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BIO. NATURAL 2021

ABSTRACT BOOK

II Bio.Natural- Bioactive Natural Products Research Meeting

November 18-19, 2021 | Virtual

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ABSTRACTS

Keynote Talks

Basic Knowledge on COVID-19 and Therapeutic Strategies against SARS-CoV-2

Thomas Efferth
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Abstract

There is no disease threatening people more than COVID-19 these days. This overview lecture provides a comprehensive overview on the basic knowledge of this novel viral disease regarding epidemiology, molecular biology, pathophysiology, immunology, and pharmacology (including natural products). In the second part of the lecture, own data are presented.

We applied a workflow of combined in silico methods (virtual drug screening, molecular docking and supervised machine learning algorithms) to identify novel drug candidates against COVID-19. We constructed chemical libraries consisting of FDA-approved drugs for drug repositioning and of natural compound datasets from literature mining and the ZINC database to select compounds interacting with SARS-CoV-2 target proteins (spike protein, nucleocapsid protein, and 2'-o-ribose methyltransferase). Supported by the supercomputer MOGON, candidate compounds were predicted as presumable SARS-CoV-2 inhibitors. Interestingly, several approved drugs against hepatitis C virus (HCV), another enveloped (-) ssRNA virus (paritaprevir, simeprevir and velpatasvir) as well as drugs against transmissible diseases, against cancer, or other diseases were identified as candidates against SARS-CoV-2. This result is supported by reports that anti-HCV compounds are also active against Middle East Respiratory Virus Syndrome (MERS) coronavirus. The candidate compounds identified by us may help to speed up the drug development against SARS-CoV-2.

Marine Natural Product Research in the Omics Era

Deniz Tasdemir
GEOMAR Centre for Marine Biotechnology, Germany.

Marine biodiscovery efforts have historically relied on macro-organisms, such as sponges and corals. However, many marine metabolites, including marine drugs and drug candidates have been shown or predicted to originate from associated microbes. This fact, in addition to realization of the role of microbiome in fitness and survival of marine holobionts have led a paradigm shift in marine natural product research towards microbes. However, microbial natural product research has many dark matters too, for example only 1% of the microbial diversity can be cultivated in artificial laboratory conditions and many biosynthetic gene clusters (BGCs) (of that 1% portion) largely remain dormant or under-expressed therein. Still, marine natural product research is currently enjoying the omics techniques, high sensitivity analytical chemistry equipment, publicly available platforms for dereplication and machine learning techniques integrated into workflow. In this presentation, I will summarize some of our work on both marine macro- and microorganisms, and highlight the importance of algorithm-based metabolomics approaches for chemical profiling and targeted isolation of the new, bioactive marine natural products.

Session I: Natural Products in Drug Discovery

Oral Talks

Diterpenoids from Plectranthus spp. as a starting tool in Drug Development

Patrícia Rijo
CBIOS – Universidade Lusófona´s Research Center for Biosciences & Health Technologies, Portugal.

Abstract
Natural products represent an important source of novel therapeutic substances to fight severe diseases including cancer. The Plectranthus genus is commonly used in traditional medicine due to its potential to treat several illnesses. Diterpenoids are commonly found in Plectranthus spp., and have a widespread spectrum of biological activity, namely anticancer properties. Protein kinase C (PKC) family isoforms are recognized as therapeutic targets in anticancer drug development and have been the focus of intense research. In this work, a small library of abietane derivatives was studied for their ability to activate PKC isoforms. Several extraction methods were tested to optimize the extraction of the lead bioactive diterpenoid 7α-acetoxy-6β-hydroxyroyleanone (Roy). The results obtained revealed potent activators of PKC family proteins, namely: a selective activator of PKC*, the 7α-acetoxy-6β-benzoyloxy-12-O-benzoylroyleanone (RoyBz). The patented RoyBz was prepared using Roy as starting material. RoyBz potently inhibited the proliferation of colon cancer cells by inducing a PKC*-dependent mitochondrial apoptotic pathway involving caspase-3 activation. The results indicate that RoyBz targets drug resistant cancer stem cells, in HCT116 colon cancer cells, preventing tumor dissemination and recurrence. These results point to promising activators of PKCs with high potency and isoform-selectivity that may emerge from the exploitation of this new family of abietane diterpenoids. Molecular docking and nanotechnology studies are currently ongoing to further identify new selective abietane diterpenoids as new PKC modulators.

Funding: This work was supported by FCT UIDB/04567/2020 and UIDP/04567/2020.

Selective Natural Product Modulators of DNA G-Quadruplex with Anticancer Potential

Mattia Mori
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In the quest for selective G-quadruplex (G4)-targeting chemotypes, natural compounds have been thus far poorly explored, though representing appealing candidates due to the high structural diversity of their scaffolds. In this regard, a unique high diversity in-house library composed of ca. one thousand individual natural products was investigated. The combination of molecular docking-based virtual screening and the G4-CPG experimental screening assay proved to be useful to quickly and effectively identify-out of many natural compounds-five hit binders of telomeric and oncogenic G4s, i.e., Bulbocapnine, Chelidonine, Ibogaine, Rotenone and Vomicine. Biophysical studies unambiguously demonstrated the selective interaction of these compounds with G4s compared to duplex DNA. The rationale behind the G4 selective recognition was suggested by molecular dynamics simulations. Indeed, the selected ligands proved to specifically interact with G4 structures due to peculiar interaction patterns, while they were unable to firmly bind to a DNA duplex. From biological assays, Chelidonine and Rotenone emerged as the most active compounds of the series against cancer cells, also showing good selectivity over normal cells. Notably, the anticancer activity correlated well with the ability of the two compounds to target telomeric G4s.

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NP3 Platform: Assisting New Discoveries from Natural Products

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Abstract
Natural substances provide new chemical scaffolds for drug discovery and can probe novel enzyme binding sites and inhibition mechanisms. However, the identification of bioactive natural products and their enzyme binding mechanisms is challenging, sample and time-consuming. We have developed an integrated approach to overcome these gaps, named the "Natural Product Cube (NP3) platform, which is based on high throughput screening of pre-fractionated NP libraries, X-ray protein crystallography and mass spectrometry techniques. All the experimental inputs for NP3 are done in miniaturized scales, using high throughput techniques. Big data management and mining are performed by designed computer algorithms, returning as outputs i) the bioactive natural product structure from the biota samples and ii) its binding site in its target protein. This information is essential for proceeding to lead development steps of drug discovery projects. The NP3 platform provides this key information in the very early stages of natural product-based drug discovery, using minimal amounts of the biota sample. The NP3 approach is empowering natural product drug discovery, as it will be exemplified by recent examples.

Chemoprevention and Therapeutic Role of Phytochemicals in Glioblastoma

Célia Cabral[1,2,3*] and Mariana Magalhães[1,2,4,5], Bruno Manadas[2,5] and Thomas Efferth[6]

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Abstract
Glioblastoma (GBM) is the most common primary tumor of the central nervous system. Current treatments available for GBM entails surgical resection followed by temozolomide chemotherapy and/or radiotherapy, which are associated with multidrug resistance and severe side effects. While this treatment could yield good results, in almost all cases, patients suffer from relapse, which leads to reduced survival rates. Thus, therapeutic approaches with improved efficiency and reduced off-target risks are needed to overcome these problems. Regarding this, natural products appear as a safe and attractive strategy as chemotherapeutic agents or adjuvants in the treatment of GBM. Besides the increasing role of natural compounds for chemoprevention of GBM, it has been proposed to prevent carcinogenesis and metastasis of GBM. Numerous investigations showed that natural products are able to inhibit proliferation and angiogenesis, to induce apoptosis, and to target GBM stem cells, which are associated with tumor development and recurrence. This talk will gives a timely and comprehensive overview of the current literature regarding chemoprevention and therapy of GBM

by natural products with a focus on essential oils and phenolic compounds and their molecular mechanisms.

Armeniaspirols, A New Class of Gram-Positive Antibiotics that Target Clp Proteases

Christopher N. Boddy
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Abstract
The emergence of multi-drug resistant bacteria in the clinic presents major challenges to managing human health and threatens the great progress that has been made in preventing morbidity and mortality in the age of antibiotics. In order to combat these pathogens, new antibiotics with diverse mechanisms of action are required. Armeniaspirols represent a novel class of natural product-based antibiotics with an unknown mechanisms of action. In this talk we will disclose the mechanism of action of armeniaspirol. Using total synthesis, analogs of armeniaspirol were synthesized and their antibiotic properties examined. A combination of chemoproteomics, quantitative proteomics, and a battery of functional assays were used to discover that aremeniaspirols directly inhibit the AAA+ proteases ClpYQ and ClpXP, leading to dysregulation of the divisome and ultimately antibiotic activity. Our in vitro biochemical results were validated by in vivo by comparisons with genetic knockouts. Sub-lethal antibiotic challenges further suggested that the development of resistance to aremiaspirol inhibition of ClpYQ and ClpXP is difficult to achieve without negative consequences for the bacteria, thus resistance does not readily arrise. Synthesis of armeniaspirol analogs, followed by characterization of their biochemical activity against Clp proteases as well as minimum inhibitor and bactericidal concentrations against multidrug resistant Staphylococcus aureus has enabled the discovery of analogs with significantly enhanced potency and bactericidal activity without detectable toxicity to mammalian cells. Our results elevate armeniaspirol and its novel mechanism of action to a highly promising position for antibiotic development.

Cannabidiol: Intriguing Target and Creative Building Block

Daniele Passarella
Università degli Studi di Milano, Italy.

Recent years have seen a dramatically increasing interest in phytocannabinoids. Isolated from Cannabis in 1940, cannabidiol (CBD) is one of the most abundant phytocannabinoids in the species of Cannabis for textile uses. CBD has a low agonist effect for cannabinoid receptors; in particular, it is considered as an allosteric negative modulator of CB1 and CB2 receptors (cannabinoid receptor type 1 and 2). Current evidence showed that CBD exerts pharmacological effects via specific molecular targets such as adenosine, glycine, opioid, serotonin, nonendocannabinoid G protein-coupled, nicotinic acetylcholine, and proliferator-activated receptors. Moreover, CBD shows anticonvulsant, antispasmodic, anxiolytic, antinausea, antirheumatoid arthritis, and neuroprotective properties. Recently, it has been demonstrated that CBD is an inverse agonist for G protein-coupled orphan receptors such as GPR3, GPR6, and GPR12, suggesting new therapeutic uses of CBD for Alzheimer's disease, Parkinson's disease, cancer, and infertility.

In the frame of our interest about the use of natural products as targets, leads and building blocks we'll report our recent results about the chemistry of CBD with particular emphasis on its conversion to trans-Δ-9-tetrahydrocannabinol (Δ^9-THC).[1]

Evaluation of Extracts Obtained from Marine By-Products using Natural Eutectic Solvent Systems for Their Therapeutic Application in The Dry Eye Disease

Maha Abdallah[1,2*], Inês Leonardo[1,2], Amalia Enríquez-de-Salamanca[3,4], Yolanda Diebold[3,4], Maria González-García[3,4], Frédéric B. Gaspar[1], Ana A. Matias[1], Maria R. Bronze[1,2,5,2] and Naiara Fernández[1]

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Abstract

Hyaluronic acid (HA) is used in pharmaceutical applications due to its biocompatibility, viscoelasticity and immunostimulatory effects. In this study, it is assessed for its application in a natural carrier formulation of a drug delivery system to treat the dry eye disease (DED). This disease has a negative effect on the quality of life of patients and its symptoms include visual impairment, discomfort and eye pain. Natural HA is extracted from the marine by-product tuna vitreous humor using natural deep eutectic solvent systems (DES), as it has shown improvements in the corneal epithelial barrier. The DES are prepared using molecules such as lactic acid, fructose and urea. These solvents, the obtained extracts and the mixture of extracts solved in DES are evaluated in vitro for their application in drug formulation to treat DED. Cell viability, anti-inflammatory and antioxidant effects are assessed in human corneal epithelial cell line. The cellular viability is evaluated using XTT test and based on the obtained results, the antioxidant and anti-inflammatory effects are analyzed at different concentrations lower than the highest viable concentration. The extracts show anti-inflammatory effect by decreasing interleukin-6 secretion. The DES, extracts and the mixtures of DES with dissolved extracts show anti-oxidant effect by decreasing the generation of reactive oxygen species. Furthermore, the DES and the mixtures of DES with extracts showed antimicrobial effect by treating DED-associated gram-positive bacteria Staphylococcus aureus and gram-negative bacteria Pseudomonas aeruginosa. Hence, the tested compounds could be used as potential ingredient in a formulation to treat the DED.

Optimization of *In Vitro* Experimental Conditions for Glycogen Phosphorylase Inhibition Assay and Demonstration of High Inhibitory Effectiveness by Chromone Derivatives

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Abstract

Glycogen phosphorylase (GP) catalyzes the breakdown of glycogen to glucose 1-phosphate. Among the high variety of pharmacological interventions for type 2 diabetes *mellitus* management, the inhibition of GP is

a promising strategy for attenuating consequent hyperglycemia. The high variability of the experimental parameters of the methods developed to evaluate the inhibitory activity of GP has created contradictory results in the literature. Thus, the aim of the present study was to optimize experimental conditions to measure GP activity, using a colorimetric microanalysis technique for high-throughput screening. The obtained results allowed the choice of the optimal concentration of enzyme (0.38 U/mL), glucose 1-phosphate (0.25 mM), glycogen (0.25 mg/mL), and temperature (37 °C). Then, using the optimized experimental conditions, the inhibitory activity of a panel of 37 structurally related chromone derivatives (flavonoids, 2-styrylchromones and 2-styrylchromone-related derivatives) were evaluated. This study highlighted that the type and position of the substituents influences the inhibitory activity of the compounds. Particularly, in 2-styrylchromones and 2-styrylchromones-related derivatives, bearing hydroxy groups at the A and B rings, and in the flavonoid group, displaying hydroxy groups at the A ring, were essential for the inhibitory activity. Additionally, the effect of high levels of glucose on the IC_{50} value of the most active compounds was investigated. Interestingly, the effectiveness of the most active compounds increased almost 4-folds with higher levels of glucose, which is particularly important to diminish the risk of hypoglycemia. The present study provides significant key findings about the chromone scaffold and their potential antidiabetic effects as GP inhibitors.

Promising novel Anti-MRSA Protein Derived from Novel Streptomyces Strain Isolated from Malaysia's Mangrove Forest

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Abstract

In the Actinobacteria, the genus Streptomyces are well known for producing many beneficial bioactiv compounds useful to humanity. These compounds include antibiotics (e.g., streptomycin), immunomodulatin drugs (e.g., rapamycin), anti-helminthic drugs (e.g., ivermectin). In recent years, studies have indicated the importance of unique environments like the desert, volcanic area, and mangrove forest for bioactiv microbial discovery.

In this study, a novel Streptomyces species strain A was isolated from a mangrove forest in Pahang, Malaysia. Its crude fermentative extract exhibited potent antibacterial activities against several pathogens, including the methicillin-resistant Staphylococcus aureus (MRSA). On the contrary, Strain B, a sibling strain of Strain

A, did not display similar antibacterial activity. An analysis based on their genomic sequences revealed a biosynthetic gene cluster in strain A responsible for producing linear azol(in)e-containing peptides (LAPs). Subsequently, one of the predicted genes (1368 bp) was selected for heterologous expression, and the purified protein – Protein A (mol wt: 51889.1 gmol-1, length: 470 a.a.) was used for anti-MRSA screening via in vitro and in vivo (rodent skin infection) models. The anti-MRSA activity of protein A at 0.25µM is far more superior (~30% inhibitory activity) than the same concentration of vancomycin (<10% inhibitory activity based on in vitro screening panel. No toxicity of Protein A was observed based on the cell-based and animal study. Preliminary results from skin infection in the rodent model also revealed improved wound healing afte treatment with Protein A. In conclusion, these findings emphasize the importance of novel strains discovery from unique habitats like mangrove forests to discover pharmaceutically critical bioactive compounds.

Challenging Organic Synthesis Inspired by Nature: From Natural Products Chemistry to Drug Discovery

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Abstract

The molecular modelling is widely recognized as a fast, efficient and cost-effective method by which to identify putative small molecule hits to submit to biological assays. At Sapienza University of Rome, we settled up an in silico screening procedure to filter a unique library of 1000 natural products (NP), mainly isolated, over the years, from plant belonging to different countries, with the aim to boost the drug discovery-oriented pipeline towards the identification of new NP leads for the treatment of several disease. In recent applications of this strategy, a number of potential NP hits/leads acting on several target proteins and disease were identified. The innovation of this approach is the opportunity to identify at the same time new potential therapeutic agents acting on different targets. For istance, we identified two NP, active against antibiotic resistance and as anticancer respectively. Antibiotic resistance limits our ability to treat lung infections in cystic fibrosis (CF). Old antibiotics, such as colistin, have been reintroduced to treat multidrug resistant gram-negative infections, which his has inevitably led to the spread of colistin-resistant strains [1]. Colistin is a cationic peptide that interacts with the lipid A moiety of the lipopolysaccharide (LPS), causing membrane destabilization and cell death. Strong experimental evidence demonstrated that in P. aeruginosa colistin resistance depends on the covalent addition of 4-amino-4-deoxy-L-arabinose (Ara4N) to lipid A, which reduces colistin affinity for LPS [2,3]. On the basis of the resolved crystallographic structure of ArnT [4], the last enzyme of the pathway leading to lipid A aminoarabinosylation, we performed an in silico molecular docking approach that allowed to identify a compound capable to potentiate colistin activity against a colistin-resistant P. aeruginosa tester strain at relatively low concentrations. Based on computational and organic chemistry studies, some analogues have already been synthesized. The toxicity and efficacy of synthesized compounds will be assessed in different in vitro and in vivo model systems.

Hedgehog signaling is essential for tissue development and stemness and its deregulation has been observed in many tumors. Aberrant activation of Hedgehog signaling is the result of genetic mutations of pathway components or other Smo-dependent or independent mechanisms, all triggering the downstream effector

Gli1. For this reason, the identication of novel molecules blocking the pathway at a downstream level, represents a critical goal in tumor biology. We clarified the structural requirements of the pathway effector Gli1 for binding to DNA and identify Glabrescione B as the first small molecule binding to Gli1 zinc-finger and impairing Gli1 activity by interfering with its interaction with DNA. Remarkably, as a consequence, Glabrescione B inhibited the growth of Hedgehog-dependent tumor cells in vitro and in vivo as well as the self-renewal ability and clonogenicity of tumor-derived stem cells. The identification of the structural requirements of Gli1/DNA interaction highlights their relevance for pharmacologic interference of Gli signaling [4].

Using Concatenated NMR and MS Analytical Data for Metabolomic-guided Production and Isolation of Bioactive Natural Products

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Abstract
Metabolomics tools that include multivariate analysis have been applied on concatenated LC-HRMS and NMR datasets to afford an intensive chemical profile of extracts from various natural sources to get a snapshot of the bioactive metabolites present in respective collections. Obtained LC-HRMS data could be processed with MZmine 2 or other MS-based metabolomics software and dereplicated using an in-house Excel Macro to couple the MS data with a database such as the Dictionary of Natural Products and/or an in-house database. However, structures of dereplicated compounds in extracts could only be validated by employing 2D NMR data and MS fragmentation that could be utilized for molecular networking. The use of fused datasets from both MS and NMR provide further structure information on the targeted bioactive metabolites along with multivariate analysis. A supervised multivariate analysis such as orthogonal partial least squares discriminant analysis (OPLS-DA) could afford statistically robust predictions to pinpoint the plausible bioactive components.

Generation of Natural Product Libraries for High Throughput Screening

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Abstract
The NCI Program for Natural Products Discovery is a newly launched, priority program for the NCI. The new initiative aims to generate pre-fractionated extracts (up to 1,000,000) for modern high-throughput targeted screening technologies and develop integrated analytical resources for the isolation and structure elucidation of biologically active natural products. Development of high-throughput, high-capacity and fully automated methodology towards the generation of the prefractionated library as well as rapid isolation and identification of the active principles will be presented. In addition, work on the creation of a bioinformatics platform capable of integrating taxonomy, biological activity, and analytical chemistry data will be discussed.

Allicin from Garlic Inhibits DNA Gyrase Activity in Bacteria

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Abstract

Allicin is a natural sulfur-containing antibiotic from garlic which is synthesized via an enzymatic reaction upon cell damage. Its antibiotic properties are based on its ability to induce oxidative stress by *S*-thioallylation of accessible thiols in peptides- and redox buffers like glutathione. In order to discover protein targets of allicin, a differential isotopic labelling method (OxiCAT) was used to characterize the oxidation status of thiol-containing proteins before and after sublethal allicin treatment in susceptible *Pseudomonas fluorescens Pf*0-1 and in *Pseudomonas fluorescens* Allicin Resistant 1 (*Pf*AR-1). In both strains 77% of the proteome was in a highly reduced state (*i.e.* less than 20% cysteine oxidation) but after allicin treatment the *Pf*0-1 proteome became more highly oxidized than the *Pf*AR-1 proteome (1). In untreated cells DNA-gyrase subunit A (GyrA) was ~6% oxidized and this increased to ~55% in *Pf*0-1 but to only ~10% in *Pf*AR-1 after allicin treatment, identifying DNA-gyrase as a major allicin target in *Pf*0-1. Furthermore, allicin inhibited *E. coli* DNA-gyrase activity *in vitro*. Reciprocal *gyrA* gene replacements between *Pf*0-1 and *Pf*AR-1 were carried out to test whether *Pf*AR-1 GyrA was responsible for increased allicin resistance of *Pf*AR-1. However, neither was *Pf*0-1 rendered more resistant, nor *Pf*AR-1 more susceptible. In conclusion, although allicin can effectively inhibit DNA gyrase activity in bacteria, *Pf*AR-1 GyrA is not *per se* resistant against *S*-thioallylation, but rather is protected by being in the *Pf*AR-1 genetic background (1,2).

1. Reiter et al. (2020) Int. J. Med. Microbiol. doi.org/10.1016/j.ijmm.2019.151359
2. Borlinghaus et al. (2020) Life Science Alliance doi.org/10.26508/lsa.202000670

ABC Transporters: From Multidrug Resistance to Cystic Fibrosis

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Abstract

The estimated number of new cancer cases is expected to grow 33% by 2030, with an estimated increase of 35% in the total number of deaths. Although having a multifactorial origin, multidrug resistance (MDR) can be achieved through efflux pump-mediated resistance in which P-glycoprotein (Pgp/ABCB1), Multidrug Resistance Protein 1 (MRP1/ABCC1), and Breast Cancer Resistance Protein (BCRP/ABCG2) were found to be markers of overall poor chemotherapy response and prognosis. Cystic fibrosis (CF) is caused by mutations in the gene encoding the CFTR/ABCC7 protein, a chloride, and bicarbonate anion channel being the most common life-threatening rare disease (median age of death ~31 years) and affects ~50 k individuals in Europe. In this communication, we will present our studies involving the molecular modeling of Pgp, BCRP and CFTR, focusing our attention on the efflux/gating mechanism, mutation impacts, and on studies aiming at optimizing the properties of novel drug-like molecules from natural sources targeting these proteins.

Ziziphus Mauritiana Lam Attenuates Airway Inflammation *via* Downregulating NFκB Pathway in Both *in-vitro* & *in-vivo* Models of Asthma

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Abstract

Ziziphus mauritiana Lam leaves were utilized in treating asthma, diabetes, inflammation, and hepatic diseases in the Indian tradition medicine. It is used for the treatment of jaundice in children. The aim is to prove the anti-asthmatic activity of methanol extract *Z. mauritiana Lam* leaves (MEZ). Terpenoids present in MEZ was quantified using UPLC analysis. For *in-vitro* activity, MEZ at 50 &100µg/mL was tested against LPS stimulated RAW 264.7 macrophage cell lines. The NO, ROS and cytokines levels were measured from the cell culture supernatants. The OVA-induced asthma in mice model was adopted for the *in-vivo* activity. MEZ at 250 & 500mg/kg was screened for airway hyper responsiveness, leukocyte counting, pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 & IL-17) and serum IgE levels. ICAM, VCAM and Muc5AC gene expressions and mechanism underlying attenuation of NFκB signaling pathway for asthma were also observed. MEZ reduced the NO, ROS and cytokines in LPS stimulated RAW 264.7 macrophages. It also reduced the total and differential leukocyte counts in asthmatic mice. MEZ 500mg/kg significantly reduced the expression levels of ICAM, VCAM and Muc5AC genes. Both the in-vitro & in-vivo analysis confirmed the suppression of the lung inflammation by inhibition of p65 mediated IκB-α translocation in NFκB signalling inflammatory pathway. These finding prove that, MEZ significantly reduced the airway inflammation by reducing oxidative stress, pro-inflammatory cytokines and inhibiting NFκB signalling pathway. Hence, this study proved that *Z. mauritiana Lam* has anti-asthmatic potential utilised in Indian traditional medicine.

Harnessing the Chemical Potential of Unprecedented Microbes from the Great Salt Lake

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Abstract

Environmental pressures have been shown to influence the structural diversity of compounds produced in Nature, and microorganisms thriving in extreme environment often produce chemical agents not observed in their terrestrial counterparts. The Great Salt Lake, also recognized as “America’s Dead Sea”, is an endorheic hypersaline lake located near the University of Utah. While seawater has an average salinity of ~3.3%, the Great Salt Lake ranges between 8-28%. Recently, our lab started a natural products drug discovery campaign aimed at interrogating halophilic bacteria isolated from this unique environment. Our preliminary data demonstrate that the unexplored hypersaline microorganisms of the Great Salt Lake produce metabolites containing molecular scaffolds never before observed, and their genomes contain unprecedented biosynthetic machinery. Thus, these microbes serve as an ideal resource for the discovery of new therapeutic agents. Fermentation studies with two of these strains led to the isolation and elucidation of the bonnevillamides and salinipeptins, which are new classes of linear nonribosomal heptapeptides and ribosomally synthesized and post-translationally modified peptides, respectively. The discovery and characterization of these new chemical entities as well as their corresponding biosynthetic machinery will be discussed.

Pharmacokinetics of Oleandrin After Administration of a Nerium Oleander Extract in Mice

Sara Abdennour[1*], Mohamed Tahar Taha Derouiche[2], Ludovic Romeuf[3], Yvan Gaillard[3], Farid Dalia[1] and Mohamed Azzouz[4]

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Abstract

Background: Nerium oleander Linn is a toxic shrub which belongs to the Apocynaceae family. It is native of Mediterranean regions of Africa and Europe where it is used in folk medical practices. The whole plant is poisonous. Some studies have shown its antimicrobial and anticancer activity, but there are few published data on pharmacokinetics.

Purpose: to determine some pharmacokinetic parameters in mice after oral and I.V. administrations of hydroalcoholic extract of Nerium oleander.

Methods: Pharmacokinetic studies of oleandrin, a cardiotonic glycoside and main active compound of Nerium oleander were conducted in mice after administration of an I.V. dose (30 mg/kg) and a P.O. dose (150 mg/kg) of an hydrolcoholic extract of Nerium oleander. Oleandrin was measured by LC-MS/MS.

Results : oleandrin was rapidly absorbed after oral administration (Cmax at 10 min). The AUC0-inf (ug/L*min) values obtained after I.V. and P.O. dosing were 34797,7 and 107222, respectively, resulting in an oral bioavailability of 61,6 %. The apparent volume of distribution Vss was 1,3 L/kg and Clearance ClT was 0,01 L/min*kg.

Poster Presentations

Evaluation of the Antidepressant Effect and Possible Mechanism of Action of the Essential Oil of Litsea Glaucescens (Kunth) (Mexican Laurel) Administered by Inhalation

Jessica Karina Díaz-Cantón[1,2*], Silvia Laura Guzmán-Gutiérrez[1,2], Mayra León-Santiago[1], Mónica Adriana Torres-Ramos[3] and y Ricardo Reyes-Chilpa[1]

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Abstract

Depression, is a mental disorder that is associated with a wide range of emotional, cognitive and physical symptoms that affects more than 350 million people worldwide and according to the World Health Organization, has been classified as the leading contributing factor to global disability (WHO, 2017; Blackburn, 2019). In addition, the COVID-19 pandemic has paralyzed mental health services in 93% of the world's countries (WHO, 2020).

In addition, brain pathologies, such as depression, are associated with a negative regulation of BDNF expression, resulting in reduced levels of this protein in the brain (Giacobbo et al., 2019). There is a hypothesis, which states that stressors reduce BDNF-mediated signaling, while antidepressant treatments increase it (Liu et al., 2019).

Antidepressant drugs, currently based on the monoaminergic hypothesis, are ineffective and have worrying side effects (Chávez-Castillo, 2019). However, alternative therapies such as aromatherapy have been sought, which has received increasing attention, because there is preclinical and clinical evidence showing that this practice can be used as a treatment in patients with depression (Díaz-Cantón, 2018; Zhang et al., 2020; Mohammed et al., 2020).

Therefore, in this work we evaluated the antidepressant effect of the essential oil of Litsea glaucescens by inhalation and explored a non-monoaminergic pathway (BDNF) as a possible mechanism of action of the effects of laurel oil.

Alkaloid Fingerprinting, Antiproliferative and Cholinesterase Inhibition Potential of Rhodophiala Advena Bulbs

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Abstract

Plants belonging to the Amaryllidaceae family are known for the biosynthesis of pharmacologically active alkaloids. Traditionally, plants extract of this family have been used as folk medicine for cancer. Galanthamine, an acetylcholinesterase (AChE) inhibitor, is well-known for being the most important Amaryllidaceae alkaloid used for the treatment of Alzheimer´s disease (AD). The structurally variety and the pharmacology properties of Amaryllidaceae alkaloids have attracted the interest for their identification and characterization. In this study, the alkaloid fingerprinting from the Chilean endemic Rhodophiala advena were investigated by high resolution mass spectrometry (HR-MS). Additionally, the cholinesterase inhibitory potential against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), and the antiproliferative effects in solid tumor cells line were assessed. Several alkaloids were detected from the methanolic extracts of the bulbs of R. advena by HR-MS. The skeleton type alkaloids identified were lycorine, homolycorine, montanine and narciclasine. R. advena showed good inhibitory activity against AChE and BChE enzymes (4.91 µg/mL and 21.12 µg/mL). In addition, R. advena showed activity in a panel of six solid tumor cancer cells (A549 (lung), HBL-100 (breast), HeLa (cervix), SW1573 (lung), T-47D (breast), and WiDr (colon) with IG50 values in the range of 13-32 µg/mL. Our findings suggest that Rhodophiala advena bulbs have several bioactive alkaloids with antiproliferative activity and inhibitory effects with potential use in neurodegenerative diseases.
Funding: C.F.-G. acknowledges Postdoctorado FONDECYT 3190794.

Phytochemical Study and Anti-Glioblastoma Activity Evaluation of Plectranthus Hadiensis (Forssk.) Schweinf. ex Sprenger var. Hadiensis

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Abstract

Glioblastoma (GBM) is the most malignant form of primary astrocytoma, accounting for more than 60% of all brain tumors in adults (1). Current treatments available entailed surgical resection followed by temozolomide chemotherapy and/or radiotherapy, which are associated with multidrug resistance, severe side effects, relapse and reduced survival rates. Therefore, new therapeutic approaches are needed to overcome these problems (2). The plant genus Plectranthus belongs to the Lamiaceae family is known to be rich in abietane-type diterpenes with have antitumor activity. Specifically, P. hadiensis(Forssk.) Schweinf. ex Sprenger has been documented to be used against brain tumours (1). Therefore, the aim of this work is to present the results concerning the extraction, fractionation, and compound isolation from P. hadiensis, and their in vitro anti-glioblastoma activity. After extraction, six fractions were obtained from P. hadiensisstems acetonic extract. The isolation results showed the presence of different diterpenes type-abietanes, such as 7α-acetoxy-6β-hidroxyroyleanone (Roy) and 6β,7β-dihydroxyroyleanone (DiRoy) which was also in agreement with the HPLC profile. A) B) Figure 1. Chemical structures of A) 7α-acetoxy-6β-hidroxyroyleanone (Roy) and B) 6β,7βdihydroxyroyleanone (DiRoy). Furthermore, the antiproliferative activity was also assessed in a panel of 5 GBM cell lines, emphasizing that, after 48 hours of treatment, Roy was able to induce a strong antiproliferative/cytotoxic effect against tumor cells, presenting low IC50 values for the different cell lines (U373 – 6.21 µg/mL; U118 – 8.84 µg/mL; H4 – 18.09 µg/mL; U87 – 23.19 µg/mL; A172 – 33.05 µg/mL). Currently, the phytochemical study is being completed and bioactivity studies of these constituents are being performed.

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New Potential Pharmacological Targets of A Plant-Derived Hydroxyanthraquinone from *Rubia Cordifolia* L.

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Abstract

The various pharmacological effects of polyphenols and their increasing use as food additives in the last years pose the need for identification of new potential pharmacological targets in their action. Recently we reported a study that estimated structural similarity between plant-derived phenolic compounds and drug molecules from the DrugBank database (https://www.drugbank.ca). A representative dataset of 75 phenols selected from the literature and a virtual library of 7770 drug compounds extracted from the DrugBank were used. Similarity was estimated by OpenEye ROCS (https://www.eyesopen.com/). Pseudopurpurin, a plant-derived hydroxyanthra-quinone from *Rubia cordifolia L.*, was found to be most similar to Gatifloxacin, a synthetic broad-spectrum antibiotic agent, suggesting two possible targets of pseudopurpurin – DNA gyrase and DNA topoisomerase IV. In the present study we extended our *in silico* research by structure-based

analysis of the interactions of pseudopurpurin in appropriate crystallographic 3D structures of the proteins. In parallel, experimental *in vitro* studies are designed to prove the relevance of the *in silico* predictions. The results are a further step in the development of *in silico* protocol for prioritisation of phenolic compounds as healthy dietary supplements.

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Mining the Chemodiversity of Diterpenoids in Scutellaria Baicalensis

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Abstract

Plants produce an enormous number of complex molecules called secondary metabolites. Many of these compounds have life-saving properties and are used in both traditional and modern medicine. Scutellaria baicalensis, from the Lamiaceae family, is important in Chinese traditional medicine has been used for over 2000 years as a common herbal remedy and as complementary cancer treatments. Labdane related diterpenoids are a large set of natural products whose chemical complexity is significantly contributed by various diterpene skeletons provided by class II and class I diterpene synthases. We identified nearly all known diterpene synthase genes involved in biosynthesis of the diterpenoid backbone in S. baicalensis. The diterpene synthase genes were cloned, and the enzymatic activity functionally identified using in vitro assay. These results assign ten of them to three different diterpene biosynthesis pathways. SbTPS2.2 and SbTPS2.3 were identified as normal-CPP and SbTPS2.5 as ent-CPP synthase. SbTPS1.1 reacts with SbTPS2.2 and SbTPS2.3 to produce miltiradiene, involved in the abietanes pathway. SbTPS1.2 reacts with SbTPS2.5 to produce ent-kaurene, involved in the gibberellin's pathway. Surprisingly, SbdiTPS1.3 reacts with SbdiTPS2.7 producing isokolavenol, revealing a novel activity isokolavenol synthase. It has emerged that the genes encoding certain natural product pathways are organized in biosynthetic gene clusters within plant genomes. The genes SbdiTPS1.1, SbdiTPS2.2 and SbdiTPS.2.3 are physically adjacent genes located in the chromosome 6 of S. baicalensis. We found that the diterpene synthase class II and class I genes involved in miltiradiene biosynthesis are part of a specific to Lamiaceae family plants biosynthetic gene cluster

Session II: Marine Natural Products

Oxy-Polybrominated Diphenyl Ethers from the Indonesian Marine Sponge Lamellodysidea Herbacea: Isolation, Reaction, Computational Study, and SAR

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Abstract

Marine natural polybrominated diphenyl ethers (PBDEs) are promising medicinal potential for anticancer and antibacterial drugs. The molecules are especially frequently found in the family of Dysideidae especially Lamellodysidea herbacea and Dysidea granulosa which showed a different chemotaxonomy marker. Recently, the marine polybrominated diphenyl ethers were expected to biosynthesize by symbiotic marine cyanobacteria Hormoscilla spongeliae, marine bacteria Pseudoaltromonas spp., or prochloron. Despite the interesting discussion of the PBDE origin within the marine organisms, we tried to reinvestigate PBDE molecules from the abundant marine sponge L. herbacea collected from Indonesian waters to see the medicinal potential for anticancer and antibacterial drugs through isolation, reaction works, and computational studies. Six PBDEs were isolated from the title of sponge L. herbacea and structure modification through transformation of phenol functional group by methylation and acetylation reaction to give seven molecules and an interesting debromination reaction with the presence of scavenger of bromine to give nine molecules. In regard with the exploration reaction, we also found that acetylation reaction could undergo with the presence of Ac2O and sonication without catalyst and solvent at room temperature for around 2-3 h. Two compounds have

new crystal structures, while some are new structures. One unpublished 13C NMR of is also reported in this presentation together with the structure-activity relationship of marine polybrominated natural products against human embryonic kidney (HEK293T) cells and Gram-positive Staphylococcus aureus as well as Gram-negative bacteria Klebsiella pneumoniae and their computational study to support their activity and rationalize the acetylation and debromination reactions.

Posters

Antineoplastic Extracts from the Giant Deep-sea Worm *Riftia Pachyptila* on Glioblastoma Cells

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Abstract

Bioactive marine compounds have been isolated with potential for pharmaceutical and biotechnological development, especially antineoplastic drugs. *Riftia pachyptila* is a marine invertebrate that lives near hydrothermal vents at 2000 meters depth, under drastic temperature changes (4-300 ° C) and reduced nutrients concentrations. The organism was separated into three parts: trophosome (Tr), wall (Pa) and, tube (Tu), and crude extracts were obtained with dichloromethane-methanol (1: 1). The extracts' cytotoxic effect was determined on human glioma cells U373 and U87 and normal fibroblasts BJ1, using 0.1, 1, 10, 50, 100, and 200 µg/mL extract for 24 h. The extracts were cytotoxic on human glioma cell lines; nevertheless, the Pa extract showed the highest concentration-dependent effect. Unexpectedly, fibroblasts did not show alterations in cell viability.

Consequently, we studied the possible molecular mechanism involved in the cell death induced by Pa's extract. We found a positive mark in the TUNEL assay and increased the cytoplasmatic p53 expression in both glioma cell lines. Furthermore, the chemical analysis of Pa by mass spectrometry and nuclear magnetic resonance showed the presence mainly of fatty acids in the extracts, being the most abundant oleic acid; however, it did not exert a cytotoxic effect on lines U87 and U373, suggesting that the antineoplastic effect by Pa is due to another component in the extract. In conclusion, crude extracts of *Riftia pachyptila* are antineoplastic in

human brain cancer, and our results suggest that the induced cell death is p53-dependent apoptosis.

Implementing Metabolomic Tools for The Isolation of Anti-Biofilm Metabolites from Endophytic *Dendryphiella Salina* Isolated from Scottish Marine *Laminaria Hyperborea*

Saif Aldeen Jaber[1,2*], Louise Young[1], Kirsty Black[3], Rothwelle Tate[1] and RuAngelie Edrada-Ebel[1]

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Abstract

Biofilm formation by pathogens has played a vital role in multi-drug resistance bacteria like those of Staphylococcus aureus and Pseudomonas aeruginosa. An increasing number of novel bioactive compounds is recently being isolated from marine endophytic fungi. In this study, metabolomic approaches have been used to aid media selection for optimum fungal growth and increase the yield of antibacterial secondary metabolites.

The endophytic fungus Dendryphiella salina obtained from marine Scottish seaweed Laminaria hyperborea was scaled-up in two different media namely: malt extract broth with sea salt and oat solid media without sea salt. Incubation of D. salina was done for 30 days for both media. Crude extracts were fractionated by medium pressure liquid chromatography. NMR and high-resolution mass spectral data of the fractions were subjected to multivariate analysis to target the possible bioactive metabolites that were prioritized for isolation work. The fractions were subjected to biofilm inhibition test by Planktonic assay.

D. salina grown on malt extract broth yielded linoleic acid, orsellinic acid, orcinol and 2,5-dihydroxy-3-(hydroxymethyl)benzoic acid. While D. salina inoculated on oat solid media afforded the acetonide and glycerylglycoside derivatives of linoleic acid along with the peptide turnagainolide A. All isolated compounds, except for orcinol and linoleic acid, displayed antibiofilm activity with MBEC (Minimum Biofilm Eradication Concentration) values of less than 35 µg/ml against biofilm forming bacteria S. aureus and P. aeruginosa. Turnagainolide A and the acetonide congener of linoleic acid gave the most potent MBEC values of 25.18 and 30.04 µg/ml against S. aureus and P. aeruginosa, respectively.

Session III: Natural Cosmetics

Oral Talk

Trends in Nanotechnology for Skin Anti-Aging Strategies and the Formulation of Bakuchiol in a Novel Nanocarrier

Duha Alamoudi and Satyanarayana Somavarapu
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Abstract

Anti-ageing compounds fight the appearance of ageing in the skin, addressing wrinkles, sagging, discolouration,

and dehydration. Currently, several compounds are available to reduce signs of ageing. This review focuses on describing the latest studies examining topical anti-ageing treatments for the skin, including both synthetic and natural products. Product formulation plays an important role in the delivery of these molecules, allowing them to bypass the primary skin barrier, called the stratum corneum. Many formulations have been explored, including simple creams and emulsions and the more complex nano formulations. Nano formulations for antiageing treatment is a particular focus of this review, representing an emerging and promising field. Although some molecules are currently being studied as topical nano anti-ageing treatments, this research area still has few studies. In the cosmetics industry, many companies have formulated products using nano formulas that claim to reduce the signs of ageing, using synthetic or natural compounds or a mixture of both. In addition, this review discusses the hypothetical formulation of a botanical compound, bakuchiol, which is known to have similar activity as retinol, and explores its potential as a novel nanocarrier. Three nanoemulgel having a positive zeta potential charge were hypothetical created one with Bakuchiol, second with retinol and a third with both compounds. The nano formulations were tested, and the hypothetical results are described here, based on the findings in this exciting new field of the literature.

Posters

Using Lipids Extracted from Black Soldier Fly Larvae for Nanoparticles Development - Preliminary Studies

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Abstract

The biomass from Black Soldier Fly (BSF) larvae (Hermetia illucens) is known to be rich in proteins, lipids, minerals, vitamins [1,2]. BSF larvae are already being used as feed in aquaculture, however, since its lipid content is mainly composed of saturated fatty acids (FA), its biomass has a great potential for new ingredients development [3]. FA are critical in skin barrier function; thus, the lipid fraction of BSF larvae biomass can be foreseen to provide barrier recovery and emollient ingredients for topical formulations. Different methods of extraction were then tested and, in general, the lauric acid (C12:0) was prevalent in all extractions, followed by the palmitic (C16:0), linoleic (C18:2), and oleic (C18:1n-9) acids. This blend of FA was used in the preliminary tests for the development of formulations containing lipid nanoparticles as a promising strategy for drugs encapsulation combined with skin protection. The lipid extract was compared with commercial lipid solids (Precirol® ATO 5, Gelucire®, Stearic Acid), and a mixture of them in different proportions. Results showed that the use of only the extract in the nanoformulations provided particles with a size under 200 nm and a polydispersity index (PDI)

Innovative Formulations Loading Phytochemicals to Tackle Skin Ageing: Preliminary Studies

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Abstract
Anti-ageing formulations aim at achieving healthy, smooth, blemish-free skin by using substances that can reverse the (epi)dermal signs associated with skin aging (1). The users' demand for innovative, natural, efficacious, and safe products has been triggering the development of formulations containing phytochemicals. Gallic acid (GA) and dehydroabietic acid (ADA) are examples of phytochemicals with anti-aging properties (2,3), however, their physicochemical properties difficult their formulation for cutaneous applications. Thus, this work aimed to develop conventional semisolid formulations, as well as nanotechnology-based formulations to load GA and ADA. Concerning conventional formulation, a O/W cream was produced to load ADA, while a gel was produced to load GA according to their lipophilic and hydrophilic properties, respectively. Moreover, solid lipid nanoparticles (SLN) were developed to load both phytochemicals. All formulations were characterized in terms of organoleptic properties, pH, viscosity, and, in the case of SLN-containing formulations, hydrodynamic diameter (Dh) and polydispersity index (PDI). The stability of the formulations was followed for 30 days upon storage at 5, 25, and 40 (± 2) °C. Overall, both semisolid formulations displayed pH and viscosity values compatible with skin applications for 30 days. It is noteworthy that the color of GA-containing gel was not stable for 30 days, from yellow to brown, suggesting that the phytochemical was undergoing oxidation. Freshly made SLN-containing formulations displayed favorable Dh (300- 400 nm) and PDI (400 nm) and PDI (<0.2) values. However, Dh and PDI values increased upon room temperature storage for 30 days, reason why these nanotechnology-based formulations must be optimized in the future.

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Session IV: Natural Products Chemistry

Oral Talks

Saponins from Raphia vinifera fruit and their Cytotoxic Activity

G. Fru Chi[1*], J. Omollo Ombito[3], A. T. Mbaveng[2,4], V. Kuete[2,4] and T. Efferth[4] and B.T. Ngadjui[1]

[1]*University of Buea, Cameroon; University of Dschang, Cameroon; University of Botswana, Botswana; Johannes Gutenberg University, Germany; University of Yaoundé I, Cameroon;*

Abstract
Cancer is an ever-rising cause of dead in the world today. According to the WHO Cancer profile 2018, over 18 million cancer cases and over 9 million cancer related deaths were recorded. The number of cancer cases keeps surging every year. It is common knowledge that feeding habits may increase the risk of developing one or another form of cancer but may actually prevent development of cancers. How this prevention occurs is in the molecules that come with these foods. Some drugs used to treat cancer such as taxol, paxitaxol were isolated from sea weeds and plants respectively. Recently, many plant-derived molecules have shown interesting anticancer potency and are worth considering for clinical trials particularly saponins. Saponins may yield the next drug against cancer. Thus, our recent focus on bio-guided isolation of secondary metabolites from food and medicinal plants by chromatographic techniques. Structure determination by NMR, MS, IR and

UV spectroscopic techniques. The cytotoxicity of extracts and isolates have been evaluated by in vitro flow cytometry assay. The mechanism of action of some of these molecules have also been elucidated to show their anticancer properties. In this lecture the isolation, structure elucidation, and bioassay of secondary metabolites from food/medicinal plants: *Raphia vinifera* (Arecaeae) will be presented. Focus will be on saponins as potential class of anticancer molecules.

Structural Elucidation of Complex Microbial Natural Peptides Aided by Biosynthetic Gene Cluster Analysis

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Abstract
Natural peptides frequently possess complex structures that make their structural elucidation a challenging task requiring the use of different spectroscopic and chemical approaches. In general, a combination of several spectroscopic techniques, including HRMS, MS/MS sequencing and NMR, combined with hydrolysis and Marfey's analysis is employed to solve the full structure and absolute configuration of complex peptides. The coexistence in the same molecule of several units of a given amino acid residue with a mixture of L and D configurations complicates this task and requires the use of additional information to successfully solve the structure.
The development in the last decade of fast and affordable next generation sequencing technologies has facilitated an enormous breakthrough in whole genome sequencing of microbial strains. Bioinformatics tools have also been developed in parallel to mine the biosynthetic potential of the sequenced genomes and, among others, they allow to predict the constituent amino acids and their absolute configuration in natural peptides. The combined use of those predictions with spectroscopic and Marfey's analysis has been successfully applied in our laboratory to the determination of the full absolute configuration of two families of natural antibiotic peptides, the krisynomycins, isolated from *Streptomyces canus* and cacaoidin form *Streptomyces cacaoi*. The rational followed in the structural elucidation of these molecules will be presented.

Use Of Statistical Tools To Accelerate The Natural Products Workflow – A Malpighiaceae Case Study

Helena Mannochio-Russo[1,2*], Paula C. P. Bueno[3], Anelize Bauermeister[2], Rafael F. de Almeida[4], Pieter C. Dorrestein[2], Alberto J. Cavalheiro[1] and Vanderlan S. Bolzani[1]

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Abstract
The natural products research field consists of studying samples from natural origin, which may be extremely complex due to the wide diversity of biosynthetic pathways involved in the secondary metabolism. This high complexity makes the natural products workflow challenging, mainly due to the high efforts one must put into the chromatographic method development to obtain proper peak separation for quantitative and qualitative analysis. This is especially critical for hyphenated techniques, such as liquid chromatography-mass

spectrometry (LC-MS), since the co-elution of compounds may lead to ion suppression or enhancement[1]. Thus, developing a proper chromatographic method will lead to more accurate representation of the chemical space of a given natural sample. Therefore, our objectives consisted of applying Quality by Design, statistical analysis, equipment automation, and commercial software to perform a computer-assisted LC method development of a mixture of nine Malpighiaceae species extracts. This approach allowed us to perform the data collection and method development procedure (screening, optimization, and robustness simulation) in only four days, resulting in very low detection and quantification limits for different classes of compounds. The chromatographic method developed was employed in LC-MS analysis, leading to the annotation of 61 compounds by molecular networking and library searches within the GNPS libraries[2,3], comprising phenolic compounds and sterols. The results obtained show that the natural products workflow can be accelerated with the methodology followed, and that time and resources were substantially reduced. Further studies are being performed to evaluate the impact of the chromatographic method in annotation rates in other Malpighiaceae species.

Antioxidant and α-glucosidase inhibitory activities of *Acacia saligna* extracts

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Abstract

Acacia saligna, a small tree that belongs to a family Fabaceae, is native to Western Australia; it is. Gradual polarity solvent-fractionation has been done on the flowers, leaves, and barks of *A. saligna* by sequential maceration in hexane, dichloromethane, methanol, and water. The fractions were screen for their antioxidant and α-glucosidase inhibition properties. We here report the inhibitory activities of the factions against 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) free radicals and α-glucosidase enzyme. Methanolic extracts were found to be the most active, and among them, the methanolic extract from the barks showed the strongest in inhibiting DPPH, $ABTS^{\bullet+}$, and α-glucosidase (IC_{50} of 94.24 ± 19.89, 49.77 ± 6.164, 4.373 ± 0.2369 µg/mL, respectively). Purification of the bioactive methanolic fractions was also performed. The isolated compounds such as flavonoid and inositol derivatives will also be discussed in this presentation. Our finding indicates that *A. saligna* could be a rich source of bioactive phytochemicals responsible for the observed activities.

Posters

New Hit Ester Derivatives to Target Protein Kinase C Isoforms using a Natural Diterpenoid

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Abstract

Natural products provide novel scaffolds with potential biological activities. Abietane diterpenoids are bioactive naturally occurring molecules isolated from *Plectranthus* spp. (Lamiaceae). The compound 7α-acetoxy-6β-hydroxyroyleanone (**Roy**, **Figure 1**) can be obtained from *P. grandidentatus* Gürke in high yields [1]. **Roy** exhibited activity in breast cancer cell lines of different malignancies and showed to be a potential lead molecule for the interaction with a promising target protein kinase C (PKC) isoforms [2].

In this work, we isolated **Roy** in high amounts to be derivatized and enhance its cytotoxic properties. In previous studies, we proved that ester derivatives from royleanones are the most stable and bioactive molecules [3]. Thus herein, we studied the adequate conditions for the preparation of new ester derivatives from **Roy** using the two hydroxyl groups suitable for derivatization (**Figure 1**). Considering the extraction and isolation of **Roy**, the acetone ultrasonic-assisted extraction (yield of 2.3 %, w/w) was performed using the aerial parts of *P. grandidentatus* affording 1 g (≈ 0.04 %, w/w) of pure **Roy**. Reactivity study pointed to the 12-OH position as the most reactive for esterification. It afforded ester derivatives, using mild conditions, with overall good yields (36-88 %). For both positions derivatization, high temperature (50 °C), excess of reagents, and higher reaction time are recommended. New ester hit derivatives are currently in preparation based on this reactivity report, focus on PKC isoforms modulation.

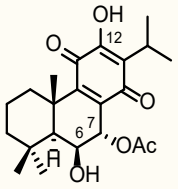

Figure 1: 7α-acetoxy-6β-hydroxyroyleanone (**Roy**)

Self-assembly Nanoparticles of Three Abietane Diterpenes from Plectranthus Species

Epole Ntungwe[1,2*], Eva María Domínguez-Martín[1,2], Catarina Garcia[1,2], Andreia Rosatella[1,5], Iris Guerreiro[1], Eleonora Colombo[3], Lucilia Saraiva[4], Ana María Díaz-Lanza[2], Catarina Reis[5], Daniele Passarella[3] and Patrícia Rijo[1,5]

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Abstract

Self-assembly drug conjugates have shown to be a promising approach for drug delivery. These conjugates can overcome drawbacks such as low drug solubility and side effects [1,2]. *Plectranthus* plants have historically been used in traditional medicine and are a known source of bioactive products particularly royleanones [3]. In this work, three molecules derived from *Plectranthus* spp. were employed as lead molecules for the synthesis of self-assembled nanoparticles. The cytotoxic royleanone 7α-acetoxy-6β-hydroxyroyleanone (**1,** Roy) isolated from *P. hadiensis* (Forssk.) Schweinf). ex Sprenger leaves, its hemisynthetic derivative 7α-acetoxy-6β-benzoyloxyroyleanone (**2,** 12BzRoy) and 6,7-dehydroroyleanone (**3,** DHR), isolated from the essential oil of *P. madagascariensis* were employed in this study. The royleanones were conjugated with squalene (sq), oleic acid (OA), and/or 1-bromododecane self-assembly inducers. Roy-OA, DHR-sq, and 12BzRoy-sq conjugates were successfully synthesized and characterized. From the obtained DLS results, Roy-OA nanoparticles (NP) assemblies show a promising size (509.33 nm), PdI (0.249), zeta potential (-46.2mV),

and spherical morphology. In addition, these NPs had a low release of Roy at physiological pH 7.4 after 24hrs. Bioactivity of DHR-sq against three human cell line and Roy-OA NPs against Vero-E6 cells was lower when compared with Roy and DHR alone, respectively, suggesting that these nanoassemblies can act as prodrugs for the release of cytotoxic lead molecules.

Multivariate Statistical Analysis to Evaluate the Effects of Environmental Factors on The Chemical Profile Of *Serjania Marginata* Casar. Leaves

Ana C. Zanatta[1,2*], Wagner Vilegas[2] and RuAngelie Edrada-Ebel[3]

[1]São Paulo State University (Unesp), Institute of Chemistry, Brazil;

[2]São Paulo State University (Unesp), Institute of Biosciences, Brazil;

[3]University of Strathclyde, United Kingdom;

Abstract

Serjania marginata Casar. is a climbing plant found in the Brazilian Cerrado region and is used in popular medicine to treat stomach pains. To better understand the influence of environmental factors on the chemical composition of this plant species, a study based on multivariate statistical analysis was conducted employing the data obtained from UHPLC-DAD-ESI-HRMS and NMR analyses. For the study, leaves of *S. marginata* were collected bimonthly for two consecutive years. The Cerrado region of Brazil has two main climatic conditions: dry period (autumn and winter) and rainy period (summer and spring). In the VIP scores for the NMR data, chemical shifts of methyl, methylene, and aromatic protons were observed. By analyzing the MS data, it was observed that the steroidal saponins and the *C*-glycosyl flavones contributed the most to the differentiation of the harvest periods. Samples harvested during the rainy season had the highest amount of saponins, while samples harvested during the dry season had the highest amount of flavones. These compounds play important ecological roles and under stress conditions, these different classes of metabolites can act in different ways in the plant's defense. Saponins are known for their good antimicrobial activity and therefore may be related to plant protection against pathogens; this can be explained by the high humidity levels in the rainy season that make plants more susceptible to microbial attack. Flavones, on the other hand, act as antioxidants to reduce oxidative stress caused by reactive oxygen species that form due to water deficiency in dry periods.

Production of Bioactive Polyphenols in *Salvia Viridis* Shoots Cultivated in Temporary Immersion System

Izabela Grzegorczyk-Karolak[1*], Paulina Staniewska[1] and Liwia Lebelt[2]

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[2]Bioorganic Chemistry Laboratory, Medical University of Lodz, Muszynskiego 1, 90-151 Lodz, Poland;

Abstract

Salvia viridis is an annual herb native to the Mediterranean countries, Anatolia and Iran[1]. In these areas for centuries it has been known in traditional medicine for its medicinal properties. It has been used as a remedy for infectious diseases and inflammatory disorders, especially affecting eyes and oral cavity[2]. *S. viridis*

is rich in chemical compounds with a wide range of biological activities. As effective upscaling of cultivation is an important step in its profitability, the aim of this study was to establish culture of *S. viridis* shoots in Plantform bioreactor and evaluation of quality and quantity of bioactive phenols in the obtained plant material. Plantform is system based on the temporary immersion technology; the culture was immersed with medium every 80 min for 10 min.

Six phenolic acids and four phenylethanoids were identified in *S. viridis* shoots extract using UPLC-PDA-ESI-MS/MS method. The level of total phenolic acids, phenylethanoids and phenols in culture grown for 3 weeks was, respectively, 18.3, 11.4 and 29.7 mg/g DW. Rosmarinic acid at a concentration of 16.6 mg/g DW and verbascoside at a concentration of 9.8 mg/g DW were the two main secondary metabolites. Ultimately, the total content of phenylethanoids and phenolic acids in shoots cultivated in the bioreactor within 3 weeks was 2 times higher than in aerial parts of 4-month-old plants grown in soil[3]. Hence, the *S. viridis* shoot culture carries out in the Plantform temporary immersion system may be an alternative efficient source of plant material rich in bioactive compounds.

Production of the Essential Oil by *Codonopsis Pilosula* (Franch.) Nannf. Transformed Roots

Ewa Skała[1*], Joanna Makowczyńska[1] and Danuta Kalemba[2]

[1]*Medical University of Lodz, Poland;*

[2]*Lodz University of Technology, Poland;*

Abstract

Codonopsis pilosula (Franch.) Nannf. (Campanulaceae) is commonly used as substitute of Panax ginseng (Zou et al. 2019). Codonopsis Radix has been used in Chinese medicine in case of weakness and fatigue or for strengthening the immune system (Gao et al. 2018). *In the natural habitat, the collection of the C. pilosula raw material requires destruction of the whole plant. Biotechnological strategies such as transformation by Rhizobium rhizogenes may be an attractive alternative method for obtaining the raw material with pharmacological properties. In this study, essential oil was isolated by* hydrodistillation *from transformed roots obtained by R. rhizogenes transformation. In the optimal condition (*cultivation in liquid Schenk and Hildebrandt medium (Schenk and Hildebrandt 1972) with 30 g/L sucrose*) after 4 weeks can be obtained* 264 g of fresh weight and 17 g of dry weight of transformed roots from 1L of medium. GC-MS analysis showed that essential oil isolated from transformed roots had very similar qualitative chemical profile to that obtained from the commercial raw material; however, considerable variation in free fatty acids content was observed. The most important group of common constituents of both oils were sesquiterpene hydrocarbons. The main among twenty two compounds in this group possessed skeletons of barbatane, thujopsane and bisabolane; the predominant ones being α- and β-barbatene, followed by β-chamigrene and thujopsene. This study presented that *C. pilosula* transformed roots can be a potential source for the industrial production of the raw material from species of the genus *Codonopsis* used in traditional Chinese medicine.

Oral Talks

Chemical Synthesis of Prenylated Phenolic Natural Products

Jakob Magolan

McMaster University, Canada.

Abstract

Many prenylated phenolic natural products of pharmaceutical or nutraceutical interest are prohibitively expensive to enable clinical development and use, or they are simply not available in sufficient quantities from any commercial source. In nature, assembly of these molecules relies on prenyltransferase enzymes that affix lipophilic side chains to aromatic scaffolds with a degree of substrate- and site-selectivity that is generally superior laboratory methods. We have recently discovered a new chemical process that enables the efficient and scalable synthesis of many of these prenylated phenolic natural products from the corresponding phenols. This phenolic prenylation reaction leverages a surface-templating effect of alumina to direct the regioselectivity of Friedel-Crafts allylation of phenols with allylic alcohols. This presentation will detail our discovery of this chemistry and its application to the efficient chemical synthesis of several rare prenylated phenolic natural products.

Session V: Other fields related to Natural Products

Poster Presentations

Morinda Perspective Use in the Treatment of Skin Conditions: A Review

Fatema Aljishi[1,2*] and Francesca Scotti[1]

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[2]Department of Natural Products and Alternative Medicine, Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia;

Abstract

Background: *Morinda* is one of the largest genera of *Rubiaceae*. It is widely distributed in tropical and subtropical countries. Some *Morinda* species like *Morinda citrifolia, Morinda coreia, and Morinda lucida* are used in traditional medicine for the management of skin conditions such as wounds and dermatitis.

Aim: To identify which *Morinda* species show the most promising medicinal properties that could contribute to the management of skin conditions.

Method: Three major electronic databases (PubMed, Web of Science and Google Scholar) were searched for original pharmacological studies on *Morinda* species related to skin ailments, or inflammatory conditions.

Results and Discussion: Among the 51 articles meeting the inclusion criteria, fruits and leaves were the most broadly used parts and *M.citrifolia* and *M.coreia* the most studied species. Different methods were used to prepare the ethanolic extracts of *M. citrifolia*. Despite these differences, they showed consistent statistically significant anti-inflammatory and wound healing effects, primarily when used orally. Both topically and orally administered aqueous extract of *M. coreia*'s leaves demonstrated wound healing properties. Orally administered ethanolic, methanolic, and aqueous extracts of the leaves showed anti-inflammatory effects. The oral administration of *M. citrifolia* fruit juice showed promising wound healing and anti-inflammatory effects in most studies. However, the chronic oral use of the fruit aqueous extract might be hepatotoxic.

Conclusion: Different *Morinda* species have been investigated and have shown promising effects, especially *M. citrifolia* and *M. coreia.* However, a wider study including more search data bases and expanding the key terms is recommended to confirm these results.

The Role of Bioderived Ionic Liquids in Topical Formulations Containing Hydroxycinnamic Acids

Ana Júlio[1,2,*], Nádia Remtula[1] and Tânia Santos de Almeida[1,#]

[1]CBIOS-Research Center for Biosciences & Health Technologies, Lusófona University, Portugal;

[2]Department of Biomedical Sciences, University of Alcalá, Spain;

Abstract

The hydroxycinnamic acids are the largest group of phenolic acids and several studies have already shown that they may be useful on the cosmetic and pharmaceutical areas, by presenting antioxidant, antiinflammatory and antimicrobial properties [1]. They are commonly present in natural sources, such as plants (e.g. bamboo shoots, eggplant), cereals (e.g. corn, rice, wheat), vegetables (e.g. radish, beans) and fruits (e.g. orange, apples) [1]. However, these compounds present low aqueous solubility, which difficult their incorporation into topical delivery systems. In this context, ionic liquids (ILs) can be innovative and multifunctional tools to ensure a higher drug solubility and even improve the properties of the topical developed systems. Two ILs derived from natural amino acids, (2-hydroxyethyl)trimethylammonium phenylalaninate [Cho][Phe] and (2-hydroxyethyl)trimethylammonium glycinate [Cho][Gly] and their impact on the incorporation of the caffeic and p-coumaric acids, on oil-in-water (O/W) emulsions [2] and gels was assessed. The incorporation of the ILs into the delivery systems improved their formulation, allowed the incorporation of higher amounts of the two hydroxycinnamic acids and led to more viscous formulations, which may improve the patient acceptance as well as the stability of the delivery systems. Furthermore, ILs also proved to be determinant to improve the stability of the O/W emulsions [2]. Our results showed that these ILs, derived from natural amino acids, may be decisive not only to the increase the incorporation of poorly soluble hydroxycinnamic acids into the delivery systems, but also to facilitate the preparation and improve the performance of topical formulations.

Biomass Accumulation and Phenolic Acid Biosynthesis in *Salvia Austriaca* Jacq. Hairy Root Culture Grown in Erlenmeyer Flasks and In Temporary Immersion Bioreactor RITA

Łukasz Kuźma[*1], Jan Gomulski[1], Renata Grąbkowska[1], Anna Kiss[2] and Ewa Skała[1]

[1]Department of Biology and Pharmaceutical Botany, Medical University of Łódź, Poland;

[2]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland;

Abstract

Salvia austriaca Jacq. (austrian sage) (Lamiaceae), is medicinal herbaceous plant native of high altitudes across Russia and eastern Europe [1]. It has been described that the roots of this species produce a medicinal valuable secondary metabolites, like abietane diterpenoids, as well as, phenolic acids [2, 3]. As a result of the genetic transformation of *S. austriaca* shoots with *Rhizobium rhizogenes* A4 strain the hairy root culture was obtained [4]. The roots were cultured in growth regulator-free Schenk and Hildebrandt liquid medium [5] for 35 days under illumination with different wavelenght of light emitting diode (LED) light (red, blue, red/blue and white) and in the dark in Erlenmeyer flasks and temporary immersion bioreactor, Rita®. The root cultures were examined in respect to the biomass accumulation and phenolic acid biosynthesis.

The highest fresh and dry biomasses of 35-day-old hairy roots grown in Erlenmeyer flasks were achieved during red/blue LED light exposure. Cultivated in (white and red/blue LED light) in Rita bioreactor the hairy roots demonstrated the highest values of the biomass.

It was noticed, that *S. austriaca* transformed roots biosynthesize caffeic acid, rosmarinic acid and salvianolic acid. The roots grown in Erlenmeyer flasks in the dark showed the highest total content of phenolic acids (about 19 mg g^{-1} dry weight). Among the cultures grown in Rita bioreactor the maximum phenolic acids content was observed in the roots exposed on blue light and it was near 9 mg g^{-1} dry weight.

Structure-Activity Relationships Reveal A 2'-Furoyloxychalcone as A Potent Cytotoxic and Apoptosis Inducer in Human U-937 Leukaemia Cells

Henoc Del Rosario[1,*], Ester Saavedra[1,2], Ignacio Brouard[3], Daniel González-Santana[4], Celina García[5], José Quintana[1] and Francisco Estévez[1]

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[3]Instituto de Productos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, La Laguna, Spain;

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[5]Instituto Universitario de Bio-orgánica AG, Departamento de Química Orgánica, Universidad de La Laguna, Spain;

Abstract

In the area of cancer, over the last 40 years, approximately 80% of all approved therapeutic agents were natural products or directly derived from these. Chalcones (1,3-diphenyl-2-propen-1-ones) are the biosynthetic precursors of flavonoids and they have attracted attention for their antiproliferative properties against various cancers. In this communication we report the synthesis of a new series of chalcones and their cytotoxicity against several human tumour cells. This series of chalcones was characterized by the absence or the presence of a furoyloxy radical on the A ring of the chalcone and the introduction of one or three methoxy groups or a methyl and two methoxy groups at positions 2,3,4 and 5 on the B ring of the chalcone skeleton. The results revealed that the most cytotoxic chalcone contained the furoyloxy radical at position 2' of the A ring, with IC_{50} values below 1 μM in human leukaemia cells and it is at least ten-fold more potent than the antitumor etoposide in U-937 cells. Human peripheral blood mononuclear cells were more resistant than leukaemia cells to the cytotoxic effects of the chalcone. This furoyloxychalcone blocked tubulin polymerization and induced G_2-M cell cycle arrest and apoptosis. Cell death was associated with mitochondrial cytochrome *c* release, caspase activation, poly(ADP-ribose) polymerase cleavage and it was dependent on reactive oxygen species generation.

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Canarian Cyanobacterias as a potential source of Fungicide Natural Products

Carolina P. Reyes[1], Nereida Rancel Rodríguez[2], Andrea García Hernández[3] and Cristina Giménez Mariño[2]

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Abstract
Cyanobacteria are emerging candidates in recent years as a valuable resource for the protection of agriculture fields. These are due to the great variety of biologically active compounds extracted from them that exhibit antifungal, antibiotic and insecticidal properties, among others. Also, cyanobacteria acquires niches in agriculture soils which are contributing to with biological nitrogen fixation and solubilization of trace elements thereby improving soil fertility and crop productivity. Cyanobacteria biomass can also be used for the scale production of biofertilizers. Therefore, in the present work, the fungicidal activity of three ethanolic extract prepared from clonal cultures of heterocyst-forming cyanobacteria was evaluated against three species of phytopathogenic fungi responsible for causing important damage in the main crops of the Canary Islands. The cyanobacteria strains were isolated from samples collected from leaves of Laurus novocanariensis Rivas-Mart., Lousa, Fern. Prieto, E. Días, J.C. Costa & C. Aguiar an endemic tree from Macaronesian Laurel forest. This represents a unique starting point for searching a natural products with potential plant fungicide products.

Canarian Cyanobacterias as a potential source of Fungicide Natural Products

Carolina P. Reyes
Universidad de La Laguna, Spain.

Abstract
Cyanobacteria are emerging candidates in recent years as a valuable resource for the protection of agriculture fields. These are due to the great variety of biologically active compounds extracted from them that exhibit antifungal, antibiotic and insecticidal properties, among others. Also, cyanobacteria acquires niches in agriculture soils which are contributing to with biological nitrogen fixation and solubilization of trace elements thereby improving soil fertility and crop productivity. Cyanobacteria biomass can also be used for the scale production of biofertilizers. Therefore, in the present work, the fungicidal activity of three ethanolic extract prepared from clonal cultures of heterocyst-forming cyanobacteria was evaluated against three species of phytopathogenic fungi responsible for causing important damage in the main crops of the Canary Islands. The cyanobacteria strains were isolated from samples collected from leaves of Laurus novocanariensis Rivas-Mart., Lousa, Fern. Prieto, E. Días, J.C. Costa & C. Aguiar an endemic tree from Macaronesian Laurel forest. This

represents a unique starting point for searching a natural products with potential plant fungicide products.

VI: Bioactivity of Natural Products

Oral Talk

Comparative Investigation of Composition, Antifungal, and AntiInflammatory Effects of the Essential Oil from Three Industrial Hemp Varieties from Italian Cultivation

Claudio Ferrante*

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Abstract

Industrial hemp is characterized by a huge amount of by-products, such as inflorescences, that may represent high-quality sources of biomolecules with pharmaceutical interest. In the present study, we have evaluated the phytochemical profile, including terpene and terpenophenolic compounds, of the essential oils (EOs) of Futura 75, Carmagnola selezionata and Eletta campana hemp varieties. The EOs were also tested for antifungal properties toward Trichophyton mentagrophytes, Trichophyton rubrum, Arthroderma crocatum, Arthroderma quadrifidum, Arthroderma gypseum, Arthroderma curreyi, and Arthroderma insingulare. In parallel, we investigated the inhibitory effects of the EOs against tyrosinase, and the production of prostaglandin E2 in isolated mouse skin exposed to hydrogen peroxide. In human H1299 lung adenocarcinoma cells, we also evaluated the influence of the EOs on the gene expression of angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2), which are involved in SARS-CoV-2 entry in human host. E-caryophyllene and α-pinene were the prominent terpenes in the EOs, whereas the cannabidiolic acid was the terpenophenol present at higher concentration. The EOs inhibited the growth of all tested dermatophytes species. In isolated skin specimens, EOs prevented the hydrogen-peroxide-induced synthesis of prostaglandin E2, consistent with the intrinsic antityrosinase activity. Finally, in H1299 cells, all tested EOs reduced the gene expression of ACE-2 and TMPRSS2, as well. Therefore, the present findings highlight the rationale for the use of the present EOs against infectious diseases.

Poster Presentations

Biological Activity of Leonotis Nepetifolia (L.) R. Br Transformed Roots Extracts Obtained Through Rhizobium Rhizogenes-Mediated Transformation

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Abstract

The plant Leonotis nepetifolia (L.) R. Br. belongs to the family Lamiaceae, is a robust annual herb, about 1–2 m tall, its inflorescence globose is whorled at upper nodes with orange flowers, and it is also known under the name of klip dagga, lion's ear or cordão-defrade [1,2]. It is widespread throughout West India, South America, and the African continent. Leonotis nepetifolia is an important medicinal plant with a long history of numerous traditional medicinal uses in various countries. It is used for cough, fever, stomachache, skin infection, rheumatism, dysmenorrhea, and kidney dysfunction [1,3]. This is the first report on establishing transformed root cultures of Leonotis nepetifolia after Rhizobium rhizogenes-mediated transformation. The preliminary phytochemical analysis showed differences in the content of phenols and flavonoids in transformed and nontransformed roots. The dominant compounds in the analyzed extracts were (+)-catechin (5464 and 6808 µg/g DW), p-coumaric acid (2549 and 4907 µg/g DW), m-coumaric acid (1508 and 2048 µg/g DW) and rosmarinic acid (1844 and 2643 µg/g DW) for nontransformed (LNNR) and transformed (LNTR4) roots, respectively. Initial biological studies carried out on LNNR, and LNTR4 extracts showed a cytotoxic effect on the A549 lung, HCC1937 breast and leukemia NALM-6 cell lines, antioxidants, as well as repair and protection against DNA damage induced by H2O2 in HUVEC cells. Due to the stronger effect of the LNTR4 root extract, which can be a relatively efficient and cheap source of bioactive secondary metabolites, further biological analyses are needed to discover in detail their potentially valuable biological properties.

Use Of Multivariable Data Analysis Comparison For Unravelling The Anticancer And Antioxidant Differences Between Two Endemic Varieties Of Chamomile

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Abstract

Terpenoids, phenolic compounds and alkaloids are examples of some secondary metabolites responsible in how plants interact with their environment. These compounds present a wide diversity of structures and functional groups, which imply several biological activities. The metabolic pathways which control its biogenesis could be easily modified by ambient conditions. The implementations of new reactions in the synthetic cascades result in novel molecules. New secondary metabolites allow a better response for the plant under different growing conditions. 1 Therefore, measuring metabolic differences between two endemic plant varieties is a useful strategy to understand better the environmental adaptation of some species. Additionally, beyond its interest for phytochemical studies, this approach can be used in new drugs discovery. Chamomile (Matricaria chamomilla) has historically been one of the most widespread used medicinal plants due to the richness in therapeutically active compounds. The dried flowers of chamomile contain many terpenoids and flavonoids some of them with antioxidant, neuro-protective, anti-allergic, anti-inflammatory, anti-microbial and anticancer activities.

How effective are natural products at improving muscle strength and function in older adults?
(A systematised review of randomized controlled trials)

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Abstract

Introduction: an age-related loss of muscle strength and function is becoming a global concern due to its harmful consequences and limited effective and safe treatments. Several medicinal plants have been indicated traditionally to improve muscle health. Therefore, this review aimed to examine the effectiveness of natural products at enhancing muscle strength and function in the elderly.

Method: a systematised review of RCTs of natural products related to muscle health in older adults was conducted by searching electronic databases and bibliographies of retrieved trials.

Results: fifteen RCTs were included on ten herbs: *Camellia sinesis*, *Kaempferia parviflora*, *Centella asiatica*, Korean mistletoe, *Withania somnifera*, *Panax ginseng*, *Cordyceps sinensis*, Liqurice flavonoids oil (LFO), *Theobroma cacao*, and Soy isoflavones. Results revealed *C.sinesis* exerted synergistic effects to exercise in sarcopenic women. It offered an 11.36% (P=0.010) improved walking ability in the exercise+*C.sinesis* group compared to the exercise alone (4.84%, P=0.020). LFO significantly improved muscle mass ($P < 0.01$) and one-leg standing (P= 0.03) in the elderly. *K.Parviflora* improved 6-min walk test in healthy elderly ($P< 0.05$). Mistletoe increased muscle strength in healthy elderly (P=0.009 for peak torque and P=0.033 for total set work). Soy isoflavones improved muscle mass (P=0.037) in sarcopenic-obese postmenopausal women. *T.cacao* enhanced handgrip strength (P=0.04), and Timed UP and GO test (P=0.03) in older people.

Conclusion: There is low-quality evidence supporting the physical benefits of *C.sinesis* ,LFO, mistletoe, *K.Parviflora*, and *T.cacao* in the elderly. However, Evidence does not support the use of *P.ginseng*, *C.sinensis, W.somnifera*, and *C.asiatica* for muscle health in the elderly.

Natural Occurring Chlorinated Guaianolide Linichlorin a Induces Apoptosis Dependent on Reactive Oxygen Species Generation in Human U-937 Leukaemia Cells

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Abstract

Guaianolides-type sesquiterpene lactones are naturally occurring compounds which have attracted attention due to their promising potential anticancer activity. We have previously evaluated the effects on viability on human tumor cells of a series of chlorinated guaianolides isolated from natural sources. Linichlorin A was one of the most cytotoxic compounds against tumor cells and displayed less cytotoxicity against normal lymphocytes. In the present study we found it is a potent apoptotic inducer on human U-937 leukaemia cells. The mechanism of cell death triggered by linichlorin A was (i) mediated by poly(ADP-ribose) polymerase cleavage and the processing and activation of initiator and effector caspases; (ii) mostly abrogated by the general caspase inhibitor z-VAD-fmk and by the selective caspase-3/7 inhibitor; (iii) associated with the release of mitochondrial cytochrome c; (iv) accompanied by activation of the mitogen-activated protein kinase pathway and inhibition of NF-κB and (iv) mostly blocked by N-acetyl-cysteine and glutathione. In conclusion, linichlorin A is a potent cytotoxic compound against human leukaemia cells and induces apoptosis involving activation of the extrinsic and intrinsic pathways, inhibition of NF-κB and dependent on reactive oxygen species generation.

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Evaluation of the Lysyl Oxidase Like 2 (LOXL2) Inhibitory Activity of a Natural Pimarane and Its Sugar Derivatives

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Abstract

Lysil oxidase (LOX) and LOXL 1-4 enzymes are amine oxidases that catalyze the elastin and collagen cross-linking in the extracellular matrix. This activity facilitates cell migration/invasion and the formation of metastases. Consequently, inhibition of these enzymes, particularly LOXL2, has been suggested as a possible therapeutic strategy to prevent breast cancer metastasis [1]. The first LOX inhibitor discovered was the phytochemical compound β-aminopropionitrile (BAPN), present in sweet peas (Lathyrus odoratus L.). Besides this plant, other species could be potential sources of novel LOXL2 inhibitors. Pimarane compounds have been previously described for their antitumor, anti-inflammatory, analgesic, and antibacterial properties [2,3]. However, their effect on LOXL2 activity was never evaluated before. The aim of this work was thus to investigate the ability of the pimarane isopimara-7,15-dien-19-ol (1), isolated from Aeollanthus rydingianus. Their derivatives with glucose 19-O-β-D-glucopyranoside-7,15- isopimaradiene (2) and mannose 19-O-α-D-mannopyranoside-7,15-isopimaradiene (3) were also evaluated. A fluorescence method based on Amplex Ultra Red [4] was implemented to assess the LOXL2 inhibitory activity, and validated using BAPN as positive control. The pimarane 1 and its mannose derivative (3) inhibited LOXL2 with a similar potency, presenting IC50 values in the micromolar range. Conversely, the glucose derivative (2) did not show inhibitory activity. Further studies will be carried out to optimize the structure of these compounds in order to increase their activity.

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Royleanone Diterpenoids from *Plectranthus* Spp. As P-Glycoprotein Inhibitors

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Abstract

The increasing number of cancer cases warrants the search for new anti-cancer therapeutics. However, the overexpression of membrane transport proteins, like P-glycoprotein (P-gp) in multi-drug resistance (MDR) cancers, continues to be a major impediment to effective therapy. *Plectranthus* species are renowned for their medicinal properties and have been reported to be rich in diterpenes, such as, 7α-acetoxy-6β-hydroxyroyleanone (Roy), which has demonstrated cytotoxicity against various cancer cell lines (1). Based on molecular docking studies (2), 10 semi-synthetic derivates of Roy, that displayed strong P-gp interactions *in silico,* were prepared. The antitumoral activity of the compounds 1-10 were assessed in resistant human cancer cell lines NCI-H460/R and DLD1-TxR. Cell viability was assessed using MTT assay and cell death induction by Annexin V/PI. The results showed that derivatives **2**, **3** and **4** have the most prominent selectivity (2.7, 2.3 and 2.6 times, respectively) towards cancer cells, compared to normal lung fibroblasts MRC5. Moreover, derivatives **2**, **3** and **4** also showed a reduction in P-gp activity in Rho123 accumulation assay and indicated P-gp inhibition in the DOX accumulation assay in resistant cell lines NCI-H460/R and DLD1-TxR. Overall, it was demonstrated that three abietane diterpenoid derivatives induced P-gp inhibition in MDR cancer cell lines, presenting novel selective compounds for the possible treatment of lung and colon cancer. Further investigations are ongoing to refine the *in silico* studies to obtain hit P-gp modulators.

Urolithin B Hinders IAPP Aggregation: A Potential Role of Diet-Derived Metabolites towards Diabetes Management

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