospray Ionisation*) mass spectra. The ESI spectra obtained with phytochemical commercial products (Mattoli et al., *J. Mass Spectrom.* **41**: 1534, 2006; Mattoli et al., *Metabolomics* **7**: 437, 2011) were submitted to fractal analysis using the "box counting" method. Subsequent cluster analysis permitted to determine a distinctive fractal dimension (D_B) for single plant extracts, as well as for mixtures of plant extracts contained in commercial herbal products. On several replicates obtained with different batches, D_B tended to display a normal distribution around a mean value, which might be suggested as a typical reference tag for that product. The fractal approach permitted to characterize the repeatability of the instrumental measure too. Changes in D_B following thermal treatment of samples, to simulate ageing, indicated the ability of the method also to identify appropriate conditions of storage and to suggest stability control interventions. In conclusion, evaluation of mass spectra D_B might be proposed as a new promising technique to be used as a summary measurement of the complexity of the overall composition of a phytochemical product.

PI39 Analysis of phenolic, polysaccharidic and lipidic fractions of mushrooms from northeast Portugal

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Mushrooms consumption continues to increase due to their functional benefits and presence of bioactive compounds. Herein, phenolic, polysaccharidic and lipidic fractions of wild mushrooms from Northeast Portugal (*Coprinopsis atramentaria*, *Lactarius bertillonii*, *Lactarius vellereus*, *Rhodotus palmatus* and *Xerocomus chrysenteron*) were analysed. Protocatechuic, *p*-hydroxybenzoic, *p*-coumaric and cinnamic acids were found in the phenolic fraction; ramnose, xylose, fucose, arabinose, fructose, glucose, manose, mannitol, sucrose, maltose and trehalose were quantified in polysaccharidic fraction; linoleic and stearic (only in *Lactarius* sp.) acids, and β- and γ-tocopherols were the main compounds in the lipidic fraction. Acknowledgements: PEst-OE/AGR/UI0690/2011, FCT BD/70304/2010 (S.A. Heleno), BPD/4609/2008 (L. Barros).

PI40 Characterization of flavonoid glycosides in traveller's tree (Ravenala Madagascariensis S.) leaves by HPLC-DAD-ESI-MS^n

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Flavonoids from the leaves of *Ravenala madagascariensis* S. were characterized for the first time by high performance liquid chromatography method coupled to electrospray ionization (ESI) and mass spectrometry (MS^n experiments). A total of seven flavonols glycosides derived from quercetin and isorhamnetin aglycones were identified. The comparison of retention time, UV and MS spectral data of standard compounds allowed us to assign: quercetin-3-O-rutinoside (rutin), quercetin-3-O-glucoside, isorhamnetin-3-O-rutinoside and isorhamnetin-3-O-glucoside. Identification of quercetin-3-O-robinobioside, isorhamnetin-3-O-robinobioside and isorhamnetin-3-O-galactoside was carried out by interpretation of the MS^2 and MS^3 spectra obtained in positive and negative ionization mode and by preliminary reported studies. Quantification was performed by HPLC-DAD and on our samples collected in Madagascar, rutin was the main compound (average 42%) and quantitative repartition of flavonols glycosides was variable depending on the date of harvest. The phytochemical profile obtained would be a powerful tool to establish analytical specifications in order to assess the quality control of traveller's tree extracts, for cosmetic applications.

PI41 From drupes to olive oil: How do bioactives variate during a single production procedure?

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It has been well established, that the beneficial effects of virgin olive oil (VOO) are related to its content in polyphenols and secoiridoid derivatives. Several factors, such as fruit variety, ripening stage, malaxation time, temperature etc, have been mentioned to play key role in the quality of the final product and literature data are contradictory. In the present study we monitored the qualitative and quantitative alterations of numerous bioactive polyphenols and secoiridoids, throughout VOO production from a rich in polyphenols olive variety Koroneiki, at a two-phase oil mill in Greece. The compounds were monitored, with the application of LC-DAD-ESI(-)-HRMS (LTQ-Orbitrap) platform, out of the four main steps of the production procedure: drupes, olive paste, first oil, final refined oil. All initial materials were obtained simultaneously, during a single production line and were similarly extracted with methanol, after de-fatting. The extracts were finally enriched through Diol SPE cartridges before the LC injections. The chemical profiles of extracts, pure compounds and internal standards, were monitored in full scan mode and by ion extraction, in a post-acquisition analysis. Results showed an significant increase in the dialdehydic derivatives, oleacin and oleocanthal from drupes to the oil with a simultaneous decrease in oleuropein and ligstroside, which were absent from the final product. Hydroxytyrosol content was also increased but a great quantity seems to be lost during the final oil refinement processing.

PI42 A monoclonal antibody-based elisa for the hedgehog inhibitors cyclopamine and cyclopamine-KAAD

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In the late 1960's cyclopamine was isolated from the plant *Veratrum californicum* and identified as the teratogen responsible for craniofacial birth defects including cyclops in the offspring of sheep grazing on mountain ranges in the western United States. More recently, cyclopamine was found to inhibit the hedgehog (Hh) signaling pathway which plays a critical role in embryonic development and is implicated in several types of cancer. Thus, cyclopamine and cyclopamine derivatives have been targeted as potential treatments for certain cancers and other