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► **Cover picture:** New York midtown skyline over the Hudson River, © Fotosearch.com

2012 International Congress on Natural Products Research

"Global Change, Natural Products and Human Health"

Date/Location:	**July 28 – August 1, 2012, New York City**
Chairmen:	Guy T. Carter, Scientific Organizing Committee Edward Kennelly, Local Organizing Committee
Issue Editor:	Mark O'Neil-Johnson

Dear Colleagues,

ICNPR 2012, the 8th joint meeting sponsored by the American Society of Pharmacognosy (ASP), and]sister societies from Europe: the Italian Society of Pharmacognosy (SIF), the Society for Medicinal Plant and Natural Products Research (GA), the Phytochemical Society of Europe (PSE) and the French Speaking Society of Pharmacognosy (AFERP), has been organized this year in New York. As Chairs of the Local and Scientific Organizing Committees, it is our pleasure to welcome you to New York. We are delighted by the response that was forthcoming for contributions to this Congress. We received nearly 1300 abstracts from scientists representing 64 countries from across the globe.

The master plan for our scientific program includes plenary sessions each morning of the Congress primarily dedicated to prestigious Award lectures and a celebration of the publication of the 75th Volume of the Journal of Natural Products. Except for Sunday these lectures are followed by parallel symposia dedicated to specialized areas of natural products research. The afternoons are devoted to contributed papers, with oral sessions launched by prominent scientists followed by extensive poster sessions arranged by topic areas. Sunday afternoon features a visit to the New York Botanical Garden for a chance to see the extensive collection in a relaxed and unhurried private showing.

The scientific program remains faithful to the traditional areas of interest to the sponsoring Societies, natural products discovery and analyses, while providing glimpses into specialized areas with strong natural products connections: Chemistry of Symbiosis, Genome Mining and Drug Discovery.

We would like to thank all of the members of the Local and Scientific Organizing Committees (listed below), for their enthusiastic contributions. We are particularly indebted to Dr. Mark O'Neil-Johnson for his efforts in gathering and editing these abstracts, which provide a foundation for the scientific discourse that will unfold during this Congress.

Edward Kennelly
Chair, Local Organizing Committee

Guy T. Carter
Chair, Scientific Organizing Committee

Invited Lectures

IL1 Chemical diversity in the plant world: Why can't we find more plant derived drugs?

Miller JS
The New York Botanical Garden, 200 Southern Blvd., Bronx, NY 10458, U.S.A.

The natural world has long been a rich source of medicinally useful compounds, yet many large scale discovery efforts in the later part of the 20th century yielded far fewer useful discoveries than had been anticipated. At the same time, only a small percentage of plant species have been evaluated against a limited number of possible therapeutic targets. The scope of plant diversity is reviewed and it is estimated that there are perhaps close to 400,000 species of plants. Study of a small percentage of these species have yielded more than 100 medically useful compounds. Extrapolating from the numbers of plant species that were likely studied to discover these compounds it is concluded that there are at least 500, and possibly many more, medically useful novel biochemical compounds that remain to be discovered in plants. The reasons that efforts of the late 20th century were not more successful are reviewed and possible alternative approaches suggested.

IL2 Biological and chemical diversity among rainforest endophytes

Strobel SA
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520

Biological and chemical diversity is an accessible entry point for novice students to make original observations about the natural world and to become engaged in the excitement of the scientific process. For the past six years we have taught the course Rainforest Expedition and Laboratory (REAL) with the goal of providing an inquiry-based educational program that gives students extensive opportunities for project design and intellectual ownership. REAL provides an integrated scientific experience that includes the fields of ecology, chemistry, microbiology, pharmacology, molecular biology and bioinformatics. Students participate in a scientific problem from field botany, through microbiology, to natural product characterization. The program inspires students regarding the diversity of life, the process of scientific inquiry, and the open-ended possibilities still available for scientific investigation. It is a capstone experience to their undergraduate training that inspires them to pursue the scientific process in their future careers. Each year we take 16 undergraduate students on an expedition to the Amazonian rainforest of Ecuador. During this two week expedition the students collect plant samples that they use as starting materials for their summer laboratory. Our program is focused on endophytes, hyperdiverse microbes living within healthy plants. There are estimated to be more than one million endophytic fungi, only a small fraction of which have been characterized to even the most limited degree. The fact that many of these organisms can be easily cultured means that it is a readily accessible entry point for novice scientists to be engaged in research. Where there is novel biology there is likely to be novel chemistry which provides opportunities for new applications. Students apply the natural products produced by these organisms to global problems of human health and sustainability, while providing opportunities for scientific collaboration with research laboratories throughout the University and other institutions. There is sufficient endophyte biodiversity that it is common for students to identify not just new species, but new genera of organisms. Rather than being a minor technical player in the science of a typical laboratory, these students have become vested in an original project over which they have full autonomy and a strong sense of ownership. Students participate in the scientific process from the mud of field biology to the quantitative precision of natural product characterization. Highlights of the biological and chemical diversity that the students have discovered as part of the REAL will be presented including biological active natural products and organisms with useful bioremediation capabilities.

IL3 Biodiversity of marine invertebrates, the benefits from their "biodiscovery" and how much do we still *not* know?

Hooper JNA
Biodiversity & Geosciences Programs, Queensland Museum, PO Box 3300, South Brisbane, QLD 4101 Australia and Eskitis Institute for Cell & Molecular Therapies, Griffith University

While only about 50% of all new approved drugs were derived directly from natural sources over the past 30 years, natural products (NPs) continue to deliver unique structural diversity, with marine invertebrates being a significant source of these (as evidenced by ~10,000 NPs discovered from marine invertebrates over this period). However, it has been estimated that these discoveries come from less than 10% of the world's (metazoan) biodiversity, and therefore many more useful natural lead compounds predictably await discovery. This is the 'Promise of Biodiversity', but how do we know it has any credence, what is the scope and distribution of the major constituent taxa, how well do we know these, and what threatens their existence – and hence the validity of this promise as a source for new bioactivity discoveries as potential sources of new drugs (marine genetic resources, or MGRs)? This presentation gives an overview of the major groups of marine invertebrates that have shown most bioactivity (basal metazoan, eumetazoan and deuterostome bilaterians), and uses some local examples of new NPs that have also revealed substantial new knowledge about marine invertebrate species. The scope of marine species diversity in both taxonomic and geographic spaces shows unequivocally huge potential, but currently faces equally huge challenges under the shadow of the 'taxonomic impediment'. This impediment includes the competing goals of achieving better, more substantial knowledge of these taxa and their unique chemical profiles, versus the diminishing taxonomic expertise capable of achieving this, the natural and anthropogenically induced threats facing the survival of this biodiversity, and further exacerbated by the "uncertainty principle" that highlights the great divide between the presently 'known', the currently 'unknown', and the 'unknowable' (the latter highly technologically dependent to move our biological resources up into the realm of the 'known'). For example, it is postulated that about 90% of marine species remain in the 'unknown' category. The presentation focuses on sponges (Phylum Porifera) – the most basal metazoan group but also the most bioactive, and historically the source of the greatest diversity of novel chemical structures (albeit many possibly of significant symbiotic microbial origin) – and on the research from the Australasian domain (containing the highest number of documented marine species, and amongst the largest of claimed marine seabed domains of all marine nations). International collaborative partnerships in marine biodiscovery have been proven to be pivotal (for both new biological and new chemical diversity discoveries), and continue to be the model for the future.

IL4 Molecular basis of plant secondary metabolites – proteins interaction

De Tommasi N
Dipartimento di Scienze Farmaceutiche e Biomediche, Università degli Studi di Salerno, Via Ponte Don Melillo,84084 Fisciano, (SA), Italia

The secondary metabolites found in plants represent an extremely rich source of novel chemical diversity for drug discovery and chemical biology programs.[1–3] One of the main problems in drug discovery from plant small molecules is the identification of their molecular targets: many compounds have been found to be more promiscuous than originally anticipated, which can potentially lead to side effects, but which may also open up additional medical uses. The drug poly-pharmacological activity can be understood only if its interactions with cellular components are comprehensively characterized. Thus the identification of target proteins and investigation of ligand-receptor interactions represents an essential step in the process of plant drug discovery and development.[4,5] However, nature is far more complex, and it is only with multidisciplinary collaborative research encompassing many disciplines that such targets can be successfully studied. Owing to our interest in the field of bioactive plant molecules, we have developed approaches to target identification based on chemical proteomics procedures, supported by spectroscopic and spectrometric data, Surface Plasmon Resonances (SPR) analyses and biochemical tests.[5,6] Chemical proteomics is a powerful mass spectrometry-based affinity chromatography approach aimed to identify a set of proteins captured by a small molecules.[6,7] The investigated molecule has to be anchored to a solid support through a flexible space arm, and then incubated with a lysate, from cell or

tissues, to obtain the interaction between the immobilized compound (bait) and its protein targets. After several washing steps, proteins tightly bound to the beads are eluted and subjected to electrophoresis or gel free separation, followed by enzymatic digestion. The obtained peptide mixtures are then submitted to MS analyses and data base search for protein identification. This study allows the description of all potential macromolecular targets of a small bioactive molecules in a single experiment, leading to a complete and selective target mapping of a drug candidate. We have recently applied this approach to the study of cellular targets of some bioactive plant small molecules showing interesting protein targets; achieved results will be described in this communication.[8,9] Koch, A.M., Schuffenhaue, A., Scheck, M., Wetzel, S., Casaulta M., Odermatt A., Ert P., Waldmann E., (2005) PNSA, 102:17272 – 17277. Clatdy, J., Walsh, C., (2004) Nature, 432:829 – 837. Li, J.W., Vederas, J.C. (2009) Scienze, 325: 161.166 Rix, U., Superti-Furga G., (2009) Nat. Chem. Biol., 5: 616 – 624. Bantscheff, M., Scholten, A., Heck. A. J., (2009) Drug Discov. Today14: 1021 – 1029. Dal Piaz, F.,Malafronte, N., Romano, A.,Gallotta, D., Belisario, M.A., Bifulco, G., Gualtieri, M.J., Sanogo, R., De Tommasi, N., Pisano C., (2012) Phytochemistry 75: 78 – 89 Dal Piaz, F.,Vassallo, A., Lepore, L., Tosco, A., Bader, A., De Tommasi, N., (2009) J. Med Chem, 52:3814 – 3828. Nigro, P., Dal Piaz, F., Gallotta, D., De Tommasi, N., Belisario, M. A., (2008) Free Rad Biol Med 45: 875 – 884. Tarallo, V., Lepore, L., Marcellini, M., Dal Piaz, F., Tudisco, L., Ponticelli, S., Lund, F.W.; Roepstoff., P., Orlandi, A., Pisano, C., De Tommasi, N., De Falco, S., (2011) JBC 286: 19641 – 19651.

IL5 New trends in the research of bioactive plant metabolites

Lanzotti V
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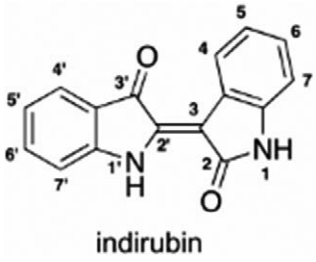
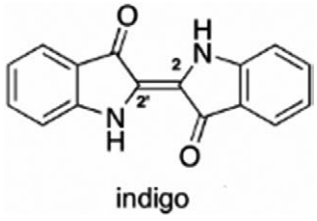
Plant-derived natural compounds received in the last years an increasing attention for the understanding of their function, the developing of new synthetic routes, and their wide range of applications [1]. Furthermore, active compounds isolated from plants are used directly in therapy or as prototype of biologically active lead to develop more active and less toxic analogues. However, one of the main reason of the failure of pharmacological treatments is the pharmacological resistance associated to the therapy. This mechanism is responsible of the failure of treatments with drugs used for humans, animals and plant diseases. Responsible of the incoming of cross-resistance phenomena to drugs, known as multidrug resistance (MDR), are a family of membrane proteins over expressed in the cells and able to pump out of the cell the drug used in therapy. Among the others, p-glycoprotein (PGP) is an interesting pharmacological target in clinical practice: to some cancers (e.g. colon and kidney adenocarcinoma, leukemia and infantile neuroblastoma). Some examples of new promising bioactive diterpenes recently discovered based on macrocyclic skeleton [2,3] and isolated from Euphorbia will be presented. These compounds add to the array of bioactive metabolites isolated from nature that to became clinically effective have to overcome the long time-consuming steps of clinical trials. Recent work opening completely new perspective of research is based on CPMAS NMR analysis [4] and it is aimed at the searching of species-specific toxicity in the living organisms. 1. Osbourn AE, Lanzotti V (eds) (2009) Plant-derived Natural Products: Synthesis, Function and Applications. Springer, Dordrecht, New York. 2. Corea G, Di Pietro A, Fattorusso E, Lanzotti V (2009) Phytochem. Rev., 8::431 – 447. 3. Lanzotti V, Fattorusso E, Taglialatela Scafati O, Di Pietro A (2012) In: Tringali C (ed) Bioactive Compounds from Natural Sources, Taylor-Fancis, Abingdon, Boca Raton. 4. Bonanomi G, Incerti G, Barile E, Capodilupo M, Antignani V, Mingo A, Lanzotti V, Scala F, Mazzoleni S (2011) New Phytologist 191:1018 – 1030.

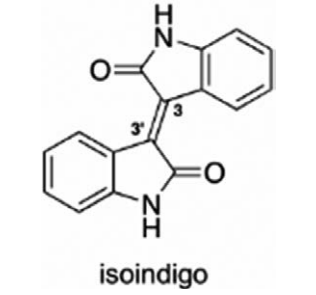
IL6 Indigoids: From natural dyeing agents to selective kinase inhibitors

Skaltsounis LA
Laboratory of Pharmacognosy and Natural Product Chemistry, Department of Pharmacy, University of Athens, Greece

Indirubin, indigo and isoindigo are the core representatives of a rather small category of bisindole alkaloids referred to as indigoids. Their fascinating history begins before even their exact chemical structure elucidation. These compounds are the colored constituents of the natural dyes indigo and the famous molluscan Tyrian purple, and were used throughout the centuries for textile dyeing. In contrast with indigo dyes, the major constituents of molluscan purple dyes are brominated indigoids. Nowadays, the use of natural dyes is very limited due to their replacement with less expensive synthetic dyes. Nevertheless, indigoids and especially indirubins have come to the foreplay due to the vast range of biological activities, which in many cases have their origin in traditional medicine. Chronic myelotic leukemia has been treated in the traditional Chinese medicine with the recipe Danggui Longhui Wan, a mixture of 11 herbal medicines. Eventually, the antileukemic activity was attributed to indirubin, which was detected in the mixture as a minor constituent. A series of derivatives has been developed during the last 15 years aiming to the investigation and amelioration of the indirubin scaffold in terms of activity, selectivity and drugability. Halogenated indirubins are by far one of the most important subcategories of indirubins, with its main representatives 6-bromoindirubin and 6-bromoindirubin-3′-oxime (**6BIO**) possessing an outstanding selectivity against GSK-3. As proposed from crystallographic studies and molecular modeling studies, the outstanding selectivity of **6BIO** to GSK-3β versus CDKs is related to minor differences in the binding pocket of the enzymes. GSK-3β with the relatively small Leu132 provides a more spacious environment for the bromine atom to be inserted in the back of the cavity, whereas in CDKs 1,2 and 5, this area is restricted due to the bulkier Phe80. This presentation attempts to summarize concisely structure/activity relationships among closely related analogues in terms of protein kinase (**PK**) inhibition and selectivity, while it also focuses on the various biological applications arising from the interactions of halogenated indirubins with molecular targets. Those include effects of halogenated indirubins on stem cells, cardiac, renal and pancreatic cells, effect on leukemia, solid tumors and neurodegeneration. In most of the cases, all of the above effects can be associated with the interaction of indirubins with important molecular targets such as members of the family of protein kinases (GSK-3, CDKs, DYRK etc). Small variations on the basic skeleton as in the case of halogenated indirubins, have been proven to modulate significantly their biological activity, leading to more active and selective PK inhibitors with fascinating applications as in the field of stem cells. All of the above along with their charming history of natural product research and development, through the ages, places them among the most promising nature-derived drug candidates.



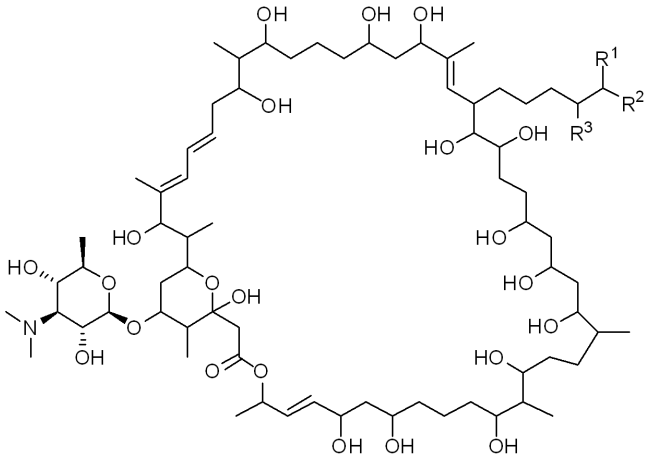




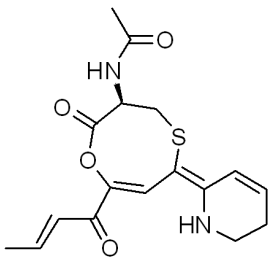
IL7 Bacterial genome mining for novel natural product discovery

Song L[1], Laureti L[2], Gomez-Escribano JP[3], Fox D[1], Yeo V[1], Corre C[1], Huang S[2], Leblond P[2], Aigle B[2], Bibb M[3], Challis GL[1]
[1]Department of Chemistry, University of Warwick Coventry CV4 7AL, UK; [2]Unité Mixte de Recherche1128 Génétique et Microbiologie, Université Henri Poincaré, F-54506 Vandoeuvre-lès-Nancy, France; [3]Department of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH, UK

Bioinformatics analyses have identified gene clusters encoding cryptic polyketide biosynthetic pathways, not associated with the production of known metabolites, in several actinomycete genome sequences. Discovery of the metabolic products of such cryptic gene clusters promises to unearth a hitherto untapped wealth of novel bioactive compounds. However, a major obstacle to the discovery of novel natural products by genome mining is that many cryptic pathways are expressed poorly or not at all under normal laboratory conditions. The discovery of the stambomycins, a new family of 51-membered macrolides with promising anti-cancer activity, as the products of a novel type I modular polyketide synthase (PKS) system identified in the partial genome sequence of *Streptomyces ambofaciens* will be described. Activation of expression of the pathway by genetic manipulation of a putative pathway-specific regulatory gene was key to this discovery. The structures of these novel macrolides suggests that their biosynthesis involves novel features, including in *trans* hydroxylation during polyketide chain assembly to provide the hydroxyl group required for offloading of the fully-assembled polyketide chain from the PKS via macrocyclisation. Experiments aimed at probing this unusual transformation will be described. Identification of coelimycin P, a pigmented metabolic product of a type I modular PKS system encoded by a cryptic gene cluster within the *Streptomyces coelicolor* genome, by exploiting a genetic engineering strategy aimed at maximising metabolic flux through the pathway will also be described. The structure of the pigment, coupled with bioinformatics analyses of the enzymes encoded by the cryptic gene cluster, suggests an unusual biosynthetic pathway with numerous novel features. Incorporation experiments with labelled precursors and biochemical experiments with purified recombinant enzymes provide experimental support for the proposed biosynthetic pathway.



stambomycins



coelimycin P

IL8 Imabing mass spectrometry and molecular network guided genome mining of microbial systems

Dorrestein PC
Skaggs School of Pharmacy and Pharmaceutical Sciences and Departments of Pharmacology, USA, Center for Marine Biotechnology and Biomedicine, Bruker Therapeutic Discovery Mass Spectrometry Center, UC San Diego, CA

In this lecture I will describe the latest mass spectrometry based tools and genome mining approaches we have developed to study the metabolic exchange of microbes.[1 – 8] The goal for visualizing molecules microbes and bridging this with genome mining is to enable the functional translation to understand their molecular language. The presentation will highlight microbial imaging mass spectrometry, the development of mass spectrometry based genome mining tools, including the utility of peptidogenomics and glycogenomics tools that will be explained by Brad Moore in the next talk, in conjunction with live colony mass spectrometry, molecular networking, molecular dendrograms, and ambient mass microscopy to study metabolic exchange and ecology of microbial communities for applications in agriculture, diagnostics and therapeutic discovery. Finally, perhaps most significantly, this lecture will introduce the development of a molecular "Genbank" and its search engines that are being co-developed with the Bandeira laboratory at the NIH Center for Computational Mass Spectrometry that will merge natural product analysis with modern genome mining. References 1. Yang, Y.L., Xu, Y., Straight, P., Dorrestein, P.C., Translating metabolic exchange with imaging mass spectrometry. Nature Chemical Biology (**2009**) 5,885 – 7. 2. Wei-Ting Liu, Yu-Liang Yang, Yuquan Xu, Anne Lamsa, Nina M Haste, Jane Y. Yang, Julio Ng, David Gonzalez, Craig D. Ellermeier, Paul D. Straight, Pavel A. Pevzner, Joe Pogliano, Victor Nizet, Kit Pogliano, Pieter C. Dorrestein. Imaging mass spectrometry of intraspecies metabolic exchange revealed the cannibalistic factors of *Bacillus subtilis* Proc. Natl. Acad. Sci. USA. (**2010**) 107,16286 – 90. 3. Jeramie D. Watrous, Pieter C. Dorrestein, Imaging mass spectrometry in microbiology Nature Reviews Microbiology (**2011**), 9, 683 – 694. 4. Yu-Liang Yang, Yuquan Xu, Roland Kersten, Wei-Ting Liu, Michael J. Meehan, Bradley S. Moore, Nuno Bandeira, Pieter C. Dorrestein, Connecting chemotypes and phenotypes of cultured marine microbial assemblages using imaging mass spectrometry. Angewandte Chemie (**2011**), 50, 5839 – 42. 6. Roland D Kersten, Yu-Liang Yang, Yuquan Xu, Peter Cimermancic, Sang-Jip Nam, William Fenical, Michael A Fischbach, Bradley S Moore, Pieter C Dorrestein, A mass spectrometry-guided genome mining approach for natural product peptidogenomics. Nature Chemical Biology (**2011**), 7, 794 – 802. 7. Vanessa V Phelan, Wei-Ting Liu, Kit Pogliano, Pieter C Dorrestein Microbial metabolic exchange-the chemotype-phenotype link. Nature Chemical Biology (**2012**), 8, 26 – 35. 8. Jeramie Watrous, Patrick Roach, Theodore Alexandrov, Brandi Heath, Jane Y. Yang, Roland D. Kersten, Menno van der voort, Kit Pogliano, Harald Gross, Jos Raaijmakers, Bradley S. Moore, Julia Laskin, Nuno Bandeirac, Pieter C. Dorrestein, Mass spectral molecular networking of living microbial colonies. Proc. Natl. Acad. Sci. USA., (**2012**), in press.

IL9 Expediting natural product discovery through genomics

Moore B
Scripps Institution of Oceanography & Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego; http://moorelab.ucsd.edu

Natural product chemicals have historically been discovered based on their molecular and biological properties. With the ease and affordability of genome sequencing today, a new era in natural product discovery is unfolding in which genomics and biosynthesis are together fostering new innovations in compound discovery. This orthogonal discovery approach takes advantage of the biosynthetic potential of a genome-sequenced organism to design hypothesis-driven experiments to uncover new chemical entities. Realizing that mass spectrometry-induced fragmentation of peptidic and carbohydrate-based molecules yields amino acid and sugar monomers that directly correlate as biosynthetic building blocks, we developed two complementary mass spectrometry-guided genome mining methods that connect diverse peptides and sugar-based metabolites directly to their biosynthetic gene clusters. In this lecture I will introduce peptidogenomics (*Nat. Chem. Biol.*, 7, 794 – 802, 2011) and glycosogenomics as powerful new discovery methods that iteratively match *de novo* MS^n structures to genomics-based structures following current biosynthetic logic. Several novel microbial natural products will

be presented to illustrate the unbiased flexibility of this concept that has the potential for automating the genome mining process.

IL10 Chemical fingerprinting for identification/ authentication of botanical materials

Harnly J
Food Composition and Methods Development Lab, Beltsville Human Nutrition Research Center, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705, USA

The identification or authentication of whole botanical materials requires a holistic approach. It is not sufficient to simply measure a marker or two. A chemical fingerprint is either the chromatographic or spectral (with no separation) pattern obtained for a solid or extract of a botanical material. Like all fingerprints, specific data points are meaningless and the whole pattern must be considered using multivariate (chemometric) methods. Botanical identification has two requirements; the unknown sample is identified by direct comparison to an authentic botanical and a collection of authentic botanicals (inclusivity panel) is available to account for expected variation with respect to species/variety, year, location, processing, and any other appropriate variable. In general, the inclusivity panel is used to construct a model using SIMCA (soft independent modeling of class analogy) or PLS-DA (partial least squares-discriminant analysis), although there are many other techniques. Fingerprints of an unknown botanical are compared to the model and yield a binary answer, YES it is authentic, or NO, it is not authentic. Targeted analyses are possible when there is an anticipated adulterant or substitute botanical. Otherwise the analyses are non-targeted. There are numerous advantages to targeted analyses. An appropriate analytical method can be selected and optimized and the authentic and adulterant botanicals can be tested and a model developed. Fingerprints of the authentic and adulterant botanicals can be digitally added to predict patterns for various levels of adulteration. And, the degree of adulteration can be quantified. Non-targeted analyses proceed in a similar manner. Fingerprints for an authentic inclusivity panel are collected and a chemometric model is constructed. Since the adulterant is unknown, any botanical whose fingerprint falls outside the specified confidence limit is judged to be adulterated. However, since the adulterant is unknown, multiple analyses may be necessary for comprehensive coverage. Until the adulterant is identified, quantification is not possible.

IL11 The use of DNA barcode techniques to identify the constituents of herbal dietary supplements

Little DP[1]
[1]*Cullman Program for Molecular Systematics, The New York Botanical Garden, 2900 Southern Boulevard, Bronx, New York 10458 – 5126 USA*

Dried fragmentary plant materials, such as those often found in herbal dietary supplements, are difficult to identify to species using morphological characteristics alone. Although often partially degraded, plant DNA can survive many common types of processing (e.g. drying, grinding). Short portions (less than 200 bp) of plant DNA from herbal supplements can usually be PCR amplified and Sanger sequenced. When compared to the growing database of publicly available DNA barcode sequences, these sequences can be used to provide reliable species-level identification. For example, black cohosh (Actaea racemosa) herbal dietary supplements are commonly consumed to treat menopausal symptoms. Accidental misidentification and/or deliberate adulteration results in harvesting other, related, species that are then marketed as black cohosh. Some of these species are known to be toxic to humans. Two nucleotides in the plant barcode region consistently distinguish black cohosh from related species. Of 36 dietary supplements sequenced, 27 (75%) have a sequence that exactly matches black cohosh. The remaining 9 samples (25%) have a sequence identical to that of three Asian Actaea species (A. cimicifuga, A. dahurica, and A. simplex).

IL12 Botanical isoscapes: emerging stable isotope tools for geographic sourcing and authentication

West JB
Department of Ecosystem Science and Management, Texas A&M University, College Station, TX, 77843, USA

The global hydrologic cycle generates predictable, spatially coherent variation in the stable hydrogen and oxygen isotopes of water (δ^2H and δ^{18}O) at local to global scales. This includes variation in precipitation along such spatial gradients as latitude and elevation. Because plants use water from soils that are recharged by precipitation or irrigation and the H and O from this water is incorporated into the organic molecules of plant tissues, there is the potential to use plant δ^2H and δ^{18}O for geographic assignment and authentication. We know that plant tissues "record" the isotopic composition of soil water, but with some important modifications. The local water signal is altered by evaporative losses from leaves (transpiration) and the biochemical processes associated with plant growth. Recent advances in modeling these process, as well as technological developments accelerating the accumulation of data on plant δ^2H and δ^{18}O have opened up new avenues of research and potential tools for authentication. I will present areas where we have modeled and mapped the spatial variation of plant-derived products, including such diverse products as wine and the drug of abuse marijuana. I will also discuss new tools that we have developed and made available online (http://isomap.org) to allow spatial modeling of stable isotopes, the generation of maps of this variation (isoscapes[1]), and statistically rigorous geographic assignment. Finally, I will discuss areas that are likely to be productive avenues for future research in geographic origin identification and authentication.

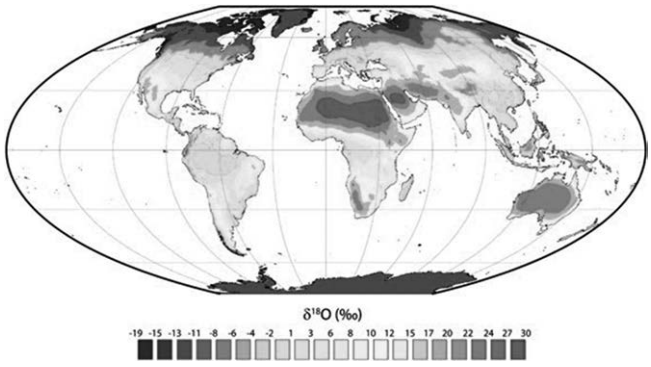


Fig. 1: Global Leaf Water Isoscape

Reference: [1]*West, JB, GJ Bowen, TE Dawson, KP Tu. 2010. Isoscapes: Understanding movement, pattern, and process on Earth through isotope mapping, 487 pgs. Springer, Dordrecht. ISBN 978 – 90 – 481 – 3353 – 6.*

IL13 Strategies and methods for a sustainable search for bioactive compounds

Bohlin L[1], *Alsmark C*[1], *Göransson U*[1], *Klum M*[1], *Wedén C*[1], *Backlund A*[1]
[1]*Division of Pharmacognosy, Department of Medicinal Chemistry, Biomedical Center, Uppsala University*

Change in global climate has been predicted to affect future society in different ways. Destruction of rain forests and pollution of the oceans are serious threats against the ecosystem and the access and sustainable use of natural resources both as food and for future discovery of bioactive compounds. A global awareness of the biodiversity predicament have resulted in several international conventions with the purpose of protecting their natural resources but also to create new possibilities for the development of knowledge-based bioeconomy by securing intellectual property rights for ethnic groups. In this lecture we want to emphasize the connection between biology and chemistry, discuss limitations in today's research, and suggest some strategies and methods for a sustainable search for bioactive natural products. The ultimate purpose being to secure molecules with natural origin as potential leads in drug development and also to reveal potential new targets. Discovery of novel structure-activity relationships in nature has an increased importance for inspiration of synthesis of natural product like compounds resulting in greater diversity but with less complexity and increased understanding of biological processes. A multidisciplinary approach is necessary in

order to discover, describe, and communicate the richness of nature to overcome threats against biodiversity and the future sustainable use of natural resources for discovery of new bioactive compounds. **References:** Bohlin L., Alsmark C. et al. (2012) Bioactive compounds from natural sources; Natural products as lead compounds in drug discovery. Chapter 1 pp 1 – 36. Ed. Corrado Tringali. CRC Press, USA.

IL14 Culturing the unculturable: Expanding the diversity of marine microorganisms for drug discovery

Fenical W and co-workers
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, Skaggs School of Pharmacy and Pharmaceutical Science, University of California, San Diego, La Jolla, CA 92093 – 0204

Although explorations of bacteria and fungi have been an exceptional source for new drug development, surprisingly only a small percentage of the genetic diversity in these groups has been accessed. In the bacteria, for example, less than an estimated 5% of the known taxa have been successfully cultivated and explored chemically. Resistance to cultivation has been convincingly demonstrated by the "Great Plate Anomaly," in which less than 5% of the cells present by direct microscopic observation can be shown to grow. This has been interpreted to suggest that the vast majority of environmental bacteria are resistant to cultivation. In the marine environment, the same situation exists; bacteria isolated from seawater or sediments are largely unable to be cultured using standard conditions. But are they really unable to be cultured? By systematically varying the culture conditions we have found that previously undiscovered bacterial strains can be readily brought into culture. These methods and new approaches now allow chemical studies of rare and previously unknown strains to become part of the drug discovery process. Several examples of new molecules isolated from these strains will be presented.

IL15 To Halaven® and beyond

Yu MJ
Eisai, Inc., 4 Corporate Drive, Andover, MA 01810

Halichondrin B (HB) is a structurally complex marine natural product that exhibits potent cell growth inhibitory activity in vitro and remarkable anticancer activity in several human tumor xenograft models. Classified as a tubulin destabilizer, HB was found to bind at the vinca domain of tubulin in a non-competitive manner, resulting in both characteristic cellular morphological changes and apoptosis. Given its promising in vitro and in vivo profile, HB was accepted by the United States NCI for preclinical development in the early 1980's. However, its future as a possible new chemotherapeutic agent remained uncertain due to significant material supply limitations that hindered the natural product's progression through the drug discovery pipeline. A total synthesis of HB by the Kishi group at Harvard University reported in 1982 offered a potential solution for solving the material supply problem, and allowed access to structurally novel synthetic intermediates for biological evaluation. The discovery at Eisai that biological activity was associated with the macrocyclic lactone region of the molecule allowed a drug discovery program to be initiated with an advanced chemical starting point and a clinically validated mechanism of action. Optimization of the chemical structure ultimately led to eribulin mesylate (E7389, NSC 707389), which inhibits microtubule dynamics in ways that differ from those of vinblastine and paclitaxel, and exhibits potent anticancer activity in a number of human tumor xenograft models. In 2010 this agent was approved by the United States FDA (trade name Halaven®) for use in patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting. Eribulin mesylate was subsequently approved for use in similar patient populations in several countries across Europe and Asia. Although optimized for biological activity and simplified relative to the natural product that inspired it, the structure of eribulin nevertheless remains a challenging synthetic target with 19 stereogenic centers distributed across a 35 carbon backbone. While the chemistry developed by the Kishi group for the total synthesis of HB was known, the presence of a macrocyclic ketone necessitated the development of alternative macrocyclic ring closing and late-stage final assembly strategies. In the first scale-up synthesis of eribulin, two of the three fragments used were identical to the Kishi route used in synthesizing the natural product, but the C.27-C.35 (halichondrin numbering) tetrahydrofuran intermediate of eribulin differed substantially from the Kishi bicyclic C.27-C.38 synthetic intermediate. Discovery chemistry approaches to the macrocyclic ketone ring formation in addition to the final fragment assembly will be discussed in the context of the first multi-gram scale synthesis of eribulin mesylate. Since that time, the process chemistry research group at Eisai has made impressive route improvements to the drug substance and made available quantities of advanced intermediates for the preparation and biological evaluation of new derivatives not explored in the original structure-activity relationship (SAR) investigation. Several new analogue series with low susceptibility to PgP-mediated drug efflux will be presented along with associated biological activity.

IL16 Lloydia and the Journal of Natural Products: 75 years of publication

Kinghorn AD[1], Powell RG[2], Ferreira D[3], Proteau PJ[4], Pearce CJ[5], Cardellina II JH[6]
[1]College of Pharmacy, The Ohio State University, Columbus, OH 43210; [2]National Center for Agricultural Utilization and Research, USDA, Peoria, IL 61604; [3]School of Pharmacy, The University of Mississippi, University, MS 38677; [4]College of Pharmacy, Oregon State University, Corvallis, OR 97331; [5]Mycosynthetix, Inc., Hillsborough, NC 27278; [6]Techical Innovation Center, McCormick & Co., Inc., Hunt Valley, MD 21031

In 2012, the *Journal of Natural Products* has reached a major milestone, through the publication of volume 75. The journal first appeared in 1938 under the title *Lloydia*, and was published by the Lloyd Library and Museum, Cincinnati, Ohio. The founding Editor of *Lloydia* was Theodor K. Just, who continued in this position until his death in 1960. The journal became affiliated with the American Society of Pharmacognosy (ASP) in 1961, with Arthur E. Schwarting as Editor. The title was changed to the *Journal of Natural Products* in 1979, under the editorship of Jack L. Beal. In 1992, while James E. Robbers was Editor, the journal moved to its present monthly publication. Since 1996, with A. Douglas Kinghorn serving as Editor, the journal has been co-published by ASP and the American Chemical Society (ACS). The journal has changed in technical scope substantially since its inception. While originally specializing in mycology, *Lloydia* began to have a phytochemical focus after its affiliation with ASP. Today, the *Journal of Natural Products* publishes papers on the biologically active constituents of terrestrial microbes and animals and marine fauna and flora as well as higher plants. Over the last 16 years, since the co-publication of the *Journal of Natural Products* by ASP and ACS, many technical changes have occurred, with the most evident being its electronic publication and the web-based submission, peer review, editing, and production processes. The journal publishes contributions from authors in countries all over the world. Since the very successful co-publication arrangement between ASP and ACS, the number of citations per year has increased substantially (16,840 in 2010 vs. 3,634 in 1997), and the ISI Impact Factor has doubled (2.872 in 2010 vs. 1.432 in 1997). The Editors of the *Journal of Natural Products* are pleased and privileged to have organized this special symposium to celebrate the 75th volume of *Lloydia*/*Journal of Natural Products*. Three speakers have been selected who have provided sustained support of the journal as an author, reviewer, editorial advisory board member, and/or editor, namely, Professors Bob Pettit (Arizona State University), Rachel Mata (National Autonomous University of Mexico), and Bill Gerwick (University of California at San Diego. This symposium is dedicated to our colleague Dr. David J. Slatkin (Chicago State University), who as ASP Treasurer since 1983 has managed meticulously many of the financial and fulfillment aspects for the *Journal of Natural Products*.

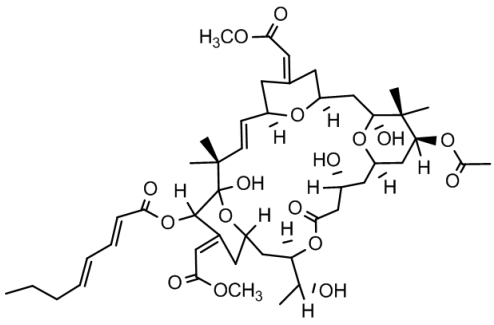
IL17 From a sea hare to global clinics

Pettit GR[1]
[1]Regents Professor and Cancer Research Group, Department of Chemistry & Biochemistry, Arizona State University, Tempe, AZ 85187

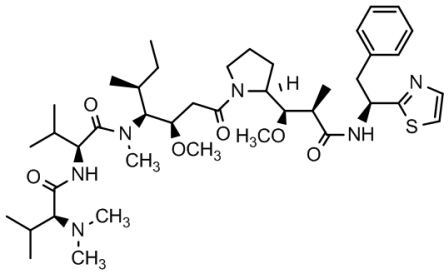
The pioneering research we directed at evaluating a broad selection of marine organisms from diverse ocean areas in 1965 forward has certainly helped accelerate interest in exploring marine microorganisms, invertebrates, and vertebrates as new sources of improved drugs for a great variety of indications. Even now, less than 0.5 percent of the marine animals, for example, have received even a cursory effort to detect

antineoplastic constituents. Thus, the most important marine animal and microorganism anticancer drugs still await discovery. Furthermore, these natural products are a result of 3.8 billion years of evolutionary biosynthetic organic reactions aimed at even more specific molecular design and targeting. The net result of these trillions and trillions of biologically directed organic reactions (biosynthetic combinatorial processes) is an astronomical number of candidates for use as anticancer drugs and as drugs necessary across most medical indications. But they need to be discovered and developed to the clinic. Our research group has been very fortunate to detect anticancer drug candidates in a variety of marine invertebrates and microorganisms. Today's summary will be focused on our discoveries of bryostatins, dolastatins, and auristatins and current status in human cancer clinical trials. Special emphasis will be placed on our advances leading to ADCETRIS™ and HALAVEN™, which were recently approved for essentially worldwide use in improving certain cancer treatments. The discovery of new drugs based on marine organism, microorganisms and plant constituents represents an exceedingly productive and exciting frontier for making great advances in medicine.

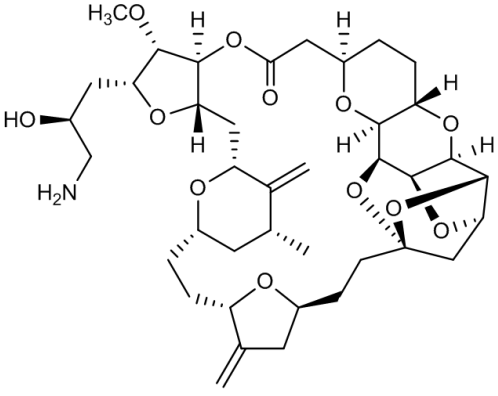
Bryostatin 1



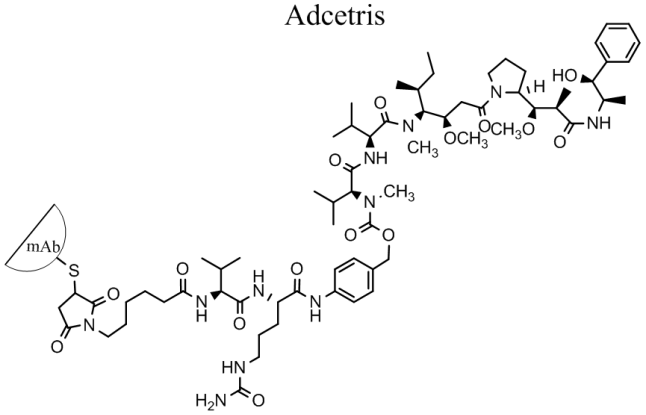
Dolastatin 10



Halaven



Adcetris



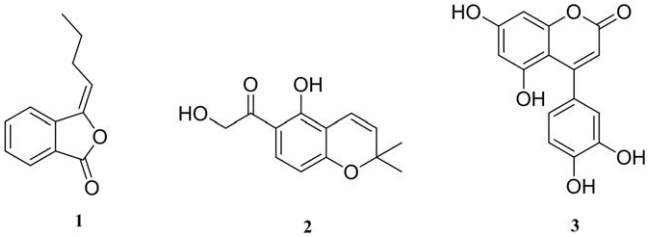
IL18

Mexican antidiabetic herbs: A valuable source of alpha-glucosidase inhibitors

Mata R[1], *Cristians S*[1], *Escandón-Rivera S*[1], *Navarrete A*[1], *Bye R*[2]

[1]*Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico;* [2]*Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico*

In Mexico, there are more than 300 hundred plants species employed for the treatment of type-2 diabetes mellitus (T2 DM), the commonest endocrine disorder affecting more than 100 million people worldwide. A few Asteraceae of the genus *Brickellia*, *Ligusticum porteri* (Apiaceae), and some Rubiaceae [*Hintonia standleyana*, *Hintonia latiflora* and *Exostema caribaeum*] are among the most highly prized species. The chemistry of the latter group of plants has been investigated by our research group since 1987 and the results published in the *Journal of Natural Products*. More recently, we have shown that the extracts and some of the hypoglycemic active principles of *H. latiflora*, *L. porteri,* and *Brickellia cavanillesii* inhibited the activity of the α-glucosidases. These findings revealed some of the molecular mechanisms underlying the antidiabetic action of these plants as well as a few novel templates for the development α-glucosidases of new drugs useful for controlling fasting and postprandial blood glucose levels. Thus, some phthalides of *L. porteri*, in particular, 3-*Z*-butylidenephthalide (**1**, 56.2 mg/kg), decreased blood glucose levels in STZ-diabetic mice after an oral sucrose load, suggesting that its antihyperglycemic effect is due to inhibition of α-glucosidases at the intestinal level. Furthermore, **1** inhibited yeast α-glucosidase activity (IC_{50} 2.35 mM) in a non-competitive fashion with a *K*i of 4.86 mM. Docking analysis predicted that **1** binds to the enzyme in a pocket close to the catalytic site but different to that for acarbose. Bioassay-guided fractionation of the active extract of *B. cavanillesii* using the same α-glucosidase inhibitory assay yielded several active compounds including 6-hydroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (**2**, IC_{50} 0.42 mM *vs.* 1.7 mM for acarbose), a new chemical entity. Kinetic analysis revealed that **2** is a non-competititive inhibitor with a *K*i of 0.13 mM. From the Rubiaceae, the most active inhibitor was 5,7,3′,4′-tetrahydroxy-4-phenylcoumarin (**3**, IC_{50} 3 μM).

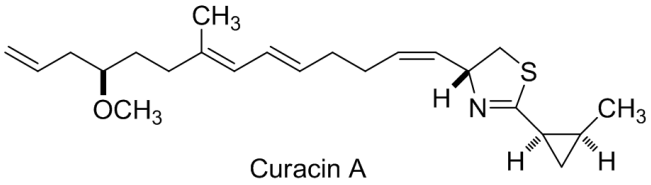


1 **2** **3**

IL19 Integrating disciplines in the natural products sciences: The story of Curacin A

Gerwick WH[1,2], Hamel E[3], White JD[4], Gerwick L[1], Sherman DH[5], Smith JL[5]
[1]Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92037; [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, UC San Diego, La Jolla, CA 92037; [3]Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, Maryland 21702; [4]Department of Chemistry, Oregon State University, Corvallis, OR 97331; [5]Life Sciences Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109

In 1993 we made collections of a filamentous marine cyanobacterium, originally identified as *Lyngbya majuscula*, from near the CARMABI research station in Curaçao. Subsequent study of these reddish hair-like bacteria and their fascinating natural products has engaged my laboratory and numerous collaborative partners and students for nearly 20 years. This was stimulated by potent cancer cell toxicity in the crude extract, and subsequently, we isolated and determined the structure of a novel active metabolite given the name 'curacin A'. Ensuing studies have probed the pharmacology of this new antimitotic agent and have used synthetic and semi-synthetic approaches to determine structure-activity features. Additionally, the biosynthetic origin of curacin A was mapped from isotope feeding studies, and genetic methods were used to locate and characterize the biosynthetic gene cluster. This latter milestone, published in the *Journal of Natural Products* in 2004 (*67*, 1356 – 1367), has spawned numerous investigations of the mechanistic biochemistry of curacin A formation, including a novel means for initial loading the Polyketide Synthase pathway with acetate, a new reaction manifold for cyclopropyl ring formation that includes cryptic chlorination, and a unique method of offloading from the megasynthase that results in terminal alkene formation. X-ray crystallographic approaches have been used to gain deep insights into the biochemical mechanisms of several of these biosynthetic enzymes. These efforts recently led to a genome sequencing project of the producing strain which gave further insights of the pathway as well as other features of its life history, including the fact that it does not fix atmospheric nitrogen. The genome sequencing project also helped recognize that the producing cyanobacterium constituted a new genus which we named *Moorea* in recognition of the pioneering efforts of Richard E. Moore in this area of marine natural products research.

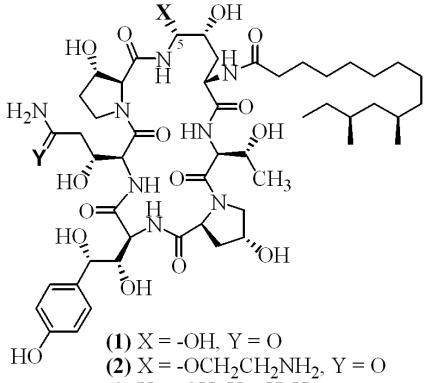


Curacin A

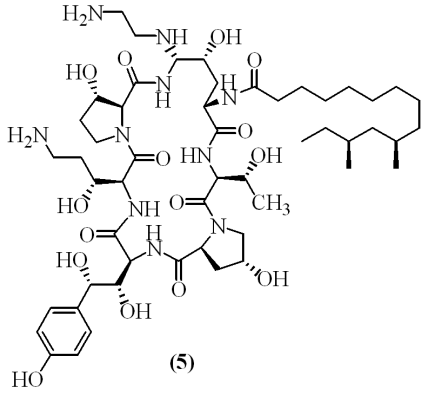
IL20 Chemical optimization of Pneumocandin B_0 and selection of the pre-clinical candidate Caspofungin

Balkovec JM[1], Black RM[2], Bouffard FA[3], Dropinski JF[4], Hammond ML[4], Zambias RA[5]
[1]ChemTract, LLC; [2]Bristol-Myers Squibb; [3]deceased Dec 14, 2008; [4]ML Hammond Consulting, LLC; [5]ThermoFisher Scientific

Pneumocandin B_0 **(1)** was a novel 1,3-β-D-glucan synthase (GS) inhibitor (GS IC_{50}= 70 nM) related in structure to the known antifungal compound echinocandin B. It was a relatively high molecular weight lipopeptide with a number of hydrogen bond donors and acceptors. As such, the compound lacked appreciable water solubility and oral bioavailability. In addition, its antifungal spectrum was limited to several *Candida* spp. Chemical modification of **(1)** led to the discovery that cationic groups could greatly improve the potency, spectrum and water solubility of the compound. Introduction of ether-linked amino groups at the C5-orn position gave rise to compound **(2)**, which was approximately tenfold more potent than the natural product and possessed potent activity against *Aspergillus* spp.. Reduction of the 3-hydroxyglutamine to the 3-hydroxyornithine gave analog **(3)** which was 15-fold more potent than **(1)**. Introduction of both modifications gave the dicationic derivative **(4)**, a compound with unprecedented potency (GS IC_{50}= 1 nM). In depth evaluation of **(4)** revealed that it had unexpectedly poor pharmacokinetics in chimpanzees and displayed unacceptable toxicity. Chemistry was developed that allowed introduction of amine-linked cationic groups with moderated basicity yielding improvements in the pharmacokinetic and toxicological profile. The aza-analog of compound **(4)** was selected as a pre-clinical candidate and after extensive evaluation was developed as caspofungin **(5)**.



(1) X = -OH, Y = O
(2) X = $-OCH_2CH_2NH_2$, Y = O
(3) X = -OH, Y = H, H
(4) X = $-OCH_2CH_2NH_2$, Y = H, H

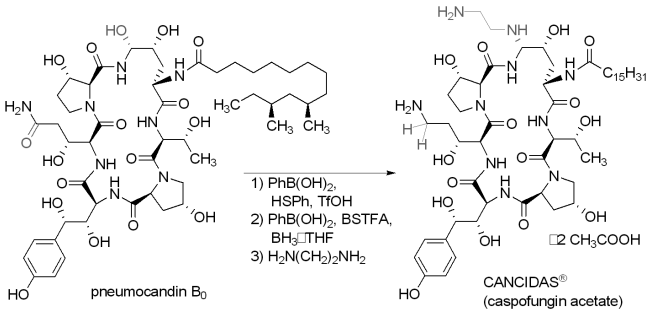


(5)

IL21 From lab to patient: Development of API and formulation manufacturing processes for Cancidas®

Hughes D, Belyk K, Leonard W, Bender DR, Nerurkar M, Hunke W, Kaufman M
Department of Process Chemistry; Department of Pharmaceutical Sciences and Clinical Supplies, Merck Research Laboratories

The design and development of API and formulation manufacturing processes for caspofungin acetate (CANCIDAS®) represented unique challenges for process chemistry and pharmaceutical sciences. From the synthetic aspect, the starting material is derived from a complex fermentation process, which is difficult to control particularly with regard to the more than 20 closely related analogs that are typically part of the fermentation product. The chemical transformations in the process require exceptional selectivity for success, as one is working on a complex, chemically sensitive natural product that is loaded with reactive functional groups. The isolation, purification and crystallization of the final product are not straightforward, as the combination of a hydrophilic core plus a hydrophobic side chain makes for unusual solubility behavior and soapy qualities which render conventional work up procedures impossible. Caspofungin and its intermediates are unstable, degrading via hydrolysis, dimerization, and oxidative pathways, which limit the reactions and isolation procedures available. Soapy compounds like caspofungin are notoriously difficult to crystallize, but a crystalline diacetate salt was ultimately discovered that provided an increase in stability that was crucial for the commercialization of this product.



The inherent instability of the API made the development of a stable IV formulation a particular challenge. A solution formulation was ruled out early due to instability across all viable pH ranges, even at refrigerated temperatures. Other options pursued included sterile crystalline API, frozen solution, and lyophilized API. Ultimately a uniquely stable lyophilized product was developed consisting of acetate buffer, sucrose, and mannitol.

IL22 The initial clinical evaluation of caspofungin in patients with fungal infections

Sable C
Merck Research Laboratories, Upper Gwynedd, PA

Caspofungin is an echinocandin antifungal with a spectrum of activity that includes *Candida albicans*, non-*albicans Candida* spp., and *Aspergillus* spp. The echinocandins have a unique mechanism of action and inhibit the synthesis of 1,3-β-D-glucan in the fungal cell wall; there is no cross resistance expected with azoles and polyene antifungals. Caspofungin has a half-life of 9 to 11 hours, and because of its very low oral bioavailability can only be administered intravenously. The initial clinical evaluation of caspofungin was in 1996. There were several considerations in selecting the first study in patients. As an IV only agent, it was important that the fungal infection that was serious enough so that intravenous therapy was appropriate. However, there were no clinical data on treatment of any fungal infection with an echinocandin, so it was important that the infection that was not life threatening in case treatment was found to be ineffective. Another consideration was that the fungal infections potentially treatable by caspofungin were relatively uncommon, so it was also important that the disease selected had standardized criteria for diagnosis and response to treatment so that a multinational trial was feasible. The initial clinical trial of caspofungin was conducted in patients with esophageal candidiasis. This was a double-blind, randomized, comparative, dose ranging study in which doses of caspofungin were compared to treatment with amphotericin B. The design and conduct of this initial trial will be reviewed and the key features of the subsequent development of caspofungin will be highlighted.

IL23 Rationale and discovery of Pneumocandins, the lead for Caspofungin

Schwartz RE, Onishi JC, Giacobbe R, Zink D, Meinz M, Wilson K
Merck Research Laboratories, Rahway, NJ

When the antifungal screening program at Merck that led to Caspofungin was initiated, two classes of antifungal therapies were being used clinically. The first was Amphotericin B a fungicidal target which involved cell-membrane perturbation, not surprisingly, with significant toxic liabilities. The second class consisted of a variety of econozoles which inhibited ergosterol synthesis, an effective fungistatic target with, however, potential for resistance development. The goal of the screening program was to discover a fungicidal natural product as a lead for a medicinal chemistry program, which was significantly less toxic than Amphotericin B and less prone to resistance development than the econazoles. The fungal cell wall became the target and the glucan synthesis inhibitor, pneumocandin B_0 the lead that was chosen, but not without some trepidation about the challenges that had to be overcome to produce this complex natural product...

IL24 Fermentation development of Pneumocandin B_0: the ultimate lead for making Caspofungin

Masurekar PS, Fountoulakis JM, Hallada TC, Sosa MS, Kaplan L
Merck Research Laboratories, Merck and Co., Inc. Rahway NJ 07065

Glarea lozoyensis, the producer fungus, biosynthesizes a family of related compounds. As described in the previous lecture eleven members were isolated and their structures were determined. The major product was named pneumocandin A_0. This compound was similar in structure and in biological, (antifungal) activity to echinocandin B, discovered and patented by Eli Lily and company scientists. Due to these similarities it was not clear at that time whether or not pneumocandin A_0 could be patented by Merck. An effort was initiated to find a pneumocandin that was significantly structurally unique and to develop a fermentation process to produce it. In the early phases of research it was noticed that one of the pneumocandins had proprietary structure; it was named pneumocandin B_0. The wild type *G. lozoyensis* produced B_0 in significantly lower amounts than A_0, so the goal of the effort was set to increasing B_0 and reducing A_0 to assist the isolation and purification of B_0. To achieve this, the mutagenesis was used to modify the genetic make-up of *G. lozoyensis*. This effort was successful and a number of mutants were isolated, latest of which produced B_0 as the main product with no detectable A_0. *G. lozoyensis* also produced a positional isomer named pneumocandin C_0, which contains 4-HO proline in place of 3-OH-proline found in B_0. With mutagenesis and medium modification levels of C_0 were reduced to less than 3% compared to B_0. In parallel with genetic modification seed and production media were developed to optimize production of pneumocandin B_0, increasing its yield several fold over that produced by the wild type *G. lozoyensis*.

IL25 50 years of NMR: The past, present and future

Keller TW
Bruker Biospin, Karlsruhe, Germany

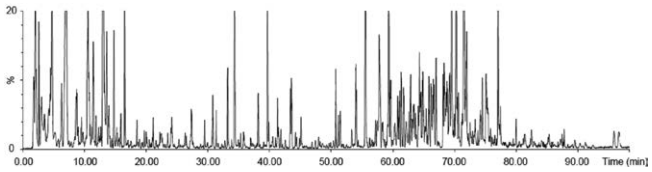
This presentation will focus on the development of NMR technology at Bruker from a 25 MHz system in 1960 to today's highest field NMR, the 1000 MHz. The introduction of the first commercial transistorized multichannel system in 1967 started the technology revolution that allowed routine access to NMR data. The introduction of FT spectroscopy as the standard method of data acquisition significantly lowered the substance requirement, thus allowing ^{13}Cspectroscopy to become a standard method for structure elucidation (1969). Incorporation of multidimensional NMR and the use of stable superconducting magnets opened up the field to protein structure determination. Automation brought to market significant productivity enhancements, while the introduction of the digital spectrometer allowed for higher reliability and reproducibility. Another important step was the introduction of hyphenated NMR, the combination of NMR in line with LC and MS. This advancement allowed for the use of NMR to analyze complex mixtures and biological samples, i.e. body fluids. This new application required a new level of reproducibility and stability. New levels of hardware and software performance brought us closer to fully automated structure determination. Bruker's introduction of commercially available cryoprobes, together with ultra high field magnets helped to overcome, at least partially, the everlasting fight for sensitivity. Pictures and comments from 50 years of experience at Bruker will enlighten everyone who has relied on an NMR for their research.

IL26 Advances in chromatography: Efficient profiling of crude extracts and isolation of natural products

Wolfender JL
School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30, quai Ernest-Ansermet, CH-1211 Geneva, Switzerland

The large chemical space occupied by natural products (NPs) is directly linked to a high variability of their intrinsic physicochemical properties that render their separation, detection and characterization challenging. The analysis of NPs in complex crude extracts requires efficient separation methods. In this respect, high-performance liquid chromatography (HPLC) has been recognized since the early 1980 s as the most versatile technique for their profiling directly in crude mixtures without the need for complex sample preparation.[1] HPLC has greatly developed through

the years in terms of convenience, speed, choice of column stationary phases, high sensitivity, applicability to a broad variety of sample matrices, and ability to hyphenate the chromatographic methods to spectroscopic detectors. The latest developments of HPLC, including the recent introduction of very pH-stable phases, sub-2-µm particles, monolith and fused core columns, have considerably improved the performances of HPLC. In particular in phytochemical analysis, the recent introduction of Ultra High Pressure Liquid Chromatography (UHPLC) systems operating at very high pressures and using sub-2 µm packing columns have allowed a remarkable decrease in analysis time and increase in peak capacity, sensitivity and reproducibility compared to conventional HPLC.[2] Such developments have a significant impact on the quality of data that can be obtained for metabolite profiling, dereplication studies and metabolomics when efficient spectroscopic detectors such as time-of-flight mass spectrometers (TOF)-MS are hyphenated to UHPLC. On the other hand, a good mastery of the chromatography parameters at the analytical scale enables efficient gradient transfer to the semi-preparative or the preparative scale based on chromatographic calculation. This efficient upscale allows high loading of crude mixture either for rapid at-line biological profiling of crude extracts with sensitive assay and/or *de novo* structure determination with microflow NMR methods with microgram of NPs. These developments, as well as other new trends in chromatography such as the renewed interest for superfluid critical fluid separation, the use of two dimensional LC or the hyphenation with additional separation efficiency in the gas phase provided by ion mobility in LC-MS hyphenation for the deconvolution of NPs in complex crude plant extracts will be discussed.



Example of a high resolution UHPLC-TOF-MS profiling of the crude leaf extract of *A. thaliana*.

(1) J.-L. Wolfender, *Planta Med.* **2009**, *75*, 719. (2) P. Eugster, D. Guillarme, S. Rudaz, J. L. Veuthey, P. A. Carrupt, J. L. Wolfender, *JAOAC* **2011**, *94*, 51.

IL27 What good is a mass spectrometer if you have an NMR?

Cech NB
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As a result of improvements in mass spectrometry ionization techniques (MALDI and electrospray) and advances in mass analyzer technology (orbitraps and q-ToFs), it is now possible to obtain accurate mass measurements (Fig. 1) of natural products even in very complex biological matrices. Nonetheless, the most common application of mass spectrometry in natural products research is for a final confirmation of molecular formula on compounds already purified and characterized with NMR. NMR has significant advantages over mass spectrometry of enabling molecular connectivity to be established, and providing data that is far less dependent on instrument parameters. Thus, it is no surprise that NMR is the technique of choice for structure elucidation of unknowns, and it will likely continue to be so for the foreseeable future. However, mass spectrometry has complementary capabilities that can be a major boon to natural products researchers. For example, it is possible using mass spectrometry to track compounds throughout the isolation process, thereby correlating their presence with biological activity (Fig. 2). This approach helps to overcome some of the biases that are inherent in the isolation process, and facilitates dereplication and the identification of analogs and synergists. Another important application of mass spectrometry in natural products research is its use for studying bioactivity and mechanism of action. This can be accomplished using targeted or untargeted metabolomics, or proteomics. This talk will include an exploration of some of the major strengths and limitations of mass spectrometry as a tool for natural products research, highlighting how recent advances in instrumentation have enabled new applications, and identifying areas with the greatest potential for future development.

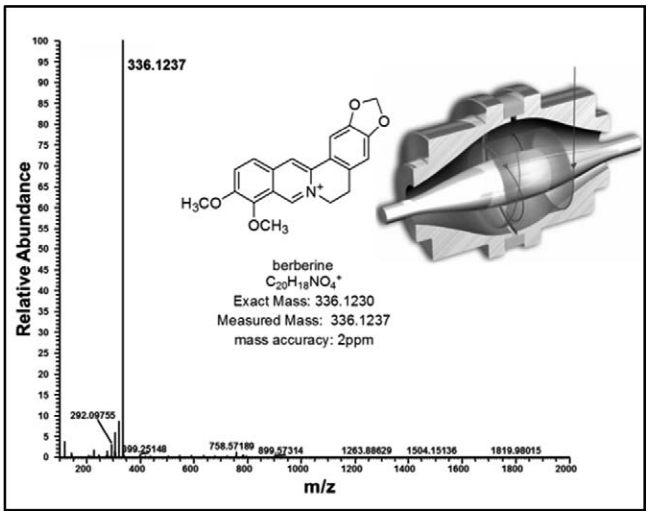


Fig. 1: Orbitrap mass analyzer superimposed on an orbitrap mass spectrum of the alkaloid berberine. Mass accuracy of 2 ppm was obtained without internal calibration.

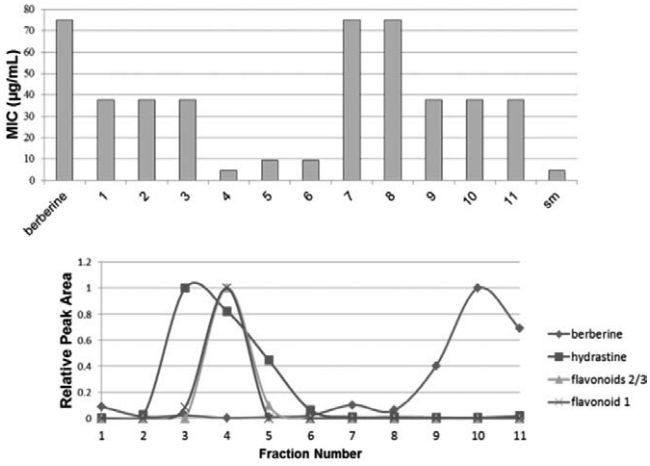


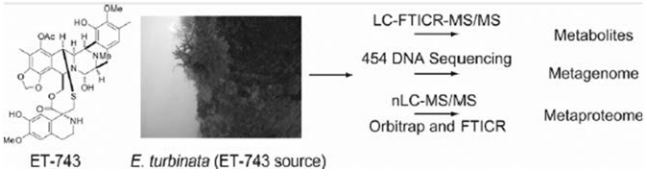
Fig. 2: Correlation of constituent profiles with bioactivity of fractions from a Hydrastis canadensis extract. The presence of flavonoids in fraction 4 correlates with its superior antimicrobial activity (low MIC).

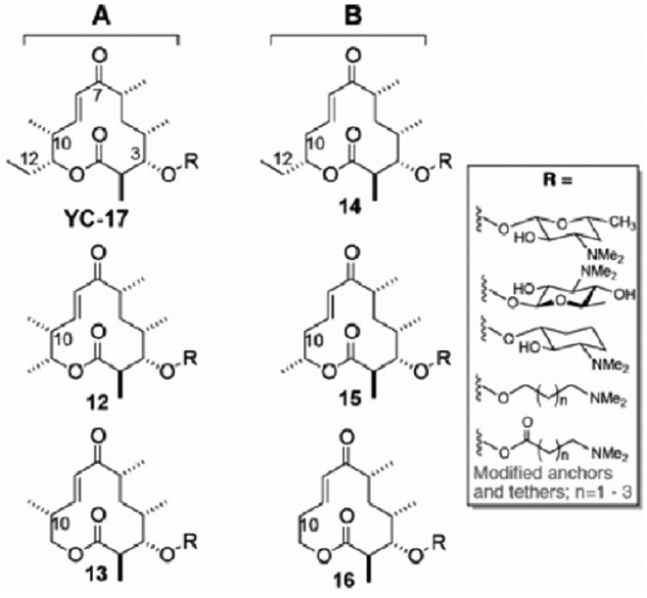
IL28 Chemical diversity and metabolic versatility in microbial natural product biosynthesis

Sherman DH
Life Sciences Institute and Departments of Medicinal Chemistry, Microbiology & Immunology, and Chemistry, University of Michigan, Ann Arbor, MI

In many marine macroorganisms (e.g. sponges, tunicates, soft corals), the ultimate source of potent biologically active natural products has remained elusive due to an inability to identify and culture the producing symbiotic microorganisms. As a model system for developing a meta-omic approach to identify and characterize natural product pathways from invertebrate-derived microbial consortia we chose to investigate the ET-743 (Yondelis®) biosynthetic pathway. This molecule is an approved anti-cancer agent obtained in low abundance (10^{-4}-10^{-5}% w/w) from the tunicate *Ecteinascidia turbinata*, and is generated in suitable quantities for clinical use by a lengthy semi-synthetic process. Based on structural similarities to three bacterial secondary metabolites, we hypothesized that ET-743 is the product of a marine bacterial symbiont. Using metagenomic sequencing of total DNA from the tunicate/microbial consortium we targeted and assembled a 35 kb contig containing 25 genes that comprise the core of the NRPS biosynthetic pathway for this valuable anti-cancer agent. Rigorous sequence analysis based on codon usage of two large unlinked contigs suggests that *Candidatus Endoecteinascidia frumentensis* produces the ET-743 metabolite. Subsequent me-

taproteomic analysis confirmed expression of three key biosynthetic proteins. Moreover, the predicted activity of an enzyme for assembly of the tetrahydroisoquinoline core of ET-743 was verified *in vitro*. This work provides a foundation for direct production of the drug and new analogs through metabolic engineering. We expect that the interdisciplinary approach described is applicable to diverse host-symbiont systems that generate valuable natural products for drug discovery and development. In another effort, we are pursuing studies of diverse natural product cytochrome P450 enzymes as biocatalysts toward C-H bond activation against native and unnatural substrates. This work was initiated by characterization of the anchor-group directed pikromycin pathway PikC monooxygenase. More recently, we have identified and probed the function and specificity of an iterative P450 enzyme that catalyzes three sequential oxidation steps including 2° hydroxylation, epoxidation and 1° hydroxymethylation. Structural studies are providing deep insights into the basis for regio- and stereoselective C-H bond oxidation, and are facilitating efforts to apply these remarkable biocatalysts for creation of structurally diverse biologically active molecules.





This work was supported by NIH grants R01 GM078553, U01 U01 TW007404 and the H. W. Vahlteich Professorship.

IL29 Ethnobotany and its role in improving primary health care delivery: An example from the pacific islands of Micronesia

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Ethnobotany, through the study and analysis of traditional medical practices based on natural substances-primarily plants but also animal and mineral products–can have a role in improving primary health delivery. This presentation considers two examples from a remote region of the tropical Pacific; however, there are great possibilities for using this approach in the urban setting. The model that has been developed first involves an inventory of the plant resources of a specific region. In the case of a small island, a geographically limited environment, the inventory work must be very precise and comprehensive, capturing knowledge of what is available both in nature as well as cultivated by local people. Next, or in parallel, an inventory is made of plants used for healing and wellness, interviewing as wide and diverse a sample of the population as possible, while establishing parameters for the information to be gathered-in our model limiting this to "common" or "generalist" knowledge and avoiding information that is considered secret or the property of a specific family or clan. These parameters are set by the traditional leaders of each island, working together with the local health care providers and strive to achieve local goals. The next step is to format the information into a condition-based primary health care manual: including general information on how to treat the conditions from a clinicians' perspective but presented in layperson's terms; when a trip to the State hospital or emergency room is mandated; how pharmaceutical products that may or may not be available can be used; and then for each condition (chapter) a series of plant-based remedies, including a photograph and description of the plant for positive identification, information on preparation, and pharmacological and toxicological data as available. Finally, the document is copyrighted in the names of the local traditional leaders' organization and/or Ministry of Health, as well as the research organizations involved as a clear statement of the ownership of the contents and rights to its utilization. To date we have published two manuals, one for the island of Pohnpei in the Federated States of Micronesia, and one for the Republic of Palau. The model has been endorsed by the Pacific Basin Medical Association as a contribution to a more sustainable and holistic approach to health care, particularly in helping to treat the current epidemic of non-communicable diseases (NCD's) on the small islands of Oceania. This work requires the active participation of a large team from many disciplines-both local and international–including traditional healers and their families, physicians, botanists, pharmacologists, toxicologists, linguists, social scientists, primary health care specialists as well as others.

IL30 Translational application of novel withanolides for cancer treatment from Physalis species and other Solanaceae

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Natural products have been the most significant source of drugs and drug leads in history. Plant biodiversity provides a resource of unlimited structural diversity for drug lead and molecular probe discovery programs such as those currently underway at the University of Kansas. Technology is gradually overcoming the traditional difficulties encountered in natural products research by improving access to biodiverse resources and ensuring compatibility of samples with high throughput procedures. Our drug lead discovery effort is aimed at uncovering bioactive molecules from plants of the U.S. Great Plains. Approaches utilized in the selection of samples consist of biodiversity-based collections and ethnobotanical information on the medicinal uses in the areas of study. In order to identify antiproliferative compounds, a library of over 1,200 plant extracts was tested for antiproliferative activity against an array of cell lines [melanoma cell lines (B16F10, SKMEL28); human head and neck squamous cell carcinomas (HNSCC) cell lines (MDA1986, JMAR, UM-SCC-2, JHU011); medullary thyroid carcinoma (MTC) cell lines (TT and DRO81 – 1), and a non-malignant cell line (MRC5)]. *In vitro* experiments included evaluation of anticancer activity and effects on cell proliferation by MTS assay and trypan blue exclusion. Apoptosis and cell cycle arrest were characterized by annexin V-propidium iodide (PI) flow cytometry and confirmed by Western Blot analysis for activation of caspase 3 and cleavage of PARP as well as cell-cycle specific proteins. Analysis of specific pathways including modulation of Akt, MAP-kinase and RET phosphorylation were evaluated *in vitro* as well as *ex vivo* in treated mice using Western blot analysis. *Physalis longifolia* Nutt. and several related members in the Solanaceae were identified as the most active of the species tested in this study. This led to the discovery of over 30 withanolide-type steroidal lactones, 16 of which are new compounds. In addition to withaferin A (WA), 14 withanolides showed IC50 values in the range between 0.067 and 9.3 μM *in vitro* by MTC with selectivity of 3 to 20 fold compared to normal cells. A mechanistic study showed that WA induced apoptosis and cell death in HNSCC and MTC cells as well as modulation of the cell cycle from G_0/G_1 arrest to a shift to G_2/M and S-phase. Western blot analysis demonstrated that cancer cells treated with WA exhibited inhibition of Akt and caspase-3 expression, and cleavage of PARP. WA was further evaluated *in vivo* in a metastaic murine model of MTC. All treated animals were alive, showing tumor regression and growth delay without toxicity or weight loss at six weeks post-treatment. Tumor cells treated with WA demonstrated inhibition of total

and phospho-RET levels by Western blot analysis in a dose dependent manner (almost complete inhibition with treatment of 5 μM WA) as well as potent inhibition of phosphor-ERK and phosphor-Akt levels. WA was shown to be a novel natural-product RET-inhibitor with efficacy in a metastatic murine model of MTC. This investigation constitutes the first report of the antiproliferative activity of WA and other withanolides against MTC and HNSCC. Procurement and screening protocols, isolation and structure determination, lead optimization, and antiproliferative activities of withanolides will be presented.

IL31 Reverse pharmacology of medicinal plants: Good luck or efficient method?

Graz B[1], Falquet J[1], Haouala A[2], Simoes-Pires C[2], Ndjoko K[2], Christen P[2], Cuendet M[2], Willcox M[3], Diallo D[4]
[1]University of Lausanne; [2]School of Pharmaceutical Sciences, University of Geneva; [3]Oxford University; [4]Traditional Medicine Department, Bamako, Mali

Reverse pharmacology has frequently been advocated in India and China (among other places), as a method based on the search for documented therapeutic effects of plants in ancient texts (Vaidya, 2006). Another definition of reverse pharmacology, inspired by ethnopharmacological approaches [Carvalho et al, 1991], begins with a documented outcome as observed by patients. Both approaches will be discussed, the latter in view of our experience with the "success story" of "improved traditional medicines" in Mali [Graz et al. 2011; Willcox et al., 2011]. In this context, the first step was a population-based ethnomedical survey which included patient accounts of recent experiences of the therapeutic itinerary (cure, worsening or adverse events after using traditional preparations) and interviews of traditional medicine practitioners [Diallo et al., 2006]. The traditional preparation based on *Argemone mexicana* (AM) appeared as the recipe associated with the best outcome among patients with presumed malaria. In subsequent clinical studies (a dose-escalating and a randomized controlled trial) in the village where the AM preparation was used, its safety and clinical efficacy was found non-inferior to the standard imported drug artesunate-amodiaquine in terms of clinical outcomes (need for second-line treatment, incidence of new episodes of malaria) [Willcox et al., 2007; Graz et al., 2010]. Several studies found that AM also has *in vitro* activity against *P. falciparum* (Adjobimey et al., 2004; Diallo et al., 2006). In terms of "evidence-based medicine", AM can now be proposed for pilot introduction in public health programs with careful evaluation of its impact. However, a problem remains: how to check the quality of the plant? Even if three alkaloids (berberine, protopine, allocryptopine) were detected by bio-guided fractionation and showed a significant *in vitro* activity [Simoes-Pires, 2009], some questions require further investigation. Are these alkaloids solely or even partially responsible for the clinical efficacy? In that case, are these alkaloids metabolized into more or less active compounds? Is there a synergy effect? Several research approaches are being explored in order to answer these questions: early ADME studies, including microsome metabolization and Caco-2 permeability assays; *in vitro* activity profiling of the alkaloids and metabolites; pre-clinical and clinical pharmacokinetics of the candidate compounds alone and/or after ingestion of the decoction. References: Adjobimey, T., Eday'e, I., Lagnika, L., Gbenou, J., Moudachirou, M., Sanni, A., 2004. C. R. Chimie 7, 1023 – 1027. Carvalho, L. H., M. G. Brandao, et al. (1991). "Antimalarial activity of crude extracts from Brazilian plants studied in vivo in Plasmodium berghei-infected mice and in vitro against Plasmodium falciparum in culture." Braz J Med Biol Res 24(11): 1113 – 1123. Diallo, D., Graz, B., Falquet, J., Traoré, A.K., Giani, S., Mounkoro, P.P., Berthé, A., Sacko, M., Diakité, C., 2006. Trans. R. Soc. Trop. Med. Hyg. 100, 515 – 520. Graz, B., Willcox, M. L., Diakité, C., Falquet, J., Dackuo, F., Sidibe, O., Giani, S., Diallo, D., 2010. Trans. R. Soc. Trop. Med. Hyg. 104, no1, 33 – 41. Graz B, Kitua AY, Malebo HM. To what extent can traditional medicine contribute a complementary or alternative solution to malaria control programmes? Malar J. 2011 Mar 15;10 Suppl 1:S6. [Epub ahead of print]. http://www.malariajournal.com/supplements/10/S1 Simoes-Pires, C. Thèse de doctorat. Université de Genève, 2009. – Sc. 4129. – 2009/07/27 Vaidya ADB. 2006. Reverse pharmacological correlates of ayurvedic drug actions. Indian J Pharmacol. 38: 311 – 315 Willcox M. L., Graz, B., Falquet, J., Sidibe, O., Forster, M., Diallo, D., 2007. Trans. R. Soc. Trop. Med. Hyg. 101, 1190 – 1198. Willcox ML, Graz B, Falquet J, Diakite C, Giani S, Diallo D. A "reverse pharmacology" approach for developing an anti-malarial phytomedicine. Malar J. 2011 Mar 15;10 Suppl 1:S8. [Epub ahead of print]

IL32 Wild tomatillos (Physalis species) as food and medicine

Kindscher K[1], Timmermann BN[2], Zhang H[2], Gollapudi R[2], Corbett S[1], Samadi A[3], Cohen M[3]
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An ethnobotanical perspective can contribute to the discovery of new medicines and drugs from plant biodiversity. With interest in withanolides, a group of anti- proliferative compounds in the Indian plant ashwaganda, *Withania somnifera*, our research team wanted to determine if any native US plant species might contain these or other compounds with similar structures. Withania is in the Solanaceae, so we considered plants in the genera of Solanum, Datura, and Physalis. As part of our Native Medicinal Plant Research Program, we have developed an extensive ethnobotanical database which lists all the edible and medicinal uses of plants in the Great Plains and Midwest. All of these Solanaceae genera had useful species, and we picked those species to explore further for biological activity. With work conducted in the Timmermann lab, we have found the longleaf groundcherry or wild tomatillo, *Physalis longifolia,* to have not only withaferin A, but also a large number of bioactive withanolides including several new structures. As a closely related group of Physalis species, the 29 wild tomatillo species found north of Mexico have been an important wild-harvested food and medicinal plant. I will discuss the traditional uses for food and medicine, and confusing taxonomic difficulties of this group. Subtle morphological differences recognized by taxonomists to distinguish these related taxa are confusing to botanists and ethnobotanists, and these differences are not recognized as important by native peoples. The importance of wild *Physalis* species is documented by at least 23 tribes using them as food, and its long history of use is evidenced by its discovery in at least 19 archaeological sites where it is often found in carbonized remains in the hearths where they were cooked. These sites, which date from the Archaic to the Classic period, stretch from the Rio Grande Valley in New Mexico and from southwest Colorado through the Great Plains and the Midwest and into Ontario, Canada and North Carolina in the east. *Physalis* species have tasty fruits and were collected from the wild and were encouraged in agricultural fields, and possibly even cultivated. In historic times and up to the present, these plants may have been cultivated by Pueblo farmers and other tribes of the US Southwest. They were used as food and medicine, but the primary use has been as food. Even today, the Pueblo people cook the wild tomatillo fruit and make them into a green sauce similar to commercial preparations. When found abundantly, they were dried for later use in soups and food. Due to the abundance of seeds in some species and in some archeological sites, it appears that the seeds might also have been dried, ground into powder and used in soups and stews. The importance of this significant food use over an extensive period of time provides some sense of the safety of use of this plant, and of its desirability. When dried, the fruits become sweeter and taste somewhat like a dried cranberry, with a slight tomato flavor. Although less important than food uses, Native American medicinal uses of *Physalis* were also common, including use by the Omaha, Ponca, and Winnebago to treat headache and stomach trouble, and as a dressing for wounds. Recent work by our research program shows that there are many withanolides within the plant and fruit, some of which have potent anti-proliferative activity. Due to the numerous, extensive, and long term uses of the fruits as food, it appears that they are safe to consume. Although this common plant is widely ignored and described as a weed, it was once an important plant for both food and medicine, and may be so again.

IL33 The development of Crofelemer: Connecting ethnobotany, conservation, biocultural diversity, global public health and indigenous knowledge

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Crofelemer is a novel first in class compound extracted, isolated and purified from the stem bark latex of the widespread pioneer tree species Croton lechleri, (Sangre de Drago). The natural distribution of this species includes the Northwest Amazon regions of Colombia, Ecuador, Peru, Bolivia and adjacent regions of the Brazil. The latex of this species has been utilized by numerous indigenous peoples of the western Amazon

basin orally for the treatment of diarrhea, topically for wound healing and many other therapeutic applications. Crofelemer is an acid-labile oligomeric proanthocyanidin. The primary monomers include (+)-gallocatechin; (-)-epigallocatechin; (+)-catechin and (-)-epicatechin. The oligomer predominantly contains 7 – 11 linked monomers in varying ratios and an average molecular weight ranges from 1800 to 2200 Da. A new drug application (NDA) for crofelemer for the treatment of HIV/AIDS related chronic diarrhea was submitted on December 5, 2011 to the US FDA and was granted a 6 month priority review designation. Crofelemer's antidiarrheal activity is mediated primarily through its action on two chloride channels. Specifically the compound reduces fluid loss and Cl- secretion through inhibition of both the cystic fibrosis transmembrane conductance regulator (CFTR) and calcium activated Cl- channels (CaCC) in the intestinal epithelium. The drug has minimal systemic absorption and has demonstrated an excellent safety profile in global clinical trials with > 2,000 patients dosed across multiple trials conducted in numerous countries. Most recently crofelemer demonstrated excellent efficacy and safety in a pivotal Phase 3 trial in HIV patients (ADVENT) in reducing secretory diarrhea with a p-value of 0.0096. Clinical trials have also been conducted at the International Center for Diarrheal Disease Research (ICDDR) in Dhaka, Bangladesh, demonstrating significant reduction in fluid loss in patients with cholera-induced diarrhea, a life-threatening condition that most recently killed 7,000 people Haiti. One goal of Napo is to make certain that crofelemer can be made available to help save lives in cholera outbreaks wherever they may occur in the world. Crofelemer cannot be produced synthetically for commercialization due to its complex chiral chemistry. The compound is extracted and isolated from latex of the Croton lechleri. We have created long-term sustainable management and harvest programs of latex in collaboration with local business partners and communities. This includes reforestation and management programs in multiple sites. Cultivation and wild harvest of this species has and will continue to provide income to local communities who are seeking to utilize tropical forest biodiversity in a sustainable long-term manner. These programs will be discussed along with our focus on the conservation of biocultural diversity and long-term benefit sharing through the Healing Forest Conservancy (HFC). Diarrhea continues to kill more then 2 million children per year and is one of the major causes of childhood illness and death. Napo is focusing on accelerating the development of a pediatric form of crofelemer that can be distributed to pediatric patients, to be used in combination with ORS and zinc, to prevent the severe dehydration and resulting death caused by acute infectious diarrhea in children.

IL34 Pharmacophore-based virtual screening – A valuable tool for the identification of bioactive natural products

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Almost half of the drugs currently in clinical use are of natural product origin and even today, in the post genomic era, plants, fungi, marine organisms, and microorganisms are still an important source for the development of new drugs[1]. The immensely high chemical and biological diversity of natural products and the possibility to fish in an infinite pool of sophisticated compounds render these chemical entities a profitable and efficient source of new hit and drug leads. However, it remains an exciting challenge to target and efficiently select the most promising bioactive compounds from the multitude and biodiversity of natural products. Pharmacophore based virtual screening is a well-established tool to assist early drug discovery and development. Among others it can be used to predict biological activities of small organic molecules and to select promising compounds for biological testing. More recently, molecular modeling is more and more integrated in natural product research[2 – 4]. It allows for a more focused isolation strategy in natural product research, compared to the classical bioactivity-guided isolation approach. In particular, virtual screening can provide suggestions for hitherto not considered pharmacological effects or mode of action elucidation of ethnopharmacologically used drugs. An extension to classical single-target focused virtual screening is virtual parallel screening, which is able to predict potential biological activities (desired or unwanted effects) of single compounds by screening them against a multitude of pharmacophore models[5,6]. To guarantee a maximum of efficacy in natural product drug discovery an integrative concept is necessary combining different strategies, classical and innovative ones, and gathering as much information as possible from different fields, e.g. traditional medicine, botany, phytochemistry and pharmacology is required. In the course of an ongoing national research network project[7] involving scientists of six Austrian universities, we aim to identify and characterise compounds capable to combat inflammatory processes specifically in the cardiovascular system. The combined use of computational techniques including NP databases with traditional knowledge delivered so far a series of promising compounds with interesting anti-inflammatory effects e.g. ganoderic acid derivatives from *Ganoderma lucidum* as farnesoid X receptor (FXR) agonists, a lignan derivative from *Leontopodium alpinum* as inhibitor of cholesteryl ester transfer protein (CETP), neolignans from magnolia as selective peroxisome proliferator-activated receptor γ (PPAR) agonists and natural PGES-1 inhibitors with IC_{50} values in the nM range. This lecture will cover some highlights of this interdisciplinary project showing the usefulness of pharmacophore based virtual screening in the identification of bioactive natural products, but revealing also limits of the *in silico* approach. *References:* [1]Newman DJ & Cragg GM: *J. Nat. Prod.* 2007, 70,461 – 477; [2]Rollinger JM et al.: Prog. Drug Res. 2009, 65, 213 – 249; [3]Li XJ et al.: *Curr. Drug Discov. Technol.* 2010, 7, 22 – 31; [4]Schuster D &, Wolber G: *Curr. Pharm. Des.* 2010, 16, 1666 – 1681; [5]Rollinger JM: *Phytochemistry Lett.* 2009, 2, 53 – 58; [6]Rollinger JM et al.: *Planta Med.* 2009, 75, 195 – 204; [7]http://www.uibk.ac.at/pharmazie/pharmakognosie/dnti/ *Acknowledgements*: This work is financially supported by grant no S 107 „Drugs from Nature Targeting Inflammation" from the Austrian Science Fund.

IL35 Searching for marine natural product inspired drug leads

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The secondary metabolites found in marine organisms represent an extremely rich source of novel chemical diversity for academic drug discovery and chemical biology programs. Our group at UBC has amassed a sizable library of crude extracts from marine sponges and cultures of marine microorganisms collected in many of the world's oceans. In collaboration with biologists, this crude extract library has been screened for activity in a variety of cell-based and pure enzyme assays designed to identify promising lead compounds for the development of drugs. Bioassay-guided fractionation of the crude extracts and extensive spectroscopic analysis is used to identify the structures of the pure natural products active in these assays. Function oriented chemical synthesis is used by our group to probe the SAR for new natural product pharmacophores that we discover and to provide material for *in vivo* testing in animal models. This lecture will present recent chemical and biological results from our academic drug discovery/chemical biology research.

IL36 Don't ditch the NMR just yet: Using atomic force microscopy as an aid to solving problematic structures

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Images of chemical structures abound in the literature, and we use different formats to explain different features of the molecules in question. Recent advances in atomic force microscopy (AFM), using a carbon monoxide terminated tips have brought about a spectacular enhancement in the resolution attainable.[1] It is now feasible to distinguish chemical features such as atoms, bond lengths and bond angles at the Ångstrom scale. The images observed are very close to textbook stick structures and we postulated that this might be useful to assist organic structure determination. To test the possibility of using this technique for this purpose, we used a planar molecule to obtain NMR and AFM data and assembled the substructures guided by the AFM image (Figure 1).[2] Two solutions were deemed possible, and one was excluded based on density functional theory calculations leaving only one structure remaining. More recent studies have dealt with non-planar structures as well as the combined use of NMR, AFM and computer assisted structure elucidation to solve structures. The presentation will include the amount of material needed to perform such studies as well as the current limitations of the method. Finally, prospects for the future will be discussed for the integration of AFM into the structure determination armoury.

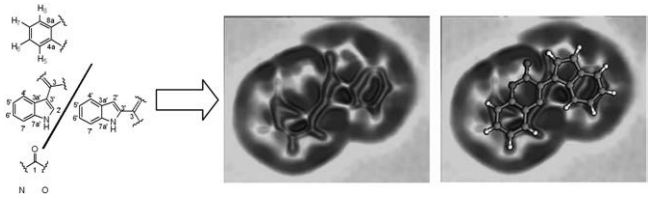

Figure 1. Substructure assembly guided by atomic force images to derive a unique solution. **References:** The chemical structure of a molecule resolved by atomic force microscopy. Leo Gross, Fabian Mohn, Nikolaj Moll, Peter Liljeroth, Gerhard Meyer, *Science*, **2009**, *325*, 1110 Organic structure determination using atomic resolution scanning probe microscopy. Leo Gross, Fabian Mohn, Nikolaj Moll, Gerhard Meyer, Rainer Ebel, Wael M. Abdel-Mageed and Marcel Jaspars, *Nature Chemistry*, **2010**, *2*, 821 – 825

IL37 Bacterial messages to Eukaryotes

Seyedsayamdost M[1], Carr G[1], Keyser S[1], Case R[2], Kolter R[2], Clardy J[1]
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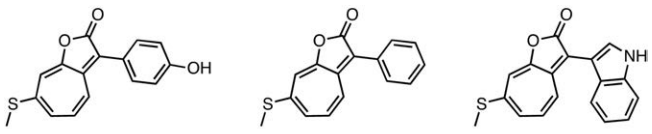
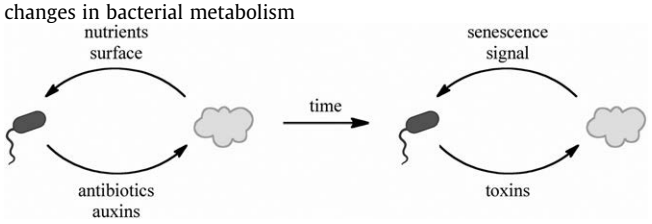
Symbiotic relationships involving microbes and eukaryotes come in many flavors as all eukaryotes have spent their entire evolutionary history on a planet teeming with microbes, and the two have evolved many ways of dealing with each other. This lecture will describe the small molecule chemistry that regulates a particularly complicated symbiotic relationship between bacteria belonging to the roseobacter clade and the microscopic alga *Emiliana huxleyi* and some of its relatives. The relationship is complicated because it changes with time as the bacterial chemistry responds to variable signals sent by their algal partner. The talk will involve three related topics, and the first is the isolation and structures of the bacterially produced roseobacticides, a group of roughly a dozen characterized molecules – and many more remaining to be characterized – with unusual structures as illustrated by roseobacticides A, B and C shown below.

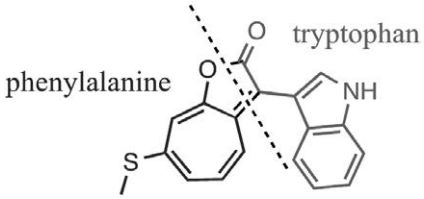


roseobacticide A roseobacticide B roseobacticide C

The second topic is the biology involved in the symbiosis, especially the roles of the newly identified roseobacticides. The relationship between bacteria and alga changes from a mutualism to an antagonism as shown below, and the changing relationship is reflected in, and caused by, changes in bacterial metabolism



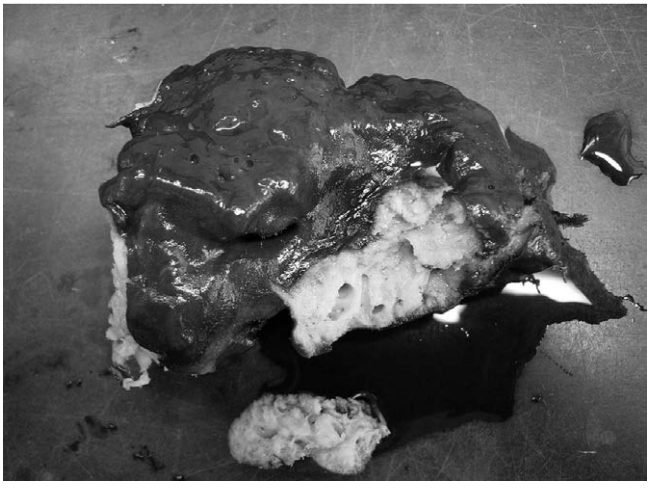
The third topic addresses how the bacteria biosynthesize roseobacticides from amino acid starting materials as outlined below.



IL38 Chemical surprises from an uncultivated sponge symbiont

Piel J[1], Freeman MF[1], Gurgui C[1], Helf M[1], Morinaka B[1], Wilson MC[1], Mori T[2], Rueckert C[3], Steffens U[1], Takada K[4], Gernert C[5], Uria AR[1], Wakimoto T[6], Abe I[6], Hentschel U[5], Kalinowski J[3], Takeyama H[2], Matsunaga S[4]
[1]Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Germany; [2]Faculty of Science and Engineering, Waseda University Center for Advanced Biomedical Sciences, Tokyo, Japan; [3]Institute for Genome Research and Systems Biology, Center for Biotechnology, Universität Bielefeld, Bielefeld, Germany; [4]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan; [5]Julius-von-Sachs Institute for Biological Sciences, University of Wuerzburg, Wuerzburg, Germany; [6]Graduate School of Pharmaceutial Sciences, The University of Tokyo, Japan

Marine sponges are a rich source of bioactive natural products and are promising sources for drug discovery and development An impressive example is the sponge *Theonella swinhoei*, which has yielded more than 120 compounds belonging to diverse structural types. Many sponges also harbor highly complex consortia of symbiotic bacteria that are suspected to be the true source of at least some of the secondary metabolites. In previous work, our group demonstrated a bacterial origin of onnamide- and psymberin-type polyketides for two different sponges (1 – 3), but there were no insights into the producer of compounds from other natural product families. In addition, the exact taxonomic identity of sponge-associated producers remained unknown. This talk will present new insights into these two issues. Isolation of genes encoding a peptide biosynthetic pathway from the *T. swinhoei* metagenome demonstrated a bacterial origin. Several genes were heterologously expressed and functionally characterized, which revealed unprecedented biosynthetic transformations. The novelty of these modifications suggests the existence of a structurally distinct natural product family, for which we propose the name proteusins. Using a strategy consisting of single-cell analysis and metagenomic sequencing, we identified the bacterial producer of onnamide polyketides in *T. swinhoei*. Surprisingly, the data suggest the symbiont to be a chemically exceptionally prolific bacterium, producing not only onnamides but most other compounds from this sponge chemotype, including the known and two previously unknown proteusins. Further biosynthetic studies and a survey of other sponges indicate that close relatives of the producer are widespread in these animals and vary with respect to their biosynthetic capabilities. These bacteria might therefore represent the first uncultivated taxon with a metabolic richness resembling that of major cultivated bacterial natural product sources. These results reveal a key role of symbiotic bacteria in the chemistry of their sponge hosts and provide new strategies to study uncultivated symbionts in a more systematic fashion.



Theonella swinhoei, onnamide chemotype

J. Piel, D. Hui, G. Wen, D. Butzke, M. Platzer, N. Fusetani, S. Matsunaga, *Proc. Natl. Acad. Sci. U.S.A.* (2004) 101:16222. T. Nguyen, K. Ishida, H. Jenke-Kodama, E. Dittmann, C. Gurgui, T. Hochmuth, S. Taudien, M. Platzer, C. Hertweck, J. Piel, *Nat. Biotechnol.* (2008) 26:225. K.M. Fisch, C. Gurgui, N. Heycke, S.A. van der Sar, S.A. Anderson, V.L. Vebb, S. Taudien,

M. Platzer, B.K. Rubio, S.J. Robinson, P. Crews, J. Piel, *Nat. Chem. Biol.* (2009) 5:494.

IL39 Chemistry of marine symbiotic interactions in ascidians and mollusks

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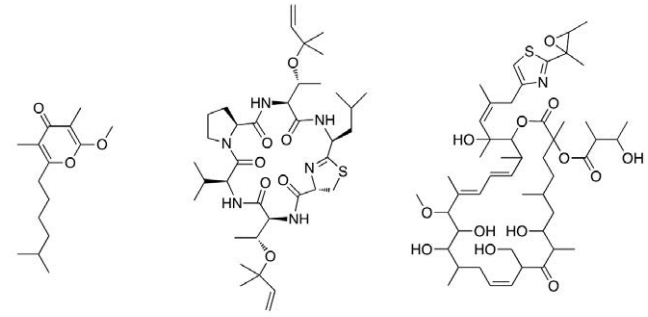
Diverse, bioactive molecules are found in marine animals. In some cases, it has been shown that symbiotic bacteria make marine animal natural products. Three different types of symbiotic associations will be described, running from casual interactions with actinomycetes to obligate, long-term symbioses. The different types of symbioses reveal different evolutionary strategies that impact the biosynthetic pathways. Application of this information to biosynthesis, pathway engineering, and natural product discovery will be described.



IL40 Recent studies on the bioavailability of dietary (poly)phenolics

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HPLC-tandem mass spectrometry is now is now used widely as an the analytical tool in studies on the bioavailability of dietary (poly)phenolics. Recent information on the absorption, disposition, metabolism and excretion of hydroxycinnamates, ellagitannins and various flavonoids that has been obtained in acute human feeding studies with teas, coffee, berries and fruit juices will be summarised. Typically, aglycones released by the action of cytosolic b-glucosidases or lactase phlorizen hydrolase in the small intestine appear in the circulatory system as glucuronide, sulfate and methyl metabolites 1 – 2 h after ingestion. Studies with ileostomists in which ileal fluid collected after ingestion was analysed indicate that substantial amounts of dietary (poly)phenols pass from the small to the large intestine where as a result of the action of the colonic microflora, a diversity of phenolic catabolites are produced. Evidence indicates that these catabolites are absorbed into the circulatory system and can be subjected to some degree of phase II metabolism before excretion in urine in substantial amounts. Of particular interest in this context are valerolactones, derived from flavan-3-ol monomers found in especially high concentrations in green tea and cocoa-based products, and urolithins which are produced in the colon from ellagitannins found in pomegranates and several berries. The potential bioactivity of colonic catabolites will be discussed. Related references Crozier, A., Jaganath, I.B., Clifford M.N. (2009). Dietary phenolics: chemistry, bioavailability and effects on health. Natural Product Reports **26**, 1001 – 1043 Stalmach, A., Mullen, W., Barron, D., Uchida, K., Yokota, T., Steiling, H., Williamson, G., Crozier, A. (2009). Metabolite profiling of hydroxycinnamate derivatives in plasma and urine following the ingestion of coffee by humans: identification of biomarkers of coffee consumption. Drug Metabolism and Disposition **37**, 1759 – 1768 Stalmach, A., Troufflard, S., Serafini M., Crozier, A. (2009). Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. Molecular Nutrition and Food Research **53**, S44-S53 Del Rio, D., Borges, G., Crozier, A. (2010). Berry flavonoids and phenolics: bioavailability and evidence of protective effects. British Journal of Nutrition **104**, S67-S90 Clifford, M.C., Crozier, A. (2011). Phytochemicals in teas and tisanes and their bioavailability. In: Teas, Cocoa and Coffee: Plant Secondary Metabolites and Health (Crozier, A., Ashihara, H. Tomás-Barbéran, F., eds.) Blackwell Publishing, Oxford, pp. 45 – 98 González-Barrio, R., Edwards, C.A., Crozier, A. (2011). Colonic catabolism of ellagitannins, ellagic acid and raspberry anthocyanins: in vivo and in vitro studies. Drug Metabolism and Disposition **39**, 1680 – 1688 Crozier, A., Clifford, M.N., Del Rio, D. (2012) Bioavailability of dietary monomeric and polymeric flavan-3-ols. In: Bioavailability and Function of Flavonoids (Spencer J.P.E., Crozier, A., eds.). Oxidative Stress and Disease, Vol. 30 (L. Packer, L., Cadenas, H., series editors) CRC Press, Boca Raton, pp. 45 – 78

IL41 Pharmacokinetics of Natural Compounds

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It is necessary for the rational use of any drug to have a good understanding of the concentrations that will be achieved in the body after its administration. Of particular interest is the question of bioavailability to assess to what degree and how fast the therapeutic agent is absorbed. Whereas there is usually detailed information available about the pharmacokinetics and biopharmaceutics of chemical drugs, this is usually not the case for natural compounds. However, in principle the same concepts apply since only with a good characterization of pharmacokinetics ('what the body does to the drug') and pharmacodynamics ('what the drug does to the body') it is possible to optimize the therapeutic use of the agent. Knowledge of the bioavailability and pharmacokinetics is essential for the correct *in-vivo* interpretation of *in-vitro* activities that are sometimes the basis of therapeutic claims. Of particular interest is the question of bioavailability to assess to what degree and how fast compounds are absorbed after administration of natural compounds. Of further interest is the elucidation of metabolic pathways (yielding potentially new active compounds), and the assessment of elimination routes and their kinetics. These data become an important issue to link data from pharmacological assays and clinical effects. Establishing the pharmacological basis for efficacy of natural compounds is a constant challenge due to their complex composition and the ever-increasing list of their putatively active constituents. In vitro assays normally are cheap and relatively easy to perform, but the relevance of the findings is based on a sufficient concentration of active constituents at the site of the action. Thus, these data become an important issue to link data from pharmacological assays and clinical effects. With increasing knowledge of putatively active compounds and availability of highly selective and sensitive analytical methods for certain natural compounds an increasing amount of data on bioavailability and pharmacokinectics have been reported recently. One common problem in the assessment of pharmacokinetic properties of natural products is that frequently the pharmacologically active agents are not known. This presents a dilemma since without clearly identified target compounds it does not make much sense to measure concentrations of the product ingredients. Only if a correlation exists between the concentration of an active component of a natural product and its efficacy and/or safety, pharmacokinetic studies of individual chemical entities are warranted. If this is not possible, an alternative approach for the characterization of natural compounds is the use of pharmacodynamic surrogates, which should be quantifiable and correlate with the therapeutic outcome. These surrogates allow evaluating the overall activity of a complex biological mixture and compare different products. Results from these pharmacodynamic studies may then also be helpful to identify the active ingredients and obtain a better scientific understanding of the pharmacological mechanisms. These studies will lead to appropriate criteria, which can be used to evaluate different natural products and their dosage forms and help to advance the field of herbal medicine from empirical experience to a more rational and safer pharmacotherapy. This presentation summarizes data available on bioavailability and pharmacokinetics of some commonly used natural compounds. Pharmacokinetic and bioavailability studies that have been conducted for some of the more important or widely used natural products are critically evaluated. A good understanding of their pharmacokinetics and bioavailability is essential in designing rational dosage regimens. Furthermore, various drug interactions are discussed which show that caution should be exercised when combining phytopharmaceuticals with chemical derived active pharmaceutical ingredients.

IL42 Mining the polyphenol metabolome to identify in population studies its components most protective against chronic diseases

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Much evidence supports the role of dietary polyphenols in the prevention of chronic diseases. However polyphenols are not all equal. Their bioavailability and biological properties differ widely according to their fine chemical structures, and the exact nature of the most protective compounds is still largely unknown. Past epidemiological studies have largely been focused on a limited number of phenolic compounds. No detailed and comprehensive view on associations between exposure to polyphenols and health is so far available and new tools are needed to develop Metabolome-Wide Association Studies (MWAS) on polyphenols and to identify *individual* phenolic compounds most strongly associated to disease risk. Based on a systematic literature search, we have built the open access Phenol-Explorer database (http://www.phenol-explorer.eu/) that includes more than 36,000 composition data for 502 polyphenols in 452 foods[1, 2]. The quality of the data was assessed. Data of sufficient quality was integrated in the Phenol-Explorer database. This data has been used to build food composition tables for polyphenols and to generate the most comprehensive data on polyphenol intake in the French SU.VI.MAX cohort[3] and the European Prospective Investigation on Cancer and Nutrition (EPIC) study et to evaluate associations with functional outcomes and disease risk. Further data on the effects of food processing and cooking on polyphenol content are now being collected from over 200 original publications to further improve accuracy of food composition tables. Retention factors for a large number of polyphenols in various foods will soon be made available to the public. The use of urinary or blood biomarkers may offer a more objective way to assess personal exposure to dietary polyphenols in population studies[4]. In order to help in the identification of the most appropriate polyphenol biomarkers, we collected data from over 200 peer-reviewed publications on 375 plasma and urinary metabolites described in both human and animal experimental. This information available on the Phenol-Explorer website is now exploited to identify polyphenol metabolites in metabolic fingerprints from population studies. This work on dietary polyphenols emphasizes the importance of data biocuration to better understand the role of plant/food bioactives in human health and diseases[5]. It could be extended to other families of plant/food constituents in a larger community effort. *References* (1) Perez-Jimenez, J.; Neveu, V.; Vos, F.; Scalbert, A. Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: An application of the Phenol-Explorer database. *J. Agric. Food Chem.* **2010**, *58*, 4959 – 4969. (2) Perez-Jimenez, J.; Neveu, V.; Vos, F.; Scalbert, A. Identification of the 100 richest dietary sources of polyphenols – An application of the Phenol-Explorer database. *Eur. J. Clin. Nutr.* **2010**, *64*, S112-S120. (3) Perez-Jimenez, J.; Fezeu, L.; Touvier, M.; Arnault, N.; Manach, C.; Hercberg, S.; Galan, P.; Scalbert, A. Dietary intake of 337 polyphenols in French adults. *Am. J. Clin. Nutr.* **2011**, *93*, 1220 – 1228. (4) Perez-Jimenez, J.; Hubert, J.; Ashton, K.; Hooper, L.; Cassidy, A.; Manach, C.; Williamson, G.; Scalbert, A. Urinary metabolites as biomarkers of polyphenol intake in humans – A systematic review. *Am. J. Clin. Nutr.* **2010**, *92*, 801 – 9. (5) Scalbert, A.; Andres-Lacueva, C.; Arita, M.; Kroon, P.; Manach, C.; Urpi-Sarda, M.; Wishart, D. Databases on food phytochemicals and their health promoting effects. *J. Agric. Food Chem.* **2011**, *59*, 4331 – 48.

IL43 Natural products as models for the conception of new active products: Benzopyran, a privileged structure

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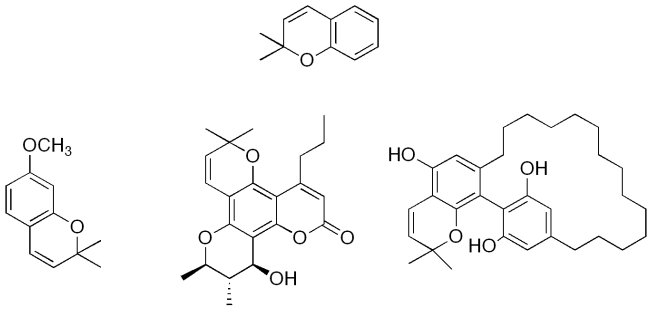
Paris Descartes University, Paris, France

Natural products are a useful source for the discovery of new bioactive products. The role of defense played by natural products in the living organisms that synthesize them most probably explains their particular interest in the discovery of antifungal, antibacterial, antiparasitic, and anticancer drugs implying novel mechanisms of action. In those therapeutic fields, most of new approved drugs are natural products, natural products derivatives, or synthetic mimics, which incorporate a pharmacophore present in a natural product. The term "privileged structures" originates from Evans and co-workers who stated "what is clear is that certain privileged structures are capable of providing useful ligands for more than one receptor". Various scaffolds have been identified as "privileged structures". Among them, benzopyran units are present in numerous bioactive natural products. Dimethylallyl pyrophosphate, which is the starter unit of terpene metabolism, appears as a particularly reactive and efficient alkylating agent involved in numerous mixed biogenetic pathways. Dimethylbenzopyrans are examplified by the insecticidal precocenes, the antiviral calanolide A and the cytotoxic kermadecins and illustrate the biological interest of fused pyrans.



This will be illustrated by examples of compounds developed in our group: antitumor pyranoacridone compounds derived from acronycine alkaloid and potential antitubercular pyranodibenzofurane derivatives.

IL44 Inspired by nature: Biomimetic synthesis of natural products

Poupon E
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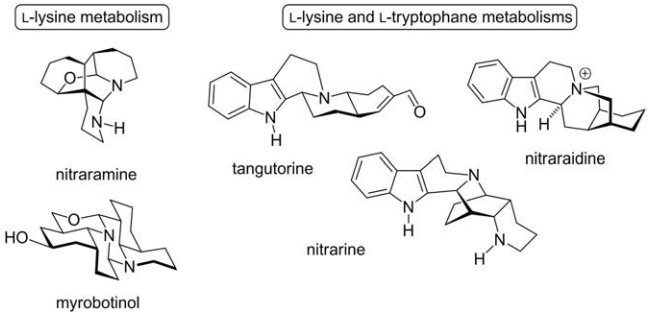
Biosynthetic pathways enable the formation of diverse and sometimes highly complex molecular architectures and, it is needless to say that they provide an inexhaustible source of inspiration to chemists. We will present recent works from our team in the field of biomimetic total synthesis of natural substances. We will particularly emphasize on examples where polycyclic molecules may, in principle, be assembled through cascade reactions from simple reactive precursors. Selected targets (mainly involving L-lysine and L-tryptophane metabolisms) that will be presented during the talk are shown in the following figure.



Selected review articles: - E. Gravel, E. Poupon, *Nat. Prod. Rep.* **2010**, *27*, 32 – 56. - E. Poupon, E. Gravel. *Eur. J. Org. Chem.* **2008**, 27 – 42.

IL45 Polyphenolic ellagitannins and gallotannins: Structures, synthesis and biological activity – from folk medicine to chemical biology via wine sciences

Quideau S
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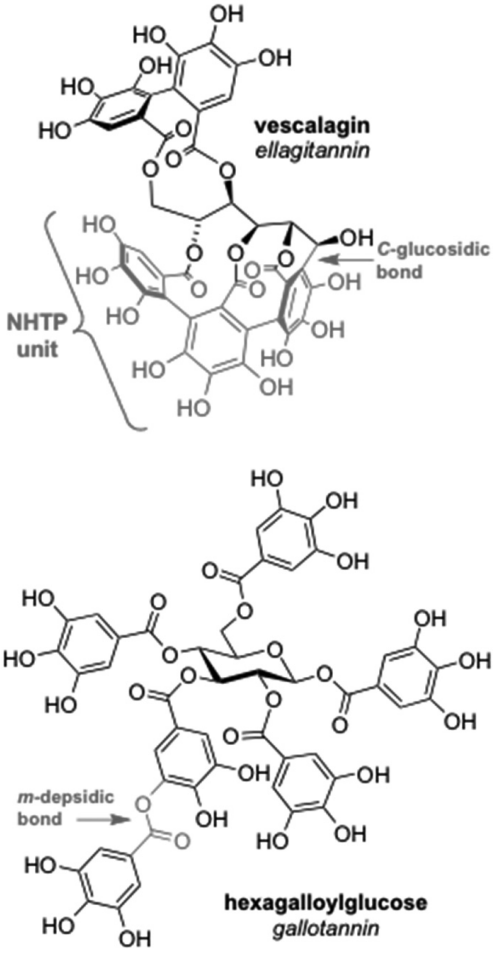
Hydrolyzable tannins constitute a class of plant bioactive polyphenols primarily composed of ellagitannins and gallotannins derived from the metabolism of gallic acid. Ellagitannins feature galloyl units esterified to a sugar core, usually glucose, and characterized by the presence of biaryl and diaryl ether bonds between some or all of their galloyl units.[1] Among ellagitannins, there exists a subclass of structurally unique non-

ahydroxyterphenoyl (NHTP)-bearing *C*-glucosidic molecules featuring an open-chain glucose core and exemplified by vescalagin. The fact that some of these *C*-glucosidic ellagitannins are found in wine as a result of aging of this beverage in oak-made barrels provided us with the impetus to examine their chemical reactivity and biological activity. Indeed, during aging in oak barrels, the hydroalcoholic and slightly acidic (*i.e.*, pH 3 – 4) wine solution enables the solid-liquid extraction of these ellagitannins. Once in the wine solution, some of these natural products such as vescalagin can capture grape-derived nucleophilic entities such as the flavanols catechin and epicatechin and the anthocyanin oenin to furnish condensation products, some of them having been postulated as active principles in Asian herbal medicines.[2] Of pharmacological importance is the fact that several of these found-in-wine ellagitannin hybrids are much more potent than etoposide (VP-16) at inhibiting *in vitro* the anticancer target DNA topoisomerase IIa.[2a,b] Furthermore, we recently discovered that vescalagin is capable of drastically perturbing the actin cytoskeleton by interacting selectively with the actin filaments, both *in vitro* and *in cellulo*.[3] Moreover, gallotannins, in which galloyl units and/or depsidically-linked chains of galloyl units are usually esterified to a glucopyranose core, and some of their simpler precursors exhibit some interesting properties with possible applications as antifibrillogenic agents. Thus, the various biophysico-chemical properties we unveiled for these hydrolysable tannins will be presented and their exploitation in the development of therapeutic agents discussed during this lecture.



[1] Quideau, S. (Ed.) *Chemistry and Biology of Ellagitannins, An Underestimated Class of Bioactive Plant Polyphenols*; World Scientific Publishing: Singapore, **2009**. [2] a) Quideau, S.; Jourdes, M.; Saucier, C.; Glories, Y.; Pardon, P.; Baudry, C. *Angew. Chem. Int. Ed.* **2003**, *42*, 6012 – 6014 (*selected for the cover*); b) Quideau, S.; Jourdes, M.; Lefeuvre, D.; Montaudon, D.; Saucier, C.; Glories, Y.; Pardon, P.; Pourquier, P. *Chem. Eur. J.* **2005**, *11*, 6503 – 6513 (*selected for the cover*); c) Chassaing, S.; Lefeuvre, D.; Jacquet, R.; Jourdes, M.; Ducasse, L.; Galland, S.; Grelard, A.; Saucier, C.; Teissedre, P.-L.; Dangles, O.; Quideau, S. *Eur. J. Org. Chem.* **2010**, 55 – 63 (*selected for the cover*). [3] Quideau, S.; Douat-Casassus, C.; Delannoy López, D. M.; Di Primo, C.; Chassaing, S.; Jacquet, R.; Saltel, F.; Genot, E. *Angew. Chem. Int. Ed.* **2011**, *50*, 5099 – 5104.

IL46 Exploring and harnessing pacidamycin biosynthesis

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Natural products represent a treasure trove of medicinally relevant compounds: over the past 3 decades over 70% of antimicrobials and over 60% of antitumor agents entering clinical trials have been based on natural products. [1] Generation of natural product analogues is an important area. A new approach to natural product analogue generation, which we have termed GenoChemetics, will be described. A genome scanning approach to determining the biosynthetic genes responsible for the construction of a highly unusual natural product and exploration of its assembly will also be discussed:- *GENOCHEMETICS a new paradigm in natural product generation* The generation of analogues of natural products is key to understanding structure activity relationships and improving physicochemical properties. Traditional approaches of analogue generation such as total synthesis and semisynthesis have limitations. We have pioneered a new concept in which a gene is introduced to an organism and coerced to work in concert with an existing biosynthetic pathway. This installs a chemical handle that enables selective derivatisation of the natural product. [2]

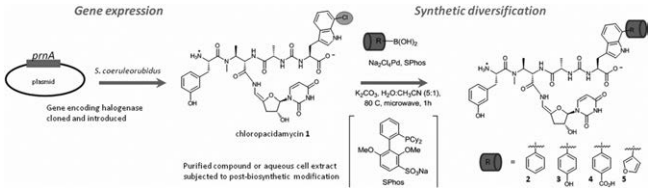

Fig. 1: Chemogenetics: gene expression enabling synthetic diversification.

Identification of the first uridyl peptide antibiotic biosynthetic cluster: Pacidamycin The first identification of the pacidamycin biosynthetic cluster and its heterologous expression, using the cutting edge approach of genome scanning will be described. [3]

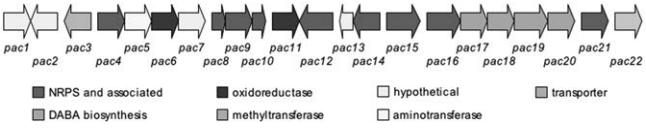

Fig. 2: Pacidamycin biosynthetic cluster.

[1] Newman, D. J. & Cragg, G. M. J. Nat. Prod. 70, 461 – 477 (2007). [2] A. Deb Roy, S. Grüschow, N. Cairns, R. J. M. Goss*, J. Am. Chem. Soc., 2010, 134, 1224 – 12245 [3] E. J. Rackham, S. Grüschow, A.E. Ragab, S. Dickens, and R. J. M. Goss*, ChemBioChem., 2010, 11, 1700 – 1709.

IL47 Lead optimisation of polyketide-based natural products: strategies, technologies and case-studies

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There is a noticeable shift in the pharmaceutical industry away from high-throughput screening of large libraries of compounds against single isolated targets, towards the application of phenotypic screening. In concert with this has been the realization that the typical small molecule libraries are insufficient to query these 'high-content' biology approaches effectively. This observation has ignited an interest in more 'high-content' chemistries to provide screening libraries, an application for which natural products are ideally suited. Results from these screening campaigns typically reveal prominent natural product hits, with polyketides well represented amongst these. These hits represent either excellent chemical genetics tool compounds or a challenge for drug discovery. Strategies and technologies for converting these polyketide leads into candidate drugs will be presented along with case studies from Biotica's work, including rapamycin and FK506. Finally the application of these technologies to sanglifehrin A will be discussed. Sanglifehrin A is a cyclophilin-binding polyketide natural product and from this lead we have discovered a series of compounds, the sangamides.

These compounds have been preclinically characterised as host-targeted therapies for chronic HCV infection.

IL48 Improving on nature:The role of nanomedicine in the development of clinical natural drugs

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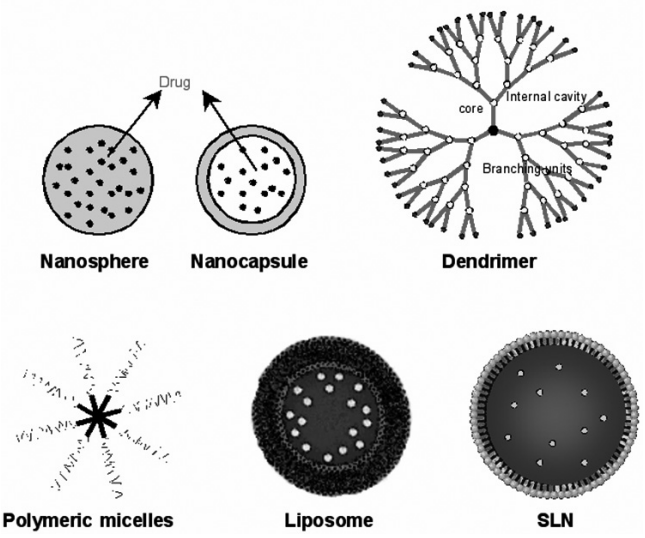
It is astonishing to observe that in spite of the technical hitches encountered in natural product research, more than 50% of drug substances are natural products or inspired by natural compounds, covering a variety of therapeutic indications with a great range of chemical structures. Several latest reviews have tinted the tangible importance of natural products to the drug discovery process, being over a hundred natural product-derived compounds which are currently undergoing clinical trials [1]. Although these natural products represent a large source of potential drugs, mostly of them have limited clinical use due to chemical instability, low absorption and biodistribution, first pass metabolism, little accumulation in the organs of the body, inadequate efficacy or safety profiles [2,3]. These characteristics strongly influence the delivery of natural products using conventional dosage forms. Different strategies have been adopted to overcome these limitations, such as development of semisynthetic compounds or synthetic analogues, or the production of prodrugs but in mostly of the cases have not been satisfactory. Nanotechnology has an enormous impact in medical technology, significantly improving the performance of drugs in terms of efficacy, safety and patient compliance. Nanosized drug delivery systems have already entered in clinical use with the most pressing challenge the design of multifunctional, structured materials able to target specific tissues or organs or containing functionalities to allow transport across biological barriers. These delivery systems can be smartly designed to tag a variety of chemical, molecular and biological entities. Researchers mainly focused priority areas such as specific target diseases such as cancer, neurodegenerative and cardiovascular diseases. A successful drug carrier system needs to demonstrate optimal drug loading and release properties, long shelf-life exerting much higher therapeutic efficacy and lower side-effects as well [4].



Promising nanoparticles, nanocapsules, lipid nanoparticles, dendrimers, micelles and liposomes of taxol and derivatives, berberin, artemisinin, huperzine, camptothecin, polyphenols such as resveratrol, curcumin, sylibin and catechins have been reported in the literature for their considerable potential in the development of new dosage forms of natural drugs. So far, the results obtained from the nanoencapsulated natural products are very encouraging generally having a sustained release and improved bioavailability at much lower doses. We have entered the era of nanomedicine with natural drugs, it is probably the most salient feature of the field and a decisive step toward the demonstration that natural drugs can continue to play a key role in modern clinic. References [1] M.S. Butler. Natural products to drugs: natural product-derived compounds in clinical trials. Nat. Prod. Rep. 25: 475 – 516 (2008). [2] M. Coimbra, B. Isacchi, L. van Bloois, J.S. Torano, A. Ket, X. Wu, F. Broere, J.M. Metselaar, C.J. Rijcken, G. Storm, A. R. Bilia, R.M. Schiffelers. Int. J. Pharm. 416: 433 – 442 (2011). [3] B. Isacchi, M.C. Bergonzi, M. Grazioso, C. Righeschi, A. Pietretti, C. Severini, A.R. Bilia. Artemisinin and artemisinin plus curcumin liposomal formulations: Enhanced antimalarial efficacy against *Plasmodium berghei*-infected mice. Eur. J. Pharm. Biopharm. 80: 528 – 534 (2012). [4] P. Couvreur, C. Vauthier. Nanotechnology: Intelligent design to treat complex disease. Pharm. Res. 23: 1417 – 1450 (2006).

Contributed Lectures

CL1 Investigating the diversity and marine natural products of bacteria associated with the gorgonian octocoral, *Eunicea Fusca*

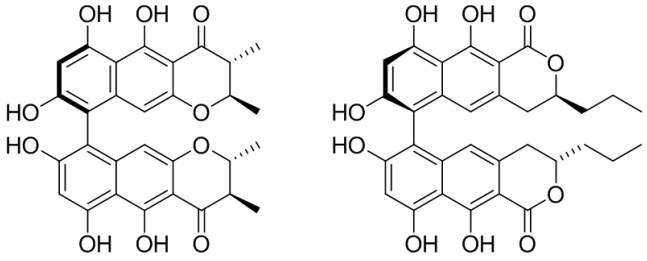
Pike R[1], Haltli B[2], Overy D[2], Berrue F[2], Kerr R[1,2]
[1]*Department of Biomedical Sciences, Atlantic Veterinary College, Charlottetown, PEI C1A 4P3;* [2]*Department of Chemistry, University of PEI, Charlottetown, PEI C1A 4P3*

Marine invertebrates, including gorgonian octocorals, are a prolific source of marine natural products (MNPs) and are also known to host diverse microbial communities. It is well-established that marine microbes are producers of bioactive secondary metabolites. Accordingly, it is hypothesized that bioactive metabolites extracted from some marine invertebrates may actually be produced by associated microbes rather than the invertebrate host. This research aims to characterize *E. fusca*'s microbial community, and to discover novel, bioactive compounds biosynthesized by associated microorganisms. *E. fusca* is the focus of this study because its microbial community has not been characterized. Moreover, *E. fusca* is the sole source of the potent anti-inflammatory diterpenes fuscol, eunicol, and the fuscosides. Investigation of the unexplored *E. fusca* microbial community may provide insights into the biosynthetic source of these diterpenes, and may also lead to the discovery of novel, bioactive MNPs. The microbiome of Floridian and Bahamian *E. fusca* samples collected in June 2009 was characterized using culture-independent and -dependent methods. The culture-independent analysis revealed that *E. fusca* hosts a diverse and geographically-dependent bacterial community. In the culture-dependent study, 140 unique bacteria were cultured, 20 of which were putative, novel species based on 16S rDNA analysis. All cultured bacteria were fermented in diverse media and analyzed for MNP production. Characterization *E. fusca*'s bacterial community and the results of MNP investigations will be presented.

CL2 Fungal BIS-Naphthopyrones as inhibitors of botulinum neurotoxin serotype A

Cardellina II JH[1], Roxas-Duncan VI[1], Montgomery V[1], Eccard V[1], Campbell Y[1], Hu X[2], Tawa GJ[2], Khavrutskii I[2], Wallqvist A[2], Gloer JB[3], Phatak NL[3], Höller U[3], Soman AG[3], Joshi BK[3], Hein SM[3], Wicklow DT[4], Smith LA[5]
[1]*Division of Integrated Toxicology, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD;* [2]*Biotechnology High Performance Computer Software Application Institute, Telemedicine and Advanced Technology Research Center, U.S. Army Medical Research and Materiel Command, Frederick, MD;* [3]*Department of Chemistry, University of Iowa, Iowa City, IA;* [4]*Bacterial Foodborne Pathogens and Mycology Research Unit, Agricultural Research Service, USDA, Peoria, IL;* [5]*Senior Research Scientist for Medical Countermeasures Technology, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD*

An *in silico* screen of the NIH Molecular Library Small Molecule Repository (MLSMR) of ~350,000 compounds and confirmatory bioassays led to identification of chaetochromin A (**1**) as an inhibitor of botulinum neurotoxin serotype A (BoNT A). Subsequent acquisition and testing of analogs of **1** uncovered two compounds, talaroderxines A (**2**) and B (**3**), with improved activity. These are the first fungal metabolites reported to exhibit BoNT/A inhibitory activity.



CL3 The discovery and biosynthesis of nucleoside antibiotics Inhibiting cell wall assembly

Van Lanen SG
College of Pharmacy, University of Kentucky, 789 S. Limestone, Lexington KY 40536, USA

The emergence of multidrug resistant bacteria has detrimentally impacted the clinical utility of several antibiotics, resulting in predictions that many previously first-line antibiotics will be rendered useless in the near future. This problem is compounded by the steady decline in the FDA approval of antibacterial drugs, particularly those considered new molecular entities. These alarming trends have provided the inspiration to search for the next generation of antibiotics, particularly those with new chemical features and/or new modes of action. We have developed a screen aimed at identifying novel antibiotics that are inhibitors of a previously unexploited cellular target, bacterial translocase I, which is an essential enzyme involved in peptidoglycan cell wall biosynthesis. This screen has led to the discovery of several uridine-based nucleoside antibiotics produced by various actinomycetes, the most prolific source of antibacterial agents. We have now identified the biosynthetic genes for five of these translocase I inhibitors, and our biochemical studies have unearthed rich chemistry with several interesting transformations highlighted by (*i*) an ATP-independent mechanism of amide bond formation, (*ii*) a novel sugar biosynthetic pathway that originates from uridine-5'-monophosphate, and (*iii*) a previously unknown mechanism of resistance of covalent modification by an arylsulfotransferase. These results, along with how the biochemical knowledge has been utilized to generate novel analogues and the potential of these metabolites as antibiotics, will be presented.

CL4 Fungal blood: Mycology and natural products chemistry of endophytes from medicinal herbs

Figueroa M[1], Raja H[1], Faeth SH[2], Horswill AR[3], Cech NB[1], Oberlies NH[1]
[1]*Department of Chemistry and Biochemistry;* [2]*Department of Biology, University of North Carolina at Greensboro, Greensboro, NC;* [3]*Department of Microbiology, University of Iowa, Iowa City, IA*

Recently, we initiated studies on the endophytic fungi of medicinal herbs, i.e. fungi that live asymptomatically within the tissues, to probe how the chemistry of such fungi may influence the chemistry (and perhaps, biological activity) of the medicinal herb. A prime example emerged with an endophytic *Penicillium* sp. (G85), which was isolated from surface sterilized stems of milk thistle [*Silybum marianum* (L.) Gaertn. (Asteraceae)] into axenic culture. Pure cultures of G85 produced a red exudate (also known as a 'guttation') on potato dextrose agar amended with antibiotics. Chemical analysis of the guttate, as well as the organic extract of a solid phase culture of this fungus, using a combination of UPLC-HRMS and NMR techniques, revealed a series of structurally related anthraquinone derivatives. Four known compounds, emodin, emodic acid, ω-hydroxyemodin, and isorhodoptilometrin, along with four new derivatives were isolated. The unsaturated nature of these compounds contributed the red color of the guttate. The pure compounds also exhibited quorum quenching activity against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA).

CL5 *Hypoxylon pulicidum* sp. nov., a pantropical insecticide-producing endophyte

Bills G[1], González-Menéndez V[1], Martín J[1], Platas G[1], Fournier J[2], Pešoh D[3], Stadler M[4]
[1]*Fundación MEDINA, Armilla, Granada, Spain;* [2]*Las Muros, Ariège, France;* [3]*Lehrstuhl für Pflanzensystematik, Universität, Bayreuth, Germany;* [4]*Helmholtz Center for Infection Research, Braunschweig, Germany*

Nodulisporic acids (NAs) are indole diterpenes exhibiting potent systemic efficacy against blood-feeding arthropods, e.g. fleas and ticks, by binding arthropod-specific glutamate-gated chloride channels. Medicinal chemistry efforts employing a NAs template has led to the development of N-*tert*-butyl nodulisporamide as a candidate for a monthly treatment of fleas and ticks. NAs originate from a monophyletic lineage of tropical asexual fungal strains, identified as a *Nodulisporium* species (Xylariales), and hypothesized to be the asexual state of a *Hypoxylon* species. Inferences from GenBank sequences indicated that other researchers have encountered the same *Nodulisporium* endophytes. Cultures from a wood-inhabiting *Hypoxylon* from Martinique belonged to the same monophyletic clade. The conspecificity of the *Hypoxylon* collections and the NAs-producing endophytes was tested by mass spectrometry of metabolite profiles, multi-gene phylogenies, and phenotypic comparisons. A new species, *H. pulicidum*, is proposed. Life cycle reconstruction now permits location of fungus populations, their study *in situ*, and investigation of how NAs insecticides aid survival of the fungus.

CL6 Bioactives products from leaves endophytes

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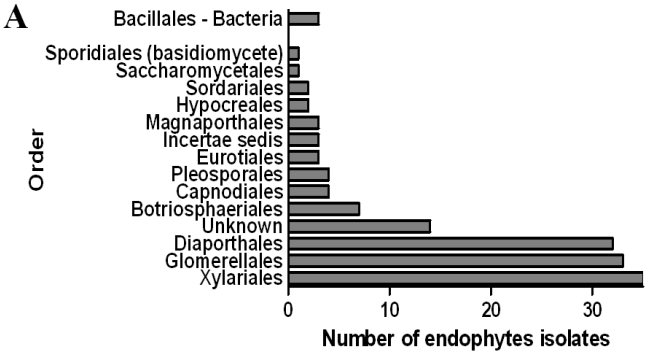
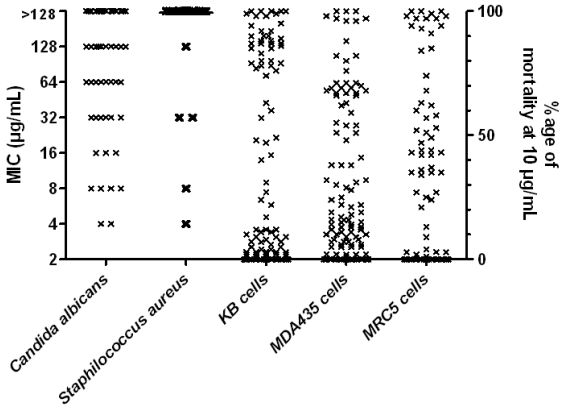
Leaves endophytes contribute to the natural defense of plant leaves, preventing both herbivory and invasion from superficial pathogens. It was therefore postulated that these microbes should produce antimicrobial and/or cytotoxic compounds. The isolated microorganisms belong to 14 different orders, the xylariales being the most represented (35 isolates, **A**). More than 20% of the extracts were considered active against at least one target (**B**) and the most active ones were produced on the large scale for bioguided fractionation, the results of which will be presented. Overall, this work demonstrates that endophytes are involved in mutualistic interactions with higher plants, contributing to their defense system. These interactions can inspire the discovery of new drug candidates.



B



CL7 Fungal co-culture as a new source of bioactive induced metabolites: A MS-based metabolomic study

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In natural product research, access to new biological sources represents a key element. A particularly large number of biologically active molecules are originating from microorganisms [1]. Very recently, the use of fungal co-culture for activation of silent genes related to metabolite biosynthesis was found successful for the induction of new compounds [2]. For such type of studies a challenge is the localization and identification of the induced metabolites in the confrontation zone where fungi interact at the petri dish level. In order to tackle this issue a high throughput UHPLC-TOF-MS based metabolomic approach has been developed for the screening of miniaturized 12-wellplates fungal co-cultures. The strategy provided a satisfactory reproducibility and was used for the identification of induced biomarkers on a large panel of pathogenic fungi. This study demonstrates the consistent induction of new metabolites through co-culture. *Acknowledgements:* This work was supported by Swiss National Science Foundation Sinergia Grant CRSII3_127187 (to J.-L. W., K. G. and M. M.) [1] Berdy J, *J. Antibiot.* **2005**, *58*, 1; [2] Glauser G *et al.*, *J. Agr. Food. Chem.*, **2009**, *57*, 1127.

CL8 NEW terpenoids from marine-derived *Penicillium* with potent anticancer activity in osteosarcoma models

Grovel O[1], Vansteelandt M[1], Blanchet E[1,2], Petit K[1], Le Bot R[2], Egorov M[2], Pouchus YF[1]
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Osteosarcomas are uncommon bone cancers affecting children and young adults for which no satisfactory treatment is available. During an *in vitro* screening of marine fungi for new antitumor compounds against osteosarcoma, a marine-derived strain of *Penicillium* belonging to a new species was selected for further investigation. Bioguided fractionation led to the isolation of four active compounds. Among them, a new chlorinated sesquiterpenoid, ligerin, is related to fumagillin and TNP470, an antiangiogenic MetAP2 inhibitor undergoing clinical trials for treatment of solid tumors. Time-lapse and flow cytometry analyses of ligerin on osteosarcoma cells showed a clear slow-down of cell division and an increase in both G2 M- as well S-phase fractions. On human and murine cell lines, activity of ligerin was higher than those of other analogs synthetized from fumagillin, and was equivalent to doxorubicin and TNP470. Activity of ligerin was also more specific, with a higher activity on osteosarcoma cells than on non-tumor cells. The results of *in vivo* studies on osteosarcoma murine models showed significant antitumor activity and a lower toxicity of ligerin *vs* TNP 470 at the same daily SC dose.

CL9 NMR-based metabolomics of *Verbascum* species and evaluation of their antioxidant and anti-inflammatory activities

Georgiev MI[1,2], Dimitrova P[3], Alipieva K[4], Ivanovska N[3], Hae Choi Y[2], Verpoorte R[2]
[1]Laboratory of Applied Biotechnologies, Institute of Microbiology, BAS, Plovdiv, Bulgaria; [2]Natural Products Laboratory, Institute of Biology, Leiden University, Leiden, The Netherlands; [3]Department of Immunology, Institute of Microbiology, BAS, Sofia, Bulgaria; [4]Institute of Organic Chemistry with Centre of Phytochemistry, BAS, Sofia, Bulgaria

Mulleins (*Verbascum* L., Scrophulariaceae) have been used in the traditional folk medicine since time immemorial for treatment of a wide range of human ailments, *inter alia* bronchitis, tuberculosis, asthma, and different inflammations. We applied an NMR-based metabolomics to study metabolic differentiations of five mullein species. ^{1}H NMR fingerprinting in combination with principal component analysis allows chemical classification of *Verbascum* species in two groups: group A (*V. phlomoides* and *V. densiflorum*) and group B (*V. xanthophoeniceum*, *V. nigrum* and *V. phoeniceum*). In addition, it was found that the plants in group B synthesize higher amounts of bioactive iridoid (*e.g.* pharmaceutically-important harpagoside) and phenylethanoid glycosides (verbascoside, forsythoside B and leucosceptoside B). The antioxidant and anti-inflammatory activities of *Verbascum* plants were evaluated using several *in vitro* and *in vivo* assays. Furthermore, the iridoid glycoside harpagoside has been thoroughly evaluated in models of acute and chronic inflammations (*e.g.* zymosan-induced arthritis in mice). Based on the obtained results it can be concluded that *Verbascum* plants could serve as attractive mines of powerful antioxidant and anti-inflammatory compounds for the food, cosmetics, and pharmaceutical industries.

CL10 Biologically active steroids from *Digitalis Ciliata*, *Tribulus Terrestris* and *Yucca Gloriosa* growing in Georgia

Kemertelidze E, Benidze M, Skhirtladze A
Laboratory of Steroids, Iovel Kutateladze Institute of Pharmacochemistry, P. Sarajishvili st. 36, 0159, Tbilisi, Georgia

30 cardenolids have been isolated from *D.ciliata* – endemic plant widely distributed in Caucasus mountain ridge. *D.ciliata* is proposed as a natural source for Digitoxin and Acetyldigitoxin. Preparations Digicilen in ampoules and Digicil in tablets have been used in cardiology for years. The seeds of *D.ciliata* are rich source of biochemical reagent Digitonin. The method of obtaining of Digitonin is patented in England, Switzerland and Germany. Steroidal glycosides from seeds exhibit antiproliferative and cytotoxic activity. Tribusponin – the remedy containing steroidal glycosides from *T.terrestris* – is successfully used for treatment/prophylaxis of atherosclerosis and as a herbal anabolic. Due to its rich chemical composition Tribusponin is superior to other preparations from *T.terrestris*. Tigogenin from the leaves of *Y.gloriosa* has been transformed into acetate-5α-androsterone and acetate-5α-pregnenolone – initial compounds for the synthesis of hormonal drugs on the basis of 5α steroids. Tigogenin has been considered as a cost-effective raw and plantations of *Y.gloriosa* have been established in eastern Georgia. Tens of steroidal glycosides have been isolated from different parts of *Y.gloriosa*, including 18 new furo-, spiro- and cholestanols. Fungicidal agent Gloriofucin is prepared from the spirostanol glycosides of the leaves. Presowing processing of seeds or spraying saplings with low concentrations (2 – 5 mg/l) of allelochemical Alexin from the flowers increases crop capacity of agricultures by 22 – 60% and makes it possible to obtain ecologically clean production. 5 new stereoisomeric stilbenes with rare spiro structure – Gloriosaols have been isolated from the roots, barks of rhizomes and stems of *Y.gloriosa*. Gloriosaols showed high antioxidant, antiproliferative and proapoptotic activities.

CL11 Alkaloids from *Grewia Paniculata* with cytotoxic and non-competitive nicotinic receptor antagonistic activities

Still PC[1], González-Cestari TF[2], Pan L[1], Chai H[1], Fuchs JR[1], Ngoc Ninh T[3], Soejarto DD[4], Yi B[2], Henderson BJ[2], McKay DB[2], Kinghorn AD[1]
[1]Division of Medicinal Chemistry and Pharmacognosy; [2]Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210; [3]Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; [4]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

Grewia paniculata Roxb. ex DC. (Malvaceae) (voucher specimen: *Soejarto et al. 14261*) is a shrub or small tree that grows in Southeast Asia. Three new piperidine alkaloids, microgrewiapines A-C, and nine known compounds, were isolated from separate chloroform-soluble extracts of the stem bark, roots and leaves of *G. paniculata*. Microgrewiapines A-C showed inhibition of the growth of the HT-29 human colon cancer cell line with IC_{50} values of 3 – 14 μM; and inhibited activation of human α4β2 or α3β4 nicotinic receptors, showing IC_{50} values in the low micromolar range. As a result of these studies, microgrewiapine A was found to be selectively cytotoxic, with noncompetitive inhibitory nicotinic receptor activity. This study represents the first report of cytotoxic and CNS modulatory piperdine alkaloids from genus *Grewia*. (Supported, in part, by grant P01 CA125066 from NCI/NIH.)

CL12 Bisindolomonoterpenic alkaloids from the stem bark of Strychnos Nux-Vomica exibiting antiplasmodial activity

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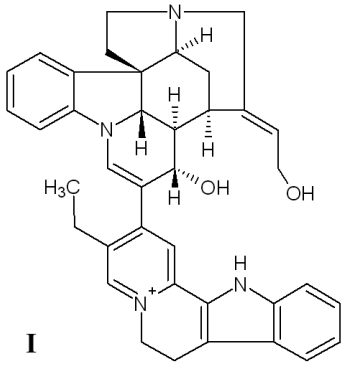
Université de Liège, CIRM, Laboratoire de Pharmacognosie, B36, 1 Avenue de l'Hôpital, 4000 Liège, Belgium

Strychnos nux-vomica L. (Loganiaceae) is famous for its monomeric alkaloids content, such as strychnine, a convulsant poison. Our laboratory investigates *Strychnos* species since five decades, and also more recently, the antiplasmodial activity of bisindole alkaloids. Strychnochrysine (I), previously isolated from the root bark of *S. nux-vomica*, together with three new bisindolomonoterpenic alkaloids have been isolated for the first time from the stem bark of *Strychnos nux-vomica*, traditionally used to treat intermittent fever in South East Asia. These longicaudatine-type alkaloids display *in vitro* antiplasmodial activity against a chloroquine resistant strain and a chloroquine sensitive strain. The most interesting is strychnochrysine showing IC_{50} around 10 μM. The alkaloids could not lead to potent antiplasmodial drug but could be used as tool to investigate original mode of action against *Plasmodium*.



I

CL13 Identification of plumericin from *Himatanthus Sucuuba* as a novel potent inhibitor of the NF-κB pathway directly targeting IKK-β

Waltenberger B[1], Fakhrudin N[2], Cabaravdic M[3], Atanasov AG[2], Heiss EH[2], Breuss JM[3], Rollinger JM[1], Bochkov V[3], Stuppner H[1], Dirsch V[2]
[1]Institute of Pharmacy/Pharmacognosy, University of Innsbruck, 6020 Innsbruck, Austria; [2]Department of Pharmacognosy, University of Vienna, 1090 Vienna, Austria; [3]Institute for Vascular Biology and Thrombosis Research, Medical University of Vienna, 1090 Vienna, Austria

Himatanthus sucuuba (Spruce) Woodson (Apocynaceae) has been traditionally used in South America to treat inflammatory diseases. Fractions from *H. sucuuba* have been shown to possess anti-inflammatory activity *in vivo*.[1] However, the active principles and involved targets remained unknown. Bioactivity-guided fractionation of bark material led to the isolation of 11 compounds.[2] By X-ray single crystal structure analysis the absolute configuration of one, plumeridoid C (**1**), was determined. Moreover, NMR experiments showed that **1** crystallizes from methanol, whereas in solution its 3C-epimer is gradually formed and coexistent. All 11 isolated natural products were analyzed for their anti-inflammatory activities. The most potent activity was exerted by the spirolactone iridoid plumericin (**2**) which was identified as a novel potent NF-κB inhibitor (IC_{50} 1.1 μM) directly targeting IKK-β. Its exceptional potency of action and *in vivo* efficacy rank plumericin among the most interesting natural products described to date as NF-κB pathway blockers. Supported by the TWF and the FWF (NFN- S 10703, S 10704, and S 10713). [1]de Miranda, A. L. P. et al. (2000) Planta Med. 66: 284 – 286. [2]Waltenberger, B. et al. (2011) Acta Cryst. C 67: o409-o412.

CL14 Cytotoxic and antibacterial beilschmiedic acids from a gabonese species of *Beilschmiedia*

Williams RB[1], Martin SM[1], Hu JF[1], Norman VL[1], Goering MG[1], Loss S[2], O'Neil-Johnson M[1], Eldridge GR[1], Starks CM[1]
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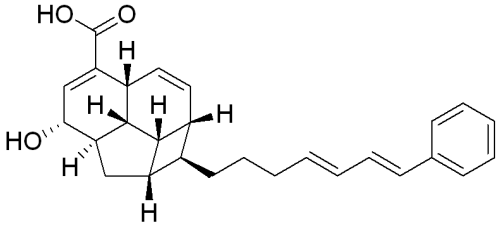
High-throughput natural products chemistry methods have facilitated the isolation of eight new and two known beilschmiedic acid derivatives from the leaves of a Gabonese species of *Beilschmiedia*. This class of compounds appears to be unique to two genera of the Lauraceae, and exhibits remarkable structural complexity for a fatty acid. Eight of the compounds were isolated in microgram quantities necessitating the use of a 1.7 mm MicroCryoProbe to obtain NMR data, including a complete HMBC, for structure elucidation and dereplication. All of the compounds were screened for cytotoxic activity against NCI-H460 human lung cancer cells and antibacterial activity against a clinical isolate of methicillin-resistant *Staphylococcus aureus*. Results from enzymatic assays provided evidence that the observed activity was not the result of membrane disruption as one might expect from lipophilic compounds such as these.

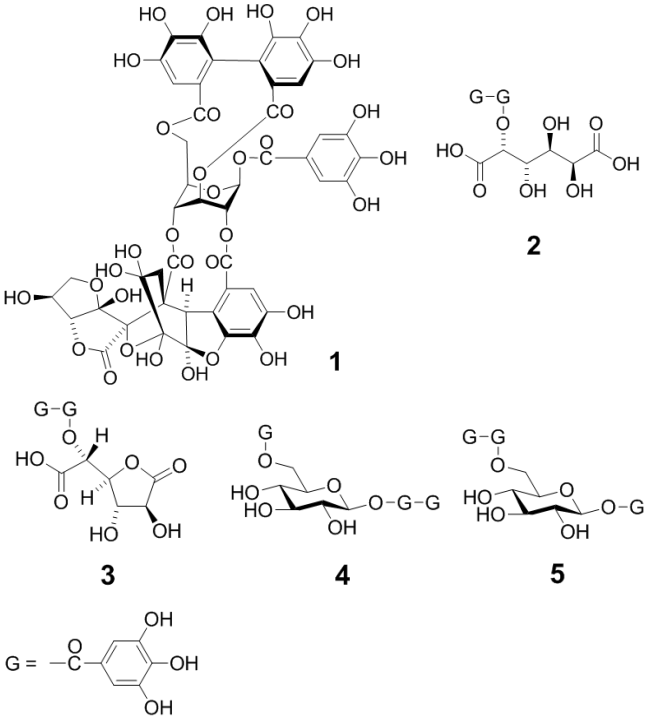


CL15

Isolation and structural identification of new phenolic compounds from *Emblica Officinalis* fruits

Arasuna H[1], Taniguchi S[1], Okuda T[2], Ito H[1], Hatano T[1]
[1]Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Tsushima-naka, Kita-ku, Okayama 700 – 8530, Japan; [2]Institute for Health Sciences, MIKI Corporation, Naruohama, Nishinomiya, Hyogo 663 – 8142, Japan

Emblica officinalis Gaertn. (=*Phyllanthus emblica* L.) is widely distributed in subtropical and tropical areas, and has been used in traditional medicinal systems such as Ayurvedic medicine. Our investigation of the constituents led to the isolation of five new compounds from an extract of the fruits. Their chemical structures were determined by spectral and chemical methods. Compound **1** was a new ellagitannin closely related to elaeocarpusin (= ascorgeraniin), which is a condensation product from geraniin and ascorbic acid. Compounds **2**-**5** were esters of organic acids or glucose with a depsidically linked digalloyl group forming mixtures due to the equilibration between the *m*- and *p*-depside linkages.

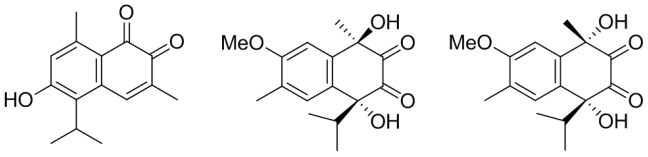


CL16

Isolation, synthesis, and bioactivity of Calamenene sesquiterpenoids from *Sterculia Capuronii* from the Madagascar dry forest

Dai Y[1], Harinantenaina L[1], Brodie PJ[1], Callmander M[2], Randrianasolo S[2], Rakotobe E[2], Rasamison VE[2], Kingston DGI[1]
[1]Department of Chemistry, Virginia Tech, Blacksburg, Virginia 24061, USA; [2]Centre National d'Application des Recherches Pharmaceutiques, B.P. 702, Antananarivo 101, Madagascar

During our ongoing research as part of the Madagascar International Cooperative Biodiversity Group (ICBG) program, an EtOH extract of the bark of the plant *Sterculia capuronii* Arènes (Malvaceae) was found to exhibit anti-proliferative activity against the A2780 human ovarian cancer cell line, with an IC_{50} value of 14 μg/mL. Liquid-liquid partition, LH-20 and silica gel open column chromatography, and C-18 reverse phase HPLC were further applied to this crude extract to obtain a known (**1**) and two novel calamenene sesquiterpenoids (**2** and **3**) which display reproducible antiproliferative activity against the A2780 human ovarian cancer cell line, with IC_{50} values of 10.2 μM, 5.8 μM and 6.5 μM, respectively. The known compound was dereplicated by comparison of its spectroscopic data with literature data, while the structures of the two novel compounds were elucidated by analysis of their 1D and 2D NMR spectra and mass spectrometric data, and confirmed by a *de novo* synthesis. The relative stereochemistries of **2** and **3** were determined by 1-D NOE difference spectra.

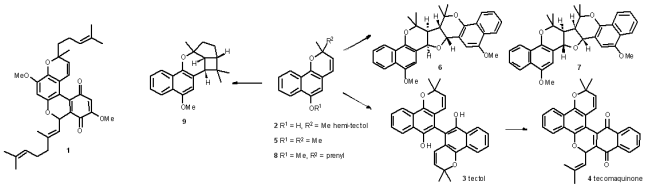


CL17

The central role of chromenols in the biomimetic synthesis of structurally diverse natural product scaffolds

Cadelis M, Barker D, Copp BR
School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

We recently reported scabellone B (**1**) to be an antimalarial metabolite of the NZ ascidian *Aplidium scabellum*. One biomimetic route we have explored to the core benzo[*c*]chromene-1,4-dione structure of **1** is via oxidative dimerisation of chromenol precursors, an example of which is the facile transformation of hemi-tectol **2**, to tectol **3**, to tecomaquinone **4**, natural products isolated from teak wood. Variation in chromenol starting material and reaction conditions yield unusual inter- (**5** to **6**, **7**) and intra-molecular (**8** to **9**) ring closure products, all of which are representative of biologically active natural products isolated from plants and microbes. The syntheses of **2**-**9** and the results of biological evaluation will be presented.



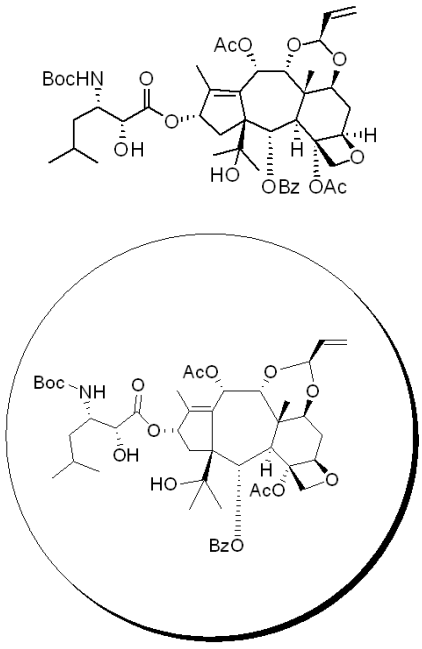
CL18

Design and development of a next generation taxane

McChesney JD
Tapestry Pharmaceuticals, Inc, Boulder, Co 80301, Current Address, Founder and Principal, Arbor Therapeutics, LLC, Etta, MS 38627

The Taxanes, Paclitaxel (Taxol) and Docetaxel (Taxotere) are among the most effective and clinically successful anti-cancer drugs ever developed. However, they show important clinical limitations in that they are subject to Multidrug Resistance (MDR) expression and some types of cancers are innately resistant to their action. Most patients relapse even after initial response, likely due to selection of multidrug resistance in the tumor cell population and regrowth of the tumor or MDR expressing metastases. Also the current clinically approved taxanes are unable to penetrate the CNS, an important site of metastases. TPI 287 has been designed to address the recognized limitations of current clinically approved taxanes. It is the first example of an *abeo*-taxane to show significant biological activity and has activity against MDR expressing tu-

mors in preclinical animal models, is orally bioavailable and penetrates the CNS in vivo. TPI 287 is now in Phase II Clinical Development.

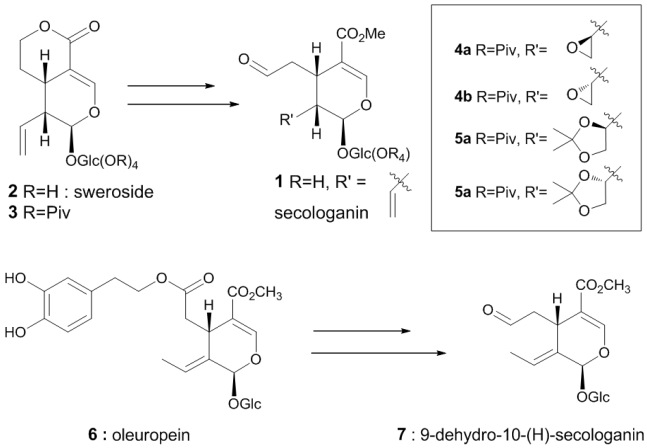


Structure of TPI-287

CL19 Synthesis of small library of secologanin analogs starting from sweroside and oleuropein

Lemoine H, Deguin B
Laboratoire de Pharmacognosie de l'Université Paris Descartes, Sorbonne Paris Cité UMR/CNRS 8638, Faculté des Sciences Pharmaceutiques et Biologiques 4, Avenue de l'Observatoire, 75006, Paris

Dedicated to the memory of Pr François Tillequin Secologanin **1** and sweroside **2** were isolated on preparative scale from *Lonicera tatarica* (Caprifoliaceae) by a new isolation process. Perpivaloylsweroside **3** was converted into secologanin **1**, via a novel sequential three-step procedure, followed by deprotection. This new methodology was successfully applied on sweroside analogs and afforded four enantiopure secologanin analogs **4 – 5**. Furthermore the abundant secoiridoid oleuropein **6** extracted from *Olea europea* (Oleaceae) was converted into 9-dehydro-10-(H)-secologanin **7**. This small library of secologanin analogs can be used as starting materials in total synthesis of natural product analogs.



CL20 Natural product derivative that cross the blood brain barrier (BBB) and protect dopamine neurons in Parkinson disease (PD) models

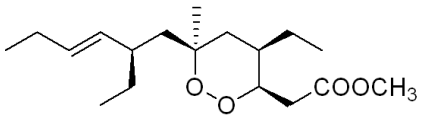
Le Douaron G[1,2], Schmidt F[1,2], Amar M[1,2], Kadar H[3], Séon-Méniel B[1], Ferrié L[1], Touboul D[3], Brunelle A[3], Raisman-Vozari R[2], Figadère B[1]
[1]CNRS-UMR 8076 BiOCIS, Université Paris-Sud, 92296 Châtenay-Malabry Cedex, France; [2]INSERM UMR 975 – CNRS UMR 7225 – CRICM, CHU Pitié-Salpêtrière, 75013 Paris, France; [3]Institut de Chimie des Substances Naturelles, CNRS UPR 2301, 91198, Gif-Sur-Yvette, France

Recently our laboratory synthesized hybrids of natural products (melatonin and fatty acid) with the aim to develop drugs possessing dual properties, namely neuroprotective and neuritogenic. Through a cell-based screening on PD model of the thus synthesized focused chemical library, we found compounds exhibiting both activities. (*Bioorg. Med. Chem.* 2010, p. 5103). The development of the lead compound brings in light its weak ability to cross the BBB. In order to increase this ability, we synthesized 2nd generation compounds, derived from amino-quinoxalines. Their synthesis would be discussed. After screening these molecules in the *in vitro* PD model, we were able to identify a compound, which exhibited both neuroprotective activity and good physico-chemical properties that allows a good BBB permeation in regards to QSARs study. HPLC/MS-MS analysis and MALDI-TOF imaging of brain treated mice tissues (*per. os.* 150 mg/kg) reach to confirmation of the QSARs prediction. Then PD MPP+ lesioned mice model was used to evaluate the *in vivo* activity of our lead compound. Finally, a 3rd generation library was synthesized and screened. Our most recent results will be presented.

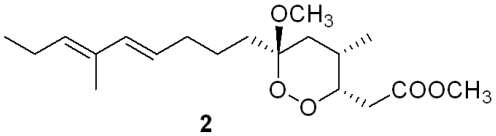
CL21 Marine endoperoxides as antiprotozoan lead compounds

Chianese G[1], Fattorusso E[1], Fattorusso C[1], Persico M[1], Taramelli D[2], Tasdemir D[3], Taglialatela-Scafati O[1]
[1]Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II", Via D. Montesano, 49 I-80131 Napoli, Italy; [2]Dipartimento di Sanità Pubblica-Microbiologia- Virologia- Università di Milano, Via Pascal 36, I-20133 Milano, Italy; [3]School of Chemistry, National University of Ireland, University Road, Galway, Ireland

Chemical analysis of Caribbean and Indonesian marine sponges yielded a family of endoperoxide polyketides, some of which showed remarkable activity against protozoans responsible either of malaria or of African trypanosomiasis. For example, we have found that plakortin (**1**) is a potent antimalarial lead, while manadoperoxide B (**2**) is an ultrapotent antitrypanosomal agent (IC_{50}= 8 nM). Resulting structure-activity relationships have been analyzed by means of experimental and computational techniques, thus gathering valuable insights into the mechanism of action of this class of compounds and information to guide the design of optimized antiprotozoan agents based on the dioxane scaffold. In this communication we will also illustrate a simple and versatile scheme of synthesis which, utilizing cheap and commercially available starting materials, afforded several structurally and stereochemically different compounds characterized by a 3-methoxy-1,2-dioxane scaffold.



1



2

CL22 In vitro and in vivo evaluation of the antidepressant activity of aplysinopsin analogs

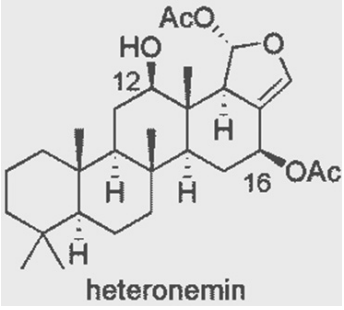
Lewellyn K[1], Bialonska D[1,3], Chaurasiya ND[4], Tekwani BL[4,5], Loria MJ[6], White SW[6], Sufka KJ[2,5,6], Zjawiony JK[1,2]
[1]Department of Pharmacognosy; [2]Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA; [3]Institute of Environmental Sciences, Jagiellonian University, 30 – 387, Krakow, Poland; [4]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA; [5]Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677, USA; [6]Department of Psychology, University of Mississippi, University, MS 38677, USA

Aplysinopsins are tryptophan-derived natural products that have been isolated from a variety of marine organisms. Previous studies have shown aplysinopsin analogs to possess a variety of biological activities, including modulation of neurotransmissions. A series of fifty aplysinopsin analogs was synthesized and assayed for monoamine oxidase A and B inhibitory activity. Several compounds displayed significant MAOI activity and selectivity. Compound **3x** possessed an IC_{50} of 5.6 nM at MAO-A and had a selectivity index of 80.24. This data, combined with previous knowledge of aplysinopsin analogs' CNS activity, suggested that these compounds may have potential as leads for antidepressant drugs. Considering this, three compounds were evaluated in a chick anxiety-depression model to evaluate their *in vivo* efficacy. Compound **3c** showed an antidepressant effect at a dose of 0.03 μM/kg in the animal model.

CL23 Derivatizations and QSAR of marine-derived antitubercular scalaranes

Thengyai S[1], Hannongbua S[2], Plubrukarn A[1]
[1]Marine Natural Products Research Units, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand; [2]Department of Chemistry, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand

A series of scalarane sesterterpenes were prepared using heteronemin as a primary starting material. QSARs for the antitubercular activity of a dataset of 22 natural and chemically derived scalaranes were assessed using 2D-QSAR and CoMFA approaches. The 2D-QSAR model suggested the importance of hydrophilic perimeters with hydrophobic cores, whereas the CoMFA approach led to the conclusion that a non-steric, positive electrostatic induction over C12, and the lengthy, negative electrostatic extensions similar to an acetoxy group from C-16 of the scalarane skeleton may positively influence the potency.



CL24 The semisynthetic modification of the antibiotic enduracidin

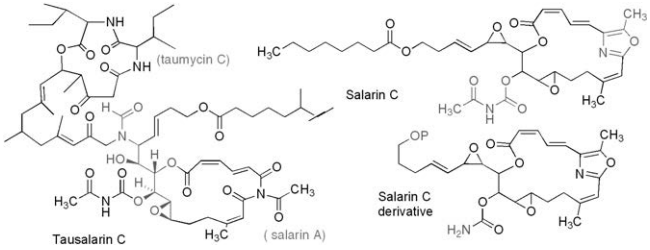
Goebel N[1], Zabriskie M[1]
[1]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331, USA

The lipopetide antibiotic enduracidin has excellent bactericidal activity against many Gram-positive bacteria including vancomycin-resistant *Enterococci sp.* and methicillin-resistant *S. aureus*. Bacterial resistance to enduracidin has not been observed. Despite having high bioactivity, enduracidin's low solubility at neutral pH limits its viability for therapeutic use. New enduracidin analogs are needed to improve solubility. The lipid tail and five hydroxyphenylglycine (Hpg) residues were targeted for semisynthentic modification in this study. A series of analogs investigating the effects of incoporating charged groups derived from *p*-aminobenzoic acid and alkyl amines into the lipid tail were prepared. The Hpg groups were modified via nitration and subsequent reduction affording both nitro and amino enduracidin analogs. The preparation and characterization of these new enduracidin analogs will be presented.

CL25 Recent Indo Pacific (Tanzania and Madagascar) nitrogenous marine natural products; structure & SAR

Kashman Y[1], Goren L[1], Bishara A[1], Aknin M[2]
[1]School of Chemistry, Tel Aviv University, Ramat Aviv 69978, Israel; [2]Laboratorie de Chimîe des Substances Naturelles et des Aliments, Faculté des Sciences et Techniques, Université de Réunion, 97715 Saint Denis, Cedex 9, France

Nitrogenous compounds isolated from the Madagascan sponge *Fascaplysinopsis* sp, namely, four groups of secondary metabolites, salarins, tulearins, taumycins and tausalarin are represented. As well as several salarin C derivatives synthesized for SAR. e.g. glycosidation of a desoctanoyl derivative.



CL26 Discovery and development of Santacruzamate A, a potent and selective histone deacetylase inhibitor

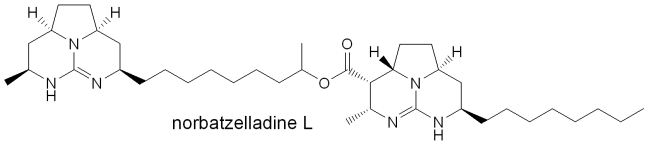
Balunas MJ[1,2,3,4], Pavlik CM[1], Wong CYB[4], Lopez DD[4], Engene N[2], McPhail K[5], Gerwick WH[2,3]
[1]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut; [2]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego; [3]Smithsonian Tropical Research Institute, Ancón, Panamá City, Panamá; [4]INDICASAT, Ciudad del Saber, Clayton, Panamá City, Panamá
[5]College of Pharmacy, Oregon State University, Corvallis, Oregon During an expedition to the Coiba National Park, a UNESCO World Heritage Site on the Pacific coast of Panama, in a unique reef habitat near Santa Cruz Island, a dark brown cyanobacterium was collected that morphologically resembled the *Symploca* genus of marine cyanobacteria. Further 16S rRNA analysis was used to determine the phylogenetic relationship with known cyanobacteria and preliminary results indicate that the genetic variation may be sufficient to propose this as a new genus. In addition, bioactivity-guided fractionation led to the isolation of the new compound, santacruzamate A, which has several structural features in common with SAHA (suberoylanilide hydroxamic acid), a clinically approved histone deacetylase (HDAC) inhibitor used to treat refractory cutaneous T-cell lymphoma (trade name Vorinostat®). As a result of this recognition of the structural similarly of santacruzamate and Vorinostat, this project was pursued by chemical synthesis of santacruzamate A as well as structurally intriguing hybrid structures blending aspects of both agents. Santacruzamate A was found to selectively inhibit Class I HDAC activity with picomolar level inhibition of HDAC2 and relatively little inhibition of HDAC4, a Class II HDAC enzyme. With a robust synthesis in place, additional analogs of santacruzamate A have been synthesized and a broader biological evaluation has been conducted.

CL27

NOR-Batzelladine L from the sponge *Monanchora* sp. displays antiviral acyivity against Herpes Simplex virus type 1

Kohn LK[1], Porto PSS[1], Bianchi BR[1], Santos MFC[2], Berlinck RGS[2], Arns CW[1]
[1]Laboratório de Virologia Animal, Departamento de Genética Evolução e Bioagentes, IB/UNICAMP, CEP 13083 – 970, Campinas, SP, Brasil; [2]Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, Brazil

Althoug marine organisms produce an incredible array of bioactive natural products, only few of such secondary metabolites display anti-viral activity. The aim of this study was to evaluate antiviral activity against Herpes simplex virus type 1 (HSV-1) and Avian metapneumovirus (aMPV) of some marine natural products. HSV-1 cause herpes labials in humans and aMPV cause respiratory disease in avian, which accounts for considerable economic losses worldwide. Fourteen pure compounds isolated and identified were tested in non-toxic concentrations for virus-infected cells. Norbatzelladine L isolated from a marine sponge of the genus *Monanchora* displayed MNTC at 2,5 μg/mL against HSV-1, with 97% of inhibition in the viral adsorption phase. Further studies are underway in order to establish the anti-viral mode-of-action of norbatzelladine L.



Financial support: FAPESP

CL28

Antifungal alkaloids from *Agelas Citrina* that decouple calcium-dependent excitation-contraction

Molinski TF[1,2], Stout EP[1], Yu LCY[1], Truong KM[2], Pessah IN[2]
[1]Department of Chemistry and Biochemistry; [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA; [3]Molecular Biosciences, University of California, Davis, CA 95616

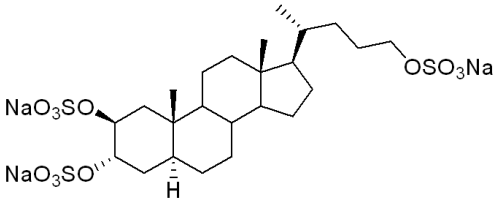
Several species of *Agelas* marine sponges produce a special class of paired diterpenoid alkaloids: C_{20} rearranged diterpenes C-linked to either hypotaurocyamine or 9-*N*-methyladeninium salts. We describe here three new alkaloids and several known compounds from *Agelas citrina*, collected in the Bahamas, that exhibit potent antifungal activity and unique properties that affect critical components of Ca^{2+}-dependent smooth muscle excitation-contraction: decoupling of the dihydropyridine receptor (DHPR) from Ca^{2+} release through the RyR-1 Ca^{2+} channel of the sarcoplasmic reticulum (SR). Structure elucidation of the new compounds was accomplished by interpretation of MS and NMR data, chemical correlation and quantitative analysis of molar rotations through van't Hoff's principle of optical superposition. The biological activity of new and known diterpenoid alkaloids will be described.

CL29

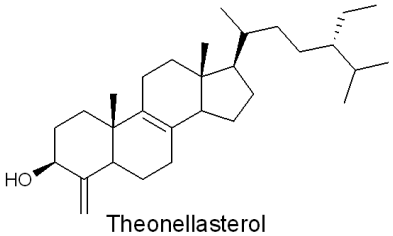
Marine natural products as modulators of nuclear receptors

D'Auria MV[1], De Marino S[1], Sepe V[1], Zampella A[1], Bifulco G[2], Renga B[3], D'Amore C[3], Fiorucci S[3], Debitus C[4]
[1]Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli "Federico II, 80131 Napoli, Italy; [2]Dipartimento di Scienze Farmaceutiche, Università di Salerno, 84084 Fisciano (SA), Italy; [3]Dipartimento di Medicina Clinica e Sperimentale, Università di Perugia, Nuova Facoltà di Medicina e Chirurgia, 06132 Perugia, Italy; [4]Institut de Recherche pour le De'veloppement (IRD), Polynesian Research Center on Island Biodiversity, BP529, 98713 Papeete, Tahiti, French Polynesia

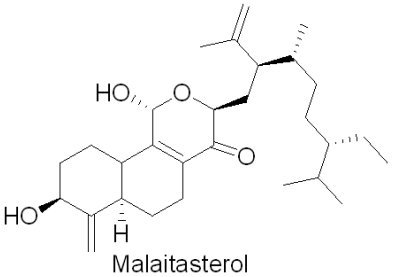
Nuclear receptors (NRs) represent one of the most important drug targets in terms of potential therapeutic application, playing a role in every aspect of development, physiology and disease in humans. In recent years many natural products were found to act as ligands for NRs. As consequence of our long standing interest in the chemistry of bioactive marine products, we found that some new natural products were endowed with modulatory activity towards FXR, PXR and PPARγ receptors. The chemistry and the biological activity of these modulators will be discussed.



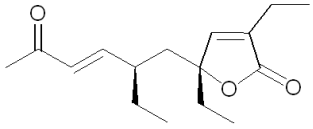
Solomonsterol A



Theonellasterol



Malaitasterol



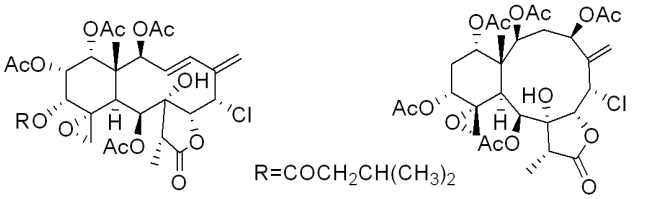
Plakilactone C

CL30

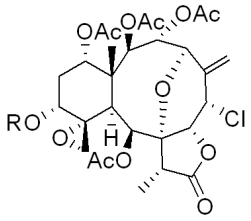
Briarane Diterpenoids: Potential tumor cell growth inhibitor

Zhang W[1], Li C[1], Jiang M[1], Kurtan T[2]
[1]Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, 325 Guo-He Road, Shanghai 200433, P. R. China; [2]Department of Organic Chemistry, University of Debrecen, P.O.B.: 20, H-4010 Debrecen, Hungary

The structural complexity of briarane diterpenoids challenges the chemical synthesis and SAR studies of the intriguing metabolites. Our systematic study on briarane deterpenoids concerning their chemistry and bioactivities led the isolation and structural elucidation of a series of briaranes, gemmacolides G-Y. The absolute configuration was determined by a TDDFT/CD approach. In *in vitro* bioassays, gemmacolide J, V, and Y (**1**, **2**, **3**), showed potent growth inhibition towards tumor cell lines of A549 and MG63, being stronger than the positive control of adriamycin. The identification of possible new targets of compound **1** was conducted using the Inverse Virtual Screening approach, which showed significant interactions in the binding sites gamma, ftase, tank1, and jmjd3. The tumor cell growth inhibitory mechanism of compound **1** is currently under investigation.



1 IC_{50} < 1.4 μM (A549) **2** IC_{50} < 1.5 μM (A549)



3 IC_{50} < 0.3 μM (A549, MG63)

CL31 First selective potentiation of Ca^{2+} current through neuronal T-type calcium channels by the marine terpene fulvol acetate

Petit K[1], Grolleau F[2], Todorovic SM[3], Joksovic PM[3], Yong Lee W[3], Biard JF[1], Hamon A[2], Pouchus YF[1], Lapied B[2]
[1]Faculty of Pharmacy, University of Nantes, F-44035 Nantes, France; [2]University of Angers, France; [3]University of Virginia Health System, Charlottesville, USA

Fulvol Acetate (Fig. 1) is a new marine homosesquiterpene isolated from the soft coral *Rhytisma fulvum*. Electrophysiological investigations on short-term cultured pacemaker dorsal unpaired median (DUM) neurons isolated from the insect central nervous system highlighted that Fulvol Acetate increased selectively T-type calcium current (Fig. 2). Fulvol Acetate used also increased T-type calcium current expressed by rat neurons from reticular thalamic and ventro-basal nuclei and dorsal root ganglion neurons. By contrast, the lack of effect of Fulvol Acetate on *Xenopus* oocytes that expressed only human cDNAs encoding Ca_V 3.1a, 3.2 or 3.3 α-subunits suggest an indirect effect of this compound on T-type current through the involvement of putative accessory proteins in such unusual activation.

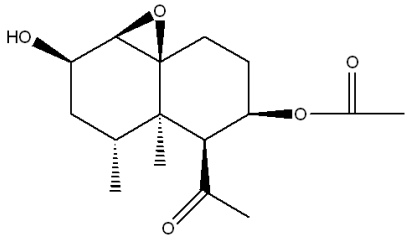


Fig. 1

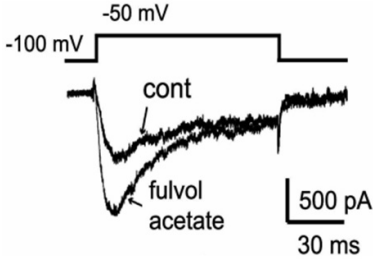


Fig. 2: LVA-Ca2+ current recording

CL32 Microalgae of different phyla display antioxidant, metal chelating and acetylcholinesterase inhibitory activities

Custódio L[1], Justo T[1], Silvestre L[1], Barradas A[1], Vizetto-Duarte C[1], Pereira H[1], Barreira L[1], Pilar Rauter A[2], Alberício F[3], Varela J[1]
[1]Centre of Marine Sciences, Marine Biotechnology Laboratory, University of Algarve, Campus de Gambelas, 8005 – 139 Faro, Portugal; [2]Faculty of Sciences, University of Lisbon, Lisbon, Portugal; [3]Institute for Research in Biomedicine, Barcelona Science Park, Barcelona, Spain

Methanol and hexane extracts from *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella minutissima* and *Rhodomonas salina* were evaluated for total phenolic contents, radical scavenging activity (RSA), metal chelating potential against copper and iron ions and acetylcholinesterase (AChE) inhibition. Only the methanol extracts contained phenolic compounds. The hexane extracts had the highest RSA. The extracts had a higher capacity to chelate Fe^{2+} ions, more pronounced in the lowest concentration of the hexane extracts with values ranging from 73.3 ± 3.3% (*R. salina*) to 97.5 ± 1.1% (*N. oculata*). The highest AChE inhibitory activity was found in the hexane extracts at 10 mg/ml of *C. minutissima* (79.3 ± 1.9%), *T. chuii* (85.7 ± 0.7%) and *R. salina* (81.5 ± 7.5%). GC-MS analysis indicated polyunsaturated fatty acids and steroids as the most abundant compounds in the hexane extracts. The species under study provide a valuable source of antioxidants, metal chelators and AChE inhibitors.

CL33 Curcumin suppresses Interleukin-6 expression in rat smooth muscle cells by inhibition of map kinase signaling pathways

Yuliani Y, Kristina L
Faculty of Biotechnology, Atma Jaya Catholic University, Jalan Jenderal Sudirman 51, Jakarta 12930, Indonesia

In atherosclerotic plaques, infectious agents from microbes may release lipopolysaccharide (LPS) and heat shock proteins that can stimulate the production of mediators, i.e. pro-inflammatory cytokines, by vascular endothelial cells and smooth muscle cells (SMCs). The elevated level of interleukin-6 (IL-6) is strongly associated with the development of atherosclerosis. Here, we investigated whether curcumin isolated from the rhizome of *Curcuma longa* affected the expression of IL-6 at protein and gene levels in rat smooth muscle cells treated with LPS *in vitro* by conducting ELISA and reverse transcriptase-polymerase chain reaction (RT-PCR) assays. LPS at 2 μg/ml activated the expression of IL-6 protein and mRNA in SMCs. Curcumin (1 – 15 μM) caused the decreased levels of IL-6 protein and mRNA in the dose-dependent manner in LPS-induced SMCs, indicating its potential antiatherosclerotic effect for cardiovascular risk management. In addition, curcumin also partially blocked the activation of LPS-induced phosphorylation of MAP kinases, i.e. ERK1/2, p38, and JNK, in SMCs, suggesting it may inhibit IL-6 expression via attenuating MAP kinase signaling pathways in LPS-induced SMCs. These data may in part explain the molecular action of antiatherosclerotic effects of curcumin.

CL34 Beyond acid suppression: mechanisms underlying the beneficial effects of STW 5 in experimental reflux esophagitis (RE)

Abdel-Aziz H[1,3], Ulrich-Merzenich G[2], Weiser D[3], Khayyal MT[4]
[1]University of Münster; [2]University of Bonn; [3]Steigerwald Arzneimittel, Darmstadt, Germany; [4]Cairo University, Egypt

Proton pump inhibitor (PPI) therapy is the most effective medical treatment for symptom relief in gastro-esophageal reflux disease (GERD). However, up to 40% of patients do not achieve adequate symptom control. STW5 was shown to relieve heartburn and concomitant reflux symptoms in patients with functional dyspepsia and to prevent inflammation in an acute model of RE, without affecting the pH of the refluxate. In the present study, the efficacy of STW5 was assessed in a subchronic model of RE, and the underlying mechanisms were investigated. After pre-treatement for 7 d with STW5 or omeprazole, esophagitis was induced surgically. Rats were treated for further 10 d with the drugs before sacrifice. The esophagi were excised and evaluated macroscopically. Tissue homogenates were used for a proteome-profiler cytokine array. Tissue samples and peripheral blood were analyzed using Agilent

whole genome microarray. Both treatments improved macroscopic parameters to similar extents. However, STW5 had a much more pronounced anti-inflammatory effect as evidenced by the cytokine array, which showed a marked increase in the number and amount of cytokines released in the esophagitis group. STW5 inhibited the vast majority of these changes dose dependently. The effect was much more prominent than that of omeprazole, suggesting a direct anti-inflammatory/mucosa protecting action. This was confirmed by microarray analysis. Since gastric acid does not seem to be the only causative agent involved in the pathogenesis of GERD, the present findings suggest that multi-target anti-inflammatory drugs like STW5 might present an alternative/additional treatment option for GERD patients not responding adequately to PPIs.

CL35 *In vitro* anti-inflammatory and wound healing activities of *Citrus* auraptene

Epifano F[1], Genovese S[1], Zhao L[2], Dang La V[3], Grenier D[3]
[1]Dipartimento di Scienze del Farmaco, Università "G. D'Annunzio" Chieti-Pescara, Chieti, Italy; [2]Department of Periodontitis, Sichuan University, Chengdu, Sichuan, China; [3]Groupe de Recherche en Écologie Buccale, Université Laval, Québec City, Canada

Auraptene is the most abundant prenyloxycoumarin that occurs in nature. It has been commonly isolated from plants mainly belonging to the family of Rutaceae, many of which are used as food in many parts of the world. In the last decade this natural product has emerged as one of the most promising biologically active compound mainly as a cancer chemopreventive agent [1]. The aim of this study was to identify novel pharmacological properties of auraptene that may offer perspective for the management of periodontal diseases. Auraptene was found to dose-dependently decrease the secretion of MMP-2 as well as key inflammatory mediators, including interleukin-6, interleukin-8, and CCL-5, by lipopolysaccharide-stimulated oral epithelial cells. Moreover, using a gingival fibroblast model, auraptene was found to promote wound healing by facilitating cell migration. In conclusion, auraptene shows promise for controlling periodontal diseases by its capacity to interfere with inflammatory mediator secretion and to promote wound healing. This study was supported by an International Association for Dental Research-GlaxoSmithKline Innovation in Oral Care Award. 1. Genovese, S.; Epifano, F. *Curr. Drug Targets* **2011**, *12*, 381; 2.

CL36 Synergistic interaction of triterpenoid saponins and plant protein toxins

Weng A[1], Thakur M[2], Beceren-Braun F[1], Gilabert-Oriol R[1], Boettger S[2], Melzig MF[2], Fuchs H[1]
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[2]Institute of Pharmacy, Freie Universitaet Berlin, Berlin, Germany. Certain triterpenoid saponins from *Gypsophila* and *Saponaria* species augment the cytotoxicity of the type-I ribosome-inactivating protein (type-I RIPs) saporin from the seeds of *Saponaria officinalis* L. in a synergistic way. Type-I RIPs (25 – 30 kDa) are widely produced by Caryophyllaceae plants, which also synthesize triterpenoid saponins. The cytotoxic synergism between type I RIPs and saponins represents an evolutionarily conserved self-defense mechanism. To investigate the specificity of triterpenoid saponins in their ability to enhance the cytotoxicity of type-I RIPs we have isolated and characterized a triterpenoid saponin (SA1641) from *Gypsophila paniculata* L. SA1641 was combined with different type-I RIPs such as gelonin from *Gelonium multiflorum* A. Juss., agrostin from *Agrostemma githago* L., RIPQ3 from *Stellaria media* (L.) Vill. and saporin from *Saponaria officinalis* L. and cytotoxicity was determined in ECV-304 cells. Toxicity of saporin was drastically augmented by SA1641. Flow cytometric evaluations showed that this effect is not based on a SA1641-mediated induction of saporin endocytosis or a modulation of the plasma membrane permeability for saporin. We further confirmed that SA1641 does modulate the intracellular distribution of internalized saporin. Confocal microscopy indicated a SA1641-mediated release of saporin molecules out of cellular organelles (late endosome) and surface plasmon resonance measurements indicated an intracellular interaction of SA1641 and saporin as a prerequisite for the synergistic cytotoxicity between triterpenoid saponins and type-I RIPs.

CL37 In a placebo-controlled study ß-Ecdysone (ECD) prevented the development of the metabolic syndrome

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Large abdominal fat depots secrete proinflammatory cytokines which lead often to a Metabolic Syndrome. In an open study 200 mg Ecd prevented this development. Ecd is produced by spinach and therefore a placebo-controlled trial was performed with an Ecd enriched spinach extract. Daily uptake of 2 x 50 mg Ecd administered with 2 x 450 mg spinach powder in slightly overweight women and men (18 placebo, 21 verum) significantly ($p < 0.05$) reduced bodyweight by 1.3%, waist circumference by 3.1% and total body fat by 7.6% while the amounts of muscles were increased by 2.9%. The serum marker for inflammatory processes, C-reactive protein, was reduced by 38% and this resulted in a reduction of serum cholesterol by 17% and of triglycerides by 37%. It is concluded that this product can be used to treat and prevent the development of the Metabolic Syndrome.

CL38 Mechanistic & functional genomic studies identify marine sesquterpene quinones that activate HIF-1 and induce VEGF expression

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The transcription factor hypoxia-inducible factor-1 (HIF-1) promotes cellular survival and adaptation under hypoxic conditions. Activators of HIF-1 show promise in the prevention and/or treatment of cardiovascular and ischemic disorders. Marine invertebrate extracts from the NCI's Open Repository were evaluated for their ability to activate HIF-1. The sponge *Dactylospongia elegans* Thiele (Thorectidae) afforded 4 new sesquiterpene quinones, a sesquiterpene phenol, ilimaquinone, and 3 previously reported analogues. The quinones that possess a 2-hydroxy-5-methoxy-1,4-benzoquinone moiety activated HIF-1 and increased vascular endothelial growth factor (VEGF) protein levels in normoxic T47D cells. A functional genomic approach was used to examine a yeast genome-wide deletion mutant library and identify mutants that exhibit either ilimaquinone hypersensitivity or resistance. Of the 31 hypersensitive mutants identified, 28 exhibited a synthetic fitness defect and 3 possessed a synthetic defect lethal to yeast cells. Golgi-related mutations and mutants that affected mitochondrial function, ubiquitin-specific proteases, and cellular iron homeostasis were identified. Mechanistic studies suggest that these quinones activate HIF-1 and induce HIF-1 target gene expression by inhibiting Fe^{++}-dependent dioxygenases known as HIF prolyl hydroxylases (HPHs). Cellular HPHs are required for the proteasome-mediated HIF-1α degradation under normoxic conditions.

CL39 Human melanoma cell growth and migration inhibitors

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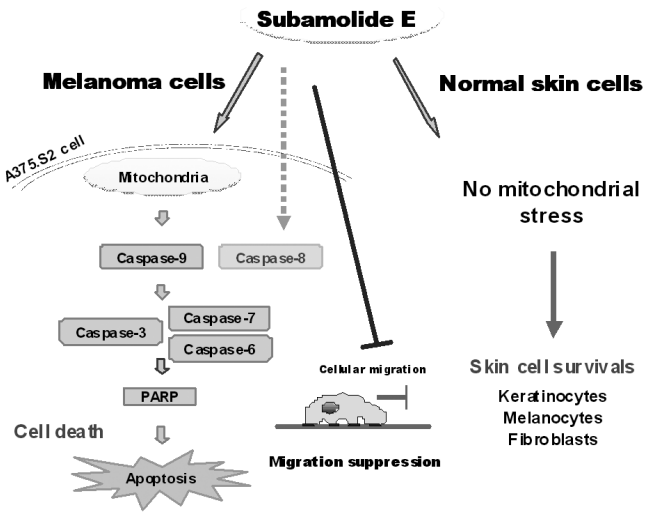
The aim of this work was to investigate the anticancer cytotoxic effects of natural compound, subamolide E, on the human skin cancer melanoma A375.S2 cells. Subamolide E was isolated from *Cinnamomum subavenium* and demonstrated cytotoxicities in cell growth assay at concentration rages from 10 – 100 µM at 24 h. Propidium iodide staining and flow cytometry analyses were used to evaluate cell cycle distribution and found that subamolide E caused DNA damage in sub G1 phase with a dose-dependent manner after 24 h of treatment. According to the Western blot result, subamolide E-treated cells were with the increasing of caspase-dependent apoptotic proteins to induce related pathway mechanisms. Subamolide E also showed that anti-migratory activities of A375.S2 cells on wound healing assay. Finally, subamolide E demonstrated minor cytotoxicities to human normal skin cells (keratinocytes,

melanocytes and fibroblasts), therefore, it is a potential chemotherapeutic agent against skin cancer.



CL40 Pulegone inhibits selectively butyrylcholinesterase and ameliorates memory in rats

Marçal RM[1], Benetti C[1], Costa AT[1], Lima JKA[1], Couto LB[2], Alves PB[1], Blank AF[1], França SC[2], Fernandes RPM[1]
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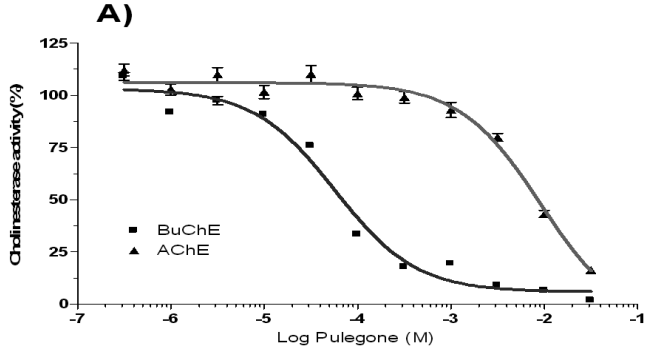
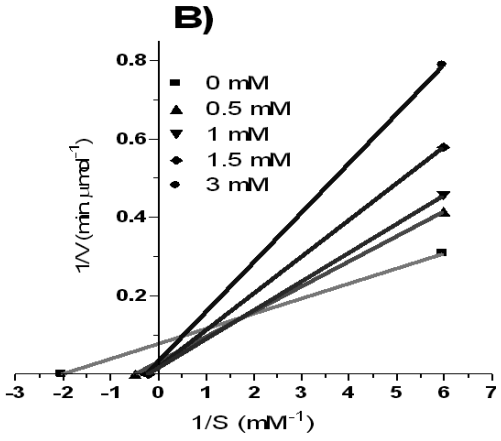
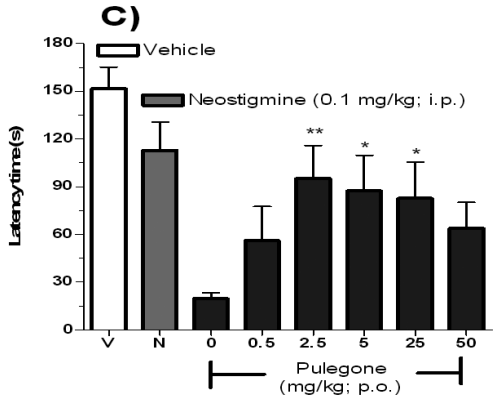
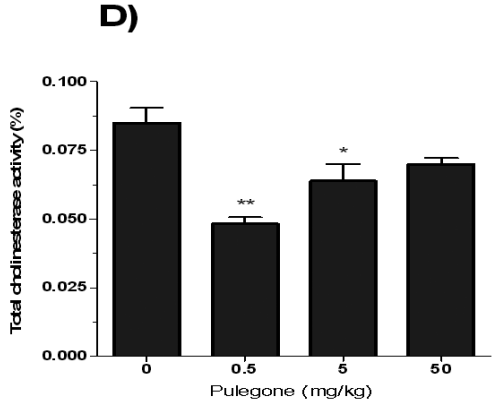
In a search for new natural compounds for treating AD cognitive symptoms, we investigated the anticholinesterase activity of pulegone, a monoterpene ketone found in several essential oils. Cholinesterase inhibition was evaluated by the Elmman's method. Pulegona potency (IC_{50}), maximal inhibition (E_{max}), and selectivity ($IC_{50AChE}/IC_{50BuChE}$) have been evaluated for AChE and BuChE inhibition *in vitro* (A). The mode of BuChE inhibition by pulegone has been investigated (B). The effect of pulegone in the scopolamine induced learning deficit in the passive avoidance test in rat (C) and the level of brain cholinesterase inhibition (D) after oral administration was also measured. The results of *in vitro*, *in vivo* and *ex vivo* experiments will be presented.









CL41 How do you make the best Caipirinha?

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Recently we discovered through metabolomics a novel type of solvents, which we called Natural Deep Eutectic Solvents (NADES). They are formed by mixtures of sugars, amino acids, organic acids and bases in certain molar ratios, e.g. malic acid and choline like well known synthetic ionic liquids. Also two neutral solids may form a deep eutectic solvent at room temperature. So we discovered that various sugars with e.g. choline or malic acid form such liquids. These NADES are medium polar like ethanol, and proved to be excellent solvents for most natural products, including proteins, DNA, RNA and polysaccharides, often with orders of magnitude higher solubility than in water. Do they occur in nature? Nectar is an example of a NADES, but we believe that NADES play an important role in nature as a third liquid phase of medium polarity between water and lipids. In resurrection plants, cacti, seeds all ingredients of NADES present, and also cold and drought resistant plants are rich in NADES ingredients. The occurrence of NADES in cells would explain how water insoluble compounds like terpenoids can be

biosynthesized, as well as very high levels of flavonoids and derivatives occur in flowers in a liquid state. NADES ingredients are found in all pro- and eukaryotic cells. When you start thinking about possible roles of NADES in nature, the possibilities are almost infinite!

CL42 Accuracy, precision and reliability in natural product analysis: mechanisms of NIH support for methods development and validation

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Reproducible biomedical and nutritional research on natural products requires precise, accurate and rugged methods of analysis. Reliable and appropriate calibration standards are indispensible for verification of product identity, for quantification of analytes of interest in products or biospecimens, and for determination of contaminants; they are thus equally critical for manufacturers and regulators. NIH supports the development and validation of methods and reference materials for natural product analysis through the Office of Dietary Supplements' Analytical Methods and Reference Materials Program (AMRM). This stakeholder-driven Program includes collaborations with other government agencies, researchers and the private sector to develop validated reference materials, standards, and analytical methods, and to disseminate them to the relevant communities. Among the projects supported by the AMRM to date are: 1) development of chemical reference materials for half a dozen botanicals and for multi-vitamin supplements, 2) publication of 16 AOAC (Association of Analytical Communities) Official Methods of Analysis, 3) development of 20 matrix standard reference materials that are available from the US National Institute of Standards and Technology and 4) publication of validation studies of more than 20 methods for botanicals or botanical components. Recently the AMRM Program has added supplemental funding for NIH awardees to validate analytical methods developed in support of a parent award. This presentation will provide a brief overview of the AMRM Program and demonstrate the effects of appropriate methodology on analytical results.

CL43 New analytical tools for quality control of natural products

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Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University Innsbruck,

Innrain 80/82, 6020 Innsbruck, Austria Phytopharmacy with its huge variety and complexity of plants, extraction procedures and ingredients is a permanently demanding challenge for the analytical chemist. The separation of active components from natural products has become increasingly important and new advancements in chromatography allow exploring inaccessible areas of natural product isolation. There is a need for highly efficient techniques to handle the problems associated with sample preparation, separation and identification. Novel enrichment and purification methods based on modern SPE technologies are applied to reduce the complexity of plant materials while μ-HPLC is used for separation, preconcentration and fractionation. Recent research in the area of solid-phase extraction led to the development of novel stationary phases based on organic polymers and fullerene-silica for enrichment and purification of natural products. Considerable progress has been made in the development of stationary phases which can be tailored to a specific application allowing endless possibilities in terms of selectivity tuning. An analytical platform which allows to profile and characterize plant materials and their extractives by vibrational spectroscopy, infrared (IR) based imaging, CE-MS and matrix-free material enhanced laser desorption ionization time of flight mass spectrometry (mf-MELDI-TOF/MS) was established. Thereby, vibrational spectroscopy offers the advantages of fast, non-invasive and simultaneous determination of chemical and physical properties being suitable for high-throughput analytical quality control.

CL44 Ambient mass spectrometry for extractionless analyses of plants: Holy Grail, useful tool or hoax?

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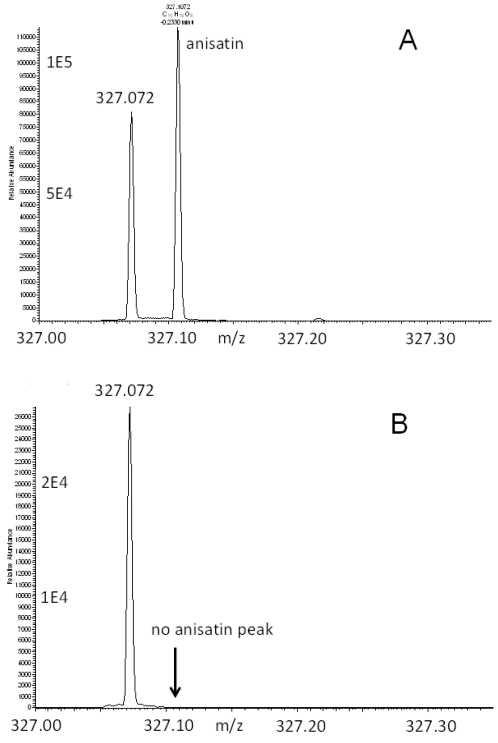
Ambient mass spectrometry allows sampling on your benchtop at atmospheric pressure and often without any sample preparation. A short overview of ambient MS is given including ionisation mechanisms and new exciting developments such as leaf spray. Direct Analysis in Real Time (DART) and Desorption Electrospray Ionisation (DESI) applications from the author's group in the field of natural products chemistry are presented. In particular DART-MS is a versatile, easy to handle and fast technique allowing even quantitative measurements of secondary plant metabolites such as alkaloids, terpenes (Ginkgo, star anise) and flavonoids. Fig. A shows the DART-MS of toxic Japanese star anise with a clear peak for the neurotoxin anisatin at *m/z* 327.107. Chinese star anise lacks this peak (Fig. B). This result was obtained in seconds without any sample preparation. In combination with high-resolution MS it can be used for quality control, adulteration detection, metabolomics and screening of herbal products. It can also be hyphenated to HPLC or TLC. A comparison of DART and DESI-MS in terms of scope, figures of merit and limitations is made.



DART-MS spectra of Japanese (A) and Chinese star anise (B)

CL45 Determination of absolute configuration of chiral molecules using vibrational optical activity

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Determination of absolute configuration (AC) of chiral molecules is an important step in any field related to chirality, especially in the pharmaceutical industry. Vibrational optical activity (VOA) has become a powerful tool for the determination of absolute configuration of chiral molecules in solution state after 30 years of evolution. VOA offers a novel alternative to X-ray crystallography, permitting AC determinations on neat liquid, oil, and solution samples. By comparing the sign and intensity of the experimental VOA spectrum with the *ab initio* DFT calculated VOA of a chosen configuration, one can unambiguously assign the absolute configuration of a chiral molecule. Comparing measured

VOA spectra with calculated VOA spectra of all the conformers can also provide solution-state conformational populations. VOA consists of vibrational circular dichroism (VCD) and Raman optical activity (ROA). VCD is used routinely by researchers of various backgrounds including basic study of chirality, asymmetric synthesis or catalysis, drug screening, pharmacology and natural products. Although the application of ROA in AC determination lags behind that of the VCD, with the recent implementation of ROA calculation method into the commercial calculation software, ROA will in the future complement VCD for AC determination. In this presentation basic principles of the application of VCD to the determination of absolute configuration in chiral molecules are described. The steps required for VCD spectral measurement and calculation are outlined followed by recent applications of AC in small organic, pharmaceutical and natural product molecules.

CL46 LCMS-based secondary metabolomics as a tool to investigate marine bacterial natural products

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The field of metabolomics has developed tools that are adaptable to secondary metabolites. We have recently shown that bacterial strains can be prioritized for drug discovery using principal component analysis (PCA) of LCMS data. LCMS-PCA is complementary to genomics, transcriptomics, and proteomics. Therefore, it can be used to assist with studies addressing a broad range of questions surrounding production of secondary metabolites including regulation and interspecies interactions. The application of LCMS-based metabolomics toward understanding the dynamics of how interspecies interactions between marine bacteria affect secondary metabolite production will be presented. Understanding the dynamics of microbial interactions represents a new forefront in systems biology and natural products discovery.

CL47 Applications of metabolimcs to medicinal plants for scientific study and drug discovery

Brown PN[1], Murch SJ[2]
[1]Natrual Health & Food Products Research Group, Centre for Applied Health & Innovation, BC Institute of Technology, Burnaby, BC V5E3C7, Canada; [2]Department of Chemistry, University of British Columbia, Kelowna, BC V1V 1V7, Canada

The term “metabolomics” was developed 10 years ago to describe the untargeted identification and quantification of all of the small metabolites in a biological sample. Over the last decade, researchers have been working to develop methodological approaches, standards, data analysis tools and new technologies that enable metabolome studies. Medicinal plants are ideal candidates for investigations by metabolomics as research questions often involve quantification multiple bioactive or marker compounds, synergy between molecules and complex interactions within a system. It has been estimated an average leaf sample could contain up to 30,000 compounds; the vast majority of which have never been isolated, identified or described. In metabolomic profiles we have observed 2,500 compounds in St. John’s wort, 4,800 in *Scutellaria baicalensis*, and 5,200 in cranberries. By comparing metabolomes of different extracts and focusing the statistical analysis on unknowns in each sample, we have identified previously undiscovered compounds required for medicinal activity leading us to propose an alternate "Metabolomics-Based Drug Discovery Pipeline". Key factors for success are production of standardized active plant extracts and statistical methods to evaluate data quality thereby minimizing potential false discovery. This approach has the advantage of providing basic chemical information on compounds not sufficiently stable to withstand purification processes and could lead to a greater understandings of chemical synergy and the interactions of medicinal plants with human metabolism.

CL48 Hyphenating countercurrent chromatography with NMR and mass spectrometry. How to enhance the range of the liquid phases

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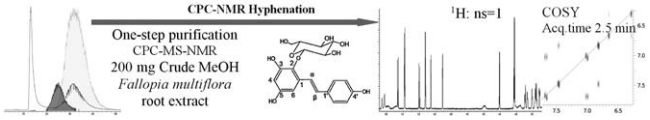
Countercurrent chromatography techniques are increasingly used in the fractionation and purification steps of natural products due to their excellent versatility and efficiency. However, one of the major bottlenecks in liquid-liquid chromatography is the solvent system selection. In this work, a NMR-based analysis of complex solvent mixtures was developed for biphasic system selection and modification. This allowed us to develop more versatile separation conditions, such as gradients. Additionally, we hyphenated our countercurrent chromatography device with NMR and mass spectrometry. Our hybrid technique afforded a time-saving approach with a significant reduction in compound handling, an asset in working with minor and/or sensitive compounds. Current results and further developments will be presented and discussed.



CL49 Two bifunctional enzymes, MTM GIV and MTM C, involved in the biosynthesis of deoxysugar moieties of the antitumor antibiotic mithramycin

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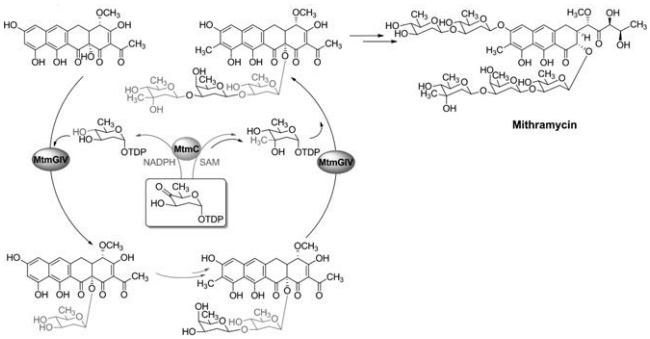
Abstract: Mithramycin is an aureolic acid-type anticancer agent consisting of a polyketide core that is decorated with disaccharide and trisaccharide units. Previous studies have shown that two gene products, MtmGIV (a putative glycosyltransferase) and MtmC (a putative *C*-methyltransferase), are indispensible for the assembly of the complex trisaccharide component. However, their exact functions remained unclear. Using *in vitro* reconstitution, these two enzymes are now shown to both have bifunctional activities and hence dual roles in the biosynthesis of mithramycin. MtmC is demonstrated to function as both a 4-ketoreductase and 3-*C*-methyltransferase, with each activity representing a branching point from TDP-4-keto-D-olivose to yield TDP-D-olivose and TDP-D-mycarose, respectively. Both sugars then serve as donor substrates for MtmGIV, a bifunctional glycosyltransferase that incorporates the first sugar (D-olivose) and the last (D-mycarose) into two distinct acceptor substrates to yield the trisaccharide component of mithramycin. The discovery of these dual enzyme functions explains two previously missing activities that are essential for mithramycin biosynthesis that were not readily predicted from bioinformatic analysis of the gene products.



(1) Wang, g.; Pahari, P.; Kharel, M. K.; Van Lanen, S. G.; Rohr, J. *J. Am. Chem. Soc.* **2012**, *134*, submitted; (2) Wang, G.; Kharel, M. K.; Pahari, P.; Rohr, J. *ChemBioChem* **2011**, *12*, 2568 – 2571.

CL50 Chemical induction of silent biosynthetic pathway for the expansion of the terpenome in *Botrytis cinerea*

Collado IG, Barua Chamorro J, Moraga Galindo J, Durán Patrón R, Aleu Casatejada J, Hernández-Galán R
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The recent availability of the *B. clnerea* genome at the Genoscope (http://www.genoscope.cns.fr/) and at the Broad Institute (http://www.broad.mit.edu/annotation/fgi/) provided the opportunity to investigate secondary metabolism gene clusters including those putatively involved in sesquiterpene biosynthesis. Although only two families of toxins have been isolated and reported, the sequencing of the genomes of the B05 – 10 and T4 strains revealed an abundance of novel biosynthetic gene clusters. Genomic data revealed that *B. cinerea* has 43 key enzymes some of them are specific of this phytopathogen. Within the genome, 6 genes coding for putative sesquiterpene cyclases have been identified, including 2 diterpene cyclases. To study them, we have undertaken different approaches: chemical- and molecular-based epigenetic studies, comparative genomic study and gene inactivation. As a result new molecules with novel structures were found. The isolation and characterization of these molecules are under study and their structures and biosynthetic pathway, using deuterated acetate and labelled glucose experiments, are shedding light on new biological targets.

CL51 Deep sequencing of secondary meta-metabolomes: A preliminary screening tool for determining natural product diversity

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Increasingly, natural product isolation strategies are circumventing culture-dependent methods for the isolation secondary metabolite genes directly from the environment. Many of these isolation strategies are undertaken with little knowledge of an environment's specific secondary metabolite potential. Next-generation sequencing technology was used to determine the diversity of non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) genes within multiple environments to a depth previously not reported. A multiplexing strategy was used to amplify thousands of ketosynthase and amino acid condensation domain sequences from over thirty different environments. Sequences were differentiated according to function and taxonomic origin, as well as their distribution within distinct environments. Similar patterns of NRPS and PKS occurrence were observed between functionally similar but geographically distinct environments. Furthermore, increases in microbial diversity between environments did not influence the occurrence of these genes. It is expected that this approach will be applied to any environment enabling for the tailoring of culture-dependent and culture-independent strategies for the isolation of novel natural products.

CL52 Genomic insights into secondary metabolism of the natural product-rich cyanobacterium *Moorea bouillonii*

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[1]Scripps Institution of Oceanography, University of California San Diego, USA; [2]St. Petersburg Academic University of the Russian Academy of Sciences, Russia; [3]Department of Computer Science, University of California San Diego, USA; [4]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, USA

Moorea bouillonii (formerly *Lyngbya bouillonii*), a marine filamentous cyanobacteria, is known to produce about 30 different natural products; however, the true natural product potential and life strategies of this *Moorea* strain remain poorly investigated. To further explore the natural product potential of a *M. bouillonii* strain and gain insights into other life history characteristics, we sequenced the genome of the type-strain, *M. bouillonii* PNG5 – 198 from Papua New Guinea. The 8.6 Mb genome was assembled using the newly developed SPAdes algorithm followed by error correction using the Bayes Hammer program. In addition to the already published Apratoxin A pathway, there is also a pathway consistent with lyngbyabellin A biosynthesis, another cytotoxic metabolite from this species. The biosynthetic pathway for lyngbyabellin A has significant similarity to the hectochlorin biosynthetic pathway found in a Caribbean cyanobacterial strain. A comparison of the *M. producin* 3L and the *M. bouillonii* genomes will also be presented. This project was funded from grants: NIH Grant 2R01 CA10887406 to W.H.G and L.G, and Russian megagrant to P.A.P.

CL53 Genome of an octopus-derived *Pseudoalteromonas* reveals unprecedented natural product biosynthesis gene clusters

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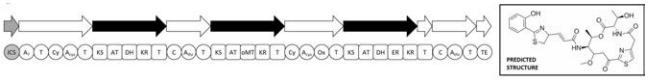
The highly toxic octopus species *Hapalochlaena maculosa* is home to a diverse community of microorganisms, some of which are proposed to give rise to small natural products involved in life-sustaining processes such as chemical defence or signalling. The *H. maculosa* derived bacterial isolate *Pseudoalteromonas* sp. HM-SA03 was selected for genome sequencing on the basis of its biosynthetic potential as indicated by targeted PCR. Genome mining of the draft sequence revealed 6 hybrid NPRS-PKS coding biosynthesis gene clusters which display little homology to known clusters. Partial characterisation of these clusters using bioinformatics techniques has allowed structures of these NRPS/PKS products to be predicted. Here we present the complement of NRPS-PKS gene clusters and report on the discovery of some compounds from *Pseudoalteromonas* sp. HM-SA03.



CL54 Characterization of a fungal thioesterase having claisen cyclase and deacetylase activities in melanin biosynthesis

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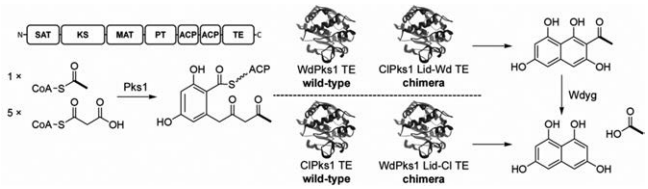
Cell wall melanins are darkly-pigmented macromolecules that contribute to pathogenicity. In fungi, melanin biosynthesis generally involves polymerization of 1,8-dihydroxynaphthalene via a 1,3,6,8-tetrahydroxynaphthalene (THN) precursor whose backbone is assembled by iterative, nonreducing polyketide synthases. Convergent routes to THN have evolved in fungi. Parallel heptaketide and hexaketide pathways utilize conventional C-terminal thioesterase/Claisen cyclase domains and discrete side-chain deacylases. In contrast, i*n vitro* characterization of Pks1 from *Colletotrichum lagenarium* establishes a true THN synthase with a bifunctional thioesterase (TE) catalyzing both cyclization and deacetylation of an enzyme-bound hexaketide. Chimeric TE domains were generated by swapping lid regions of active sites between classes of melanin-related TEs to gain insight into this unprecedented catalysis of carbon-carbon bond making and breaking by a single α/β-hydrolase fold enzyme.



CL55 Biosynthesis of prenylated fused-ring aromatic polyketides in fungi: From viridicatumtoxin to the immunosuppressive neosartoricin

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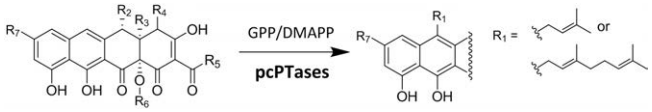
The prenyltransferase (PTase) gene *vrtC* was proposed to be involved in viridicatumtoxin biosynthesis in *Penicillium aethiopicum*. Targeted gene deletion and reconstitution of recombinant VrtC activity in vitro established that VrtC catalyzes a regiospecific Friedel-Crafts alkylation of the naphthacenedione carboxamide intermediate with geranyl diphosphate (GPP). Genome mining using the VrtC protein sequence leads to the identification of a homologous group of polycyclic aromatic PTase (pcPTase) genes in the genomes of human and animal-associated fungi. Three enzymes from this new subgroup of pcPTases were shown to be able to catalyze transfer of dimethylallyl to several tetracyclic substrates in vitro. In total, seven C_5- or C_{10}-prenylated naphthacedione compounds were generated. Furthermore, activation of the silent gene cluster containing the pcPTase gene in *N. fischeri* led to the isolation of a tricyclic prenylated polyketide – neosartoricin. Neosartoricin exhibits T-cell antiproliferative activity with an IC_{50} of 3 µM. The discovery of this new subgroup of PTases extends our enzymatic tools for modifying polycyclic compounds and enables genome mining of new prenylated polyketides.



CL56 Apllication of epigenetic tools in mycogone species for the enrichment of their fungal secondary metabolome

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Fungi are known for their ability to produce bioactive small molecules. Production of fungal secondary metabolites is a special process that occurs only under specific environmental conditions or at a certain stage in their life cycle. Many microorganisms harbor significant numbers of secondary-metabolite-encoding biosynthetic pathways, but only a fraction of their small-molecule products are detected in the laboratory. The potential for using epigenetic modifying agents to induce changes in fungal secondary metabolism have only recently come to light [1]. In a continuation of our studies for the discovery of novel metabolites from filamentous fungi, the effect of application of DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors has been investigated, as well as the use of synthetic adsorbent resins in the production of secondary metabolites in strains of the mycophilous fungus *Mycogone,* in liquid fermentations. The strains have been cultivated in the presence and absence of the epigenetic chemical agents. The metabolic profiling of the extracts were compared using HPTLC and HPLC methods and it was concluded that the epigenetic tools enhanced in the strains both the over expression of specific secondary metabolites, as well as the production of novel secondary metabolites. References: 1. Cichewicz R.H. Nat. Prod. Rep., 2010, 27, 11 – 22

CL57 Novel elastase inhibitors from marine cyanobacteria: Structural diversity, target-bound crystal structures and cellular effects

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We report the isolation and structure determination of six new dolastatin 13 analogs given the trivial names symplostatins 5 – 10, the molecular basis of their anti-proteolytic activity, and characterization of their cytoprotective effects. IC_{50} determination for symplostatin 5 against a panel of 26 serine proteases revealed potent and selective anti-proteolytic activity against elastase with low-nanomolar IC_{50}, and to a lesser extent against chymotrypsin, while IC_{50}s for the other serine proteases tested occurred only at high-micromolar concentrations. Crystallization of the elastase-inhibitor complex at 1.5 Å resolution offered insights on key pharmacophores for this class of serine protease inhibitor, with the 2-amino-butenoic acid (Abu) moiety being central, while other functionalities can be tuned to achieve further improvements in potency and selectivity. The structural basis of elastase inhibition was also corroborated by structure-activity relationship studies derived from *in vitro* enzyme inhibition assays. This family of compounds can also alleviate the effects of exogenous elastase on mammalian cells, and elastase-induced cell injury was attenuated with inhibitor treatment.

CL58 Implications of Nagoya protocol implementation for the pharmaceutical and cosmetic industries

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Legal access to biodiversity is a key point for natural product chemists, academic and industrial researchers. The fair and equitable sharing of the benefits arising from the utilization of genetic/biological resources was reaffirmed in Nagoya in October 2010. The Nagoya ABS regulations will enter into force only when the protocol has been translated into national laws. It has to be operational by 2015 but many states are aiming for an earlier entry into force. However, the new access laws should not be overly restrictive for both academic and industrial researchers in order to avoid paradoxical effects on pharmaceutical and cosmetics industries. The presentation will describe the new practical processes which are going to be in force to legally access to national and international biodiversity samples (PIC, MAT,). It will focus on the main issues of the Nagoya protocol implementation: clarification of the scopes, definition of commodities, interpretation of obligations, establishment of clear, fair, transparent and efficient rules for access, dispute settlement and multilateral benefit sharing mechanism ... In fact, without possible and secure access, no valorisation and no benefit sharing will be possible toward the source countries by the pharmaceutical and cosmetics industries. Sustainable access should be facilitated for the fair, equitable benefit of all (source countries, industries, patients and users of cosmetics) and, of course, biodiversity preservation.

CL59 Sedative effects of *Passiflora Edulis* F. *Flavicarpa* and *Passiflora Alata* extracts in mica measured by telemetry

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Several *Passiflora* species have been used widely as a folk medicine due to their sedative and anxiolytic activities. In Brazil, a number of native plants of the genus *Passiflora* exist but only *Passiflora edulis* f. *flavicarpa* (PE) and *Passiflora alata* (PA) are of commercial value. Thus, the aim of the present study was to investigate the sedative effects of aqueous extracts obtained from the pericarp as well as from the leaves of PE and PA in mice using radiotelemetry. Aqueous extracts from PE and PA

were tested for effects on locomotion over 180 minutes in 300 mg/kg, 600 mg/kg and 1200 mg/kg, in male C57BL/6J mice after oral administration. For validation of the telemetry system, caffeine (negative control) and midazolam (positive control) were used. All tested extracts decreased locomotor activity in a dose-dependent manner in comparison to the control group. The two lower concentrations of each extract showed the highest decrease in locomotion after 24 minutes, while 1200 mg/kg had a significant sedative effect already after 18 minutes. Interestingly, aqueous extracts of PA were more active in comparison to aqueous extracts of PE and the pericarp extracts of both plants showed more pronounced effects on locomotor activity if compared to leaf extracts. In conclusion, the present study represents an innovative, objective approach to measure sedative effects of plant extracts with minimized handling-related stress and remote data collection.

CL60 Breast feeding, hypogalactia and molecular exploration of herbal galactogogues

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Research has shown that breast milk is the personalized health food and immune protection for infants. Breast milk carries the nutrients required by infants and provides anti-infective properties to newborns. Breast milk contains immunoglobulins, proteins like lactoferrin, lysozyme and casein, lipids, oligosaccharides, enzymes, prostaglandins, growth factors, hormones which provide many mechanisms to prevent infections and modulate the immune system. This protection is personalized as the mother would likely be exposed to the same microorganisms that cause infections in the infants. However, many mothers experience insufficient milk production called hypogalactia. Prolactin plays a primary role in lactation and estrogens act as regulators of milk production. High levels of estradiol can decrease milk secretion by changing the mammary secretory epithelial morphology. Galactogogues are molecules that increase lactation. Galactogogues function either by increasing prolactin secretion or by inhibiting estradiol. Hypogalactia is treated by medications like domperidone, metoclopramide and sulpiride which increase endogenous prolactin production but are limited in use due to side effects. Herbal galactogogues known and used since ancient times may provide new leads. This study is an effort to identify components of traditionally used galactogogues in Asian and Middle Eastern countries, the seeds of *Nigella sativa*, *Trachyspermum copticum* and *Trigonella foenum-graecum*. The seeds were extracted by various solvents and analyzed by Gas Chromatography and Mass Spectrometry for either analogues of the prolactin inducing medications mentioned above or those which may inhibit estradiol.

CL61 Prokinetic and antispasmodic constituent discovered in Perilla Frutescens – Development of a gut health ingredient

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Perilla frutescens (L.) Britton is an annual herbaceous plant native to Asia[1]. Perilla leaves are used as food and in traditional medicine. During our phytochemical investigation of Perilla leaves, we discovered the compound, Vicenin 2, which has not been described as key constituent for Perilla leaves before. Furthermore, we elucidated novel potential biological activity for Vicenin 2. First, a relaxant effect on isolated adult male Wistar rat's ileum contraction was investigated. Both a special extract and Vicenin 2 demonstrated antispasmodic effects, inhibiting neurotropic and musculotropic activity. Second, an acute neuroactive effect on the neuronal activity of murine frontal cortex networks was tested by means of electrophysiological multi-channel recording (Figure 1). Vicenin 2 as well as the special Perilla leaf extract showed prokinetic properties and are potential novel active ingredients to prevent and improve functional gastrointestinal discomfort and disease, like IBS.

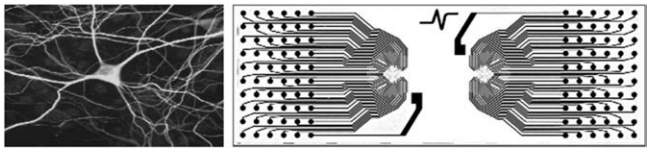

Figure 1: Primary cultures of murine embryonic cells and microelectrode Array byNeuroProof GmbH

CL62 Analgesic effects of a standardized bioflavonoid composition – UP446 From Scutellaria Baicalensis and Acacia Catechu

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Current use of commonly prescribed or over the counter non-steroidal anti-inflammatory drugs (NSAIDs) for pain and/or osteoarthritis have been limited due to their untoward gastrointestinal and cardiovascular related side effects, as a result the need for a safe and effective alternative has become unequivocally crucial. Analgesic activity of UP446, a standardized bioflavonoid composition with primarily baicalin and (+)-catechin which has been used in both joint supplements and a prescription medical food was evaluated. This natural flavonoid composition reduces production of eicosanoids through dual inhibition of COX and LOX enzymes and also decreases gene expression and protein levels of pro-inflammatory cytokines, IL-1β, IL-6, and TNF-α. Carrageenan and microbial adjuvant induced paw edema, formalin test and abdominal constriction assays were used to evaluate antinociceptive activity of UP446 at 50, 100, or 150 mg/kg administered orally. Ibuprofen was used as a reference compound. Pre-treatment of carrageenan or adjuvant evoked hyperalgesic rats with UP446 showed 39.5% and 34.8% reduction in pain sensitivity, respectively. Similarly, a single dose of UP446 at 100 mg/kg, exhibited 58% and 71.9% inhibition in pain sensitivity compared to vehicle treated control in Writhing's and formalin tests, respectively. These findings suggest that the anti-inflammatory standardized bioflavonoid composition, UP446, could also be employed to inhibit nociception as a safe and effective alternative of botanical origins.

CL63 Anti-mycobacterial natural products from canadian traditionally used medicinal plants and their endophytic fungi

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Whilst multi-drug resistant (MDR) and extensively drug resistant (XDR) tuberculosis have been recognised as serious global health concerns for some time, recent media reports of "extremely drug resistant" (XXDR) and "totally drug resistant" (TDR) tuberculosis have confirmed that new anti-mycobacterial drug leads are still urgently required. We have identified 12 medicinal plants traditionally used by the First Nations of the Canadian Maritime Provinces that have reported ethnopharmacological uses relating to the treatment of tuberculosis or its related symptoms. A total of 97 strains of endophytic fungi have been isolated from the 12 plants and extracts of the plants and the fungi have been screened for anti-mycobacterial activity against Mycobacterium tuberculosis $H_{37}Ra$. This presentation will highlight some of the research currently being conducted by the UNB Natural Products Research Group focused on the isolation of new anti-mycobacterial natural products from these Canadian traditionally used medicinal plants and their associated fungal endophytes.

CL64 Australian flora – A source of new compounds for wound healing?

Wohlmuth H[1], Banbury L[1], Shou Q[1], Renshaw D[1], Griesser H[2], Lambley E[1], Mon H[2], Jones E[1], He X[1], Heinrich M[1,3]
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Forming part of the Australian Wound Management Innovation Cooperative Research Centre, this project focuses on identifying plant compounds, in particular from native Australian plants, with potential application in wound healing. *In vitro* testing for anti-inflammatory (inhibition of TNF-α, nitric oxide, PGE_2), antibacterial and antioxidant activity is used to identify candidate extracts, while bioassay-guided fractionation and structural elucidation using NMR and mass spectroscopy are employed to isolate and characterise active lead compounds. Around 130 native plant species were identified in the literature as having been used by Australian indigenous people for wounds and sores, and more than 250 species have been screened in the project so far. New natural compounds isolated include dibenzofurans, coumarins, flavonoids and anthranilic acid derivatives. Australia's unique biodiversity provides a rich source of natural products, some of which may have potential as new wound healing agents due to their ability to inhibit microorganisms, modify the inflammatory response, or modulate other aspects of the wound healing process.

CL65 Heterologous biosynthesis as a route to complex polyketide and isoprenoid natural products

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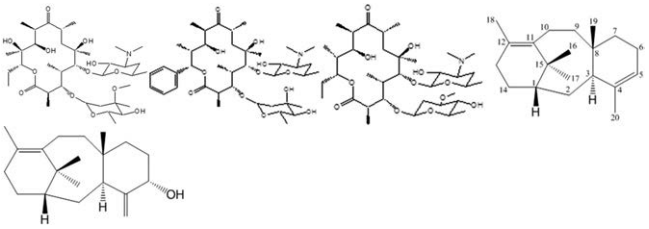
Department of Chemical and Biological Engineering, State University of New York at Buffalo, Buffalo, NY 14260

This presentation will detail recent work to produce therapeutic and chemically complex natural products using *Escherichia coli* as a heterologous host. Broadly, our laboratory strives to establish and optimize natural product formation using heterologous host systems. The motivation for doing so is two-fold. First, there is the desire to continually access the tremendous medicinal value associated with natural products. Second, challenges in over-producing and/or manipulating biosynthesis through native hosts have spurred the emergence of heterologous biosynthesis as a means to exert control over the processes responsible for natural product formation. In this presentation, specifics will be provided regarding the logic and success in producing the polyketide antibiotic erythromycin A, erythromycin analogs, and early-stage intermediates of the isoprenoid anticancer agent paclitaxel. The presentation will also feature multi-scale engineering strategies our group has used in an effort to enable, maximize, and alter heterologous production.



CL66 Antiviral lectins from red and blue-green algae show potent *in vitro* and *in vivo* activity against Hepatitis C virus

Takebe Y[1], Saucedo CJ[2], Lund G[3], Uenishi R[1], Hase S[1], Tsuchiura T[1], Knetman N[3], Ramessar K[4], Tyrrell DLJ[3], Shirakura M[5], Wakita T[5], McMahon JB[4], O'Keefe BR[4]
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Hepatitis C virus (HCV) infection is a significant public health problem with over 170,000,000 chronic carriers and infection rates increasing worldwide. Current treatment options for HCV infection are limited to PEG-ylated interferon alpha, the nucleoside ribavirin and the recently approved HCV protease inhibitors telaprevir and boceprevir. Although showing significantly improved efficacy over the previous therapies, treatment with protease inhibitors has been shown to result in the rapid emergence of drug-resistant virus. Here we report the characterization of two novel proteins, originally isolated from natural product extracts, which demonstrate low or sub-nanomolar *in vitro* activity against both genotype I and genotype II HCV. These proteins inhibit viral infectivity by binding to the HCV envelope glycoprotein E2 and blocking viral entry into human hepatocytes. In addition, we demonstrate that the most potent of these agents, the 25 kDa protein griffithsin, comprised of domain-swapped homodimer of 12.7-kDa subunits, is bioavailable after subcutaneous injection and shows significant *in vivo* efficacy in reducing HCV viral titers in a mouse model with engrafted human hepatocytes.

CL67 Discovery of plant proteins and protein scaffolds

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Division of Pharmacognosy, Department of Medicinal Chemistry, Uppsala University, BMC Box 574, SE 75123 Uppsala, Sweden

Ribosomally produced proteins and peptides represent a class of natural products that still is vastly underexplored. Our research is focused on discovery and applications of novel proteins and protein scaffolds, and in particular, on the family of cyclic and cystine knotted plant proteins known as cyclotides.[1,2] This peptide family defines an ultra-stable peptide scaffold that may be considered ideal for peptide engineering. Currently, we are exploring the antimicrobial activity of cyclotides and their mechanism of action. We have recently shown that specific cyclotides have potent effect against gram-negative bacteria,[3] and that they act by membrane thinning.[4] However, recent results demonstrate that the membrane is not the only target of these peptides. At sub-cytotoxic concentrations, cyclotides demonstrate a different mode of action: they also cross over the cell membrane. **1**. Göransson U, Burman R, Gunasekera S, Strömstedt AA, Rosengren KJ (2012) J Biol Chem, *In press*. **2**. Craik DJ, Daly NL, Bond T, Waine C (1999) J Mol Biol, 294:1327. **3**. Pränting M, Lööv C, Burman R, Göransson U, Andersson DI (2010) J Antimicrob Chemother. 65:1964. **4**. Burman R, Strömstedt AA, Malmsten M, Göransson U (2011) BBA-Biomembranes, 1808:2665.

CL68 Analyzing outcomes from a library-based screening against diverse targets

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To what extent plant selection based on ethnomedical information contributes to the outcomes of library-based natural product lead discovery programs is a matter of debate. Therefore, we have performed a retrospective analysis of the outcomes from screening campaigns and follow-up discovery of active compounds with our in-house extract library. Using functional as well as mechanism-based assays, we screened for $GABA_A$ receptor modulators, antiprotozoal activity against *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense* and *Leishmania donovani*, anti-retroviral activity, and inhibition of DYRK1A, and we compared an ethnomedicine-based subset of the library with a subset of "randomly" collected plants. Very few samples gave hits in more than one assay. In

the subset of traditional Chinese medicinal plants, the hit rates and potentiation of GABA-induced chloride currents was significantly higher than in the rest of the library. When analyzing the data according to plant parts, however, no significant difference was seen between the two subsets. In the other assays we did not find statistically significant differences in hit rates between the two subsets. Selected molecules with *in vivo* activity and drug-like physicochemical properties from this screening campaign will be presented and discussed.

CL69 NMR- and UHPLC-MS correlation for identification of biomarkers from woods of *Vitis Vinifera* cultivar resistant to pathogens

Halabalaki M[1], Bertrand S[2], Stefanou A[1], Boccard J[2], Kostidis S[1], Rudaz S[2], Gikas E[1], Skaltsounis AL[1], Gindro K[3], Wolfender JL[2]
[1]Laboratory of Pharmacognosy and Pharmaceutical Chemistry, School of Pharmacy, Panepistimioupoli, Zografou, 15771, Athens, Greece; [2]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, quai Ernest-Ansermet 30, CH-1211 Geneva, Switzerland; [3]Mycology group, Agroscope Changins ACW, Route de Duillier, CH-1260 Nyon, Switzerland

Vitis cultivars exhibit different susceptibility to pathogens such as *Botrytis cinerea* or *Plasmopara viticola* (downy mildew of grape). Therefore the selection of resistant species is important for a sustainable wine production without use of pesticides. The wood extracts from three cultivars with different resistance to pathogens were profiled by HR-NMR, UHPLC-(NI)-ESI-TOF-MS and UHPLC-(NI)-ESI-orbitrap-MS in order to highlight relevant biomarkers accumulated in one complete season. Differenzial metabolomics showed interesting features related to resistance with each analytic method. The comparisons of the selected features (RT and m/z for LC-MS and chemical shift for NMR) showed high convergence which was confirmed by multiple factorial analysis. The use of covariance calculation between LC-MS and NMR data was used in order to help dereplication of highlighted LC-MS features. The pseudo NMR spectra generated in this way were interestingly similar to pure compound spectra of the major biomarkers.

CL70 Anti-diabetic and toxicological studies of the alkaloids of *Acanthus Montanus* (Acanthaceae) leaf

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Department of Pharmacognosy and Enviromental Medicine, Faculty of Pharmaceutical Science, University of Nigeria, Nsukka

This study was conducted to determine the anti-diabetic effect and safety profile of alkaloids of *Acanthus montanus* leaf (AAML). The anti-diabetic study was carried out using alloxan-induced diabetic rats by daily intraperitoneal administration of AAML at doses of 100, 200 and 400 mg/kg for 4 weeks and 8 weeks. The toxicological assessment of AAML was also done by determining the haematological, biochemical and urinary parameters in blood, serum, and urine samples respectively at the end of the tested periods. In the acute toxicity test, mice were administered intraperitoneally with AAML up to 5000 mg/kg. Animals were then observed for behavioural changes, signs of toxicity, and mortality within 24 h. Surviving mice were monitored for 7 days for signs of delayed toxicity. Result of the anti-diabetic study shows significant ($P < 0.05$) dose-dependent reduction (42.68%) in blood sugar levels of the hyperglycemic rats when compared with glibenclamide (standard drug). In the acute toxicity test, extract was practically non-toxic showing no mortality and visible signs of delayed toxicity. Administered for 4 weeks, the extract did not produce any significant ($P < 0.05$) effect on haematological and biochemical parameters. In the 8 week study, the extract elicited significant ($P < 0.05$) increases in platelet and WBC count and reductions in levels of liver enzymes (AST, ALT, and ALP), total cholesterol, HDL, triglycerides, and total protein. Results obtained in this study suggest that the alkaloids of leaf *Acanthus montanus* leaf is safe when administered intraperitoneally with potential beneficial effects as immunostimulant, hepatoprotective, and hypocholestrolemic agent, when administered over a long period of time.

CL71 The diabetic wound healing effect of a two-herb formula and its mechanisms of action

Lau CBS[1,2], Lau VKM[1,2], Liu CL[1,2,3], Lai PKK[1,2], Tam JCW[1,2], To SMH[1,2], Kwok FHF[1,2], Lau CP[1,2], Ko ECH[1,2], Chan JCN[4], Poon SKS[5], Leung PC[1,2], Fung KP[1,2,3]
[1]Institute of Chinese Medicine; [2]State Key Laboratory of Phytochemistry and Plant Resources in West China (CUHK); [3]School of Biomedical Sciences; [4]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong; [5]School of Information Technologies, The University of Sydney, Australia

The dried roots of *Astragalus membranaceus* (AR) and *Rehmannia glutinosa* (RR) are two herbs commonly used in Chinese medicines for relieving diabetes and its complications. The objective of present study was to examine the wound healing effect of formula NF3 (AR and RR in the ratio of 2:1) in diabetic rats, and its potential mechanisms of action in fibroblast proliferation, angiogenesis and inflammation control *in vitro*. Using diabetic foot ulcer animal model, NF3 (0.98 g/kg) was found to significantly promote wound closure when compared to control (water) group. In the *in vitro* mechanistic studies, NF3 could significantly i) stimulate fibroblast (Hs27) proliferation; ii) increase cell migration and tubule formation of endothelial cells (HUVEC and HMEC-1); and iii) inhibit nitric oxide production from LPS-stimulated macrophage cells (RAW 264.7). In conclusion, herbal formula NF3 could enhance diabetic wound healing through actions of tissue regeneration, angiogenesis and anti-inflammation. The diabetic wound healing effect of NF3 was attributed to the synergistic interaction between its two component herbs. The roles of these two herbs in different underlying mechanisms of action, as well as the clinical efficacy of NF3 will also be presented.

CL72 Antibiotic mode of action profiling (Biomap) as a platform for the discovery of novel natural product antibiotics

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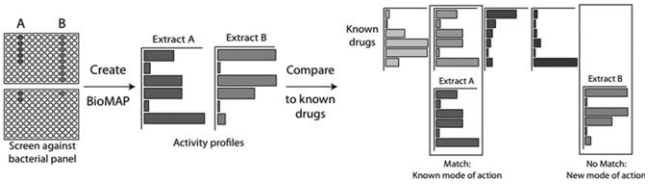
[1]Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA, 95064, USA; [2]Molecular Structure Facility, Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

This study establishes a new antibiotic profiling strategy using a panel of clinically relevant bacterial strains to create biological fingerprint profiles for all the major classes of antibiotics. BioMAP screening has been shown to effectively cluster antibiotics by structural class based on biological activities. Using this approach we have accurately predicted the presence of known antibiotics in natural product extracts and have discovered a naphthoquinone-based antibiotic from our marine natural products library that possesses a unique carbon skeleton. We have demonstrated that bioactivity fingerprinting is a successful strategy for profiling antibiotic lead compounds, and that BioMAP can be applied to the discovery of new natural product antibiotics leads.



Award Lectures

AL1 Thirty years of evolution in an academic drug discovery program: Then and now

Ireland CM
College of Pharmacy, University of Utah, 30 S. 2000 E., Skaggs Hall room 201, Salt Lake City, UT, United States, 84112

The search for drugs from the sea has progressed slowly and often with frustration over the past thirty years with a number of compounds showing early promise but ultimately failing in the clinic. However, the recent success of Prialt, Yondelis and Halaven, along with a robust pipeline of candidates in clinical trials has reinvigorated academic-based drug discovery. Our program has focused on creating a pure marine invertebrate compound library which we have named MICL and utilizing this library in assays targeting cellular components or pathways commonly over-expressed or selectively expressed in tumor cell lines. This presentation will provide a 30 year perspective on my research program as well as our newest results from several exciting projects including our recent exploration of combining natural product in vitro and virtual screening methodology.

AL2 Role of Quercetin on iron homeostasis

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Iron is crucial in processes such as oxygen binding in haemoglobin, electron transport in mitochondria, cell proliferation and differentiation, etc. Insufficient iron regulation can lead to either accumulation of iron that enhances oxidative stress or iron deficiency leading to altered cell growth and metabolism. As there is no regulated physiological mechanism for excretion of iron, iron homeostasis is maintained primarily by regulating iron absorption. Dietary iron content and other dietary constituents can have a major impact on iron homeostasis. Flavonoids are a group of compounds found in the diet either naturally or added as food additives. Therefore interaction of these compounds with iron is of interest. Quercetin, one of the most abundant flavonols in the diet, is an effective iron chelator and also acts as an antioxidant by modulating expression of antioxidant enzymes. Both of these properties of quercetin therefore can have an effect on iron absorption. The aim of this work was to elucidate the effect of quercetin administered by gavage or intraperitionally (IP) on iron uptake. Sprague Dawley male rats, fed on an iron deficient or iron replete diet for two weeks, were used as the animal model. Gene expression of selected intestinal proteins (DMT1, FPN, Dcytb, GLUT1 and SGLT1) was quantitated by Real-time PCR. Intestinal iron absorption was measured in another group of animals using the *in situ* loop method and radioactive iron. Additionally, levels of iron and transferrin saturation in serum, and nonheme iron in duodenum, liver and spleen, were determined. Results were obtained during 13 different treatments on animal models. Study showed that quercetin affects iron homeostasis by changing expression of proteins involved in iron absorption in greater amount when administrated orally than IP. Furthermore, after longer oral treatment with quercetin, animals became iron deficient as both liver and spleen iron levels decreased significantly, 1.2 and 1.1 fold, respectively. These results confirmed negative effects of polyphenols on iron adsorption when consumed in a diet for a longer time. Additionally, when administrated orally, quercetin significantly upregulated SGLT1 transporter, namely 1.8 and 1.6 fold, 18 hours and 5 hours before being sacrificed. These results indicate that quercetin is probably transported by SGLT1 in the duodenum. Moreover, in uptake experiments where quercetin was introduced into the duodenum together with radioactive iron, there was a significant difference in levels of radioactive iron accumulated by duodenal mucosa (increase 2.7 fold) or transferred to serum (decrease 7.3 fold), compared to controls. The most probable explanation is that quercetin chelates iron, and that the complex is transported into enterocytes, but is not further transported into circulation. However, other uptake experiments showed no change in concentration of absorbed iron. Bearing in mind our results and the fact that there was no change in concentration of absorbed iron in the mucosa and circulation during uptake studies, further experiments are needed in order to get a more complete picture of how quercetin affects iron absorption. Acknowledgements: This study was carried out in the Laboratory of Professor Surjit Kaila Singh Srai, supported by a BBSRC project grant awarded to Professor Srai, UCL, London UK. This work was kindly supported by Dr. Willmar Schwabe Research Scholarship for Young Scientists awarded by the Society for Medicinal Plant and Natural Product Research and by the Ministry of Education and Sciences Republic of Serbia (Grant No. 172058).

AL3 Targeting inflammation and Influenza: Integration of computational methods into lead finding from natural sources

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Currently the search for drug leads from natural sources is undergoing an enormous revival which is reflected by an increasing interest in developing optimized time- and cost-saving strategies guiding this endeavor. Since nature offers an infinite pool of complex scaffolds with a high chemical and biological diversity sophisticated rationales to prioritize isolation and purification efforts are of utmost importance. Here, the successful application of a combination of several approaches including in silico techniques, ethnopharmacological knowledge, and bioactivity-guided isolation is demonstrated by two examples:

1. the pharmacophore based discovery of anti-inflammatory and farnesoid X receptor (FXR)-inducing lanostane-type triterpenes from the fruit body of the traditional Chinese medicinal fungus Ganoderma lucidum (Curtis) P. Karst. (Ganodermataceae), and
2. the identification and in silico binding mode studies of a diarylheptanoid isolated from the seed extract of Alpinia katsumadai Hayata (Zingiberaceae) as an innovative influenza neuraminidase (NA) inhibitor.

Finally, pitfalls and challenges of these strategies will be discussed in respect to future applications.

AL4 Targets, tools and leads – The different roads natural products can take us

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Nature provides a valuable source of bioactive small molecules which becomes evident in the number of drugs derived from nature or natural precursors and the efficacy of (traditional) herbal medicines. Our research is focused on bioactive natural products alleviating vascular and metabolic dysfunction. Major attention is hereby drawn (i) to the elucidation of the mode of action of a given compound (target-finding), (ii) to deciphering novel cellular cross-talks by the use of natural compounds (chemical biology) and (iii) to the identification of the active principle within complex mixtures (lead finding). In this talk some of our recent findings will be highlighted: Center of one project was vitamin C (ascorbate) which has been reported to result in vasodilation in patients immediately after infusion, however, with so far undisclosed mechanism. We unraveled that ascorbate leads to modulation of the phosphorylation state of endothelial nitric oxide (NO) synthase (eNOS) and, thus, rapidly enhances production of NO, a vasodilatory gas, in endothelial cells. In another project we employed a derivative of oleanolic acid as a tool in order to activate nuclear factor erythroid 2-related factor 2 (Nrf2), a cytoprotective and redox sensitive transcription factor. We investigated the impact of Nrf2 activation on endothelial function when it is challenged by aberrantly high glucose levels, a common feature in diabetic patients. Like this, pharmacologically activated Nrf2 could be shown to prevent endothelial dysfunction under hyperglycemia, in part by a so far unknown mechanism beyond mere detoxification of reactive oxygen species. Finally we will present data demonstrating that a derivative of indirubin, a component of the traditional Chinese recipe Danggui Longhui Wan, inhibits vasculoproliferative complications, such as atherosclerosis and restenosis, both in vitro and in vivo. By identifying the transcription factor signal transducer and activator of transcription (STAT) 3 as molecular target we provided (i) a molecular explanation for the action of indirubin, (ii) a promising but in the context of vascular dysfunction so far neglected molecular target and (iii) a potential lead structure for the development of new drugs in the battle against vasculoproliferative disorders.

AL5 Screening of the ICSN chemical and plant extract libraries for the discovery and optimization of natural products targeting kinases and anti-apoptotic proteins – Dedicated to the memory of Pr. François Tillequin

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ICSN has a collection of natural compounds and plant extracts thanks to collaborations we have maintained for several years with our colleagues from Africa and Southeast Asia. Screening of these libraries against anti-apoptotic proteins and different protein kinases led to the discovery of new bioactive natural products. From the chemical library, flavonoid alkaloids were thus identified as potent inhibitors of the CDK1 and CDK5 kinases. From the extract library, we have selected several plants whose extracts inhibited the binding of the Bak BH3 peptide to Bcl-xL. The study of these plant species has led to the isolation of a novel chemical series of pentacyclic polyketides and sesquiterpene dimers. Structure, synthesis, molecular modeling and bioactivities will be presented. *Acknowledgments: V. Dumontet, M. Litaudon, B. Morleo, C. Poullain, F. Roussi, T. Sévenet (members of my team), L. Meijer (ManRos Therapeutics, Roscoff), B. Iorga (ICSN, Gif-sur-Yvette), K. Awang (Univ. Kuala Lumpur, Malaysia), N.V. Hung (Institute of Chemistry, Hanoi, Vietnam), P. Rasoanaivo (IMRA, Madagascar), B. Kiremire (Univ. Kampala, Uganda) and colleagues of AFERP (Association Francophone pour l'Enseignement et la Recherche en Pharmacognosy)*

AL6 Stictamides – Isolation, synthesis, and biological evaluation of cell invasion inhibitors

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γ-Amino-β-hydroxy-acid units are structural motifs often encountered in natural products distributed across diverse taxa. Since their discovery in the microbial secondary metabolite pepstatin there has been widespread interest in this moiety due to its ability to inhibit a broad range of enzymes, primarily by mimicking the transition state of peptide hydrolysis. Classic examples of marine natural products containing this moiety are the didemnins[1] that were isolated from a *Tridemnum* sp., and related metabolites, which continue to be evaluated in clinical trials as anticancer agents. As observed in the structure determination of didemnin A though[2], this densely functionalized unit has a manifold of reaction pathways available during the acid hydrolysis protocol, commonly used to determine the absolute and relative configuration of this unit, and many of these either destroy or invert the configuration of the β-hydroxyl group. This ultimately leads to the question "Under what circumstances is the configuration determined from analysis of the hydrolysis reflective of the configuration of the γ-amino-β-hydroxy-acid units?"

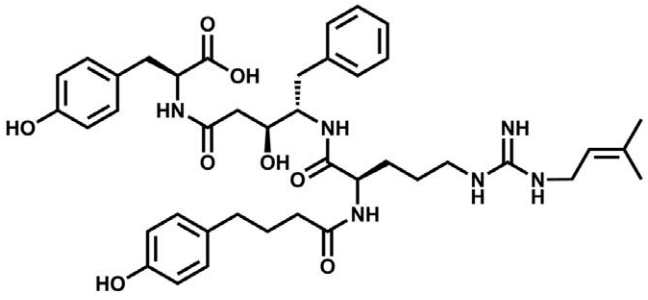

Fig. 1: Stictamide A

This presentation will discuss our data demonstrating the extent to which this stereochemical erosion of statine units is an issue, our development of a simple ^{1}H NMR method to determine the relative configuration of these units[3], and procedural recommendations for their absolute configuration analysis. The utility and validity of the methodology will be demonstrate using a series of recently isolated natural products inhibitors of cell invasion, stictamides A-C[4], of which we have recently completed a total synthesis. References: 1. Rinehart, K. L.; Gloer, J. B.; Cook, J. C.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857 – 1859. 2. Rinehart, K. L.; Sakai, R.; Kishore, V.; Sullins, D. W.; Li, K. M. *J. Org. Chem.* **1992**, *57*, 3007 – 3013. 3. Preciado, A.; Williams, P. G. *J. Org. Chem.* **2008**, *73*, 9228 – 9234. 4. Liang, Z.; Sorribas, A.; Sulzmaier, F. J.; Jiménez, J. I.; Wang, X.; Sauvage, T.; Yoshida, W. Y.; Wang, G.; Ramos, J. W.; Williams, P. G. *J. Org. Chem.* **2011**, *76*, 3635 – 3643.

AL7 The American herbal pharmacopoeia -The marriage of traditional herbalism and classical pharmacognosy

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The origins of pharmacognosy span from before the first century to the present day. Throughout most of its history pharmacognosy paralleled the history of herbal medicine beginning with the roots, barks, leaves, and seeds that made up the traditional crude herb materia medica to relatively recent innovations that led to isolation, purification, and standardization of modern plant-based pharmaceuticals. Worldwide there is a resurgence in traditional herbal medicine which makes the traditional plant-based knowledge of yesteryear's pharmacognosist equally as important as the reliance on chemical and molecular technologies used in modern drug development. This presentation will trace the roots of pharmacognosy from its historical beginnings to the needs of the natural medicine market today.

AL8 Phytochemistry: Templates for new antibacterials and 'legal highs'

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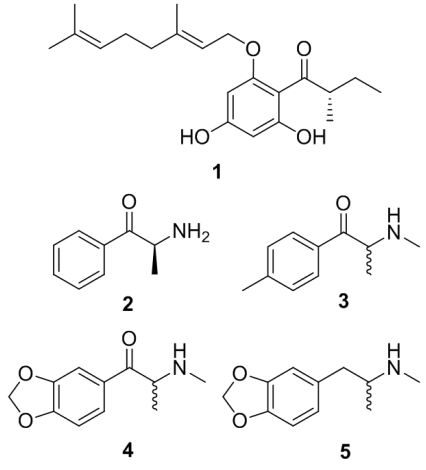
Plants are exceptionally adept at producing antimicrobial and psychoactive compounds, which are presumably biosynthesized as a chemical defense strategy against fungi, bacteria and herbivory (including Man). Our work has focused on the characterization of various chemotypes that are antibacterial (e.g. **1**)[1] and inhibitors of bacterial multidrug-resistance. Plants also produce phytochemicals with exquisite CNS activities from the tryptamine (*N,N*-dimethyl-tryptamine), tropane (cocaine) and indole (mitragynine) alkaloid, diterpene (salvinorin A), and amphetamine (cathinone, **2**) classes. The last example, cathinone (**2**) from Khat (*Catha edulis*), has recently served as a template for synthetic compounds known as 'Legal Highs' or Novel Psychoactive Substances (NPS), such as mephedrone (**3**) and methylone (**4**). These compounds can be bought without legal restriction and are often strikingly similar in structure to existing controlled and harmful drugs of abuse such as ecstasy (MDMA, **5**). This award lecture will broadly cover our work on plant antibacterials and NPS and discuss their potential.



References: 1. Shiu, W.K.P., *et al*., (2012). *Journal of Natural Products* **75:** 336 – 343. 2. Gibbons, S. and Zloh, M. (2010) *Bioorganic Medicinal Chemistry Letters* **20:** 4135 – 4139.

Poster

Topic A: Biodiversity

PA1 Biochemical contents of mulberry (*Morus spp.*) fruits

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Morus alba and *Morus nigra* are native to Far East and Eurasia, respectively. Fruits and leaves of both species are widely used in folk medicine. Both species are mostly used in Turkey to consume as fresh or dried fruits, juice and jam. Fruits of *M.alba* are white, purple or black in color whereas *M. nigra* has only black colored fruits. Study was conducted to compare fruits of *M.alba* and *M. nigra* fruits for natural biochemicals and antiradical activities. All fresh fruits were obtained from Southern Turkey and total phenols were analysed by Folin-Ciocalteu method. While spectrophotometric method were used for analysing of total flavonoids. Antiradical activity were measured by DPPH method. *M.nigra* has the highest phenolic content (368,2 mgGAE/100 g FW) when compared to fruits of *M.alba*. White colored fruits in *M. alba* has the lowest phenolic content (267,5 mgGAE/100gFW). Amount of total flavonoids (mg CTE/100 g FW) ranged from 214,5 in *M. alba*'black' to 170,0 in *M. Alba* 'white' but *M. nigra* (247,9) has more flavonoid than that of *M. alba* genotypes. Amount of total phenols and flavonoids increased with rising color intensity. *M. nigra* had superior values for both biochemicals tested when compared to *M. alba*. Antiradical acivity ($1/EC_{50}$) was higher in *M. nigra* (2.81) than all kind of fruits of *M. alba* ranging from 2.65 in black and 2.46 in white colored fruits. Natural antiradical activity of *M. nigra* has nearly equal to synthetic antioxidant known as BDH (2.91). *M. nigra* have superior numbers for all biochemicals tested when compared to *M. alba*.

PA2 Biodiversity of Tahitian vanilla highlighted by its sensory properties

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V.x tahitensis (Tahitian vanilla) exhibits a typical flavour compared to other vanillas and shows relative biodiversity regarding genetic, morphologic and chemical profiles. In this work, *V.x tahitensis* biodiversity was investigated through its sensory properties. Vanilla samples from French Polynesia and Papua New Guinea were evaluated using i) sensory evaluation by Quantitative Descriptive Analysis and ii) detection of odor-active compounds by GC-Olfactometry. Sensory profiles clearly differentiated the origin of *V.x tahitensis*, Papua New Guinea vanilla being more "spicy", "fruity", and "rum" compared to Polynesian varieties, which were characterized by stronger "caramel" and "anise" notes. Moreover, sensory variations were detected among Polynesian varieties. The sensory biodiversity of Tahitian vanilla is promising and could be used as a tool for i) discrimination, ii) valorisation and iii) preservation of vanilla origins and varieties.

PA3 Medicinal plant conservation in Thailand using reintroduction of micropropagules

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An attempt to conserve 10 Thai medicinal plants using micropropagation and reintroduction systems has been performed. The reintroduction of plants becomes an increasingly utilized strategy in medicinal plant conservation. These combined techniques can increase number of selected medicinal plants in their habitats. We established rapid micropropagation systems for 10 medicinal plants, i.e. *Stemona hutanguriana* W.Chuakul, *Grammatophyllum speciosum* Blume, *Rauvolfia serpentina* (L.) Benth. ex. Kurz, *Afgekia mahidoliae* Burtt et Chermsir, *Kaempferia roscoeana* Wall., *K. rotunda* L., *K. parviflora* Wall. ex Baker, *K. larsenii* Sirirugsa, *K. candida* Wall., *Zingiber gramineum* Noronha, to produce *in vitro* plantlets, developed methodology for transferring plants to *ex vitro* growth conditions, and then reintroduced to the forest. In August, 2010, *K. roscoeana* Wall. and *K. rotunda* L. regenerants were reintroduced to a forest in Kanchanaburi. *K. roscoeana* Wall. could survive up to 95.00%, whereas, the survival rate of *K. rotunda* L. was 10.98%, observed one year later (August, 2011). Our results can be developed to longer-term strategies for the reintroduction of micropropagated medicinal plants into natural habitats.

PA4 Chemical and biological evaluation of native bamboo species from atlantic rain forest

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Brazilian bamboo species should be considered a forest resource and its industrial application should be improved. The aim of this work was to study the chemical composition and evaluate the allelopathic, anticholinesterase (AChE) and free radical scavenging (DPPH) activities of two endemic bamboo species. The culm and leaf extracts of *Apoclada simplex* and *Merostachys magelanica* were prepared with H_2O (60°C) and ethanol (90%). The aqueous (A) and ethanolic (E) extracts from the leaves (LA) and culms (CE) were successively partitioned with H_2O/n-hexane (H), ethyl acetate (E) and n-buthanol (Bu). Allelopathic assay showed that the aqueous extract leaves of *M. magelanica* and *A. simplex* (AE) and the acetate fraction of *M. magellanica* (FC) were active inhibiting around 55% the germination of native tree species. TLC (F_{254}, Merck, CH_3OH:$CHCl_3$, 4:6) with *M. magellanica* inhibited the AChE at *Rf*= 0.6, 0.80, 0.32 and DPPH at 0.7, 0.46 and 0.48. Determination of the EC_{50} (microplate, 96 vials, DPPH, 412 nm and *Ginkgo biloba* 31.2 μg/mL) showed *A. simplex* at 73.3 ± 2.6% (LA) and 72.7 ± 0.8 (CA) and to *M. magelanica* at 68.93 ± 2.6% (LA) and 53 ± 1.8 (LE). Data from TLC, HPLC and GC/MS after derivatization (BSTF) of the extracts and fractions identified main phenolic acids (vanilic, cinamic and ferulic). The biological activities of native bamboo species and the presence of the phenolic compounds showed the economic potential of these species.

PA5 Clonality and protein diversity of *Bacillus pumilus* isolates from different sources and geographic regions

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The constant increase of multi-drug resistant pathogens stimulates research to identify and develop new antibacterial compounds. Nature and its huge biodiversity harbors an endless source of compounds containing unique chemical structures, being several medicines originated from various natural sources including terrestrial microorganisms. Recent ad-

vances in genome sequencing have highlighted *Bacillus* genus as an unexpected source of antibiotic-like compounds'. Nevertheless, descriptions of compounds with antibacterial activity from *Bacillus pumilus* are scarce and with chemical/biochemical/microbiological features insufficiently described. *B.pumilus* is associated with a wide range of biotechnological activities, however, its diversity is also scarce characterized and few studies compared the genomic content derived from PFGE (Pulsed Field Gel Electrophoresis) with proteomic analysis by SDS-PAGE (Sodium Dodecyl Sulfate Polycrylamide Gel Electrophoresis). In this study, using PFGE and SDS-PAGE, we assessed genomic and protein diversity of *B. pumilus* isolates (n = 24) from a variety of sources and geographic regions, which includes food medicines and cosmetics' contaminants; cucumber roots and gastropods' normal flora. Clonal relationship evaluated by *Apa*I-PFGE, defined 18 PFGE-types, being particular pulsotypes associated with isolates'origin. An ability to produce a large variety of proteins was also observed, suggesting its important role in the maintenance and adaptation of *B.pumilus* to different niches as well as that the toxigenic and biotechnological potential of this species is insufficiently explored. Moreover, clonal host specificity from gastropods isolates highlights the plasticity of *B.pumilus* and stresses the relevance of studying symbiotic associations, as a source of unrecognized strains able to produce new compounds of pharmaceutical and/or biotechnological interest.

PA6 Ex-situ conservation of medicinal plant genetic resources managed by national agrobiodiversity center of Korea

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In South Korea, systematic seed germplasm management has begun at the Rural Development Administration (RDA) in 1985. A total 173,217 accessions of plant seed germplasm has preserved at National Agrobiodiversity Center (NAC), RDA, in 2011. Among them, RDA genebank maintains the medicinal germplasm colletion of 5,905 accessions of 390 species, comprising 374 developed varieties (6.3%), 1,267 landraces (21.5%), 742 wild relatives (12.6%), and 3,532 others (59.8%). In aspect of PGR status, landraces are dominant part of conserved accession, followed by developed varieties, wild relatives, and cultivated materials. Beside this, the medicinal germplasm is preserved in the sub-genebanks located separately in 9 administrative provinces of South Korea. The conserved accessions are 7,110 belonged to 1,216 species. Recently, characterization and evaluation of medicinal gerplasm have been activated for 3 years in NAC, which are useful to breeders and users who are interested in their exploitations of functional components or the efficacy of the respective accessions. It will promote utilization of medicinal germplasm and development of medicinal crop breeding program for both human being and sustainable agriculture in the near future.

PA7 Development of a statistical tool to predict anticandidal activity of essential oils

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There is a general agreement that natural volatile compounds of essentials oils from plants provide a great potential for discovery of new antimicrobial drugs such as anticandidal ones. In this work, 60 commercial essential oils were analysed by GC/MS and their inhibitory activity against the growth of three *Candida* strains was evaluated. We then conducted a chemometric analysis and proposed a tool to predict their capacity to inhibit the *Candida* sp. developpment. Raw data were pretreated and normalised using MZmine2.2 software. It was possible to identify some of the natural compounds with anticandidal properties using two statistical tools in XLstat7.5: hierarchical clustering analysis (HAC) and principal component analysis (PCA). We highlighted that essential oils containing limonene, 1,8-cineole, linalool and linalyl acetate were mostly inactive, whereas eugenol, thymol, carvacrol, geraniol, geranial and neral as major components of the oils seems to be linked to an interesting anticandidal activity. Linear regressions showed a high correlation between the concentration of these molecules and their antifungal activity. Exceptions to these general tendencies could be characteristic of synergistic or antagonistic interactions.

PA8 Genetic diversity analysis of Siberian ginseng collection using SSR markers and their transferability to other species

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Acanthopanax senticosus, commonly called as "Siberian ginseng", is distributed in northeast Asia region including Korea, China and Japan. Diverse *A. senticosus* accessions were collected and preserved in ginseng & medicinal plants experiment station of Korea. In this study, we constructed simple sequence repeat (SSR)-enriched genomic DNA library of *A. senticosus*, and sequenced a total of 711 clones. Among the designed 190 primer pair flanking SSR motif, we finally acquired 21 polymorphic SSR markers and these were transferable other *Acanthopanax* species (*A. sessiliflorus* and *A. gracilistylus* var. *gracilistylus*). The allele number ranged from 2 to 16 with an average of 8.8 alleles, and average polymorphism information content (*PIC*) was 0.527 ranged from 0.200 to 0.840 in *Acanthopanax* accessions. Phylogenic tree based on SSR profiles revealed the divergent clade according to the species. The 21 polymorphic and transferable microsatellite markers will be useful for genetic evaluation and classification of *Acanthopanax* species.

PA9 Antimicrobial potential, antioxidant activity and total phenolic contents of EtOH extract of aerial part from Korean mint (*Agustache rugosa*) germplasm

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Korean Mint (*Agustache rugosa*), one of the minor crops grown in Korea belonging to the mint family (Labiatae) is a perennial herb widely distributed in East Asian countries. The antimicrobial potential, antioxidant activity and the total phenolic contents of ethanol extract of Korean Mint (*Agustache rugosa*) was investigated by electrochemical measurements. The purpose of this study is to analyse the functional activity and content of Korean Mint (*Agustache rugosa*) 53 accessions in the National Agrobiodiversity Center. The antimicrobial potential against *Bacillus subtilis* were assayed by a disc diffusion test, antioxidant activity was determined as DPPH radical scavenging activity (RSA) and total phenolic contents were evaluated using a spectrophotometric technique based on the Folin-Ciocalteau reagent according to the method of Spanos and Wrolstad and calculated as gallic acid equivalents GAE/g dw. Inhibition zone diameters of ethanol extract (5g/Ml), antioxidant activity of ethanol extract (100μg/Ml) and total phenolic content from Korean Mint (*Agustache rugosa*) 53 accessions ranged from 9.67 to 25.33, 17 to 66% and 2.31 to 9.61 mg gallic acid/g dw.

PA10 Labdanum from mediterranean *Cistus* species: GC-MS fingerprints and relative quantification of antispirochaetal manoyloxides

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The oleoresin labdanum from *Cistus creticus* was used in ancient Greece as incense, anti-infective, and wound treatment [1]. On Crete, the main production center since antiquity, it is brushed off the leaves with long textile strings. After the Ottoman conquest of Crete 1645, Western Europe imported Spanish labdanum prepared by hot water extraction of aerial parts of *Cistus ladanifer*. Shortly there- after, labdanum fell out of pharmaceutical use [2]. Presently, *C. creticus* leaf extracts from Turkey are applied by German self-help groups for borreliosis therapy [3]. Our results indicate that this anti- spirochaetal activity is mainly due to manoyloxides in the essential oil [3,4]. Here, 8 labdanum sam- ples were analyzed by GC-MS for these active constituents, revealing exceptionally high contents of 13-epi-manoyloxide, 2-keto-manoyloxide, ent-3β-hydroxy-13-epi-manoyloxide, manoyloxide, sclareol, and acetoxy-manoyl-

oxide in the Cretan ones. In other eastern Mediterranean samples, the concentration of these compounds was several orders of magnitude lower, whereas Spanish labda- num is dominated by simple alkanes with only trace amounts of manoyloxide and 13-epi-man- oyloxide. Thus, discontinuation of medicinal use of labdanum in Western Europe is understandable as "labdanum" from *C. ladanifer* is clearly not equivalent to the traditionally harvested *C. creticus* drug. Rumors that *C. creticus* contains psychotropic THC were refuted. **References:** 1. Aufmesser, M (2002) Dioscurides. Olms Verlag. Hildesheim. 2. Husemann, T (1889) Archiv der Pharmazie 227: 1075 – 1092 / 1105 – 1132. 3. Hutschenreuther, A. et al. (2010) Pharmazie 65: 290 – 295. 4. Grötzinger, K. et al. (2010) Planta Med 76: 245.

PA11 Evaluation of natural products as potential cosmetic agents with tyrosinase inhibition activity

Vontzalidou A[1], Chaita E[1,2], Aligiannis N[1], Makropoulou M[1], Kalpoutzakis E[1], Termentzi A[1], Guldbrandsen N[3], Hamburger M[3], Dumontet V[4], Pamlard O[4], Guéritte F[4], Skaltsounis AL[1]
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Skin whitening effects of a library of plant derived natural products were evaluated for their potential as new cosmetic agents. Specifically, 480 compounds with structurally diverse scaffolds and decoration patterns were screened for their anti-hyperpigmentation properties. The ability of the compounds to inhibit the oxidation of L-DOPA to dopaquinone and subsequently to dopachrome by the enzyme tyrosinase in a cell-free system was examined. The produced amount of dopachrome was measured at 475 nm, and kojic acid was used as positive control. Four compounds were found to be very active (IC_{50}< 50 μg/ml), 3 compounds showed a moderate activity (75 μg/ml>IC_{50}> 50 μg/ml), and 10 compounds were found to be weak inhibitors (150 μg/ml>IC_{50}> 75 μg/ml). Esculetin was the most potent inhibitor of tyrosinase. Its IC_{50} value was 3.7 μM which was lower than that of kojic acid (IC_{50}= 11.9 μM). On the basis of this screening an extract library derived from approx. 1800 plant species of the worldwide flora has been established.

PA12 Investigation of medicinal plants diversity of south slopes of touchal elevations

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This study was conducted in Touchal mountain region located in north of Tehran. The highest altitude is Touchal peak with 3960 meters above the sea level. The present study was carried out to introduce the medicinal plants, particularly anticancer ones with their life forms. Medicinal plants were collected during 2006 – 2009 frequently, then they were identified again exactly by local flora and other reliable resources. The gathered list of medicinal plants was adjusted with pharmacognosy handbooks and herbal medicines guides. Anticancer ones were derived and classified in the other list. 40 anticancer plants were identified among of 144 medicinal species from 132 genera belong to 53 families. The largest plant family was Lamiaceae with 28 species. The life forms spectrum classified according to the Raunkaier classes revealed that they include Phanerophytes 12%, Chamaephytes 20%, Hemicryptophytes 55%, Geophytes 2%, Helophytes 1% species and Therophytes 16%. High frequency of Hemicryptophytes in this region is a reason to existence an environmemt with cold and temperate climate, also absence or low frequency of geophytes indicated a deficit and shallow soil. Enough precipitations and existence of ever rivers and waterfalls lead to presence of high frequency of lamiaceae species and some of Fern species, particularly near the rivers and wet parts. Dracocephalum, Melissa, Thymus, and Ziziphora were the most important of medicinal species. 14 species were in threatened status; among of them, *Dracocephalum kotschyi* Boiss. Is an endemic and rare species, has endangered status that distribute on mountainous areas; rocky, stony slopes and calcareous rocks.

PA13 Cosmetic properties of plant derived natural products: antioxidant and UV-protection effects

Chaita E[1,2], Aligiannis N[1], Argyropoulou A[1], Boka BI[1], Kalpoutzakis E[1], Pratsinis H[3], Kletsas D[3], Guldbrandsen N[4], Hamburger M[4], Dumontet V[5], Pamlard O[5], Guéritte F[5], Skaltsounis AL[1]
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The present work aims to identify new promising plant sources which could be exploited for their cosmetic properties. 480 natural products were selected for screening against their antioxidant activity. DPPH assay revealed that 5 compounds were very active, namely esculetin (IC_{50}= 33.7 μM), ellagic acid (IC_{50}= 26.5 μM), nordihydroguaiaretic acid (IC_{50}= 33.1 μM), mearnsetin (IC_{50}= 45.1 μM) and rosmarinic acid (IC_{50}= 41.6 μM) and 11 compounds showed moderate activity. The 16 most promising compounds were tested for their capacity to reduce intracellular ROS and to protect skin fibroblasts from UV-induced damage. Twelve compounds with putative UV-protective capacity were identified. The concentration range in which the compounds exert cytotoxic effects was estimated with MTT-method. On the basis of these findings a collection of 450 plant species of the Greek flora was established, and the resulting extract library was tested in the assay panel.

PA14 Evaluation of natural products as potential angiogenesis modulators

Dimitrakoudi S[1], Angelis A[1], Aligiannis N[1], Karamitri A[2], Michailidou M[1], Loutrari E[1], Topouzis S[2], Papapetropoulos A[2], Skaltsounis AL[1]
[1]Department of Pharmacognosy & Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis Zografou, Athens, 15771, Greece; [2]Laboratory for Molecular Pharmacology, Department of Pharmacy, University of Patra, Rio/Patra, 26504, Greece

Small molecules derived from species of the Greek flora were evaluated as lead preclinical candidates. The aim was to identify potential innovative compounds and molecular targets in the rapidly growing therapeutic field of angiogenesis-related diseases (solid tumors and ischemic heart disease). Specifically, 170 compounds with structurally diverse scaffolds and decoration patterns were screened for their anti- or pro-angiogenetic properties by endothelial cell-based functional assays. The initial screening in an immortalized HUVEC (human umbilical vein endothelial cells) cell line (EA.hy926) at 10 μM and 100 μM, showed that flavonoids, alkaloids and phenolic compounds exhibited both pro- and anti-angiogenic activity, while terpenes, coumarins, xanthones and anthraquinones provoked inhibition of angiogenesis. The most active inducer was the alkaloid 4-{(E)-2-[4-(dimethylamino)phenyl]-1-ethenyl}-1-ethylpyridinium chloride, while the phenylpropanes, bisphenol and 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)propane, and the isoflavon 5,4'-dihydroxy-7-methoxyisoflavon showed strong inhibition. Based on these data, a collection of 200 plant extracts obtained from Greek flora was compiled and evaluated in order to discover new natural compounds as promising angiogenetic modulators.

Topic B: Biosynthesis

PB1 Initiation of callus culture of *Juniperus communis* L. Horstman

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Juniperus communis, belonging to the family of Cupressaceae, is an evergreen tree with needle -like leaves. Juniperus species are known to produce podophyllotoxin, a lignan, which is valuable precursor of anticancer and antiviral medicines (Canel et al. 2000, Eyeberg et al. 2006). Podophyllotoxin itself is too toxic for the therapeutic use but its semisynthetic derivatives are important antitumor medicines used in the treatment of various cancers. Since natural sources of podophyllotoxin become scarce and the demand continuously increases alternative ways to obtain podophyllotoxin are needed (Farkya at al. 2004). Lignans, produced by terrestrial plants, are biosynthetically derived from the phenylpropanoid pathway. However, the biosynthetic formation of podophyllotoxin is not fully known. In order to study biochemical pathways of podophyllotoxin formation we initiated a callus culture of twigs of *Juniperus communis* L. Horstmann growing in Rombergpark in Dortmund, Germany. To optimize the callus formation we cultivated the twigs on agar plates supplemented with various growth hormones and coal, various temperatures and light cycles. The results showed that callus was formed most effectively when the twigs were cultivated in the media supplemented with 2,4- dichlorophenoxyacetic acid at 25 °C in 6000 lux, with light cycle of 16 h light and 8 hours dark without activated charcoal. (Canel, C. et al. (2000). Phytochemistry 54, 115 – 120., Eyberger, A.L. et al. (2006). J. Nat. Prod. 69, 1121 – 1124., Farkya, S. et al. (2004). Appl. Microbiol. Biotechnol. 65, 504 – 519.)

PB2 Jasmonates-induced expression of farnesyl diphosphate synthase gene in aquilaria microcarpa

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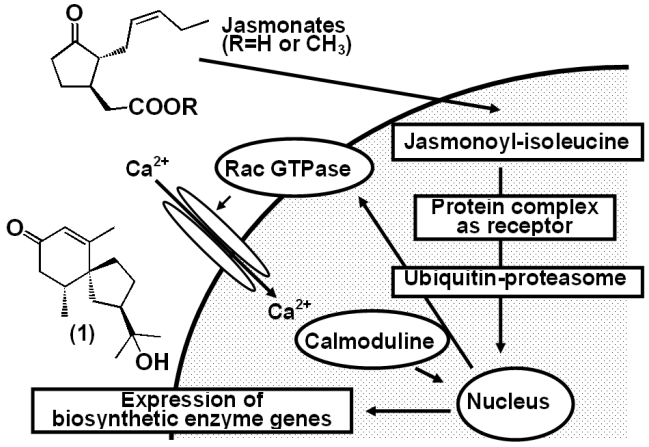
Lab of Medicinal Bioresources, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Sugitani, Toyama 930 – 0194, Japan

Biosynthesis of a unique spirovetivane sesquiterpene compound (1) in *Aquilaria microcarpa* is triggered by the treatment of the plant with jasmonic acid or methyl jasmonate (MJ). Expression of farnesyl diphosphate synthase gene of *A. microcarpa* (*FaPS1*) was markedly enhanced by the addition of MJ to the cell culture. Calmodulin (CAM) and Rac/Rop GTPase genes were also transcriptionally activated in MJ-treated cells. In addition, *FaPS1* expression in *A. microcarpa* transformed with the GTPase or CAM gene appreciably elevated even in the absence of MJ. These results suggest that the GTPase and CAM play important roles in MJ-induced sesquiterpene biosynthesis.



PB3 Protein and metabolic profiles of *Peperomia Obtusifolia* (Piperaceae)

Batista ANL[1], Batista Jr. JM[1], Zocolo GJ[1], Zanoni MVB[1], Kato MJ[2], López SN[3], Furlan M[1]
[1]Instituto de Química, Universidade Estadual Paulista – UNESP, Araraquara, SP 14800 – 900, Brazil; [2]Instituto de Química, Universidade de São Paulo – USP, São Paulo, SP 05508 – 900, Brazil; [3]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario S2002LRK, Argentina

Proteomics has been used for many applications in plant sciences, including the study of biosynthetic pathways leading to secondary metabolites. Phytochemical studies on *Peperomia obtusifolia* have led to the isolation of several meroterpenes, including prenylated chromanes with potent trypanocidal activity [1] as well as novel monoterpene chromane esters [2]. Such biosynthesis would require the involvement of PKS and prenyltransferases for the formation of aromatic (orsellinic acid derivatives) and the lipophylic moieties, respectively. Since these types of compounds are of limited occurrence in nature, *Peperomia* species represent an interesting model to investigate these keys biosynthetic steps. Herein we report comparative protein and metabolic profiles of roots, stems, and leaves from adult plants of *P. obtusifolia*. The protein profiles were obtained using SDS-PAGE and 2-DE while the metabolic ones were determined by HPLC-PAD-MS analyses. A possible correlation between the protein and metabolic profiles will be presented. [1] *Planta Med.* **2009**, *75*, 620; [2] *J. Org. Chem.* **2011**, *76*, 2603.

PB4 Synergistic reductive methyltransferase GILM and its crucial role in gilvocarcin biosynthetic pathway

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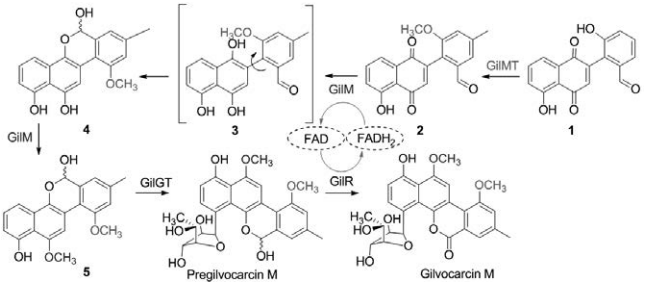
The missing links in the biosynthetic pathway of gilvocarcin have been identified. The intermediate (**1**) in the pathway was synthetized chemically and fed to the two most dubious enzymes GilM and GilMT. The chemo-enzymatic synthesis revealed that GilMT is an *S*-adenosylmethionine- dependent methyl transferase that catalyzes the first methylation after the GilOII mediated C-C bond cleavage providing substrate (**2**) for GilM. GilM then couples with GilR, an FAD-dependent oxidoreductase that finishes gilvocarcin biosynthesis by converting pregilvocarcin to gilvocarcin, and utilizes reduced flavin produced in the GilR reaction to catalyze the necessary reduction of the quinone (**2**) to hydroquinone (**3**) which is then followed by either GilM assisted or spontaneous ring-closing hemiacetal (**4**) formation. S-adenosylmethionine bound GilM then further catalyzes a second *O*-methylation to form defuco-pregilvocarcin (**5**).



PB5 Towards the sustainable *in-vitro* production of plant derived anticancer compounds

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One aspect which could explain the limited number of complex molecules entering clinical trials and reaching the patient is the restricted supply chain of the plant raw material, thus limiting the availability of Active Pharmaceutical Ingredients (API). Still today, most of the raw

materials needed for the extraction of the active ingredients are harvested from cultivated or wild plant populations, posing a threat to the bioavailability of certain medicinal plants and strong variability in the yield of API. We have developed a new *in-vitro* propagation method based on the use of temporary immersion bioreactors that allows for the rapid and abundant generation of a leafy-biomass from medicinal plant cell cultures. Examples with tobacco and *Hypericum perforatum* will be described. This technology provides a unique opportunity for the sustainable production of complex APIs which require plant cell differentiation.

PB6 Gloriosa superba-made anticancer molecules

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Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401, USA

In the United States, cancer is the second common cause of death, which cost several billion dollars each year. Colchicine analogs have potential anticancer properties with significant regulatory effects on the progress of the tumor cell cycle. Plant-made colchicine is one of the high commodity bioactive alkaloid molecules. *Gloriosa superba* biosynthesizes high levels of colchicine. New anticancer mechanisms of colchicine dimers have been introduced as potential anticancer drug candidates. We have established a *G. superba* bioproduction technology which allows for scale-up in pilot-scale bioreactor to produce colchicine and its analogs for anticancer molecules production. I will present the preliminary bioprocess engineering results to produce *G. superba*-made anticancer molecules.

PB7 Elucidating the biosynthetic pathway of the saccharomicins – New antibiotics from Saccharothrix Espanaensis

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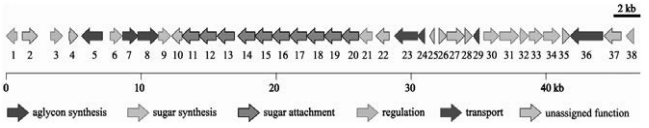
Institute of Pharmaceutical Science, Albert-Ludwigs University, Stefan-Meier Straße 19, 79104 Freiburg

The discovery of new antibiotics is an essential approach to effectively combat multidrug-resistant pathogens. The saccharomicins A and B, produced by the soil-dwelling bacterium *Saccharothrix espanaensis*, comprise a new class of heptadecaglycoside antibiotics with activity against MRSA and VRE. The complete genome sequence of *Saccharothrix espanaensis* revealed the entire gene cluster of the saccharomicins **1**. Annotation of the cluster led to a putative pathway for the synthesis of the exceptional aglycon N-(3,4-dihydroxycinnamoyl)-taurine. To verify this hypothesis, genes from the cluster were expressed in a heterologous host leading to the partial synthesis of the aglycon. Additionally, the genes responsible for the synthesis and attachment of the sugar moieties were heterologously expressed and checked for biotransformation of the fed caffeic acid, a component of the aglycon. **1**



PB8 Heterologous expression and elucidation of the biosynthesis of Rishirilide A and B

Wunsch-Palasis J, Yan X, Welle E, Bechthold A
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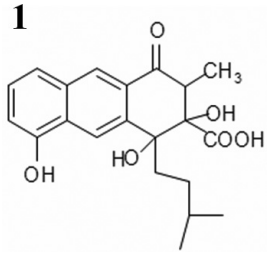
Streptomyces bottropensis was found to produce the potent α_2-macroglobulin inhibitors rishirilide A and B **1** by screening the cosmid library for type II PKS genes. After heterologous expression of the *rsl*-biosynthetic gene cluster **2** in *Streptomyces albus* rishirilide B could be detected. Inactivation experiments revealed RslO1 catalyzing a Favorskii-like rearrangement during the biosynthesis. Currently feeding experiments with ^{13}C-labeled acetates are carried out to support this finding. Furthermore, the results of inactivation experiments of different tailoring oxygenases and the starter unit selection gene *rslK4* will be presented.

1



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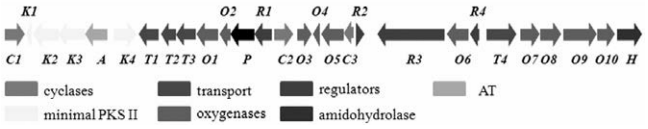

PB9 The biosynthesis of mensacarcin and the importance of MSNO8

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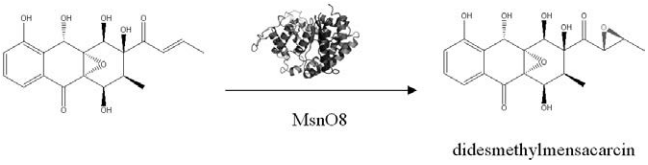
Mensacarcin, a hexahydroanthracene, is produced by *Streptomyces bottropensis* and shows cytotoxic activity. Subsequent knockouts of different genes in the PKSII biosynthetic gene cluster gave more insights into the biosynthesis of mensacarcin. Recently we could show, that MsnO8 catalyzes the epoxide formation in the side chain of mensacarcin [1]. The structure of this protein was solved by X-ray crystallography. To complete the elucidation of the mensacarcin biosynthesis we are currently inactivating further genes. Eventually, we will be able to use this knowledge for combinatorial biosynthesis to produce new anticancer agents.



1. Probst, K., *PhD thesis.* **2011**, *University of Freiburg.*

PB10 Improvement of tropane alkaloids production in transgenic *Anisodus Acutangulus* by genetic engineering

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Tropane alkaloids (TA) including hyoscyamine, anisodamine, scopolamine and anisodine, are used medicinally as anticholinergic agents with increasing market demand, so it is very important to improve TA production by metabolic engineering strategy. Here, we report the simultaneous introduction of genes encoding the branch-controlling enzyme tropinone reductase I (TRI, EU424321) and the downstream rate-limiting enzyme hyoscyamine-6β-ydroxylase (H6 H, EF187826) involved in TA biosynthesis into Anisodus acutangulus hairy roots by *Agrobacterium-mediated* gene transfer technology. Transgenic hairy root lines expressing both TRI and H6 H (TH lines) produced significantly higher ($P < 0.05$) levels of TA compared with the control and single gene transformed lines (T or H lines). The best double gene transformed line (TH53) produced 4.293 mg/g TA, which was about 4.49-fold higher than that of the control lines (0.96 mg/g). As far as it is known, this is the first report on simultaneous introduction of TRI and H6 H genes into TA-producing plant by biotechnological approaches.

PB11 Induction of specialized metabolites in hairy rootS of *Scutellaria lateriflora* treated with cyclodextrin and methyl jasmonate

Marsh Z[1,2], Nopo-Olazabal L[1,2], Yang T[1,2], Joshee N[3], Medina-Bolivar F[1,2]
[1]Arkansas Biosciences Institute; [2]Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401, USA; [3]Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030, USA

Scutellaria lateriflora (American skullcap) is a plant in the mint family (Lamiaceae) which produces biologically active flavonoids exhibiting antioxidant and anticancer properties. In order to develop a bioproduction system to study the biosynthesis of these compounds, hairy root cultures were developed using *Agrobacterium rhizogenes* and line SL-4 was selected for further studies because of its growth performance in liquid medium. In the present work, we studied the effect of methyl jasmonate and cyclodextrin on production of specialized metabolites in these hairy root cultures. Thirty-day-old hairy root cultures were treated with 0.75, 7.5 or 15 mM of cyclodextrin alone or combined with 100 μM of methyl jasmonate (MeJA). As controls ethanol (solvent of MeJA), cyclodextrin and MeJA alone were used. After 24 hours of treatment, the roots and culture medium were collected and the metabolites were extracted with ethyl acetate. The extracts were further analyzed by HPTLC and HPLC. The HPTLC system was developed in order to rapidly detect the presence of known and unknown compounds. The levels of the known *Scutellaria* flavonoids baicalin, baicalein and wogonin did not vary significantly in the roots or medium upon the above treatments, however at least 10 novel compounds were induced upon treatment with MeJA and cyclodextrin. Our results suggest that this strategy could be used to induce and identify novel bioactive compounds in this medicinal plant.

PB12 Bioproduction and purification of prenylated resveratrol analogs from hairy root cultures of peanut

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[1]Arkansas Biosciences Institute; [2]Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72467, USA

The natural prenylated resveratrol analogs arachidin-1 and arachidin-3 exhibit anticancer activity and affinity to cannabinoid receptors. In order to study the biosynthesis of these important bioactive compounds, we established hairy root cultures of peanut as a sustainable and inducible bioproduction system for these compounds. We showed that the stressors methyl jasmonate and cyclodextrin were highly effective to induce the biosynthesis these polyphenols in 1 liter hairy root cultures. High Performance Counter Current Chromatography (HPCCC) was used to purify arachidin-1 and arachidin-3 from the culture medium. Scalable injection and decreased fraction volumes in the HPCCC system were shown to improve purification efficiency as demonstrated by HPLC and MS analysis of the purified fractions. Our results highlight the benefits of this scalable bioproduction and purification system for producing these natural products and discovering bioactive compounds with potential uses in human health.

PB13 Establishing a new methodology for genome mining and biosynthesis of natural products through fungal molecular genomics

Noguchi H
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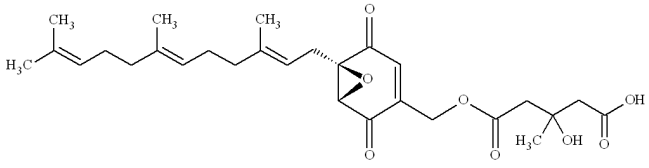
Polyketides (PKs) and nonribosomal peptides (NRPs) have been isolated from *Streptomyces* and many other source organisms. In recent years, gene clusters encoding PK synthases (PKSs) and NRP synthetases (NRPSs) have been discovered through fungal genome sequencing. While on average 50 gene clusters are identified in a single fungal genome, fewer fungal PK and NRP products can be isolated from a fungal culture grown under a typical growth condition. Thus, simple artificial reactivation of the cryptic gene cluster may be insufficient for an efficient natural product biosynthesis. To circumvent these obstacles, we examined upregulation of 60 gene clusters encoded in chromosomal DNA of four fungal species, *Aspergillus fumigatus, A. flavus*, *A. oryzae* and *Chaetomium globosum,* using the aforementioned fungal molecular genetics. Thus far, we have isolated seven new PK and NRP compounds successfully. Subsequently, we used our recombination cloning-based yeast expression system to reconstitute these biosynthetic gene clusters quickly and efficiently. Our preliminary results clearly demonstrate successful expression of seven *C. globosum* PKS gene clusters in *Saccharomyces cerevisiae*, three of which led to the production of new natural products whose identities have been characterized spectroscopically. Our methodology will facilitate the efforts in isolating novel natural products and rationally engineering in the biosynthetic pathways for production of analogs possessing comparable if not more potent bioactivity.

PB14 Elucidation of the biosynthesis of meroterpenoid yanuthone D in *Aspergillus Niger*

Holm DK, Petersen LM, Klitgaard A, Jarczynska Z, Larsen TO, Mortensen UH
Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Soltofts Plads – Building 223, DK-2800 Kgs. Lyngby

We have elucidated the mode of biosynthesis of the meroterpenoid compound Yanuthone D in *Aspergillus niger*. We have successfully deleted all cluster genes, and identified a number of intermediates. Structures of the intermediates were solved using a combined approach comprising classical 1D- and 2D-NMR and tandem mass spectrometry (MS/MS). In this study we have confirmed that Yanuthone D is of meroterpenoid origin, and we have identified an unexpected precursor, which has not before been reported for *Aspergillus niger*.



Yanuthone D

PB15 Activation of oxidative burst induces antioxidant response and indole alkaloid production in *Uncaria Tomentosa* root cultures

Huerta-Heredia AA[1], Vera-Reyes I[1], Ponce-Noyola T[1], Cerda-García-Rojas CM[2], Trejo-Tapia G[3], Ramos-Valdivia AC[1]
[1]Departamento de Biotecnología y Bioingeniería; [2]Departamento de Química. Cinvestav-IPN. Mexico City 07360, Mexico; [3]Departamento de Biotecnología, CeProBi, Instituto Politécnico Nacional, Yautepec, Morelos, Mexico

Uncaria tomentosa (cat's claw), an indigenous plant from the Amazon rainforest, is the source of monoterpenoid oxindole alkaloids (MOA) with immunomodulatory and antitumor activities. In cell cultures, production of these alkaloids has been stimulated by hydrodynamic stress via oxidative burst, while in root cultures, the role of reactive oxygen species in alkaloid production has not been fully examined. Roots were grown in 200 mL airlift bioreactors using a 4% (w/v) inoculum, reaching a total biomass of 21.1 ± 0.8 g dry wt L^{-1} after 65 days. Within the first hour of culture, a H_2O_2 peak (0.34 μmol g^{-1} dry wt) was observed, indicating that oxidative burst occurred. In order to register the root redox state, activity of antioxidant enzymes was monitored. SOD and POD showed activity peaks of 340 ± 5 U mg $prot^{-1}$ and 546 ± 8 μM min^{-1} mg $prot^{-1}$ 5 days after inoculation, while GR and GPx activities remain constant. The oxidative burst and enzyme response were associated with 14 and 6-fold increase of MOA and 3α-dihydrocadambine (DHC) at day five (1.4 ± 0.1 and 6.2 ± 0.7 mg L^{-1}), also preceded by a two-fold increase of strictosidine synthase and strictosidine glucosidase activities. Enhancement of both activities showed correlation with mRNA transcript level. MOA and DHC production was 12 and 14-fold higher than those found in roots grown in Erlenmeyer flasks.

PB16 Genomics-guided new insights on natural product biosynthesis in filamentous cyanobacteria

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Filamentous fresh water cyanobacteria are prolific producers of bioactive natural products, including antimicrobial, antitumor indole terpenoids and allelochemical tetramic acids. However, the molecular, genetic and biochemical basis for the biosynthesis of these molecules remains unknown. In this presentation, we detail a genomics-guided approach to understand natural product biosynthesis in several species of filamentous cyanobacteria.

PB17 Does nitrarine form spontaneously in non-enzymic conditions?

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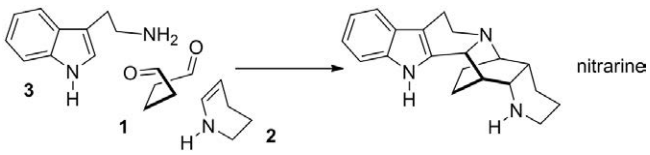
Nitrarine, a polycyclic indolic alkaloid isolated from several species of *Nitraria* (Nitrariaceae), may biosynthetically arise from an usual L-lysine metabolism implying reactive C_5 units such as glutaraldehyde (**1**) and tetrahydropyridine (**2**). We have investigated the self-condensation of these units with tryptamine (**3**) in biomimetic non-enzymic conditions. The resulting reaction medium is complex and contains numerous closely related compounds. Isolation of target compounds from the prepared mixture has been performed using multi-dimensional chromatography including pH-Zone refining centrifugal partition chromatography.



PB18 Comparison of fungal diterpene synthases – subtle differences lead to substantially different ring systems

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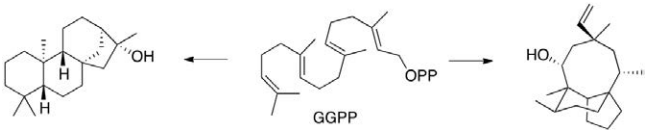
We have been studying the biosynthesis of the diterpene antibiotic pleuromutilin, which is produced by the fungus *Clitopilus passeckerianus*. The diterpene synthase (DS) that produces the alcohol core, pleuromutol, has been cloned and characterized. The DS catalyzes a two-step rearrangement of the linear precursor GGPP, with the first step being a protonation-initiated cyclization and the second step being promoted by ionization of the allylic diphosphate. Although the sequence of the pleuromutol DS is related to sequences of other bifunctional fungal DS enzymes, the tricyclic core that is formed is unique. A BLAST search with the pleuromutol DS revealed an uncharacterized protein from the dry-rot fungus *Serpula lacrymans* as having the highest identity (39%; 59% similarity). The *S. lacrymans* DS gene has been cloned and the product of the cyclase has been identified as *ent*-kauranol. While *ent*-kauranol is also a tricyclic diterpene, the overall structure is quite distinct from the pleuromutol skeleton. Attempts are being made to discern the sequence differences that lead to these divergent structures.



PB19 Biosynthesis of bioactive piperamides in *Piper Tuberculatum* (Piperaceae)

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Piper tuberculatum species belongs to the Piperaceae family and is an herb that typically grows in tropical areas of Brazil[1]. Previous chemical studies of the leaves and seeds from *P. tuberculatum* described the occurrence of isobutyl, piperidine and piperidone amides, which showed a potent trypanocidal, antifungal and insecticide activity. In general plant alkaloids comprise an important group of secondary metabolites because of their potent biological activity. Nevertheless, the biosynthesis of amides at the enzyme and gene levels is virtually unknown. In order to unravel this biosynthetic pathway, we have investigated the participation of malonic acid and *L*-valine and *L*-lysine amino acids as precursors. Additionally, the leaves and seeds protein profile of *P. tuberculatum* were investigated aiming to understand the amides formation as well as the regulatory process at the enzymatic level. The proteomic analysis indicated the presence of enzymes involved in primary and secondary metabolism, including a variety of defense proteins. All of these information will contribute significantly for further biosynthetic studies at gene levels.

PB20 First steps on MALDI-IMS images generation of protein maps on Arnica-da-Serra leaf cuts

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Lychnophora ericoides (Vernonieae: Asteraceae), known as "arnica-da-serra", is used as an anti-inflammatory and wound healing remedy. One of our latest works determined the "wild-type" chemical fingerprint of *L. ericoides* leaves volatile fraction, as made of bisabolane-type sesquiterpenes. Our current focus is to reach protein maps through the performance of proteic maps by using proteomics tools like. Protein maps are main players of our searching for the enzymes involved with the volatile sesquiterpenes biosynthesis. In parallel of this classic approach, we are showing here the first results of parameters stipulation on MALDI Imaging of leaf cuts that aims to reach terpene synthase identification, which are hypothetically responsible for the richness of bisabolane-type molecules. By using microtome to reach 50 μm thin transversal sections of leafs, DHB as a matrix, Laser beam operation at medium frequency range and software post-production manipulation of images, our first steps on MALDI-IMS of plant proteins have shown to be a good way to reach protein maps of *L. ericoides* since the spectra acquired ≈ 94KDa peaks. Further steps comprise TOF/TOF analysis of "LIFTed" ions to confirm, through MASCOT database usage, similarity with reported sesquiterpene synthase.

Topic C: Chemical Ecology/Symbiosis

PC1 Effects of fungicides on galanthamine and metabolite profiles in *Narcissus* bulbs

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Large-scale plant cultivation usually involves the use of a wide range of pesticides. Apart from eliminating the target organism, those external chemicals may affect the metabolism of the crop plant. This may have implications for plants cultivated for specific medicinal compounds. In this study the effects of diverse fungicides on the metabolism of *Narcissus pseudonarcissus* bulbs were investigated. *Narcissus pseudonarcissus* is being cultivated for the extraction of the alkaloid galanthamine. Fungicides typically used in *Narcissus* cultivation were applied in a field experiment. The aim was to determine whether fungicide applications

changed the concentration of galanthamine in the bulbs. ^{1}H NMR spectroscopy allowed quantitative analysis of galanthamine and other metabolites in bulb extracts. Multivariate data analysis revealed changes in bulb metabolite patterns caused by fungicides. Bulbs treated before planting generally had higher levels of alkaloids, while foliar field applications caused lower alkaloid levels but altered carbohydrate metabolism. Within these groups, certain fungicide treatments caused changes in specific metabolites. This study shows that the fungicides used in *Narcissus* cultivation can cause a change in the metabolome still detectable in the bulbs after harvest. The standard cultivation practices in terms of fungicide treatment were found suitable for the production of *Narcissus* as raw material for galanthamine extraction. In the cultivation of medicinal plants for secondary metabolites the potential effect of pesticides and other agrochemicals should be taken into account.

PC2 Isolation of malassezia metabolites with powerful AHR activity from human skin. is there implication in skin cancer development?

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Malassezia is a genus of human symbiotic yeasts that can become pathogenic under insufficiently understood conditions and have been correlated with skin diseases like seborrheic dermatitis, pityriasis versicolor, dandruff etc affecting a major part of the global population. We have recently proposed that Malassezia could be a factor that promotes basal cell cancer due to the production of Aryl hydrocarbon Receptor (AhR) inducers that can modify the immune system response and hyperactivate the CYP enzymes. When we investigated skin extracts from patients they showed 100 – 1000 times stronger AhR inducing activity than the skin extracts of healthy volunteers. Chemical analysis of the patients' extracts by LC/MS/MS revealed for the first time significant amounts of compounds like 6-formylindolo[3,2-b]carbazole (FICZ), indolo[3,2-b]carbazole (ICZ), malassezin, indirubin and pityriacitrin in human skin. The same compounds were also identified and isolated from *Malassezia furfur* extracts revealing their unequivocal origin. Indirubin and FICZ were found to be the two most active known AhR ligands even stronger than dioxin. Evaluation of their AhR inducing activity in human HepG2 cells transfected with a luciferase reporter gene at 6 h showed EC50 s 3.8×10^{-11} and 9.9×10^{-11} M respectively, in comparison with 5.2×10^{-10} M for dioxin.

PC3 Study of metabolites from lichen-associated bacterial communities

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Lichens are complex organisms resulting from the symbiosis between fungus, microalga and/or cyanobacteria and are source of metabolites of interest. As other living organisms harboring bacterial communities they could be considered as a mini-ecosystem. These bacterial communities most often belong to different phyla: *Actinobacteria*, *Proteobacteria*, with a dominance of *Alphaproteobacteria* and *Firmicutes*. In this study, we focused on the bacterial communities present on six lichens from Brittany coast (France) (*Roccella fuciformis, R. phycopsis, Lichina confinis, L. pygmaea, Xanthoria aureola and X. calcicola*). Abundance and diversity of these communities are dependent on several extrinsic factors (environmental) and/or intrinsic parameters including the chemical composition of their substrates (lichens). So, our aims are to elucidate the chemical composition of the studied lichens (extraction, isolation and structural identification) as well as those of associated bacterial communities. Some bacterial species were isolated from these lichens, identified by molecular fingerprints and their culture were optimized (media composition, pH and temperature). Due to the existence of chemical interactions between symbionts we target molecules with antibiotic properties.

PC4 Ilimaquinone and minor metabolites from Polynesian marine sponges: Isolation and ecology

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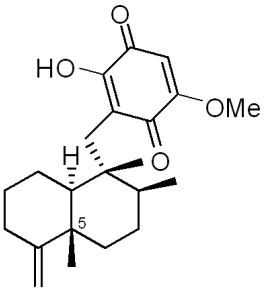
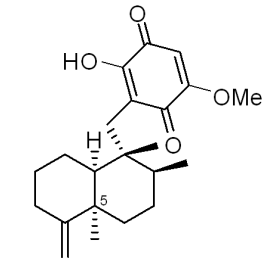
Sesquiterpene quinones are the most common class of metabolites isolated from *Dactylospongia* sponges. Samples from several biotopes from French Polynesia have been collected. Intriguing data have been brought together concerning the ratio between ilimaquinone and 5-*epi*-ilimaquinone. A metabolomic study of the minor secondary metabolites is also being conducted and will also be presented.



ilimaquinone (1)



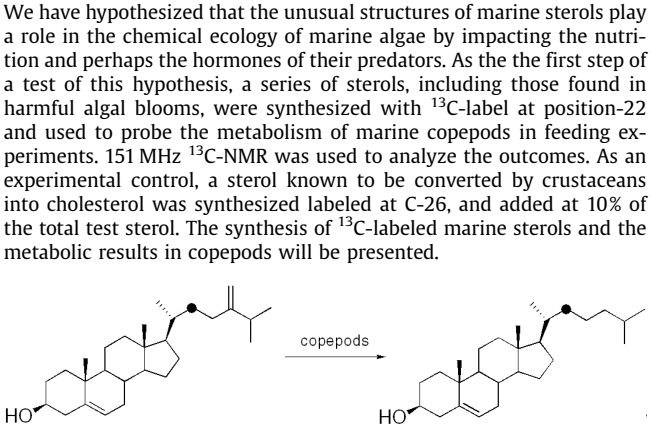
5-*epi*-ilimaquinone (2)

Marina Gordaliza, *Mar. Drugs*, 2010, 8, 2894 – 2890

PC5 Metabolic studies of marine sterols by Copepods

Giner JL[1], Wikfors GH[2], Hassett RP[3]
[1]Department of Chemistry, SUNY-ESF, Syracuse, NY 13210; [2]NOAA-NMFS, Milford, CT 06460; [3]Department of Biology, Ohio University, Athens, OH 45701

We have hypothesized that the unusual structures of marine sterols play a role in the chemical ecology of marine algae by impacting the nutrition and perhaps the hormones of their predators. As the the first step of a test of this hypothesis, a series of sterols, including those found in harmful algal blooms, were synthesized with ^{13}C-label at position-22 and used to probe the metabolism of marine copepods in feeding experiments. 151 MHz ^{13}C-NMR was used to analyze the outcomes. As an experimental control, a sterol known to be converted by crustaceans into cholesterol was synthesized labeled at C-26, and added at 10% of the total test sterol. The synthesis of ^{13}C-labeled marine sterols and the metabolic results in copepods will be presented.



PC6 Effect of light intensity on chemical composition and essential oil content in *Piper aduncum* L. leaves and roots

Ferreira MI[1], Gonçalves GG[2], Ferreira AB[2], Haber L[3], Marques MOM[3], Chau Ming L[2], Lima GPP[1]
[1]Department of Chemistry and Biochemystry, Biosciences Institute, São Paulo State University, Botucatu, SP, Brazil; [2]Horticulture Sector, Department of Plant Production, Agronomical Sciences College, São Paulo State University, Botucatu, SP, Brazil; [3]Center for Research and Development of Plant Genetic Resources, Natural Products Laboratory, Agronomical Institute of Campinas, Campinas, SP, Brazil

This study aims to evaluate the content, yield and quality of *Piper aduncum* leaves and roots essential oil (EO) under different conditions of Photosynthetically Active Radiation (PAR). The experimental design was completely randomized with three treatments and seven replicates of five plants each. The treatments were three levels of shading: 0% (1661 ± 180), 50% (1089 ± 118) and 70% (598 ± 52 μ mol m^{-2} s^{-1} PAR). The content and yield of EO leaves did not differ by Tukey test at 5% of significance level, but the Principal Components Analysis (PCA) showed different ratios of the constituents among treatments. The compounds of leaves EO with higher relative percentage (RP), asaricin and safrole, showed a strong relation with 70 and 50% of shading, respectively, while α-humulene was related to the light and spathulenol had no effect on the light conditions. In the roots asaricin and safrole had a higher RP than in leaves and a strong correlation to 50% of shading and full sunlight, respectively, whereas guaiol showed a strong relation to 70% of shading. The EO content of the roots did not differ, however, higher EO yield was observed in full sunlight plants, which also had greater root biomass.

PC7 Black fly attractants and the control of Onchocerciasis

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[1]Department of Chemistry and Center for Drug Discovery and Innovation, University of South Florida; [2]Department of Entomology, University of Georgia; [3]Department of Global Health, University of South Florida

Onchocerciasis or river blindness disease is a parasitic disease caused by infection from the nematode *Onchocerca volvulus*. The parasite is transmitted to humans by black fly vectors of the genus *Simulium*. Most of the infections occur in central Africa, with significant incidence also in Central and South America. The World Health Organization (WHO) estimates 18 million people suffer from onchocerciasis. However, due to a prolonged period from infection to hosts becoming symptomatic it is difficult to accurately access infection. The current method for monitoring the spread employs human bait, which is neither optimal nor ethically sound. The need for a new monitoring method is very important. It was noticed that anthropophilic gravid flies are attracted to both egg masses recently deposited by other flies of the same species as well as human scent. This paper will describe our efforts to isolate and identify the compounds responsible for this attraction using a variety of analytic methods such as LC-QToF, GC-MS and EAG. The identified compounds will be developed as bait for a field trap for monitoring vector pressure in both Latin America and Africa. In the long term, field traps may be useful in eradication of the disease.

PC8 Shade levels on yield and chemical composition of the leaf essential oil of *Pothomorphe umbellata* (L.) Miquel

Chau Ming L[1], Souza Mattana R[1], Aparecida Ribeiro Vieira M[1], Balbino Ferreira A[1], Ortiz Mayo Marques M[1,2], Abramo Marchese J[3]
[1]UNESP – Agronomical Sciences College – Department of Plant Production, Horticulture Sector, Botucatu, SP, Zip Code 18.603.970 [2]IAC -Center of Research and Development of Genetic Resources, Campinas,; [3]UTFPR – Biochemistry and Plant Physiology Laboratory – Pato Branco, PR – Brasil

Pothomorphe umbellata (L.) Miquel, is a Brazilian medicinal species that belongs to the Piperaceae family and popularly called as "pariparoba" and "caapeba". It grows in regions rich in humus, humidity and under the shade of trees, wich contributes for its best growth as it is considered as a sciophyte species. The present work aims to study the effect of shade on the yield and chemical composition of essential oil of *P. umbellata* leaves. Nine-month-old seedlings were planted and subjected to three shade levels (30%, 50%, 70%) and full sun; the experimental design was in randomized blocks, with 4 treatments and 6 replications, in split-spot in time scheme. Two following harvests of aerial part were taken (seven and nineteen months after planting). Essential oil was extracted by hydrodistillation and chemically analyzed by gas chromatography-mass spectrometry (GC-MS). The highest essential oil yield was observed in second harvest and plants under 30% shade. Twenty-six chemical substances were identified, of which trans-nerolidol was predominant.

PC9 T-RFLP profiling of the microbial communities associated with the marine Tunicate Mogula Manhattanensis

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Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut 06269, USA

Tunicates are generally sessile, filter feeding marine members of the Chordata phylum. Recently, interest in these animals has increased as they have been shown to be a rich source of natural products. In addition, tunicates have also been shown to be host to a large numbers of associated microbes, although these host-associated microbes have not been extensively studied. Investigating tunicate-associated microbial communities can help answer many questions about their relationship with their host: when did the tunicate acquire these bacteria and how are the microbial communities transmitted to offspring, what types of microbes are sequestered and where are they localized, and what function do they serve for the host and/or for the microbes? In this study, we consider a tunicate species native to the Long Island Sound, *Mogula manhattanensis*, and attempt to answer questions related to locations of microbial sequestration via genomic studies, in particular, evaluation of community 16S rRNA using terminal restriction fragment length polymorphism (T-RFLP) to characterize the associated microbial communities.

Topic D: Drug Discovery: Medicinal Chemistry, Pharmacology, Screening and other

PD1 Leucettines, a family of pharmacological inhibitors of DYRKs & CLKs kinases derived from the marine sponge Leucettamine B

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[1]C.N.R.S., Station Biologique, Roscoff, France; [2]Structural Genomics Consortium, Oxford, U.K; [3]Université de Rennes, France; [4]Université de Montpellier, France; [5]ManRos Therapeutics, Roscoff, France

We here report on leucettines, a family of kinase inhibitors derived from the marine sponge leucettamine B. Stepwise synthesis of analogues, followed by activity testing on 8 purified kinases led to highly potent inhibitors of CLKs & DYRKs, two families of kinases involved in pre-mRNA splicing and Alzheimer's disease. Leucettine L41 was co-crystallized with DYRK1A, -2, CLK3 and PIM1. Leucettine L41 inhibits phosphorylation of pre-RNA splicing regulating Ser/Arg-rich proteins. Leucettine L41 modulates alternative pre-mRNA splicing in a cellular systems. The selectivity of Leucettine L41 was extensively characterized. Leucettine L41 provides protection against glutamate-induced cell death in cultured HT22 hippocampal cells. It also provides neuroprotection against APP-induced cell death in mouse brain slices. Finally it prevents in vivo cognitive impairments due to icv injection of amyloid-β 25 – 35. Leucettines should be further explored as pharmacological tools to study and modulate pre-RNA splicing. Leucettines should also be investigated as potential therapeutic drugs in Alzheimer's disease and in diseases involving abnormal pre-mRNA splicing.

PD2 Screening for naturally occurring oxyprenylated secondary metabolites as cancer cell growth inhibitory agents

Epifano F[1], Genovese S[1], Lullo P[1], Fiorito S[1], Trivisonno G[1], Bruyere C[2], Kiss R[2]
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Oxyprenylated plant products are emerging as a novel class of naturally occurring biologically active secondary metabolites. These compounds are typically extracted from plants belonging to the Rutaceae, Apiaceae, Compositae, and Leguminosae families. As a continuation of our ongoing studies aimed to investigate the anti-cancer properties of such compounds, a series of oxyprenylated natural phenylpropanoids and polyketides were synthesized, and their growth inhibitory activities were evaluated in vitro. The compounds were tested on six human cancer cell lines (U373, A549, Hs683, SKMEL-28, PC3, and LoVo) using MTT colorimetric assays. The data reveal that of the chemical groups studied, *O*-prenylanthraquinones were the most effective agents. Compounds belonging to other classes, like the oxyprenylated chalcones exhibited only a moderate activity.

PD3 Two novel inclusion complexes of colon cancer chemopreventers, Auraptene and 4'-Geranyloxyferulic acid

Genovese S[1], Epifano F[1], Colamarino M[1], Curini M[2], Soares de Melo VH[3], Tasic L[3], Burgos M, de Azevedo M[3]
[1]Dipartimento di Scienze del Farmaco, Università "G. D'Annunzio" Chieti-Pescara, Chieti, Italy; [2]Dipartimento di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, Perugia, Italy; [3]Biopharmaceuticals and Hormones, Center of Biotechnology, Instituto de Pesquisas Energéticas e Nucleares (IPEN), Sao Paulo, Brazil

In recent years we have demonstrated that two prenyloxyphenylpropanoids, namely the geranyloxycoumarin auraptene, widespread in edible fruits of plants belonging to the genus *Citrus*, and 4'-geranyloxyferulic acid, extracted from the Australian shrub *Acronychia baueri* Schott (Rutaceae), exert valuable effects as dietary feeding colon cancer chemopreventive agents.[1] The same was seen for the well known *i*NOS inhibitor *N*-nitro-L-arginine methyl ester (L-NAME).[2] As a continuation of our studies about physico-chemical and pharmacological properties of cyclodextrin-inclusion compounds of prenyloxyphenylpropanoids, we describe herein the synthesis and structural characterization of complexes between auraptene, 4'-geranyloxyferulic acid, each coupled to L-NAME, and β-cyclodextrin to be used as novel prodrugs of the title cancer chemopreventers. 1. Tanaka, T.; de Azevedo M.B.; Durán, N.; Alderete, J.B.; Epifano, F.; Genovese, S.; Tanaka, M.; Tanaka, T.; Curini, M. *Int. J. Cancer*, **2010**, *126*, 830, 2. Yu, L.B.; Dong, X.S.; Sun, W.Z.; Zhao, D.L.; Yang, Y. *World J. Gastroenterol.* **2005**, *11*, 6385.

PD4 Multifunctional small molecular weight compounds from Peltiphyllum Peltatum: From antioxicant to neuroprotection and antidiabetic effects

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Pharmacognosy Research Laboratories, Medway School of Science, University of Greenwich, Central Avenue, Chatham-Maritime, Kent ME4 4TB, UK

An imbalance between the production of reactive oxygen species (ROS) and antioxidant defences is considered to be an important pathogenic factor for the development of a variety of disease conditions including cancer, neurodegenerative diseases, aging, diabetes and inflammation [1]. While antioxidant compounds offer some benefit in these disease conditions, multifunctional compounds acting at multiple targets of these diseases have tremendous therapeutic potential. In this communication, a case study on one ornamental and edible plant native the USA, *Peltiphyllum peltatum*, is presented. The leaves [2,3] and rhizomes of this plant are source of various antioxidant flavonoids (aglycones and glycosides) and gallic acid derivatives. The differenzial activity of these isolated compounds in cellular and cell free antioxidant assays together with potential neuroprotection and antidiabetic effects are discussed.

PD5 Evaluation of the cytotoxic effects from carvacrol and two new analogues

Sardella TB[1], Silva VB[2], Cavalcanti SCH[2], Fernandes PD[1]
[1]Federal University of Rio de Janeiro, Institute of Biomedical Science; [2]Federal University of Sergipe, Pharmacy Department, Medicinal Chemistry Laboratory. Brazil

The *Origanum vulgare* is popularly known as "oregano" with several activities described. From the essential oil can be obtained the carvacrol (5-isopropyl-2-methylphenol), a phenolic monoterpene. Studies have shown various effects of carvacrol (i.e., antibacterial and antifungal). The aim of this work was to evaluate the cytotoxic effects of carvacrol and two new analogues (carvacryl acetate [CA] and carvacryl trichloroacetate [CTA]) against tumor cell lines (U-87, brain; K562, leukemic; Lucena, a leucemic cell line resistant to multiple drugs; Hep.G2, hepatomas) after 48 h in culture, using the MTT method. Statistical analyses was performed by ANOVA and Bonferroni's test ($^*p < 0.05$). Results of IC_{50} (in μM) after 72 h incubation are:

Cell Line	Vincristine	Carvacrol	CA	CTA
Hep.G2	0.03	> 100	> 100	> 100
K562	1.3	0.9	0.97	0.94
Lucena	5.6	12.3	81	85
U-87	0.1	12	9	5

Carvacrol and analogues demonstrated a significant cytotoxic effect against some cell lines indicating that these molecules can be candidates for future studies in other models.

PD6 Determination of D-Pinitol in a decoction of *Desmodium Adscendens* by means of a newly developed GC-method

van Dooren I[1], Dhooghe L[1], Naessens T[1], Vermeylen R[2], Claeys M[2], Vlietinck A[1], Pieters L[1], Apers S[1]
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A decoction of the leaves and stems of *Desmodium adscendens* (Fabaceae), a herb occurring in Africa and South America, is used in traditional medicine for the treatment of asthma, hepatitis, muscle spasms, pain, fever, and seizures. Previous phytochemical research revealed that flavonoids, soyasaponins, β-phenylethylamines, and an indol-3-alkylamine were present in *D. adscendens*. Our investigations have led to the identification of D-pinitol, a low molecular weight carbohydrate with antihyperglycemic, hepatoprotective and anti-inflammatory effects, as a potentially active compound. In order to prepare a quantified extract of *D. adscendens* with a known level of D-pinitol to be used in *in vivo* experiments, an analytical GC-method was developed. Several parameters were studied, i.e. the derivatisation reagent, the internal standard and sample preparation. The final method was validated and shown to be linear, specific, precise (RSD% < 1.3%) and accurate (recovery of 103.4% – 105.8%). The amount of pinitol in lyophilized decoctions from *D. adscendens* used in *in vivo* experiments was about 5%. Also different food supplements were analyzed in which the amount ranged from 1.8 mg/capsule to 30 mg/capsule and was 2.0 mg/ml solution.

PD7 Targeting proteasome and autophagy with *Fragaria Vesca* L

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The dysfunction of protein degradative pathways has been associated with the development of a number of important diseases including cancer. Thus, the aim of this work was to evaluate the effect of a *Fragaria vesca* L. leaves extract in ubiquitin-proteasome system and autophagy, the two major intracellular degradation pathways. *Fragaria vesca* extract was obtained by successive extractions with ethanol and 50% aqueous ethanol. Using the fluorogenic peptide Suc-LLVY-AMC as substrate, we demonstrated that the extract significantly reduced the chemotripsin-

like activity of proteasome at different time points, in a macrophage cell line. Furthermore, and through western blot assay, we observed an increase in ubiquitin conjugates and an increased conversion of LC3-I to LC3-II, a marker of autophagy. In conclusion, the extract reduces proteasome activity, increases autophagy and could be a valuable source of lead molecules with anti-carcinogenic activity. Acknowledgements: Research supported by FCT PhD fellowship SFRH/BD/72918/2010, and FCT project PTDC/SAU-FCF/105429/2008 and FEDER/COMPETE (FCOMP-01 – 0124-FEDER-011096).

PD8 Antioxidant activity of polyphenol-enriched fractions from *Agrimonia Eupatoria* L. infusion

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Agrimony (*Agrimonia eupatoria* L.) is used in traditional medicine as infusion, decoction and tincture for oxidative stress-related diseases. In our previous works, we found that agrimony infusion has significant scavenging capacity against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, eventually related to their phenolic compounds, namely tannins and flavonoids, such as flavan-3-ols, flavonols and flavones derivatives[1]. The aim of this work was to evaluate the contribution of the phenolic compounds for the antioxidant activity, by using two different radical scavenging assays: DPPH and superoxide anion. A polyphenol-enriched fraction (AePEF) and two sub-fractions (FI, containing phenolic acids and tannins, and FII, containing flavonoids) were obtained from the infusion fractioning. Both AePEF and FII exhibited a marked activity against the DPPH and superoxide radicals. In conclusion, our results point that the agrimony flavonoids significantly contribute for antioxidant activity, which could be good candidates for the treatment of inflammation and cancer.

PD9 Immunostimulant activity of *Uncaria Tomentosa* and its tannins

Francisco V[1,2], Liberal J[1,2], Ferreira J[1,3], Costa G[1,3], Lopes MC[2,3], Garcia-Rodríguez C[4], Cruz MT[2,3], Batista MT[1,3]
[1]Center of Pharmaceutical Studies; [2]Center for Neurosciences and Cell Biology; [3]Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; [4]Instituto de Biología y Genética Molecular, Universidad de Valladolid – CSIC, Valladolid, Spain

Uncaria tomentosa (Ut), commonly known as Cat's claw or "uña de gato", is a medicinal plant with immunostimulant, cytotoxic and antioxidant properties. The aim of this work is to investigate the immunostimulant activity of Ut bark decoction and its tannin-rich fraction (TF) on human macrophages. Ut decoction and TF were obtained as previously [1]. The production of inflammatory-related cytokines, namely interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12 (p70), granulocyte-macrophage colony-stimulating factor (GM-CSF), chemokine (C-C motif) ligand (CCL) 2, CCL3, CCL4, and tumor necrosis factor (TNF)-α, in the culture medium of human macrophages treated with Ut decoction and TF, were determined by Bio-Plex suspension array system (Bio-Rad, Hercules, CA). We found that Ut decoction increases the expression of all the mentioned cytokines, revealing an immunostimulant action. Tannins partially contributed to this activity. In conclusion, our data supports the traditional use of *Uncaria tomentosa* as immunostimulant plant and points its tannins as immunomodulating compounds that could play a significant role in human disease prevention and treatment. Acknowledgements: Research supported by FCT fellowships (SFRH/BD/46281/2008 and SFRH/BD/72918/2010), FTC project (PTDC/SAU-FCF/105429/2008) and FEDER/COMPETE (FCOMP-01 – 0124-FEDER-011096). [1] C. Gonçalves et al., Phytochemistry 2005; 66: 89 – 98.

PD10 4'-Aminochalcones as novel inhibitor of the chlorinating activity of myeloperoxidase

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Excessive activation of neutrophils generates reactive oxygen species (ROS) and secretion of primary granular enzymes, such as myeloperoxidase (MPO), which is implicated in numerous inflammatory diseases. The purpose of this study has been evaluating the activity of chalcones as inhibitors of the chlorinating activity of MPO. Besides, cytotoxic properties, scavenger capacity and oxidation potential were measured. Neutrophils were isolated from the blood from healthy donors by Ficoll-Paque density gradient centrifugation. Several natural chalcones were tested and were inactive. However the synthetic 4'-aminochalcone (**1**); 4'-amino-4-fluorochalcone (**2**); 4'-amino-4-methylchalcone (**3**) were potent inhibitors of MPO activity, as potent as 5-fluorotryptamine, a compound considered an excellent inhibitor of the chlorinating activity of MPO: (**1**) IC_{50}= 0.265 ± 0.036 μM; (**2**) IC_{50}= 0.250 ± 0.081 μM; (**3**) IC_{50}= 0.250 ± 0.012 and 5-fluorotryptamine IC_{50}= 0.192 ± 0.012 μM. These compounds were not toxic to neutrophils at concentrations lower than 100 μM as evaluated by the trypan blue exclusion assay. The compounds **1 – 3** presented high oxidation potential (E_{pa1}0.80V) and low scavenger capacity against DPPH and HOCl. Hence, we propose that 4-aminochalcones, a derivative of non-toxic chalcones, could provide the basis designing novel and specific inhibitors of MPO, a target for the development of anti-inflammatory agents. *Acknowledgements: FAPESP and CNPq.*

PD11 Cytotoxic esterified diterpenoid alkaloid derivatives with increase selectivity against a drug-resistant cancer cell line

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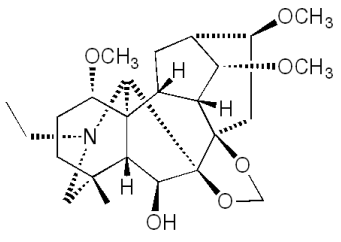
C-6 Esterifications of delpheline (**1**) were carried out to provide 20 new diterpenoid alkaloid derivatives (**4-22**, **24**). Three natural alkaloids (**1-3**) and all synthesized compounds (**4-25**) were evaluated for cytotoxic activity against lung (A549), prostate (DU145), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KB-VIN) cancer cell lines and interestingly, showed an improved drug resistance profile compared to paclitaxel. Particularly, 6-(4-fluoro-3-methylbenzoyl)delpheline (**22**) displayed 2.6-fold greater potency against KB-VIN cells compared with the parental non-drug resistant KB cells. 6-Acylation of **1** appears to be critical for producing cytotoxic activity in this alkaloid class and a means to provide promising new leads for further development into antitumor agents.



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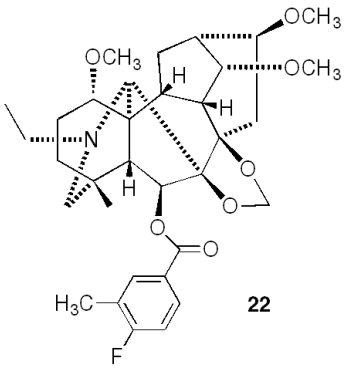

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PD12 Effect of synthetic betulinic acid derivatives on mitochondrial membrane potential ($\Delta\Psi m$) in *Plasmodium falciparum* strains

Silva GNS[1], Innocente AM[1], Schuck DC[2], Nakabashi M[2], Maria NRG[1], Gosmann G[1], Garcia CRS[2], Gnoatto SCB[1]
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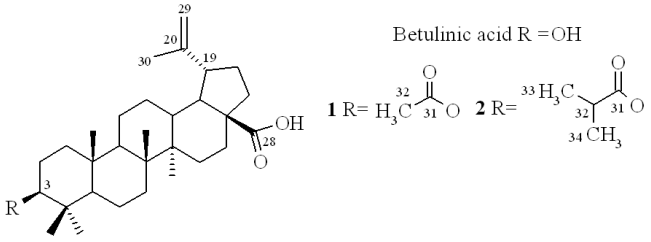
Malaria is caused by a protozoan parasite, and the most virulent agent in humans is *P. falciparum*. Since the energy metabolism of *Plasmodium* is quite different from that of the mammalian host, the energy-transducing enzymes of the parasite are promising anti-malarial drug targets. Furthermore, due the importance of the *Plasmodium* mitochondria to many physiological activities, such as the metabolism of molecules and Ca^{2+} homeostasis, it has been validated as a drug target. In this work, we obtained betulinic acid (BA), a triterpene, from barks of *Platanus acerifolia*, and the modification at C-3 resulted in ten compounds. These derivatives were evaluated against the chloroquine-sensitive strain (3D7) of *P. falciparum*. The action mechanism of the derivatives **1** (IC_{50} 4 μM) and **2** (IC_{50} 8 μM) was investigated through the evaluation of $\Delta\Psi m$ using a technique described by Srivastava et al (1997). Only **1** had a collapse in $\Delta\Psi m$ at IC_{50} 2 mM and showed a displacement histogram indicating the dissipation of $\Delta\Psi m$. Our data showed that BA derivatives can be used as agents against the malaria protozoan, and the investigation of the action mechanism of these compounds can support the design and development of more potent derivatives.



PD13 Semisynthesis of Guttiferone A analogs

Fromentin Y[1,2], Grellier P[2], Michel S[1], Buisson D[2], Lallemand MC[1]
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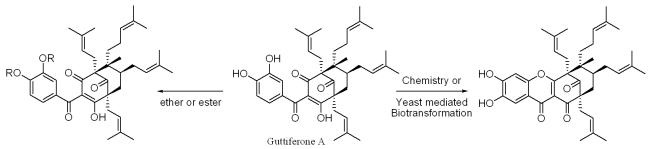
Guttiferone A isolated from *Symphonia globulifera* trees belongs to the PPAPs family (Polycyclic Polyprenylated Acyl Phloroglucinols). This natural compound exhibits several interesting biological activities, such as anti-HIV, cytotoxic, trypanocidal, antiplasmodial and leishmanicidal properties. Our aim is to develop several analogs in order to increase the activity and selectivity toward antiparasitic targets and to decrease the cytotoxicity. To start the SAR study of guttiferone A, we performed the synthesis of catechol analogs and some intramolecular cyclisations using both yeast biotransformation and chemistry.



PD14 Antiproliferative withanolides from the solanaceae: A structure-activity study

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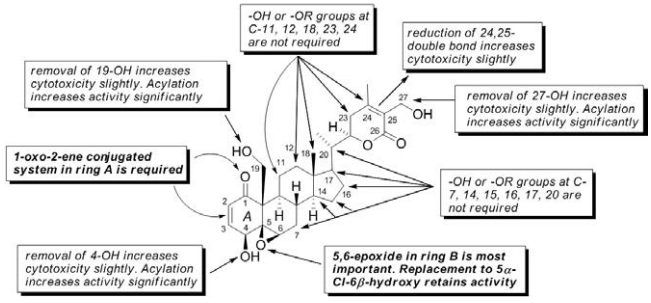
The antiproliferative activities for all published withanolides from members of the family Solanaceaewere summarized. The structure-activity relationship analysis (SARA) confirmed the importance of the presence of 2-en-1-one in ring A; 5β,6β-epoxy or 5α-chlorine-6β-hydroxy in ring B; and nine carbon side chain with a lactone moiety for cytotoxic activity. Conversely, the SARA indicated that the -OH or -OR groups at C-4, 7, 11, 12, 14, 15, 16, 17, 18, 19, 20, 23, 24, 27 were not contributors to the observed activities.



PD15 Synthesis and trypanocidal activity of a furofuran lignan against trypomastigote and amastigote forms of *Trypanosoma Cruzi*

Pereira AC[1,2], Esperandim VR[2], Ferreira DS[2], Magalhães LG[2], Cunha WR[2], Silva MLA[1,2], Dhammika Nanayakkara NP[3], Bastos JK[1]
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Chagas disease, which affects approximately 8 million people in Latin American and more than 25 million people are at risk of this infection, is a neglected tropical disease caused by the protozoan *Trypanosoma cruzi*. Drugs currently used to treat it, such as benznidazole, do not completely eliminate the trypomastigote forms (present in the blood), are ineffective against the intracellular stage of the parasite and cause some side effects. In this work, a furofuran lignan was obtained by oxidative coupling of ferulic acid with $FeCl_3$ in presence of O_2, according to Ahmed, 1973. Then, it was evaluated *in vitro* against trypomastigote and amastigote forms of *T. cruzi*. The results showed that this lignan presents potent tripanocidal activity since it induces trypomastigote and amastigote lysis with IC_{50} values of 9.3 and 7.3 μmol/L, respectively. On the other hand, the direct effect of benznidazole, the positive control, was also evaluated and it showed an IC_{50} value of 1.9 μmol/L on trypomastigotes and an IC_{50} of 69.2 μmol/L against the other form tested. These results indicate that this lignan is promising compound that could be used for the development of a new trypanocidal agent, therefore further studies are in progress to elucidate it mechanism of action. The authors thank FAPESP and CNPq for their fellowships. Ahmed, R., Lehner, M., Stevenson, R. Tetrahedron, 29, 3753 – 3759, 1973.

PD16 Reactivation of HIV latency by ingenol esters isolated from *Euphorbia Tirucalli*

Delvecchio R[1], Monteiro Abreu C[1], Pandeló D[1], Santana Aguiar R[1], Gliński JA[2], Kinkade P[2], Douglas J[3], Tanuri A[1], Pianowski LF[4]
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During the early stages of HIV infections, the virus establishes latency in a portion of the population of infected long-lived memory cells CD4+. Such latent viral reservoirs are inaccessible to standard antiretroviral drugs and result in an inability to eradicate the infection. A reactivation of the latent virus would offer an opportunity to expose it to the action of antiretroviral drugs (HAART) and consequently lead to full elimination of the virus. We have found that esters of ingenol found in *E. tirucalli* act as reactivators of HIV. They were isolated by Centrifugal Partition Chromatography (FCPC Kromaton) followed by preparative HPLC and their structures were characterized by NMR as three E/Z isomers of ingenol-3-dodecatrienoate (**1**). We used an antiviral assay in MT-4 cells and flow cytometry studies, in the presence of **1**, were to elucidate its mechanism of antiviral action. The MT-4 assay demonstrated that **1** inhibited viral replication until cellular toxicity was observed at 40 μM. This molecule was able to down-regulate CD4 expression in MT-4 as well as in human PMBCs. Interestingly, this compound was also capable to activate human CD4+ cells, as shown by double marked CD4/CD38 and CD4/CD69. Nevertheless, **1** was not able to induce cellular proliferation. By acting through proviral activation and by blocking viral entrance through receptor down regulation, **1** can be used in a shock-and-kill strategy to be placed together with HAART therapy to eliminate viral reservoir.

PD17 Inhibitory effect of *Copaifera langsdorffii* on 1,2-Dimethylhydrazine induced genotoxicity in rat colon

Morais Alves J[1], Marques Senedese J[1], Tinti de Castro P[1], Eleutério Pereira D[1], Ambrósio SR[1], Kenupp Bastos J[2], Crispim Tavares D[1]
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Copaiba oils are produced by exudation from the trunks of trees belonging to the genus *Copaifera*. *Copaifera langsdorffii* known as "copaiba", "capaiva" or "pau-de-oleo" belongs to the Leguminosae family. The effects attributed to copaiba oils in folk medicine include anti-inflammatory, anti-tetanus, anti-tumour, anti-blenorrhagea and urinary antiseptic activities. In the present study, we evaluated the effects of *C. langsdorffii* oil on the formation of 1,2-dimethyl-hydrazine (DMH)-induced aberrant crypt foci (ACF) in the colon of the male Wistar rat. The animals received subcutaneous (sc) injections of DMH (40 mg/kg body weight, b.w.) twice a week for two weeks to induce ACF. *C. langsdorffii* oil was administered to the rats five times a week for four weeks by gavage at doses of 12.5, 25 and 50 mg/kg b.w/day each, during and after DMH treatment. All animals were sacrificed in week 5 for the evaluation of ACF. The results showed a significant reduction in the frequency of ACF in the group treated with the *C. langsdorffii* oil plus DMH when compared to those treated with DMH alone, suggesting that *C. langsdorffii* oil suppress the formation of ACF and have a protective effect against colon carcinogenesis. Financial Support: Foundation for Research Support of São Paulo State (FAPESP, grants number 2009/17237 – 8 and 2011/13630 – 7).

PD18 Antimutagenic activity of essential oils from Schinus *Areira L.* (Anacardiaceae)

Rodriguez SA[1,3], Sueiro RA[2], Murray AP[1], Leiro JM[3]
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Schinus areira L. (syn. *Schinus molle* L. var. *areira* (L.) DC.) is a native species known by its medicinal value. The essential oils (EOs) of fruits and leaf from *S.areira* L. and the major compound from fruit oil have been studied for their antimutagenic activity. The chemical composition of the oil of the aerial parts of *S. areira* obtained by hydrodistillation was determined by GC-MS. Fruit and leaf oils of *S. areira* were analyzed separately. The experiments were carried out using the Ames test [1] with *Salmonella typhimurium* strains TA100, TA98, TA102, TA 1535 and TA 1537. The result determined that EOs do not exhibit mutagenic effect, at concentrations between 5 and 0.05 μg/mL. The EOs also showed antimutagenic activity against sodium azide, 9 amine acridine, 2,4,7-trinitro-9-fluorenone and Mitomycin C. The inhibition of the mutagenic effect from leaf and fruit oils ranged from 30.2 – 78.3% to 37.1 – 87.9% respectively, in a concentration-dependent manner. The essential oil from S. areira fruit was more active, this could be associated with the presence of myrcene, the main compound in the oil which is reported to have antimutagenic properties. [1] Maron, D.M. and Ames, B.N. (1983), Mutation Res., 113, 173 – 215

PD19 Inhibition of mutagenicity by Atriplex Undulata (Moq) D. Dietr. (Chenopodiaceae)

Rodriguez SA[1,3], Sueiro RA[2], Murray AP[1], Leiro JM
[1]INQUISUR, Departamento de Química, UNS, Pcia. Bs.As., Argentina; [2]IIAA, Laboratorio de Microbiología, USC, Santiago de Compostela, Spain; [3]IIAA, Laboratorio de Parasitología, USC, Sgo de Compostela, Spain

Atriplex undulata, commonly known as Zampa crespa or Cachiyuyo, is an endemic species. It originates from Patagonia Argentine and is used as astringent and antiequimotic. Aerial parts from *A. undulata*, were extracted with ethanol. The ethanolic extract and its ethyl acetate partition were evaluated the direct mutagenic and antimutagenic effects. The ethyl acetate partition, which has presented a promising antimutagenic activity, was fractionated according to previous reports [1]. The experiments were carried out using the Ames test with *Salmonella typhimurium* strains TA100, TA98, TA102, TA 1535 and TA 1537. Both extract did not induce relevant increases in the number of revertant colonies in any of the tester strains assayed, at concentrations between 50 and 0.05 mg/mL. These extract also showed antimutagenic activity against sodium azide (NaN_3), 9 amine acridine (9AA), 2,4,7-trinitro-9-fluorenone (TNF) and Mitomycin C. The inhibition of the mutagenic effect ranged from 47.16% to 81.29% in a concentration-dependent manner. This antimutagenic effect could be explained, at least in part, by the presence in this fraction of quercetin, which showed significantly inhibition of mutagenicity. [1] Rodriguez S. and Murray A. P., XVIII SINAQO, Mendoza, Argentina, 14 – 18th November 2009

PD20 Protective activity of S*tyrax camporum* extract against genotoxicity induced by doxorubicin and methyl methanesulfonate

Francielli de Oliveira P, Andrade Furtado R, Oliveira Acésio N, Leandro LF, Montanheiro G, Corrêa de Pádua F, Beltrame Corrêa M, Guedes Braguini C, Mendonça Pauletti P, Crispim Tavares D
University of Franca, Franca, São Paulo, Brazil

Styrax camporum Pohl is a tall shrub or a tree with small white flowers, which grows in the states of São Paulo and Minas Gerais and is popularly used for the treatment of gastroduodenal diseases. The aim of this study was to evaluate the genotoxic potential of *S. camporum* hydroalcoholic extract and its influence on genotoxicity induced by doxorubicin (DXR) and methyl methanesulfonate (MMS) in Swiss mice using the micronucleus and comet assays, respectively. The animals were treated with different doses of the extract (250, 500 and 1000 mg/kg body weight). For antigenotoxicity assessment, different doses of the *S. camporum* extract were administered simultaneously with DXR and MMS. The results showed that the *S. camporum* extract itself was not genotoxic. The number of micronucleus was significantly lower in animals treated with the *S. camporum* extract and DXR when compared to animals treated only with DXR. In the comet assay, the *S. camporum* extract significantly reduced the extent of DNA damage in liver cells induced by MMS. The antioxidant activity of the active compounds of *S. camporum* extract may explain the effect. Financial support: FAPESP; Grant number 2011/21310 – 2

PD21 Anticarcinogenic potential of *Solanum lycocarpum* fruits glycoalkaloid extract in male wistar rats

Carolina Munari C[1], Francielli de Oliveira P[1], Leandro LF[1], Mara Pimenta L[1], Aparecida da Silva D[1], Kenupp Bastos J[2], Crispim Tavares D[1]
[1]University of Franca, Franca, São Paulo, Brazil; [2]Faculty of Pharmaceutical Sciences of Ribeirão Preto – USP, Ribeirão Preto, São Paulo, Brazil

Solanum lycocarpum St. Hill. (Solanaceae) is a hairy shrub or small much-branched tree of the Brazilian Cerrado, popularly known as "fruit-of-wolf". Besides the current use for the control of diabetes and obesity, the green fruits are topically applied on snakebites and the hot baked fruit is used in the treatment of tissue atrophy. Plants of genus *Solanum* are known for their high alkaloid concentration and part of their toxicity may be due to these alkaloids. Solasonine and solamargine are two major glycoalkaloids found in at least 100 *Solanum* species. Studies on the activities of solamargine demonstrated that it inhibits the growth of human tumor cells, eg. colon, prostate, breast and hepatoma cells. In this sense, the aim of present study was to evaluate the anticarcinogenic potential of *S. lycocarpum* fruits glycoalkaloid extract (SL) on the formation of 1,2-dimethylhydrazine (DMH) induced aberrant crypt foci (ACF) in the colon of male Wistar rats by ACF assays. Animals were treated by gavage with doses of 15, 30 and 60 mg/kg body weight/day. Also, two doses subcutaneous injection of 40 mg/kg of DMH were administered for two weeks, and animals were sacrificed two weeks after the last injection for evaluating ACF development in rats' colon. The results showed a significant reduction in the frequency of ACF in the group treated with the SL plus DMH in comparison with those treated with DMH alone, suggesting that SL suppressed the formation of ACF, as well as had a protective affect against colon carcinogenesis.

PD22 Synthesis and anti-cancer activity of alpha-santonin derivative

Arystanova TA, Serikbayeva AD, Orynbassarov YK
South Kazakhstan State Pharmaceutical Academy, Shymkent, Kazakhstan

Synthesis is based on cooperation of equimol quantity of chloralhydrate thiosemicarbazid with sodium acetate, adding alpha-santonin, extracted from Artemisia cina Berg. ex Polyak, in spirit. The extraction of composition is 90%. The construction of extracted santonin thiosemicarbazon proved by the methods of IR-, H^{-1}NMR-spectroscopy. Santonin thiosemicarbazon ($C_{16}H_{21}O_3N_3S$) obtained as chromatographically clean, colorless substance, easily soluble in dimethylsulfocside and dimethylformamide, but badly soluble in methanol and chloroform. UV-spectrum has maximum absorption at 270 ± 2 nm wave lengths in neutral environment (95% ethyl spirit). At IR-spectrum (cm^{-1}) there are stripes of absorption at 2942.15, 2898.02, 2870.33 (C-H, CH_2, CH_3 groups), 1772.99 (–C = O in γ-lactone cycle), 1225.751 (C = S). At NMR-spectroscopy of santonin thiosemicarbazon there are signals of protons of the third methyl group at 1.02 m.d (3 H, singlet C 10-CH_3), the methyl group at γ-lactone cycle at 1.13 ppm. (3 H, doublet, J = 6.5 Hz, C 11-CH_3). The lactone proton is presented as doublet at 5.59 ppm. (1 H. doublet, J = 5 Hz, C 6-H). The proton signal of N-H group is presented as singlet at 11 ppm. The anti-cancer activity of santonin thiosemicarbazon is detected against these strains of cancers: solid Ehrlich carcinoma, adenocarcinoma of the breast glands (Ca755), melanoma B16, Lewis lung carcinoma (LL), Walker carcinosarcoma, Pliss lymphosarcoma (LSP), sarcoma 45 (S-45).

PD23 Effect of macelignan on inhibiting biomarkers of gouty inflammation in rat chondrocytes *in vitro*

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Gout is an inflammatory disease caused by the increased production of pro-inflammatory cytokines and enzymes in cartilage cells, i.e. macrophages, chondrocytes, and osteoclasts, that lead to the accumulation of monosodium urate crystal within the joint tissues. Most conventional gout treatments using non steroidal anti-inflammatory drugs (NSAIDs) have been commercially used, however, they posted side effects on hepatic functions and hypoactive immune functions. We examined the potential macelignan isolated from nutmeg seed extract (*Myristica fragrans* Houtt.) on decreasing biomarker expression of gouty inflammation at protein and gene levels, i.e. interleukin (IL)-6, tumour necrosis factor (TNF)-α, cyclooxygenase (COX)-2, and matrix metalloproteinase (MMP)-9, in rat chondrocytes treated with lipopolysaccharide (LPS) *in vitro* by conducting ELISA, Western blot, and reverse transcriptase-polymerase chain reaction (RT-PCR) assays. Macelignan was isolated from the ethyl acetate fraction of *M. fragrans* methanol extract using silica gel column chromatography and identified by ^{13}C-NMR, ^{1}H-NMR, DEPT and FAB-MS. Macelignan (1 – 10 μg/ml) did not affect cell morphology and cell growth, suggesting it may be safely used for further treatment. LPS at 2 μg/ml activated the protein and mRNA expression of IL-6, TNF-α, COX-2, and MMP-9 in chondrocytes. Macelignan dose-dependently blocked the expression of IL-6, TNF-α, COX-2, and MMP-9 at protein and gene levels in LPS-induced chondrocytes, indicating its potential effect for prevention of inflammation-related gout. Hence, it may be used as an alternative natural therapeutic agent for management of gout.

PD24 Analysis of essential amino acids in lasso peptide lariatins for anti-mycobacterial activity by single amino acid substitution

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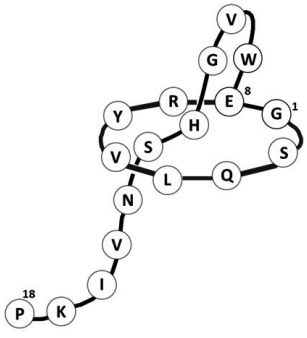
Lariatin A and B discovered as an anti-mycobacterial peptides are ribosomally synthesized by Gram positive *Rhodococcus jostii*. The compounds are unique cyclic peptides, consisting of 18 and 20 L-amino acid (aa) residues with an internal linkage between the α-amino group of G1 and the γ-carboxyl group of E8. The C-terminal tail passes through the ring to form the rigid 'lasso' structure. Production of lariatins in *R. jostii* is dependent upon a five-gene cluster, *lar*A to *lar*E. We established a simple production system of amino acid substitutions of lariatin by *R. jostii* K01-B0171Δ*larA*, Using this system, we have performed mutational scanning of lariatin, constructing and analyzing about 30 singly substituted derivatives of lariatin and defined aa residues important for production of lariatin and anti-mycobacterial activity. The results showed that three aa residues (G1, R7 and E8) in the circle and three aa residues (W9, V10 and G11) in the threaded segment of the tail are important for lariatin production, and that one aa residue (Y6) in the circle and three aa residues (V10, N14 and K17) in the threaded segment of the tail are important for anti-mycobacterial activity. These findings will open the way for design and construction of more potent lariatin-based anti-mycobacterial agents.



Structure of lariatin A

PD25 Structure-activity investigations of Santacruzamate A, a potent histone deacetylase inhibitor isolated from a Panamanian cyanobacterium

Pavlik CM, Balunas MJ
Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut 06269, USA

Recently a new natural product, santacruzamate A, has been isolated from a dark brown cyanobacterium closely related to the genus Symploca. Santacruzamate A contains a unique scaffold analogous to several

other potent histone deacetylase (HDAC) inhibitors. Initial in vitro HDAC2 enzyme inhibition bioassay data has shown that the natural product is selective for class I HDAC proteins with an IC_{50} in the picomolar range. Comparative analysis with the clinically approved HDAC inhibitor, Vorinostat®, demonstrated that the isolated natural product was found to be approximately 1000-fold more potent. Similar to Vorinostat and many other known HDAC inhibitors, santacruzamate A contains three specific regions: a zinc-binding group (ZBG), an alkyl-linker, and a hydrophobic-cap group. Our initial SAR investigation involved modification of our presumed ZBG terminus and subsequent evaluation for class I and II HDAC enzyme inhibition and cell cytoxicity.

PD26 Antigenotoxicity of *Solanum lycocarpum* glycoalkaloid extract and its majority compounds, solamargine and solasonine, in V79 cells

Crispim Tavares D[1], Carolina Munari C[1], Francielli de Oliveira P[1], Mota de Souza Lima I[1], Kenupp Bastos J[2]
[1]University of Franca, Franca, São Paulo, Brazil; [2]Faculty of Pharmaceuticals Sciences of Ribeirão Preto – USP, Ribeirão Preto, São Paulo, Brazil

Solanum lycocarpum A. St.-Hil. (Solanaceae) are commonly used in traditional medicine for the treatment of diabetes and obesity, as well as for controlling cholesterol levels. Solasonine and solamargine are two major glycoalkaloids found in at least 100 *Solanum* species. In this sense, the aim of the present study was to evaluate the cytotoxic, genotoxic and antigenotoxic potential of *S. lycocarpum* fruits glycoalkaloid extract (SL), solamargine (SM) and solasonine (SS), in V79 cells. Cytotoxicity was evaluated by the colony forming assay. Genotoxic and antigenotoxic potential were evaluated by comet assay. Different concentrations of SL (4, 8, 16 and 32 μg/mL), SM (7.1 μg/mL) and SS (14.4 μg/mL) were used for the evaluation of their genotoxic potential in V79 cells. In the antigenotoxicity assay, the different concentrations of SL, SM and SS were combined with methyl methanesulfonate (MMS). SL, SM and SS were cytotoxic at concentrations up to 32 μg/mL, 7.1 μg/mL and 14.4 μg/mL, respectively. The comet assay revealed that SL, SM and SS displayed no genotoxic activity, but they significantly reduced the extent of DNA damage induced by MMS. Financial support: Foundation for Research Support of São Paulo State (FAPESP, grants number 2009/15871 – 1 and 2011/05732 – 4).

PD27 Antioxidant, anti-inflammatory and neuroprotective activities of plastoquinones from the seed fat of *Pycnanthus Angolensis*

Gustafson K[1,2], Giurleo D[1,2], Ho CT[2,3], Dang W[4], Pan MH[5], Wu Q[1,2], Simon JE[1,2]
[1]New Use Agriculture & Natural Plant Products Program, Department of Plant Biology, Rutgers University, New Brunswick, NJ 08901; [2]Department of Medicinal Chemistry, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ 08854; [3]Department of Food Science, Rutgers University, New Brunswick, NJ 08901; [4]Department of Cell Biology & Human Anatomy, School of Medicine, University of California, Davis, Shriners Institute, Sacramento, CA 95817; [5]Department of Seafood Science, National Kaohsiung Marine University, Kaohsiung 811, Taiwan

Pycnanthus angolensis (African nutmeg), native to several West African countries, was investigated for new medicinal uses. Sustainably harvested in Ghana, the seed fat is processed into kombo butter by farmer cooperatives. This plant-based butter is rich in myristoleic acid, a precursor to cetyl myristoleate (CMO) which is currently marketed as a supplement in the treatment of joint pain. Separate from the myristoleic acid and as a waste product from the processing of kombo butter into CMO, the effluent was found to contain high concentrations of the plastoquinones sargaquinoic acid (KB-1), sargachromenol (KB-2) and sargahydroquinoic acid (KB-3). These three major phytochemicals, along with kombo butter and plastoquinone-enriched extract, were tested *in vitro* for antioxidant activity in the ABTS and DPPH assays, and for anti-inflammatory activity using RT-PCR and Western blot analysis to measure inhibition of both COX-2 and iNOS mRNA and protein expression in LPS-induced RAW 264.7 mouse macrophages. Additionally, KB-3 and an acetylated derivative exhibited neuroprotective activity *in vivo* using the pMCAO model, demonstrating their potential for use following ischemic stroke.

PD28 Easy transformation of indoles to substituted tryptanthrins with powerful AHR activity

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Malassezia furfur isolates from diseased skin preferentially biosynthesize compounds which are among the most active known Aryl-hydrocarbon Receptor (AhR) inducers, such as indirubin and tryptanthrin. In our effort to study their production from *Malassezia* spp., we investigated the role of indole-3-carboxaldehyde (I3A), the most abundant metabolite of *Malassezia* when grown on tryptophan agar, as a possible starting material for the biosynthesis of both alkaloids. Treatment of I3A with H_2O_2 and use of diphenyldiselenide as a catalyst resulted in the simultaneous one-step transformation of I3A to indirubin and tryptanthrin in good yields. The same reaction was first applied on simple indole and then on substituted indoles and indole-3-carboxaldehydes, leading to a series of mono- and bi-substituted indirubins and tryptanthrins bearing halogens, alkyl or carbomethoxy groups. Afterwards, they were evaluated for their activity on AhR in four different cell lines stably transfected with a luciferase reporter gene. Among them, 3-bromotryptanthrin interestingly showed 10 times higher activity than dioxin in the human HepG2 7.5 cell line at 6 h. In conclusion, I3A could be the starting material used by *Malassezia* for the production of both indirubin and tryptanthrin through an oxidating mechanism. Additionally, some of the prepared tryptanthrins showed significantly increased activity and selectivity against the human cell line.

PD29 Anti-leukemia activity of cranberry A-PACS is mediated by disruption of iron metabolism

Bystrom L[1], Hsu HT[1], Patel K[2], Yiantsidis E[2], Neto C[2], Guo M[2], Rivella S[1], Guzman M[1]
[1]Weill Cornell Medical College New York, New York 10021; [2]University of Massachusetts-Dartmouth, Dartmouth, Massachusetts 02747

Some of the health effects of cranberries are associated with a unique class of compounds known as A-type proanthocyanidins (A-PACs). Preliminary studies of the biological effects of A-PACs indicated these activities may be associated with their ability to function as iron chelators. Several studies indicated that iron chelators have anti-tumor activities. This information prompted us to investigate the effects of A-PACs on iron metabolism and leukemia cell survival. Isolation techniques were developed to obtain A-PAC fractions from cranberry cultivars of *Vaccinium macrocarpon* Ait., or from the cranberry powder CystiCran-40. Phytochemical analysis indicated that the A-PAC fractions consisted mostly of small A-PACs and that CystiCran-40 showed a similar profile as A-PACs, but with additional flavonoids such as quercetin derivatives. The survival and iron metabolism effects of leukemia cells treated with A-PAC fractions or CystiCran-40 were assessed using flow cytometry. A-PAC fractions proved more effective than CystiCran-40 at inducing apoptosis, whereas an A-PAC dimer was the least effective. Among the cell lines tested, REH and K562 were the most sensitive while OCI-AML-3 and KG-1 were the most resistant. A-PAC fractions also increased reactive oxygen species and upregulated the transferrin receptor. These results suggest cranberry A-PACs have potential therapeutic value for the treatment of leukemia and that their anti-tumor mechanisms may involve effects on iron metabolism.

PD30

In vitro cytotoxic activity of novel protoflavone analogs-selectivity against a multi-drug resistant cancer cell line

Hunyadi A[1,2], Chuang DW[3], Wang HC[3], Chang FR[3], Wu YC[3,4,5]
[1]Institute of Pharmacognosy, University of Szeged, Szeged, Hungary; [2]COST Action CM0804 (Chemical Biology with Natural Products) of the European Commission, Brussels, Belgium; [3]Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan; [4]School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan; [5]Natural Medicinal Products Research Center, China Medical University Hospital, Taichung, Taiwan

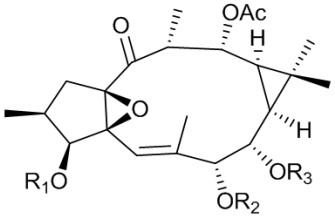
Protoapigenone (PA), a natural flavonoid possessing an unusual p-quinol moiety on its B ring, is a prospective novel anticancer lead compound currently in development, together with WYC0209, a potent synthetic PA analog. Previously, an increased cytotoxicity was found when a 3 – 4 carbon long aliphatic side-chain was present at C-1' for analogs of PA, while this was not the case when WYC0209 derivatives expressed the same moiety. Fifteen 1'-*O*-alkyl protoflavone derivatives were synthesized from genkwanin or 4'-hydroxy-6-methylflavone, thirteen of which are new compounds. All compounds, as well as PA, WYC0209 and fourteen of their previously reported analogs were also tested on a multidrug resistant sub-cell line (MDR) of L5178 mouse T-cell lymphoma and on its parental counterpart (PAR). In general, derivatives expressing a free hydroxyl group at C-1' exerted the strongest activities, while C-1' substituted compounds were found much weaker. Derivatives of 6-methylflavone showed mild, but statistically significant selectivity against the MDR cell line. The results are in agreement with our previous findings for fundamental structure-activity relationships on protoflavones. 6-methylated protoflavones may serve as valuable leads for developing selective compounds against MDR cancer. Identical activity of other derivatives on the PAR and MDR cell lines suggests that cancer cells cannot confer resistance to protoflavones by ABCB1 efflux pump over-expression.

PD31

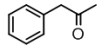
Diterpenes isolated from *Euphorbia Sp* that promote neurogenesis from neural stem cells

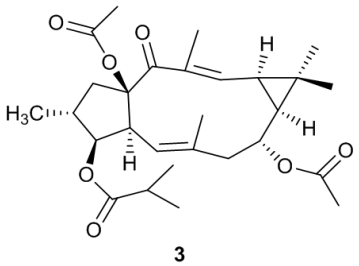
Flores-Giubi ME[1,2], Geribaldi N[1], Murillo-Carretero M[1], Macías-Sánchez A[2], Daoubi M[2], Echeverri F[3], Castro C[1], Hernández-Galán R[2]
[1]Área de Fisiología, Facultad de Medicina. Universidad de Cádiz, Spain; [2]Departamento de Química Orgánica, Facultad de Ciencias. Universidad de Cádiz, Spain; [3]Grupo de Química Orgánica de Productos Naturales, Instituto de Química. Universidad de Antioquia, Medellín, Colombia

Protein kinase C isozymes (PKCs) are important signal transducers through an endogenous or exogenous activating compound. As they are involved in many biological events, PKCs are attractive targets in drug design for treatment of cancer, AIDS or Alzheimer's disease. Based on previous results obtained by our research group and on structural similarities with non-tumor promoter diterpenes such as prostratine, compounds with lathyrane skeleton have been designed as molecules agonist of proteins kinase C (PKC). Ingols **1-2** and lathyranes **3-5** were isolated from *Euphorbia sp.* Their isolation and their capability to promote neural stem cells proliferationwill be presented.



1 R_1 = Ac R_2 = H R_3 = Tig

2 R_1=  R_2 = R_3 = Ac



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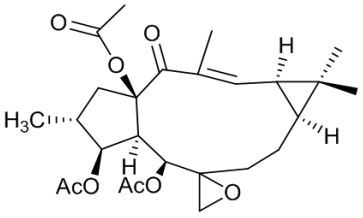

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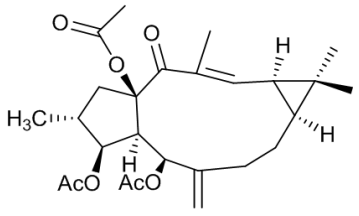

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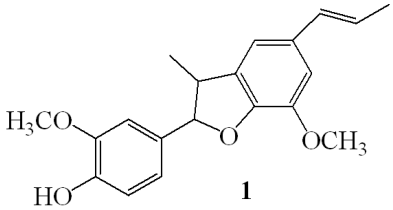
PD32

(±)-Licarina-A loaded PLGA nanoparticles: Preparation and *in vitro* schistosomicidal activity for controlled release

Lima TC[1], Cunha WR[1], Magalhães LG[1], Bastos JK[2], Pereira AC[1,2], Luz PP[1], e Silva MLA[1]
[1]Universidade de Franca, Franca, SP, Brazil; [2]Universidade de São Paulo, Ribeirão Preto, SP, Brazil

The aim of this work was incorporation of (±)-licarina-A **1** into the PLGA particles were carried out by emulsion/solvent evaporation method. The obtained particles were centrifuged and dried out by lyophilization. The nanoparticles were characterized by FESEM and the amount of **1** was quantified by UV-Vis spectroscopy. Preliminary bioassay results indicate that **1** possesses *in vitro* schistosomicidal activity against adult worms couples of *Schistosoma mansoni* at 200 μM, leading to 100% of couples separation and completely motor activity reducing. *In vitro* schistosomicidal activity of (±)-licarina-A-loaded PLGA nanoparticles are in progress in order to elucidate a possible improved and prolonged schistosomicidal action. Sponsors: **FAPESP, CNPq and CAPES**

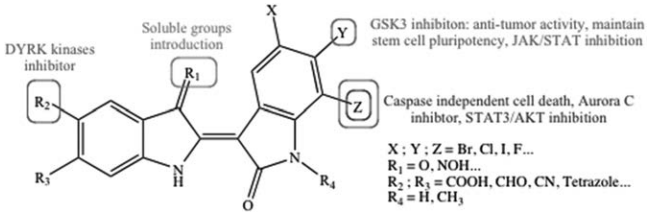


1

PD33 Versatile selective kinase inhibitors: Chemistry and biology of indirubins

Gaboriaud-Kolar N[1], Magiatis P[1], Brivanlou A[2], Greengard P[3], Knapp S[4], Nam S[5], Jove R[5], Meijer L[6], Mikros E[1], Skaltsounis AL[1]
[1]Departments of Pharmacognosy and Pharmaceutical Chemistry, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15771, Athens, Greece; [2]Laboratory of Molecular Vertebrate Embryology; [3]Laboratory of Molecular and Cellular Neuroscience, The Rockfeller University, 1230 York Avenue, New-York, NY10065, USA; [4]Nuffield Department of Clinical Medicine, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Headington, Oxford, OX37DQ, UK; [5]Molecular Medicine Beckman Research Institute, City of Hope Comprehensive Cancer Center, 1500 East Duarte Road, Duarte, California, 91010, USA; [6]Cell Cycle Group, Station Biologique de Roscoff, BP 74 29682 Roscoff Cedex, France

In an effort to identify new kinases inhibitors, natural and synthetic indirubins have been tested. This full SAR study led to the discovery of versatile selective kinases inhibitors according to the substitutions degree. This inhibition selectivity led to diverse biological activities e.g. stem cell pluripotency preservation[1] or *in vivo* anti-tumor activities.

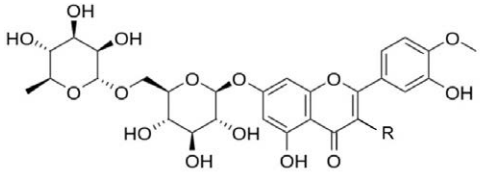


1: N. Sato et al, *Nature Med*, **2004**, 10, 55 – 63.

PD34 Emergence of high aqueous solubility for some flavone glycosides by disruption of molecular planarity

Lewin G[1], Maciuk A[1], Moncomble A[2], Cornard JP[2]
[1]UMR 8076 CNRS, Faculty of Pharmacy, University Paris-Sud, France; [2]UMR 8516 CNRS, University Lille 1, Villeneuve d'Ascq, France

Balance between aqueous solubility and hydrophobicity is an important parameter for drug design. Improvement of aqueous solubility by introducing hydrophilic groups often results in a decrease of oral bioavailability. At the same time, poor hydrosolubility can be observed for polar compounds like flavones. For these compounds, molecular planarity, symmetry and subsequent molecular associations play a significant role[1]. Therefore an alternative strategy to increase aqueous solubility consists of disrupting molecular planarity. The phlebotropic drug diosmin, a flavone glycoside, is known to be greatly insoluble in water. We describe the dramatic 10^5-fold increase of aqueous solubility concomitant with increase of lipophilicity by the introduction of a single halogen substituent at the C-3 position. Computed optimized structures show that the value of the twist angle between the chromone moiety and the B ring is closely related to the aqueous solubility. Also, steric hindrance in position 3 is more important than electronic factors to increase aqueous solubility.



R	Aqueous Solubility (g/L)	LogP	Retention time C_{18} HPLC (min)
H	0.0012	-0.10	18.6
Br	> 100	0.61	20.9

[1]Ishikawa & al., J. Med Chem 2011, 54, 1539.

PD35 Synthesis of imperatorin analogoues and their evaluation as acetylcholinestarase and butyrylcholinesterase inhibitors

Granica S[1,2], Kiss AK[1], Czarnocki Z[2]
[1]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Banacha 1, 02 – 097 Warsaw, Poland; [2]Faculty of Chemistry, University of Warsaw, Pasteura 1, 02 – 093 Warsaw

In this study we analyzed inhibitory activity of some synthesized furocoumarins towards acetyl- and butyrylcholinesterase. We used fruits of Archangelica angelica L. obtained after cultivation for medical purposes, as a source of imperatorin and other linear furocoumarins. Apart from isolated imperatorin we used commercially available xanthotoxin as a substrate for chemical transformations leading to several derivatives differing in the structure of a side chain of furocoumarin rings. Anticholinesterase activity was evaluated in *in vitro* experiments according to the modified Ellman's method using 96-well plate reader. For each of synthesized compounds IC_{50} value for both enzymes was established basing on concentration inhibition curves. Galantamine hydrobromide was used as a positive control in enzymatic experiments. All active compounds showed significant selectivity towards butyrylcholinesterase rather than acetylcholinesterase. The most active were 8-(3-methylbutoxy)-psoralen and 8-hexoxypsolaren with IC_{50} for BuChE around 16.5 and 16.4 μM respectively. The results of our study may be considered as the beginning of a search for potential anti-Alzheimer's disease drugs based on the structure of natural furocoumarins.

PD36 Structure activity-relationships of P-Glycoprotein modulation using a small library of macrocyclic lathyrane diterpenes

Reis M[1], Ferreira RJ[1], Santos MMM[1], dos Santos DJVA[1], Molnár J[2], Ferreira MJU[1]
[1]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Lisboa, Portugal; [2]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

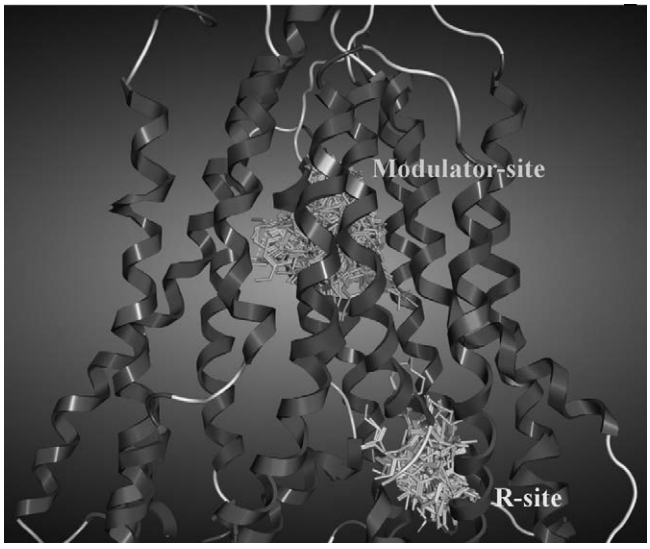
Multidrug resistance is a major hurdle for the success of cancer chemotherapy. The main cause for this phenomenon is attributed to the overexpression of the efflux pump, P-glycoprotein (P-gp). A small library of novel bioactive macrocyclic lathyrane-type diterpene derivatives was developed to discover a selective P-gp reversal agent. In this way, a macrocyclic diterpene was acylated with different alkanoyl and aroyl anhydrides/chlorides. In order to explore the role of the substitution pattern on the modulation of P-gp efflux, the anti-MDR effects of compounds were evaluated in human *MDR1*-gene transfected mouse lymphoma cells, through the rhodamine-123 exclusion assay. Furthermore, some of the compounds synergistically enhanced the antiproliferative effect of doxorubicin. The combination of these results with physicochemical descriptors allowed inferring some structure-activity relationships. Strong correlations were found between activity and molecular weight, accessible solvent areas or octanol/water partition coefficient. Acknowledgements: This study was supported by FCT, Portugal (project PTDC/QUI-QUI/099815/2008; PhD grant SFRH/BD/72915/2010; PEst-OE/SAU/UI4013/2011).

PD37 Modulation of P-Glycoprotein activity: Insights from docking studies

Ferreira RJ[1], Reis M[1], Santos MMM[1], Molnár J[2], dos Santos DJVA[1], Ferreira MJU[1]
[1]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, Lisboa, Portugal; [2]Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary

P-glycoprotein is one of the best studied transmembranar proteins directly related with multidrug resistance phenomenon. Using a refined P-gp structure recently published by our group, new docking studies have identified three distinct binding sites: two for substrates (H- and R-sites) and one for modulators (Modulator-site), which are in perfect agreement with experimental studies described in literature. From these docking studies, it was observed that the acylation pattern at the pen-

tacyclic ring of lathyrane-type macrocyclic diterpenes influences the affinity towards the Modulator-site.



PD38 Pharmacomodulation of Meiogynin A, a dimeric sesquiterpenoid inhibiting BCL-XL and BAK interaction

Dardenne J[1], Pujals A[2], Colas C[1], Birlirakis N[1], Wiels J[2], Dumontet V[1], Geny C[1], Iorga B[1], Guéritte F[1], Roussi F[1]
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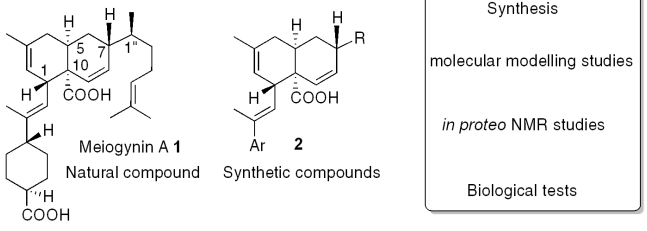
A new dimeric sesquiterpenoid, meiogynin A **1**, was recently isolated in our team. This compound acts as an antagonist of the Bcl-xL/Bak association. We performed its biomimetic total synthesis as well as those of analogues guided by molecular modelling and *in proteo* NMR studies. The chemical and biological results will be presented.



PD39 Synthesis and antimalarial activity of fagaronine

Rivaud M[1], Mendoza A[1,2], Sauvain M[1,3], Valentin A[1], Jullian V[1]
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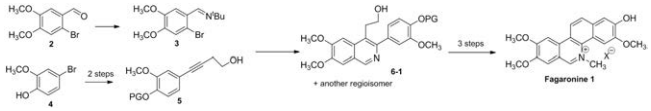
Fagaronine **1**, a benzo[c]phenanthridine first isolated from *Fagara zanthoxyloïdes* in 1972 is known for its *in vitro* activity against *P. falciparum* in the nanomolar range. In order to study its antimalarial potential further, we performed a short synthesis for this molecule, inspired from the synthesis already published by Luo for nornitidine. This allowed us to obtain fagaronine **1** in 7 steps with a 10% yield. The key step is a nickel catalyzed annulation reaction between alkyne **5** and bromobenzaldehyde **3**.



An antimalarial activity *in vivo* on mice comparable to the activity of chloroquine was evidenced. Fagaronine appears as a good potential lead for the design of new antimalarial molecules.

PD40 Chemopreventive effects of *Lawsonia Inermis* L. (Henna) leaf powder and its pigment artifact, lawsone on skin carcinogenesis in mice

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The medicinal plant *Lawsonia inermis* L, commonly known as 'henna' and lawsone (2-hydroxy-1,4-naphthoquinone), the red-orange pigment artifact formed during the extraction or preparation of the dye from henna leaves, were evaluated for chemopreventive effects against skin carcinogenesis in three mouse models. In the two-stage mouse skin carcinogenesis model using UV-B radiation for initiation and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for tumor promotion, oral feeding of henna leaf powder (0.0025%) in drinking water *ad libitum* decreased tumor incidence by 66% and multiplicity by 40%. Similarly, in the above mouse model, orally fed lawsone (0.0025%) decreased tumor incidence by 72% and multiplicity by 50%. The tumor inhibitory trend continued throughout the 20-week test period. Similar antitumor activities were observed when henna leaf powder (0.5 mg/ml) was applied topically on the back skin in the UV-B initiated, TPA promoted and peroxynitrite initiated, TPA promoted mouse skin carcinogenesis models. Topically applied lawsone (0.015 mg/ml) also exhibited similar protection against tumor formation in the 7,12-dimtehylbenz(a)anthracene induced and TPA promoted skin cancer in mice. Also, there was a delay of 1 to 2 weeks in tumor appearance in both henna leaf powder and lawsone treated groups in all three test models. This study ascertains the skin cancer chemopreventive activity of henna leaf powder and lawsone when administered by either oral or topical routes. Further, the results suggest that lawsone, the pigment artifact is primarily responsible for the observed chemopreventive effect of henna leaf powder in the mouse skin carcinogenesis models used.

PD41 Synthesis and biological activity of some C(9)-hydroxymethyl-5,11-dimethylellipticine derivatives

Mexia N[1], Michel S[2], Rocha VPC[3], Meira CS[3], Macedo TS[3], Carvalho NC[3], Nonato FR[3], Soares MBP[3], Skaltsounis AL[1]
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The alkaloid ellipticine, first isolated from the Australian *Ochrosia elliptica* Apocynaceae, exhibits interesting antineoplastic properties. Due to this great antitumoral activity, numerous compounds have been prepared to overcome the lack of selectivity of this series. The moderate cytotoxic activity of some hydroxymethyl derivatives led us to evaluate their antiparasitic properties against commonly drug resistant diseases. So, starting from ellipticine isolated from *Strychnos diklangei* [1], we have synthesized the 9-formyl derivative and its reduction to 9-hydroxymethylellipticine permitted to achieve new analogues, such as carbamates and acyl esters. These compounds were evaluated for their *in vitro* activity against *L. amazonensis*, *T. cruzi* and *P. falciparum* and also, their cytotoxicity on splenocytes from BALB/c mice. The results were ex-

pressed as IC_{50} and all the tested derivatives demonstrated good activity against the parasites. Especially carbamates and acyl esters, caused the inhibition of the microbes in lower concentrations, without affecting splenocytes. 5,11-dimethylpyridocarbazole = ellipticine References: 1. Michel S. et al. (1980) J. Nat. Prod. 43:2:294 – 295

PD42 Ginkgo Biloba extract modulates proteasomal activity and reduces polyglutamine aggregation in vitro and in vivo

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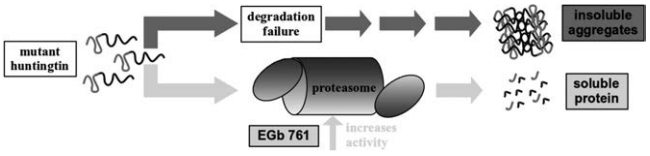
The *Ginkgo biloba* leaf extract EGb 761 has a wide range of neuroprotective activities and has been proposed as a potential neuroprotectant for several neurodegenerative disorders. Our present study describes a novel modulatory effect of EGb 761 in the maintenance of protein homeostasis in cell culture and *C. elegans* models of polyglutamine protein aggregation of mutated huntingtin (mhtt). Analyzing proteasomal activity, which is reduced in mhtt expressing cells, we find that EGb 761 treatment leads to an increase of activity independently of the extent of mhtt aggregation. In addition, EGb 761 treatment is shown (i) to reduce the size of mhtt aggregates and (ii) to increase the soluble cytosolic mhtt fraction. Strengthening these observations, we find that EGb 761 alleviates motility defects in *C. elegans* bearing polyglutamine aggregates and reduces their aggregation tendency. These findings suggest that EGb 761 treatment can have modulatory properties on mhtt aggregation *in vitro* and *in vivo*, presumably by enhancing proteasomal activity.



PD43 Microtubule associated tau protein reducing activity of polyhalogenated monoterpenes

Barhate C[1], Maschek A[1], Jinwal U[2], Zhang J[2], Amsler C[4], McClintock J[4], Baker BJ[1]
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Accumulation of tau protein is a hallmark of several neurodegenerative diseases collectively known as tauopathies. A major tau related disorder is Alzheimer's disease and to a lesser extent Parkinson's disease. Screening of organic extracts of *Plocamium cartilagineum* showed considerable reduction in tau protein level. Further purification of extract yielded two acyclic monoterpenes, anverene and (*1E,3R,4R,5E,7S*)-1,8-Dibromo-3,4,7-trichloro-3,7-dimethyl-1,5-octadiene and one cyclic monoterpene (*1R,2R,4R*)-2,4-dichloro-1-*((E)*-2-chlorovinyl)-1-methyl-5-methylenecyclohexane. Structures and absolute stereochemistry of acylic monoterpenes were confirmed by single crystal X-ray analysis. Our data on drug screening in cell culture models for tau revealed one halogenated monoterpene as a potential treatment for tauopathies.

PD44 The effect of auraptene on folfox-resistant HT-29 colorectal adenocarcinoma cells

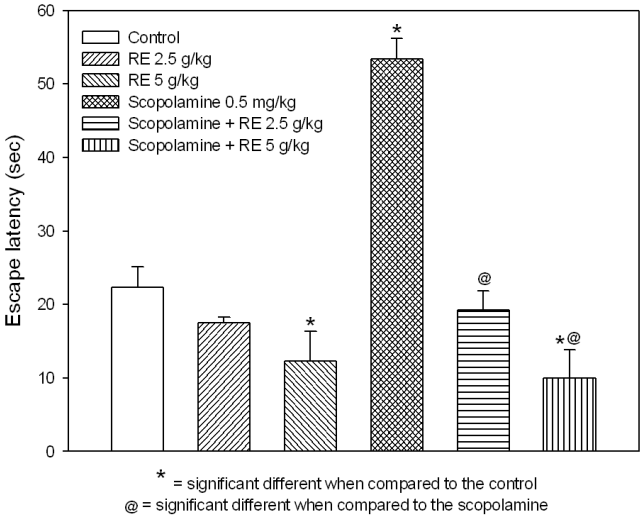
Epifano F[1], Genovese S[1], Miller R[2], Majumdar AN[2]
[1]Dipartimento di Scienze del Farmaco, Università "G. D'Annunzio" Chieti-Pescara, Chieti, Italy; [2]VA Medical Center, Karmanos Cancer Institute, Department of Medicine,Wayne State University, Detroit, Michigan, USA

Investigations on cancer stem cells (CSCs) represent a novel challenge in cancer therapy. Our recent studies seem to indicate that auraptene (AUR), a geranyloxycoumarin extracted from fruits of edible plants belonging to the Rutaceae family, may represent a novel lead compound for dietary colon cancer chemoprevention.[1] Previously, we reported that AUR inhibits the growth of primary colon tumors in rodents.[2] However, nearly 50% of patients with colorectal cancer show recurrence of tumors that exhibit resistance to chemotherapy primarily due to the presence of CSCs. The current investigation was undertaken to determine whether AUR would prevent the growth and sphere (surrogate tumors) formation of FOLFOX-resistant colon cancer cells that are highly enriched in CSCs. Indeed, our results demonstrate that AUR (10 μM) inhibits the growth and formation of colonospheres of FOLFOX-resistant colon cancer HT-29 cells in vitro. The corresponding parental cells were also similarly affected by AUR. The reduction in the growth and colon spheres formation in FOLFOX-resistant HT-29 was also associated with a concomitant decrease in phospho-epidermal growth factor receptor (pEGFR). These observations suggest that AUR could prevent the re-emergence of CSCs. 1. Genovese, S.; Epifano, F. Curr. Drug Targets 2011, *12*, 381. 2. Kohno, H.; Suzuki, R.; Curini, M.; Epifano, F.; Maltese, F.; Prieto Gonzales, S.; Tanaka, T. Int. J. Cancer 2006 118, 2936.

PD45 Unpolished thai purple sticky rice (variety Luem Phua) aqueous extract enhancing spatial memory in mice

Sattayasai J[1], Puapairoj P[1], Arkaravichien T[1], Simasathiansophon S[1], Na Lampang Noenplab A[2]
[1]Department of Pharmacology, Faculty of Medicine, Khon Kaen University, 40002, Thailand; [2]Phitsanulok Rice Research Center, Phitsanulok, 65130

In Thailand, aromatic and indigenous purple sticky rice variety Luem Phua is a variety enriched with flavonoids and many other substances. This study investigated the effect of unpolished Thai Luem Phua rice aqueous extract (RE) on spatial memory in mice. Mice were fed with water or RE, 2.5 or 5 g/kg and SC injected with water or scopolamine (0.5 mg/kg), and subjected to the Morris water maze test. Compared to the control, mice treated with 5 g/kg RE showed a significant decrease, while 0.5 mg/kg scopolamine had a significant increase in escape latency. Pretreatment with either 2.5 or 5 mg/kg RE could ameliorate the scopolamine-induced learning deficit as the escape latency times were significantly less when compared to the scopolamine-treated group. Only 2.5 g/kg RE-treated mice presented a significant longer time in right quadrant than the control. However, when compared to the scopolamine-treated group, mice received RE (2.5 or 5 g/kg) prior to scopolamine treatment had a significant longer time in right quadrant. Thus, this aqueous extract of unpolished Thai Luem Phua rice, with enhancing spatial memory activity, should be potentially useful as dietary supplement.



PD46 Anxiolytic effect of unpolished thai purple sticky rice (variety Luem Phua) aqueous extract in mice

Simasathiansophon S[1], Sattayasai J[1], Puapairoj P[1], Arkaravichien T[1], Na Lampang Noenplab A[2]
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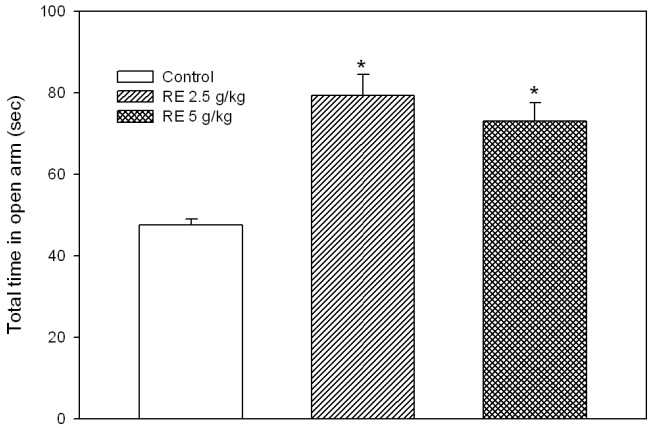
Purple or black sticky rice variety Luem Phua, one of the aromatic and indigenous varieties in Thailand, is a good source of antioxidant polyphenols. In this study an aqueous extract of purple sticky rice variety Luem Phua (RE) was tested for the anxiolytic effect in mice using elevated plus maze and dark-light box tests. Mice were fed with water or RE, 2.5 or 5 g/kg and, after 45 minute, were placed in the elevated plus maze and dark-light box for 5 min each. Duration time in open arm elevated plus maze and time in light part (dark-light box) were determined. In elevated plus maze, total time in open arm during 5 min interval of mice receiving water (control gr.) or 2.5 or 5 g/kg RE were 47.5460 ± 1.5050, 79.3590 ± 5.0320 and 73.0190 ± 4.5860, respectively. The significant differences were existed among the RE-treated groups and the control. No difference could be observed among the two groups of RE-treated. In the dark-light box test, no significant effect of RE treatment could be seen. The results showed that the aqueous extract of unpolished Thai Luem Phua rice possessed anxiolytic effect in mice. Thus, this special rice might be potentially useful as dietary supplement.



PD47 4'-Geranyloxyferulic Acid-L-name prodrug inhibits inflammation-related colorectal carcinogenesis in mice

Tanaka T[1], Genovese S[2], Kochi T[3], Epifano F[2], Scauri L[2], Curini M[4], Shimizu M[3], Moriwaki H[3]
[1]Departments of Tumor Pathology; [3]Internal Medicine, Gifu University School of Medicine, Gifu, Japan; [2]Dipartimento di Scienze del Farmaco, Università "G. D'Annunzio" Chieti-Pescara, Chieti, Italy; [4]Dipartimento di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, Perugia, Italy

We previously reported cancer chemopreventive ability of the naturally occurring cinnamic acid derivative 4'-geranyloxyferulic acid (GOFA) and two β-cyclodextrin inclusion compound of GOFA in colorectal carcinogenesis.[1,2] In this study, potential cancer chemopreventive ability of a newly synthesized prodrug, GOFA-L-NAME, was investigated using a colitis-associated mouse colorectal carcinogenesis model with azoxymethane (AOM) and dextran sodium sulfate (DSS).[3,4] The dietary administration of GOFA-L-NAME significantly reduced the multiplicity of adenocarcinomas (inhibition rates: 100 ppm, 84%, $p < 0.001$; and 500 ppm, 94%, $p < 0.001$), when compared with the AOM+DSS group. Subsequent short-term experiment revealed that dietary GOFA-L-NAME lowered mRNA expression of pro-inflammatory cytokines and inflammatory enzymes in colonic mucosa of mice that received 1.5% DSS in drinking for 7 days. Our findings will be presented. **References** 1. Miyamoto, S. et al. *Nutr. Canc.* **2008**, *5*, 675; 2. Tanaka, T. et al. *Int. J. Cancer*, **2010**, *126*, 830; 3. Kawamori et al., *Cancer Lett.* **2000**, *148*, 33; 4. Tanaka, T. et al. *Cancer Sci.* **2003**, *94*, 965; 5. Tanaka T. *Int. J. Inflammation* **2012**, Article ID 658786. .

PD48 Unpolished thai purple sticky rice (variety Luem Phua) aqueous extract antagonizing scopolamine-induced dementia in mice

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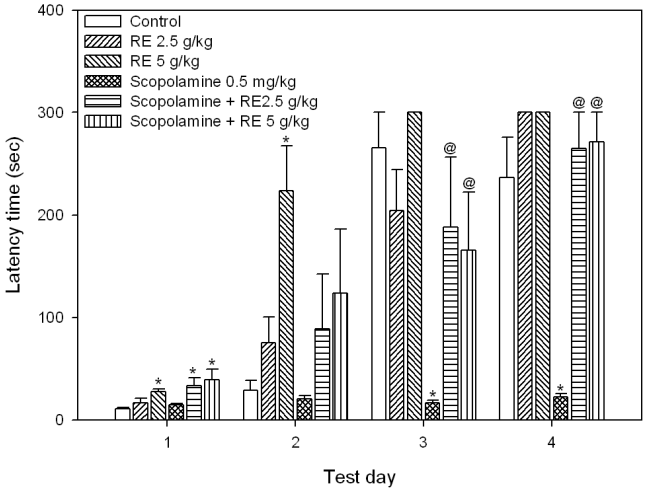
[1]Department of Pharmacology, Faculty of Medicine, Khon Kaen University, 40002, Thailand; [2]Phitsanulok Rice Research Center, Wangthong, Phitsanulok, 65130

Purple or black rice is one of the good sources of anthocyanins, a group of antioxidant polyphenols. In this study an aqueous extract of purple sticky rice variety Luem Phua (RE) was tested for the antagonistic effect against scopolamine-induced amnesia in mice using passive avoidance test. Mice were fed with water or RE, 2.5 or 5 g/kg and subcutaneously injected with water or 0.5 mg/kg scopolamine, and received electrical foot shock in passive avoidance apparatus. Latency time was determined as a training trial followed by 4 test trials on 4 consecutive days. On the test day 1 and 2, a significant increasing in latency time could be observed in mice treated with 5 g/kg RE when compared to the control. The latency time of the animals treated with 0.5 mg/kg scopolamine was significantly seen on test day 3 and 4. Pretreatment with either 2.5 or 5 mg/kg RE could ameliorate the scopolamine-induced memory deficit as the latency times were significantly longer when compared to the scopolamine-treated group on the test day 3 and 4. The results showed that the aqueous extract of unpolished Thai Luem Phua rice could enhance memory in mice, thus, this special rice might be potentially useful as dietary supplement.



PD49 Effect of hydro-alcoholic extract from *Brunella* and *Adiante Capillaire* on calcium oxalate crystallization in-vitro

Mesbah A[1], Goodarzvand M[2], Zamani H[2], Saeidinia A[3], Keihanian F[3], Basirjafari S[3]
[1]Pathology Department, Guilan university of medical science, Rasht, Iran, [2]Laboratory Scineces student and Member of Medicinal Plants research center of student Basij, Guilan University of Medical Science, Rasht, Iran, [3]Medical Student and Member of Medicinal Plants research center of student Basij, Guilan University of Medical Science, Rasht, Iran

Use of herbal medicine is a remedial method for treatment of kidney stones in Iranian traditional medicine. In order to being a therapeutic-hygienic problem for treatment of kidney stone, using herbal medicine will be effective. *Brunella and Adiante capillaire* are two medicinal plants which had been used for kidney disorders in traditional references. In this study effect of hydro-alcoholic extract of these two herbs on crystallization of calcium oxalate in vitro were investigated. Extracts of every herb were provided in the concentrations of 0.25, 1 and 2 mg/ml, at first. Urine samples of 10 adult and healthy persons devided in two groups (by and without adding of extracts). Presence of calcium oxalate crystals were evaluated immediately and 24 hours after induction. Extracts of *Brunella* has been decreased number of crystals against control group in 0.25, 0.5 and 1 mg/ml concentrations ($P < 0.05$) and increased number of mono hydrate calcium optical but *Adiante capillaire* has not ($P > 0.05$).

Index of turbidity by spectrophotometry in none of extracts showed significant difference ($P > 0.05$). Extract of *Brunella* lead to prevention of composition of crystals and it can inhibit creation of kidney stone probably by increase in number of mono hydrate crystals. Animal studies for confirmation of this effect will be offered.

PD50 Ethnopharmacology of two plants used in the treatment of Type 2 Diabetes in Yucatan, Mexico

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Diabetes mellitus type 2 (DMT2) is defined as an elevated blood glucose level associated with an absent or inadequate pancreatic insulin secretion. This may be expressed with or without concurrent impairment of insulin action. DMT2 is one of the most prevalent health problems in Mexico, and common treatment options include a wide variety of both medicinal products and health food plants. We developed a mathematical tool for analysing ethnopharmacological field data, Disease Consensus Index, with the ultimate aim to select species with the most prominent impact in a community to treat a single disease, as a result of the application of this tool *Malmea depressa* R. E. Fries, (Annonaceae) (MD), and *Cecropia peltata* L. (CP) were selected as the prominent species traditionally used in the Mayan communities of south-eastern Mexico to treat the disease. The acute hypoglycemic effect of both plants was confirmed, thereafter was determined whether CP or MD would reduce hepatic glucose production by targeting gluconeogenesis. The effects of the plants extracts on gluconeogenesis (in vivo) and the activity of Glucose-6-Phosphatase (in vitro) were examined. Furthermore, the phytochemical composition of the plants was analysed. The results suggest that administration of the plants can improve glycemic control by blocking hepatic glucose production, especially in the fasting state. These data support its traditional use as an infusion consumed continually throughout the day.

PD51 Identification of the antidiabetic and antihyperlipidenic principles of *Cassia auriculata*

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Cassia auriculata is a popular Asian beverage and medicinal plant widely used in traditional medicine for treating diabetes and hyperlipidemic conditions. The scientific evidence for the folklore uses of the plant have been provided in recent years but the active principles remains to be isolated and identified. In the present study, an alpha-glucosidase and pancreatic lipase enzyme inhibition studies as *in vitro* model of diabetes and obesity assays respectively were used. The clinically used drugs including acarbose and orlistat were used to serve as positive controls. It was found that the crude extract of *C. auriculata* has significant effect in both assay systems. Bioassay-guided fractionation of the crude extract resulted in the identification of kaempferol-3-*O*-rutinoside along with many related compounds. The activity profile (including synergism), therapeutic potential and structure-activity-relationship of the isolated compounds are discussed

PD52 Dehydroeburicoic acid, an antileukemic triterpene from the fruiting bodies of dish-cultured *Antrodia Cinnamomea*

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[1]*Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan;* [2]*Graduate Institute of Marine Biotechnology, National Dong Hwa University, Pingtung, Taiwan;* [3]*Yung Peng Biotechnology. CO., LTD, Taiwan;* [4]*School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan*

Antrodia cinnamomea (AC), an endemic species, has long been used as a chemopreventive mushroom in traditional Taiwanese medicine. In this current study, we reported a standardized triterpenoids-dependent analysing platform for marketing AC products. Twelve different AC products were collected and evaluated for their triterpenoids content. The results revealed that fruiting bodies from dish-cultures (AC-DC2) had a similar triterpenoids profile compared to wild AC. The AC-DC2 ethanolic extract was subjected to bioactivity-guided isolation and yielded five major cytotoxic triterpenes (**1**-**5**). Among them, dehydroeburicoic acid (**5**) was the most potent cytotoxic component against HL-60 cells while it activated DNA damage, apoptosis biomarkers and also inhibited topoisomerase II. In the xenograft animal model, **5** resulted in a marked decrease of tumour weight and size without any significant decrease in mice body weights. Taken together, our results showed that fruiting bodies from the dish-cultured AC, which had the same major active triterpenoids compared to wild AC, could be considered as one of alternative AC sources.

PD53 Antihypertensive and vascular protective effects of subchronic treatment with a standardized fraction of Hancornia Speciosa Gomes

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Aim: to investigate the antihypertensive and vascular protective effects of a standardized fraction from leaves of *Hancornia speciosa* (SFH) in hypertensive mice. **Methods:** Male normotensive (SHAM) and hypertensive Doca-salt (DOCA) Swiss mice were used. Systolic blood pressure (SBP) was measured by tail pletismography. SFH was given on drinking water (0.1 mg/kg/day) for 35 days to SHAM and DOCA. Small mesenteric artery was used for vasodilatation experiments. Nitrite and lipid peroxidation levels were measured by spectrophotometry. Protein expression was analyzed by Western-blot and mRNA levels by real-time PCR. **Results:** Treatment with SFH brought SBP in DOCA to normotensive level (192 ± 4 to 131 ± 2 mm Hg; untreated and treated, respectively). Improved the endothelium-dependent vasodilation induced by acetylcholine (Emax = 44.3 ± 1.2 and 73.0 ± 2.0%, untreated and treated, respectively). In addition, SFH also blunted the increased sensitivity to contractile agents (Emax = 8.5 ± 0.8, 4.2 ± 0.2 and 4.1 ± 0.4 mN/mm, in untreated, treated DOCA and SHAM mice, respectively). Treatment with SFH prevented lipid peroxidation (0.188 ± 0.03 μM and 0.044 ± 0.01 μM, in untreated and treated DOCA, respectively). Inasmuch, restored the levels of plasmatic nitrite in DOCA (19 ± 2 μM and 48 ± 3 μM, in untreated and treated, respectively) compared to SHAM (46 ± 3 μM). Levels of total eNOS and phosphorylated Ser1177 were improved in DOCA after treatment. ET-1 and pre-pro-ET-1 mRNA levels were reduced after treatment with SFH. Conclusion: SHF presents a strong antihypertensive effect at low dose accompanied by a significant protective effect in the cardiovascular system.

PD54 Vasorelaxation induced by 1-Nitro-2-Phenylethane in rat aorta involves guanylate cyclase stimulation

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1-Nitro-2-phenylethane (NPE), a hypotensive substance found in the essential oil of *Aniba canelilla*, has vasodilator properties. Here, the underlying mechanism of such effect was studied in rings of rat aorta under isometric recording conditions. In endothelium-intact aortic rings, NPE (1 – 300 μg/mL) relaxed the phenylephrine (PHE)-induced contractions with IC_{50} values of 35.0 [23.3 – 52.6] μg/mL, effect significantly ($n = 22$, $P < 0.05$, Mann-Whitney) decreased by ODQ (10 μM, $n = 6$) or methylene blue (10 μM, $n = 9$) but not by endothelium removal ($n = 7$) or by pretreatment with L-NAME (100 μM, $n = 6$), indomethacin (10 μM, $n = 6$), MDL-12,330A (3 μM, $n = 7$), KT5823 (0.5 μM, $n = 7$) or KT5720 (1 μM, $n = 8$). In Ca^{2+}-free medium, in presence of K^+ (60 mM, $n = 10$) or PHE (1 μM, $n = 7$), $CaCl_2$-induced contractions were almost abolished by NPE at 100 μg/mL. In Ca^{2+}-free medium, containing EGTA, the contractile response of PHE was significantly reduced by NPE (100 μg/mL, $n = 6$), an effect prevented by treatment with ODQ (10 μM, $n = 6$) and was inert on caffeine-induced contraction ($n = 6$). Similar results were obtained with sodium nitroprusside ($n = 6$). In silico (docking) simulation revealed clusters of interactions of NPE with the guanylate cyclase molecule. Thus, the vasorelaxant activity of NPE on rat aorta appears due to its stimulatory properties on guanylate cyclase.

PD55 Reversal of multidrug resistance by purgin ii in human breast cancer cells

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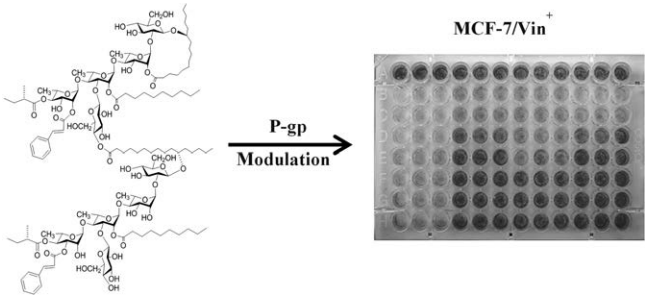
Reversal of multidrug resistance (MDR) by purgin II, a new resin glycoside isolated from *Ipomoea purga*, was evaluated in vinblastine-resistant human breast carcinoma cells (MCF-7/Vin). The effects on the cytotoxicity and P-glycoprotein (P-gp)-mediated MDR were estimated with the sulforhodamine B colorimetric assay. Purgin II enhanced vinblastine activity > 1906-fold when incorporated at 25 μg/mL and increased the intracellular accumulation of rhodamine 123 based on flow cytometry. Incubation of MCF-7/Vin cells with tested compound notably lowered the efflux rate of rhodamine 123.



PD56 Antifungal activity of turmeric creams at different concentrations

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Curcuma longa L. or turmeric of the family Zingiberaceae is widely used for treatment of rash, itching, tinea and ringworm. Turmeric oil possesses effective antifungal activity against dermatophytes, a group of fungi that causes skin diseases. The creams containing 6 and 10%w/w turmeric oil promoted effective antifungal activity with mild irritation[1]. In this study, the 6 and 10%w/w turmeric creams stored for 6 months under 3 conditions i.e. in a refrigerator (4 – 8 °C), at room temperature (25 – 28 °C) and at 45 °C were tested for their antifungal activities by broth dilution method for the minimum fungicidal concentration (MFC) compared to cream base and Daktarin® cream in solution form (0.07 g/mL) as negative and positive controls, respectively against 4 dermatophytes (*Trichophyton mentagrophytes*, *T. rubrum*, *Epidermophyton floccosum* and *Microsporum gypseum*). After 6 months of storage, 6%w/w turmeric cream kept at 4 – 8 °C and 25 – 28 °C showed stable antifungal activity while only 10%w/w turmeric cream stored under 4 – 8 °C showed the stable activity. Six percent turmeric cream showed better antifungal activity than the 10%w/w cream and should be used for further clinical trial tested. **Reference** 1. Pitakvongsaporn P. The study of antifungal activity stability and skin irritation of turmeric oil. M.Sc. Thesis, Mahidol University. Thailand, 2000.

PD57 Oral administration of dietary scallion extract suppresses colorectal tumor growth in murine model

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Colorectal cancer is a common malignancy and a leading cause of cancer death worldwide. Diet is known to play an important role in the etiology of colon cancer and dietary chemoprevention has recently received increasing attention for prevention and/or alternative treatment of colon cancers. Allium fistulosum L., also commonly known as scallion, is used as a popular spice or vegetable worldwide, and as traditional medicines in Asian cultures for treating a variety of diseases. The possible beneficial effects of dietary scallion on chemoprevention of colon cancer are evaluated in this study, using a subcutaneously inoculated CT-26 colon tumor model in BALB/c mice. Tumor lysates were subjected to western blotting for analysis of key inflammatory markers, ELISA for analysis of cytokines, and immunohistochemistry for analysis of inflammatory markers. Metabolite profiling of scallion extracts were analyzed by LC-MS/MS. Scallion extracts, particularly the hot-water extract, orally fed to mice at 50 mg (dry weight)/kg body weight resulted in a significantly suppression of tumor growth and enhanced the survival rate of test mice. At the molecular level, scallion extracts inhibited the key inflammatory markers COX-2 and iNOS, and suppressed the expression of various cellular markers known to be involved in tumor apoptosis (apoptosis index), proliferation (cyclin D1 and c-Myc), angiogenesis (VEGF and HIF-1α), and tumor invasion (MMP-9 and ICAM-1), when compared with vehicle control-treated mice. Our findings may warrant further investigation for the use of common scallion as a chemopreventive dietary agent to lower the risk of colon cancer.

PD58 Isolation of a rhamnogalacturonan polysaccharide from *Spilanthes oleracea* with gastroprotective and healing effects

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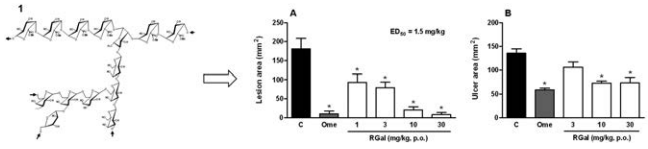
The aim of this study was to investigate the gastroprotective and healing effects of rhamnogalacturonan polysaccharide [RGal **(1)**] isolated from *S. oleracea*. Oral treatment of animals with RGal (1 – 30 mg/kg) reduced the ethanol-induced acute gastric lesions with ED_{50} of 1.5 mg/kg **(A)**, without alter the protective factors of gastric mucosa, mucus and reduced glutathione (GSH). Besides, i.p. administration of RGal (0.01 – 1 mg/kg) also inhibited the gastric lesions induced by ethanol, increasing the mucus, but not the GSH levels of gastric mucosa. Furthermore, RGal (10 and 30 mg/kg) showed healing property in the model of chronic gastric ulcer induced by 80% acetic acid **(B)**. These results showed that RGal has an interesting gastroprotective and healing activity; however, more studies must be carried out to identify the mechanisms of action.



PD59 Mechanisms involved on the antihypertensive effect from leaves of a standardized fraction from Hancornia Speciosa

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Aim: to investigate the antihypertensive effect and its mechanism of action of a standardized fraction of Hancornia speciosa leaves (SFH) in hypertensive mice. **Methods:** Male Swiss mice were submitted surgery doca-salt. Systolic blood pressure (SBP) was measured by tail pletismography. Each mouse (SBP 183 ± 6 mm Hg) received single dose of SFH by oral (po) route and SBP was monitored every hour until 5 hours. First branch of small mesenteric arteries mounted in myograph were used for vasorelaxation procedures. Nitrite levels were measured by spectrophotometry in the serum 1 hour after po administration of SFH (1 mg/kg) or L-NAME (20 mg/kg, ip). **Results:** SFH at doses from 0.03, 0.1 and 1 mg/Kg induced significant and long-lasting reductions (5.1 ± 2, 25 ± 5, 60 ± 6 mm Hg, up to 4 hours) on SBP compared to saline. In resistance vessel SFH produced a concentration-dependent vasorelaxation (IC_{50}=–4.58 ± 0.16 μg/mL) in mesenteric arteries pre-contracted with phenylephrine (3 μM). SFH induced a significant increase on plasmatic content of nitrite in mice (98 ± 8 μM). Pre-treatment of mice with L-NAME blunted the effect of SFH. The relevance of this effect was confirmed by the inhibitory effect of L-NAME on the antihypertensive effect of SFH. **Conclusions:** we showed that a SFH is an antihypertensive drug, reduced the SBP through vasodilatation in resistance artery and by increase on plasmatic level of NO. Therefore, these results strongly suggest

that SFH possess a great potential for its use on the treatment of hypertension.

PD60 Selective Inos and COX-2 inhibition of *Morinda Citrifolia* (Noni) fruit extract in rat gastric ulcerated mucosa

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Gastric nitric oxide synthase (nNOS, iNOS) and cyclooxygenase (COX-1 and COX-2) inhibitory activity of *Morinda citrifolia* (noni) aqueous fruit extract (AFE) and ethanolic fruit extract (EFE), two common forms of noni fruit extract used in traditional medicine, was studied in rats treated with topical application of acetic acid. The gastric ulcerated area was excised and the mRNA expression of NOS and COX was analyzed using one-step RT-PCR analysis. Treatment with AFE and EFE at the dose that contain 0.5 – 1 mg/kg of active ingredient: scopoletin for 10 d after ulcer induction markedly accelerated ulcer healing that related to their inhibitory activity on an increased mRNA expression level of both pro-inflammatory enzymes (iNOS and COX-2) at the transcription level and at only the excess expression level, but had no markedly inhibitory effect on the mRNA expression level of constitutive nNOS and COX-1 which produce gastric mucosal protective NO and PGs respectively. This indicated that both the fruit extracts at the dose use exerted an optimal oral dose for further clinical study on treatment of human chronic gastric ulcer.

PD61 Effects of peat distillates in adjuvant arthritis of rats

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In continuation of our studies on efficacy of peat distillates [1], anti-inflammatory effects in model of adjuvant arthritis (AA) were studied. Samples of peat were collected in October 2010 by Dr. N. Demidova. The peat was distilled with steam. AA was induced by a single injection of 0.l mL of Freund's adjuvant in the aponeurosis of right paw of rats. After 5 days rats were injected with peat distillates (1.5, 3.0 and 4.5 mL) during 21 days. Ibuprofen was used as positive control. The edema volume was determined using oncometric method, antipyretic and analgesic effects were registered [2]. Anti-edema effect was not registered. The antipyretic effect of peat distillate (3.0 mL) was registered after 9 days of application and after 21 days the temperature was practically normalized. The response time of rats in hot plate test was increased 2 – 2.5 -fold compared to the control group after 21 days. Arthritic lesions found in roentgenographic examinations were also inhibited in peat distillate-treated animals. The findings suggest that peat distillates may used for the treatment of AA. References: 1. Makarova et al. (2011), Planta Med., 77, 1411; Shikov et al. (2010) Phytomedicine, 17, 463 – 468. Acknowledgements: the study was supported by FORESTSPECS project, grant agreement 227239.

PD62 Evaluation of the cytoprotective effects of individual and combined constituents of the combination drug STW 5

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We investigated the effects of individual herbal extracts of STW 5 (Iberogast®) for cytoprotective action in CaCo-2 cells. Possible synergistic effects were determined by isobologram analysis of LPS (10 ng/ml, 2 h)-induced cell toxicity using a commercially available LDH kit. STW 5 significantly inhibited LPS-induced LDH-release. Comparable effects were found with lemon balm, whereas in equivalent concentrations to STW 5 the effects of *Iberis amara*, peppermint, chamomile, angelica and milk thistle were less potent. Caraway and celandine had stimulatory effects. Isobolgram analysis indicated that a 2-h exposure to peppermint and Iberis amara (ratio 0.5/0.5) had a synergistic effect. Less pronounced synergy was observed using the component ratio 0.75/0.25. An additive effect was present in the ratio 0.25/0.75. Application of peppermint together with milk thistle (ratio 0.5/0.5) showed ratio-independent synergistic effects whereas the combined application of chamomile and *Iberis amara* had ratio-independent additive effects. The combination of chamomile and angelica (ratio 0.5/0.5) exerted an additive effect, the ratio 0.75/0.25 showed an antagonistic effect which increased using the ratio 0.25/0.75.The observations confirm that the components of STW 5 contribute differently to the cytoprotective effect in CaCo-2 cells and clearly indicate that synergistic, additive and antagonistic effects exist between the extracts which are ratio dependent.

PD63 Protective effect of suberin against CCl_4-induced hepatotoxicity

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Suberin is a natural lipidic biopolymer found in the cell walls of normal and wounded external tissues of aerial and subterranean organs of plants. In frame of ongoing project FORESTSPECS effects of suberin against CCl_4 induced hepatotoxisity was studied for the first time. Suberin was extracted from outer bark of birch according to the literature procedure [1]. Hepatotoxicity was induced by intragastric administration of CCl_4 for 4 weeks (0.5 mg/kg, every 4 days). Suberin in dose 5, 10 and 15 mg/kg was administered by gavage during 10 days starting on 21 day of experiment. Extract of *Silybum marianum* (18 mg/kg) was used as positive control. Serum aminotransferase levels, bilirubin, alkaline phosphatase and malondialdehyde were increased by CCl_4. Suberin significantly decreased the level of ALT, bilirubin and malondialdehyde in dose of 15 mg/kg, and alkaline phosphatase in dose of 5 and 10 mg/kg. Histopathological examination for rat livers observes positive improvements of liver tissues. This study provides evidence that suberin is potent candidate for treatment of hepatocellular damage of liver. Reference: Ekman et al., US Pat. 4732708 Mar. 22, 1988 Acknowledgements: the study was supported by FORESTSPECS project, grant agreement 227239.

PD64 Antiviral effect of polyphenol-enriched extract of *Rumex Acetosa* L. against Influenza A virus

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Although effective antiviral drugs are available for the treatment of influenza A virus (IAV) infection, the need for intensified drug discovery towards antiviral active compounds is obvious. A polyphenol-enriched extract of *Rumex acetosa* L. (Polygonaceae), standardized on galloylated oligomeric proanthocyanidins, was tested for antiviral activity against IAV *in vitro* using MTT, plaque reduction and hemagglutination assay. The extract inhibited IAV replication in a dose-dependent manner. IC_{50} and CC_{50} values, as determined by MTT assay, were 2.2 µg/mL and 79.2 µg/mL, respectively, indicating a CC_{50}/IC_{50} selectivity ratio of 36. This antiviral activity was confirmed by plaque reduction assay. To determine the mechanism of this antiviral effect, the extract was added at different stages during the viral replication cycle. The strongest antiviral effect was observed when the extract was added before adsorption of IAV to the cells, but in high concentrations an effect was also detected when extract was added after adsorption of IAV to and even after penetration of IAV into the cells, indicating the antiviral effect being associated not only with viral adsorption and penetration. Structure-activity relation of flavan-3-ols and oligomeric proanthocyanidins indicated that trihydroxylation of ring B or alternatively galloylation at position O-3

potentiate the antiviral activity. Detailed studies using the model compound procyanidin-B2-digallate indicated this compound as a potent inhibitor of IAV.

PD65 Bioassay-guided fractionation of *Eupatorium Perfoliatum* L. towards anti-Influenza A-activity leads to dicaffeoyl quinic acids

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Ethanolic extracts from *Eupatorium perfoliatum* L. (Asteraceae) are traditionally used for fever treatment. Furthermore reports on the use against viral-associated common colds are documented (Contramutan®). An ethanol-water extract (62%) from *E. perfoliatum* was found to be active in MTT and plaque reduction assay against influenza A virus (IAV) H1N1v 2009 with an IC_{50} of approx. 55 µg/mL due to an inhibition of viral entry. Bioassay-guided fractionation towards the active compounds led to the isolation of active fraction B (IC_{50} approx. 48 µg/mL). Further fractionation of B into EtOAc- and MeOH-soluble subfractions yielded 2 antiviral multicomponent fractions C and D, resp. The antiviral activity found in the ethanolic extract was due to compounds interacting with polyvinylpyrrolidone (PVP). Treatment of fraction D with PVP and subsequent LC-MS studies revealed a drastic reduction of peaks, which should hence comprise the antiviral compound. The respective peaks were assigned to dicaffeoyl quinic acids and flavonole glycosides. Isolation and *in vitro* testing of these compounds to verify this result towards the development of a new class of antiviral compounds leads to 3 yet unidentified dicaffeoyl quinic acids. Summarizing, the traditional use of extracts from *E. perfoliatum* against viral respiratory diseases seems to be justified, due to the presence of polyphenolic components with antiviral activity.

PD66 Flavonoids with xanthine oxidase inhibitory activity isolated by guidance of bioassay from *Artemisia asiatica* Nakai

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Artemisia asiatica has been used in the traditional oriental medicine for the treatment of cancer, gastritis, ulcers and other inflammatory disorders. Previous *in vivo* and human studies demonstrated the antioxidant and anti-inflammatory effects of its formulated EtOH extract DA-9601 on gastro-intestinal injuries. It was stated, that its therapeutic effect is mediated partly through the inhibition of gastric xanthine oxidase (XO) activity, which is a late enzyme of purine catabolism, well known as a major source of reactive oxygen species generation in the pathogenesis of various diseases. In our experiment the XO inhibitory activity of *A. asiatica* was investigated, followed by a bioactivity guided fractionation aiming the isolation of the components responsible for the activity. The MeOH extract, and its flavonoid-containing fractions obtained by CC on polyamide, significantly inhibited the XO induced uric acid production. The pure flavonoids were isolated using VLC, CPC and PLC and identified as eupatilin, jaceosidin, hispidulin, chrysoplenetin, cirsilineol, 5,7,4'-trihydroxy-6,3',5'-trimethoxyflavon and 5,7,4',5'-tetrahydroxy-6,3'-dimethoxy-flavon by means of UV, NMR and MS. With except of chrysoplenetin and cirsilineol, all compounds exerted remarkable XO inhibitory effect with IC_{50} values between 1.18 – 16.69 µM. Acknowledgements: This work was supported by the New Hungary Development Plan projects TÁMOP-4.2.1/B-09/1/KONV-2010 – 0005 and TÁMOP-4.2.2/B-10/1 – 2010 – 0012.

PD67 Discovery of antiasthmatic natural products from Mexican medicinal plants

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Diseases such as asthma and COPD (chronic obstructive pulmonary disease) involve bronchoconstriction of airways. Several natural products and medicinal plants have shown the ability to produce relaxation of airway smooth muscle. The flavones gnaphaliin A and B were identified as tracheal smooth muscle relaxant active principles from the *n*-hexane extract of *Gnaphalium liebmannii* Sch. Bp ex Klatt (family *Asteraceae*). The main relaxant mechanism of action of these flavones is the inhibition of PDEs with a preference to inhibit the degradation of cGMP. The docking study suggested that these flavones bind with high specificity to the same binding site of sildenafil at PDE type 5. The content of gnaphaliins varies widely with the particular *Gnaphalium* species. On the other hand the alkaloid berberine (as carbonate) was identified as one of the active relaxant principles in the dichloromethane extract from the aerial parts of *Argemone ochroleuca* (Papaveraceae). The relaxant effect of berberine on tracheal muscle is due to its antagonistic effect on muscarinic acetylcholine receptors. Tymol has been identified as active principle in other Mexican medicinal plants and aqueous extracts of *Bougainvillea glabra* potentiates the relaxant effect of salbutamol and ipratropium. *Acknowledgements*: This work was supported by grant of Dirección General de Asuntos del Personal Académico DGAPA-UNAM IN210112.

PD68 Beta-ecdysone (Ecd) prevents visceral, bone marrow and joint fat accumulation and has positive effects on serum lipids, bone and joint cartilage

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Ecd prevents obesity and osteoporosis following ovariectomy (ovx) of rats. Whether it also protects joint cartilage was compared with the effects of estradiol/E2). Furthermore the effects of Ecd in 20 slightly overweight male and female persons were also determined. In ovx rats Ecd reduced the amount of abdominal, bone marrow and joint fat ($p < 0.05$) and this resulted in better trabecular and joint cartilage architecture. In the open clinical trial with slightly overweight persons a daily intake of 200 mg Ecd reduced waist circumference by 2.7 cm, serum cholesterol by10,2%, LDL by 13.9% and triglycerides by 31.1% whereas HDL were increased by 10.8%. It is concluded that Ecd has similar osteo- and chondro-protective effects as E2 and prevents fat accumulation in the abdomen, bone marrow and joints. Hence, Ecd may prevent the Metabolic Syndrome and the accompanying osteoporosis and osteoarthritis.

PD69 *In vitro* attenuation of acrolein-induced toxicity by phloretin, a phenolic compound from apple

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In the current study, the protective effects of phloretin were investigated in acrolein-challenged amino acid, protein, and cell models. It was found that the formation of acrolein-lysine adducts FDP-lysine was strongly inhibited with the presence of phloretin and the remaining electrophilic site in FDP-lysine was also blocked by phloretin. Moreover, direct trapping of acrolein by phloretin was found to be responsible for inhibiting the incorporation of carbonyl groups into BSA and oligomerization in RNase A. Subsequently, the reduction of LDH release in human neuroblastoma SH-SY5Y cells under acrolein challenge suggested the cytoprotective effects of phloretin. Such protection might be mediated through inhibiting the increased cellular protein carbonyl level as revealed by Western blotting analysis. Taken together, findings of the present study highlighted an apple phenolic compound, phloretin as a promising candidate in prevention or treatment of acrolein-associated human diseases.

PD70 Cytotoxic activity, acute and sub-acute toxicity of root extracts of *Corrigiola Telephiifolia Pourr*

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C. telephiifolia is an herbal plant commonly used in Moroccan folk medicine for treatment of many disorders. In the present study, we investigated cytotoxic activity by an *in vitro* assay system of growth inhibition against a human cancer cell line (HeLa), the results demonstrated that CH_2Cl_2 extract show a moderate cytotoxicity. The acute and sub-acute toxicity of the methanolic extract was evaluated. For acute toxicity, a single oral administration was performed at a dose of 2 g/kg body weight (6 females, 6 males mice). The study of sub-acute toxicity was evaluated by daily oral (5 females, 5 males) with doses from 10 to 500 mg/kg/day for 45 days. No mortality or signs of toxicity were observed in the acute study. Mice were analyzed for final body and organ weights, necropsy, and blood chemical and histopathological parameters. In the 40 day study in mice the extract at 10, 50 mg/kg/day showed no toxicity, mortality, macroscopic or microscopic change of internal organs or tissues in comparison with the control group. The extracts of *C. telephiifolia* showed a cytotoxic activity with low toxicity. They are good candidates for further investigations in the fields of new anticancer drugs discovery.

PD71 Reversal of P-Glycoprotein-mediated multidrug resistance by anthraquinones through cyclooxygenase-2 inhibition

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We assessed the effects of anthraquinone derivatives from Rhubarb on LPS-induced RAW 264.7 macrophages in order to determine their anti-inflammatory potential. Also, the derivatives were tested in Caco-2 cell lines to evaluate the inhibition of P-gp expression. Among them, emodin significantly inhibited NO production, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression, and prostaglandin E2 (PGE_2) secretion. Furthermore, emodin reduced paw swelling in the carrageenan-induced paw edema mice model. In Caco-2 cells, emodin elevated the accumulation of R-123 and decreased the efflux ratio of R-123, which indicates P-gp inhibition. The inhibition of COX-2 protein by emodin paralleled the decrease in P-gp expression. In addition, MAPK expression was decreased through the prevention of AP-1 DNA binding, which leads to down-regulation in the expression of P-gp. Our data indicate that the inhibition of P-gp function is caused by the decreased expression of COX-2 through the MAPK/AP-1 pathway. In addition to its anti-inflammatory potential, emodin is a novel P-gp inhibitor.

PD72 Wound healing activity of Triterpenes from birch bark – insights into the molecular mechanism

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Birch bark extract (TE), which consists of triterpenes such as betulin, lupeol and betulinic acid, was shown to exert promising wound healing effects in patients [1]. Here, we report studies on the underlying molecular mechanisms. We demonstrate that TE and betulin influence the inflammatory phase of wound healing by upregulating varieties of pro-inflammatory cytokines, chemokines and cyclooxygenase-2 (COX-2) in human primary keratinocytes. These mediators play a crucial role in cell migration, proliferation and angiogenesis. We provide evidence with COX-2 that its increase is due to a mRNA stabilizing effect, a process in which p38 MAPK and HuR are essentially involved. In the new tissue formation phase, controlled migration of keratinocytes at the wound edge is a crucial step in wound healing and requires a coordinated reorganization of the actin cytoskeleton. We demonstrate that TE, betulin and lupeol increase the formation of actin filopodia and lamellipodia, a process which is dependent on the activation of Rho GTPases. [1] Metelmann et al., in preparation. Acknowledgement: Financial support from the Federal Ministry of Economics and Technology is gratefully acknowledged.

PD73 Chemical investigation and evaluation of analgesic and anti-inflammatory activies of *Copaifera langsdorffii* leaf extract

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Copaifera langsdorffii Desf. (Leguminosae-Caesalpinioideae) is found in northern and northeastern of Brazil. Aqueous ethanolic extract of *C. langsdorffii* was subjected to solvent-solvent partitioning followed by counter-current chromatography (HSCCC), gel filtration and HPLC, to yield quercetin-3-*O*-α-L-rhamnopyranoside, kaempferol-3-*O*-α-L-rhamnopyranoside, 6'-methoxy,4''-methoxy-3,4-di-*O*-galloylquinic acid and 6'''-methoxy-3,4,5-tri-*O*-galloylquinic acid. Analgesic and anti-inflammatory activities were evaluated using acute toxicity, open field, acetic acid-induced writhing response, hot plate, formalin, λ-carrageenan and dextran-induced paw edema and LPS-induced cell migration assays. The extract was found to be non toxic to mice and did not interfere with their normal behavior. It showed activity in writhing, formalin (2^{nd} phase) and paw edema induced by λ-carrageenan and dextran assays. The compounds, quercetin-3-*O*-α-L-rhamnopyranoside and kaempferol-3-*O*-α-L-rhamnopyranoside were inactive in writhing assay. Kaurenol, previously isolated by our group from the aqueous ethanolic extract of leaves of *C. langsdorffii*, displayed activity in writhing, formalin (2^{nd} phase) and edema paw induced by λ-carrageenan and dextran assays. These data suggest that the activity of the aqueous ethanolic extract of leaf of *C. langsdorffii* may be related to modulation of the inflammatory process.

PD74 Akebia saponin PA induces autophagic and apoptotic cell death via MTOR and MAPK pathways in gastric cancer cells

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Akebia saponin PA (AS), a natural product isolated from *Dipsacus asperoides*, exhibits cytotoxicity in several cancer cell lines through a mechanism that is not yet defined. To better understand AS-induced cell death and its underlying mechanism, experiments were performed in human gastric cancer cells. The present studies illustrate that AS-induced cell death is caused by autophagy and apoptosis. The autophagy-inducing effect of AS was observed at an early stage, as indicated by the formation of cytoplasmic vacuoles and microtubule-associated protein 1 light chain-3 II (LC3-II) conversion while apoptosis-induction was characterized by DNA fragmentation, flow cytometric analysis, caspase-3 activation and the cleavage of poly(ADP-ribose)polymerase-1 (PARP-1). The autophagy inhibitor bafilomycin A1 decreased AS-induced cell death and caspase-3 activation, but caspase-3 inhibitor Ac-DEVD-CHO did not affect LC3-II accumulation or AS-induced cell viability, suggesting that AS induces autophagic cell death and autophagy contributes to caspase-3 mediated apoptosis. Molecular mechanism studies indicate that AS-induced autophagy is regulated by the p53/AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) and Akt/mTOR signaling pathways and enhanced autophagic flux promotes mitogen-activated protein kinases (MAPKs) modulated apoptosis.

PD75 Ursolic acid, a potential PPARγ agonist, suppresses Ovalbumin-induced airway inflammation and hyperresponsiveness

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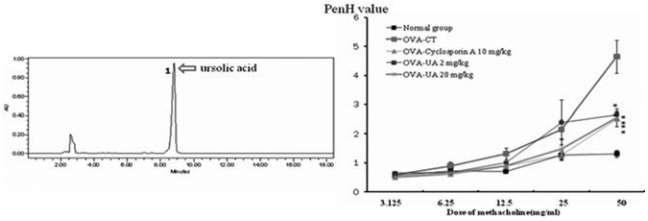
Ursolic acid (UA) was known for its pharmacological effects, such as anti-tumor, anti-inflammatory and antimicrobial activities. In BALB/c mice, we found that UA-treated groups had been suppressed eosinophil infiltration, allergic airway inflammation and AHR. Our data suggest that the therapeutic mechanism by which UA effectively treats asthma is based on reductions of Th2 cytokines (IL-5, IL-13), OVA-specific IgE production and eosinophil infiltration through Th2-STAT6 pathway and IL-17-NF-κB pathway.



PD76 Effect of pomegranate juice, punicalagin and ellagic acid on fructose mediated glycation of albumin

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Non-enzymatic glycation of proteins results in the formation of advanced glycation endproducts (AGE), leading to the crosslinking of proteins. The process is believed to be responsible for the mobidity and mortality associated with the diabetic state. Currently, there is no FDA approved therapeutic agent to inhibit protein glycation, however numerous natural products have been shown to inhibit this process. In this study, we examined the effect of pomegranate juice and its constituents (punicalagin and ellagic acid) on protein glycation and compared them to other commercially available fruit juices. Bovine serum albumin (10 mg/ml) was incubated with 250 mM fructose for 72 hours and the extent of glycation determined fluorometrically at the wavelength pair of 370/440 nm. Pomegranate juice, at 10 μL/ml, inhibited protein glycation by 98%; this inhibition was much greater than that observed with other juices. The greater inhibition by pomegranate juice was also observed when the juices were added at the same phenolic concentration and the same FRAP (antioxidant capacity) values. Both punicalagin and ellagic acid inhibited protein glycation by approximately 90% when present at a concentration of 5 μg/ml. These results demonstrate that pomegranate juice and its constituents are robust inhibitors of fructose mediated protein glycation.

PD77 Effect of *Arrabidaea chica* verlot ethanolic extract on epithelial cells viability exposed to a bisphosphonate

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Bisphosphonates alter bone and oral epithelial cells growth and proliferation. *Arrabidaea chica* (H&B.) Verlot is a common tropical American plant with a wound healing activity previously described. This study aimed the evaluation of *A.chica* extract effect on the viability of human keratinocytes (HaCat) previous or after exposure to Zoledronic Acid (ZA). Cells (3×10^4cel/mL, RPMI/FBS 5%, 100 μL/well) in 96-well plate were incubated (37 °C, 5%CO_2). After 24 h, they were submitted to ZA (10 μM) or *A.chica* (AC; 50, 100 or 150 μg/mL) treatment, both in RPMI/FBS 0.3%. After another 24 h, these treatments were switched and cells were incubated for 48 h. Cells were fixed with 50% Trichloroacetic acid and had their viability determined by Sulforhodamine B method. All experiments were done in triplicate and 3 plates, for each incubation period, used for comparison of the results. Data was expressed as media ± SD. HaCat cells exposed to ZA for 72 h had shown 31.9%(± 4.4) of cell viability. AC treatment previously to ZA resulted on cell viability of 79.4%(± 7.7), 88.2%(± 0.5) and 94%(± 4.3) whereas AC treatment after 24 h ZA exposition promoted a viability of 30.4%(± 4.3), 23.7%(± 1.0), 35.8%(± 19.8), respectively to 50, 100 and 150 μg/mL of AC. These data showed that *A. chica* extract treatment of epithelial cells previously to ZA exposure was able to protect these cells against the harmful effects of this bisphosphonate.

PD78 Might paretin have effect against tau protein?

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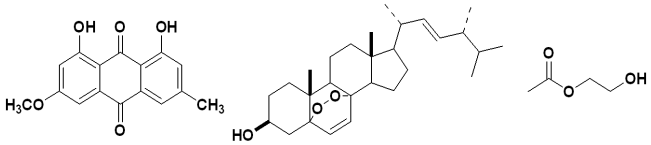
Alzheimer disease (AD) is the most common neurodegenerative disorder involving amyloid-β and neurofibrillary tangles, composed by hyperphosphorylated tau protein. We investigate parietin **1** in the context of their action to prevent tau alterations in the etiopathogenesis of AD. *Xanthoria ectaneoides* and *Arthopyrenia saxicola*, two marine lichens, were studied for the first time given parietin as the major compound, ergosterol peroxide **2** and 2-hydroxyethylacetate **3**. The effects of parietin against the aggregation process of tau protein will be presented and discussed.



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PD79 Transport in Caco-2 cell monolayers of flavone *C*-glycosides from a neotropical blueberry

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We previously reported the isolation of two flavone *C*-glycosides, orientin and vitexin, from the neotropical blueberry *Anthopterus wardii*, and reported their anti-inflammatory and inhibitory activities against IL-8 and MMP-1, respectively. The objective of this study was to investigate the transport of these flavone *C*-glycosides using a two-compartment transwell human intestinal epithelial cell Caco-2 monolayer system, simulating the intestinal barrier. Orientin and vitexin transport in the apical to basolateral direction across Caco-2 cell monolayers were determined. The amounts of the *C*-glycosides were quantitatively measured by HPLC-TOF-MS, and the apparent permeability coefficient (P_{app}) values were also calculated. Our results showed that both orientin and vitexin could permeate to the basolateral side. The P_{app} values for orientin and vitexin were ranged from 6.58 to 29.9×10^{-5} and 1.58 to 2.58×10^{-5} cm/s, respectively. Also, a small amount of vitexin was absorbed inside the epithelial cells. This is the first report of the transport of the anti-inflammatory components orientin and vitexin in human intestinal epithelial cell monolayers. Our findings provide scientific evi-

dence suggesting the possible bioavailability of these components *in vivo*.

PD80 Vasorelaxant effects of 1-nitro-2-phenylethene in isolated rings of rat aorta

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Nitro-2-phenylethane (NPEa) is a vasorelaxant constituent of the essential oil of *Aniba canelilla*. Here, we investigated whether 1-nitro-2-phenylethene (NPEe), a derivative molecule obtained synthetically, has similar properties in isolated rings of rat aorta. Trace recordings were obtained isometrically. NPEe (0.1 – 100 μg/ml) had no effect on aortic rings under resting tonus, but relaxed sustained contractions induced by phenylephrine (PHE, 1 μM) in endothelium-intact preparations (IC_{50}= 6.01 ± 1.62 μg/ml; n = 6). This vasorelaxation remained unaltered by vascular endothelium removal (IC_{50}= 4.56 ± 0.75 μg/ml; n = 5), but was significantly reduced by pretreatment with 10 μM ODQ (IC_{50}= 26.18 ± 7.54 μg/ml, n = 9). NPEe also relaxed the contractions evoked by 60 mM K^+ with the same potency for relaxing PHE without influence of endothelium removal. In Ca^{2+}-free medium, NPEe (1 – 3 μg/ml) inhibited contractions induced by exogenous Ca^{2+} (0.1 – 20 mM) addition in preparations depolarized by K^+, but was inert against PHE-induced phasic contractions in presence of verapamil (1 μM; n = 9). NPEe inhibited contractions caused by extracellular Ca^{2+} restoration in thapsigargin-treated aorta in Ca^{2+}-free medium. Thus, as NPEa, NPEe has vasodilator properties in rat aortic rings, which seem potentially mediated through a guanylate cyclase pathway.

PD81 Transport in Caco-2 cell monolayers of cucurbitane triterpenoids from *Momordica charantia*

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Bitter melon, *Momordica charantia*, is a widely-used treatment for diabetes in traditional medicine systems throughout the world. The compounds responsible for this biological activity are still under investigation, but curcurbitane triterpenoids are thought to be paramount. We investigated the gastrointestinal transport of a saponin-enriched *n*-BuOH fraction of *M. charantia* using a two-compartment transwell human intestinal epithelial cell Caco-2 monolayer system, simulating the intestinal barrier. Eleven triterpenoids in this extract were transported from the apical to basolateral direction across Caco-2 cell monolayers and were identified or tentatively identified by HPLC-TOF-MS. Cucurbitane-type triterpenoids permeated to the basolateral side with apparent permeability coefficient (P_{app}) values for 3-β-7-β,25-trihydroxycucurbita-5,23(*E*)-dien-19-al, momordicines I and II at 15.8 x 10^{-6}, 7.03 x 10^{-6} and 4.34 x 10^{-6} cm/s, respectively. Also, small amounts of these triterpenoids were absorbed inside the epithelial cells themselves, suggesting additional bioactivity for these compounds. To our knowledge, this is the first report of the transport of the reputed anti-diabetes cucurbitane-type triterpenoids in human intestinal epithelial cell monolayers; these results therefore point to a novel mechanism for the possible bioavailability of these bitter melon compounds *in vivo*.

PD82 *Echinacea Purpurea* L. modulates human t-cell cytokine response

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The study evaluates the chemical composition of an Echinacea purpurea (EchNWA) extract previously shown to modifiy the course of influenza infection in a mouse model and assessed immunomodulatory effects on human T-cells. EchNWA extract was obtained from fresh aerial parts extracted with water, followed by ethanolic precipitation, and size-exclusion chromatography. The chemical profile was characterized by chromatographic techniques, including size-exclusion, HPLC, GC-MS and HPLC-PDA. Jurkat T-cells were pretreated with doses of EchNWA followed by activation with phorbol 12-myristate 13-acetate plus ionomycin (PMA+I). Interleukin-2 (IL-2) and interferon gamma (IFNγ) cytokine secretion as well as CD25 expression were measured. EchNWA contains 80% poly-saccharides and phenolic compounds, but alklyamides were not detected. The extract, but not phenolic compounds, mediated dose-dependent enhancment of high-density T-cell production of IL-2 and IFNγ response to PMA+I. EchNWA also enhanced mean fluorescence intensity of IL-2 in Jurkat T-cells activated by PMA+I or ionomycin alone. The extract alone did not stimulate T-cells. Conversely the extract supressed low-density T-cell production of IFNγ and percentage of CD25+ T-cells. Conclusions are that E. purpurea polysaccharides have dose-related adjuvant effects on human T- cell cytokine responses that are characterized by enhancing and suppressive effects and regulated by T- cell density.

PD83 Anti-angiogenesis and immunomodulatory activities of an anti-tumor sesquiterpene bigelovin

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Bigelovin is a sesquiterpene lactone isolated from the plant *Inula helianthus-aquatica* which was traditionally used in cancer treatment in Yunnan, China. We previously demonstrated the potent apoptotic activities of bigelovin. The objective of this study was to investigate the anti-angiogenic and immunomodulatory effects of bigelovin using transgenic zebrafish *Tg(fli1a:EGFP)y1* with fluorescent blood vessels and human peripheral blood mononuclear cells (PBMCs), respectively. The growth of subintestinal vessels and gene expression in angiogenesis signaling pathways of bigelovin-treated zebrafish embryos were examined. The proliferation and cytokines production of bigelovin-treated PBMCs were also evaluated. Our results showed that bigelovin could significantly inhibit the growth of subintestinal vessels and down-regulate the expression of Ang2 and Tie2 of zebrafish embryos. Besides, bigelovin could significantly suppress the proliferation and production of Th 1 cytokines (IFNγ, IL-2 and IL-12) in human PBMCs. This is the first report of the anti-angiogenic and immunomodulatory activities of bigelovin in zebrafish and human lymphocytes. Our findings suggest that bigelovin may exert multi-target function against tumor in animal model.

PD84 Antimetastatic activity of halichondramide for human prostate cancer cells via modulation of PRL-3 signaling

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Halichondramide, a trisoxazole-containing macrolide, was isolated from the marine sponge *Chondrosia corticata*. Halichondramide (HCA) has been shown to exhibit cytotoxicity and antifungal activity. Recent our study exhibited the growth inhibitory activity against a variety of cancer cells. However, the precise mechanism of action of HCA in the antitumor activity remains to be identified. In the present study, the antimetastatic activity of HCA was elucidated in human prostate cancer cells. HCA was found to exhibit the potent growth inhibitory activity of the highly metastatic PC3 prostate cancer cells with an IC_{50} value of 0.81 μM. Additional mechanism of action studies revealed that HCA suppressed the expression of a potential metastatic biomarker phosphatase of regenerating liver-3 (PRL-3) in the PC3 cells. The suppression of PRL-3 by HCA sequentially down-regulates the expression of p85 and p110. The antimetastatic effect of HCA was also correlated with the down-regulation of matrix metalloproteases (MMPs) and N-cadherin, and with the up-regulation of E-cadherin in PC3 cells. HCA also inhibited the migration and invasion of PC3 cells in a dose-dependent manner. These findings suggest that the antimetastatic activity of halichondramide is associated with the inhibition of PRL-3 activity in PC3 cells and thus might be served as a potential candidate for development of cancer chemotherapeutic agents from marine organisms.

PD85 Anti-obesity role of Aster glehni extract: *in vivo* and *in vitro* effects

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Aster glehni (AG) is a kind of Korean traditional herb which only grows in Ulleung-do (Island), Korea. Although there are several reports on Aster glehni, the effect of AG on anti-adipogenesis was not officially reported. The primary aim of this study was to investigate whether AG attenuates 40% high-fat diet (HFD)-induced adipogenesis in the epididymal fat tissues of mice. Male C57BL/6J mice at the age of 3 – 4weeks were divided randmly and equally into four groups; normal diet group, HFD group, HFD including 1% AG extraction (AG1) and HFD including 5% AG extraction (AG5). Experimental animals were fed with HFD for 7 weeks respectively. Compared to the HFD group, mice fed a HF with AG showed comparatively lower body weight gains, visceral fat-pad weights and lipid plasma level such as total cholesterol and glucose. Moreover, AG inhibited the expression of key adipogenic genes, such as PPARγ, C/EBPα, SREBP-1c, FAS, LPL, aP2, and leptin in the epididymal adipose tissues of mice fed with AG1 and AG5. *In vitro*, AG blocked the differentiation of 3T3-L1 preadipocytes in a concentration-dependent manner and suppressed expression of adipogenic relative gene such as PPARγ, the master regulator of adipogenesis. Taken together, these findings indicate that AG exhibits the anti-adipogenesis and anti-obesity effects and suggest therapeutic potential of AG in obesity and obesity-related diseases.

PD86 Anti-inflammatory activity of handelin through the modulation of NF-κB signaling and pro-inflammatory cytokine productions

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Handelin, a guaianolide dimer, is a constituent of the flower of *Chrysanthemum boreale* Makino (Compositae). *C. boreale* have been used as traditional medicines for the treatment of vertigo and fever in Asian countries. The present study was designed to investigate the anti-inflammatory potential of handelin *in vitro* and *in vivo*. Handelin inhibited LPS-stimulated production of NO and PGE_2 in cultured mouse macrophage RAW 264.7 cells. The suppression of NO and PGE_2 production by handelin was correlated with the down-regulation of mRNA and protein expressions of iNOS and COX-2. Further study revealed that handelin suppressed the induction of pro-inflammatory cytokines including TNF-α and IL-1β as well as microRNA-155 in LPS-induced RAW264.7 cells. Activation of the transcriptional activity of NF-κB by LPS was also alleviated by handelin, which was well coincided with its inhibitory effect on IκB degradation. In addition, the activation of MAPKs such as ERK and JNK signaling was suppressed by handelin. In *in vivo* animal model, oral administration of handelin inhibited carrageenan-induced paw edema and TPA-induced ear edema, respectively. The analysis of cytokine production in serum showed that handelin dose-dependently inhibited the production of IL-1β in a carrageenan-induced paw edema. These findings suggest that handelin might be an active ingredient of *C. boreale* with the anti-inflammatory activity by inhibiting NF-κB activation and pro-inflammatory cytokine productions.

PD87 Berberine: An old drug but new use for liver diseases

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Berberine (BBR) is naturally occurring alkaloid from many plants. It is firstly isolated and identified as a plant isoquinoline alkaloid in the early nineteenth century. BBR has been used in modern medicine with multiple pharmacological activities, including anti-inflammation, anti-hyperlipidemia and hyperglycemia as well as anticancer effects. It is an over-the-counter medicine for diarrhea in China. Recent Clinical studies have shown that BBR causes significant reduction of blood glucose, cholesterol as well as triglyceride in patients with type 2 diabetes and hyperlipidemia with no side-effect on liver and muscle. It was reported that BBR has preventive effect, but not curative effect in liver damage models. To clarify this issue, we tested the effect of BBR on CCl_4-induced liver damage models or liver cancer in animal and cell models. The results showed that BBR possesses liver protective effect which has not only preventive, but also curative effects in rats with CCl_4-induced acute and chronic liver injury. The liver protective effect is associated with anti-oxidative effect and anti-apoptotic effect. Using liver cancer cell and mice models, we found that BBR has anti-liver cancer effect in cancer cell lines and cancer cell graft mice by machineries of cell death, anti-cell migration and anti-angiogenesis. These results suggested that BBR could be used as a potential new drug for liver injury and liver cancer. This study was financially supported by grants from the research council of the University of Hong Kong (Project Code: 201111159223) and The Research Grant Committee (RGC) of Hong Kong (Project Code: 10500362).

PD88 Oleacein protects endothelial progenitor cells against angiotensin II – induced oxidative stress

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Oleacein is a secoiridoid from *Olea europaea* L. (*Oleaceae*) occurring mainly in olive oil. The latest *in vitro* studies show that oleacein is a potent antioxidant, and thus may prevent oxidative stress, LDL oxidation and oxidative DNA damages. Endothelial progenitor cells (EPC) constitute a basic repair mechanism of damaged blood vessels, but these cells are especially sensitive to oxidative stress. The aim of the study was to examine whether oleacein may protect EPCs against impair of function due to oxidative stress induced by angiotensin II. EPCs were isolated from peripheral blood of young healthy volunteers and cultivated on fibronectin-coated plates in EGM-2 medium in the presence or absence of angiotensin II (1 μM) and oleacein (2 – 50 μM). Cell senescence was measured using a β-galactosidase staining kit; ROS generation was measured using a ROS/Superoxide detection kit; expression of heme oxygenase-1, nitrotyrosine formation and oxidative DNA damages were measured using ELISA kits. Angiogenic potential was measured by tube formation in Matrigel™. Oleacein reduced cell senescence increased by angiotensin II and inhibited ROS formation. These effects resulted in reduction of nitrotyrosine and oxidative DNA damages formation. Moreover, oleacein increased the angiogenic potential of EPC *in vitro*. This activity of oleacein was correlated with the increase of heme oxygenase-1 expression. Our results suggested that oleacein may protect EPC against angiotensin II-induced oxidative stress.

PD89 Shikonin inhibits colitis-associated colorectal dysplasias in a mouse model of azoxymethane/dextran sulfate sodium colitis

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Shikonin (Shk) inhibits the development of colorectal dysplasia and the colitis induced in a mouse model of azoxymethane (AOM)/dextran sulfate sodium (DSS), as demonstrated both by macroscopic and biochemical determinations. The oral administration of shikonin prevents weight loss and colorectum shortening. Histological analysis revealed a decrease both in the severity and extent of inflammation, together with an amelioration of the colonic architecture and the protection from the appearance of dysplasia. This was associated reduction in MPO activity together with the inhibition of COX-2 and iNOS expression. A possible mechanism of action which would be responsible for this protection is the inhibition of NF-κB activation, since we demonstrated the inhibition of the translocation of NF-κB-p65 subunit to the nucleus in the colon homogenate. As a positive control we examined the effect of oral administration of sulfasalazine (Ssz).

Group	Blank	Controls		AOM-groups				DSS
		AOM	DSS	DSS	DSS-Ssz	DSS-Shk 3.5	DSS-Shk 7	Shk 7
AOM (7.5 mg/kg i. p.)	-	✔✔	-	✔✔	✔✔	✔✔	✔✔	-
1.5% DSS	-	-	✓	✓	✓	✓	✓	✔✔
Treatment	-	-	-	-	100 mg/kg Ssz	3.5 mg/kg Shk	7 mg/kg Shk	

Introduction: The biomedical development and the general improvement of the living conditions in Western societies has led over the last century to a change in epidemiological patterns of the most relevant diseases. Thus, allergies, autoimmune disorders, chronic inflammatory diseases, such as inflammatory bowel disease (IBD) and cancerous processes, have replaced infectious diseases as the main cause of mortality. [1] Patients with a long-term established IBD have a higher risk of developing colorectal cancer (CRC), which depends on the preexisting colitis, its duration, anatomic extent and degree of inflammation. Thus, the risk of colon cancer is between 2 and 3 times higher than that of the general population, showing an increased incidence of 2, 8 and 18% after 10, 20 and 30 years of suffering from IBD, respectively. [2] **Experimental approach:** The Azoxymethane (AOM)/dextran sodium sulfate (DSS) model of colitis-associated CRC employed in this study was based on that of Tanaka *et al.* (2003). Female Balb/C mice were distributed in 8 groups (6 – 10 animals/group) as following:

Group	Blank	AOM	DSS	AOM DSS	AOM DSS SSZ	AOM DSS Sh 3,5	AOM DSS Sh 7	DSS Sh 7
AOM (7.5 mg/kg i. p.)		✔		✔	✔	✔	✔	
1,5% DSS			✔	✔	✔	✔	✔	✔
Treatment					100 mg/kg sulfasalazine	3,5 mg/kg shikonin	7 mg/kg shikonin	

The experimental protocol is outlined in the following table:

		1,5% DSS	Water	1,5% DSS	Water	1,5% DSS	Water
	AOM injection	SSZ and Shikonin treatment (as corresponding to each group)					
Week	0	1	2	3	4	5	6

DSS, sulfasalazine and shikonin were administered in the drinking water of the corresponding groups. Body weights were recorded at the time of AOM injection and every day until the end of the experiment. Animals were sacrificed at the end of the experiment. At that time the entire colon and rectum were measured, excised, cut longitudinally, and rinsed in PBS. Following groß examination, some colons were frozen in liquid nitrogen and processed in their entirety for histopathologic evaluation. Others were processed for myeloperoxidase activity analysis and for COX-2, iNOS and p65 determination by Western blot. RESULTS: [1] Danese S, Fiocchi C. Etiopathogenesis of inflammatory bowel diseases. World J Gastroenterol. 2006;12:4807 – 12.

PD90 Marine sponge-derived xestoquinones suppress HIF signaling and disrupt cellular respiration in human breast tumor cells

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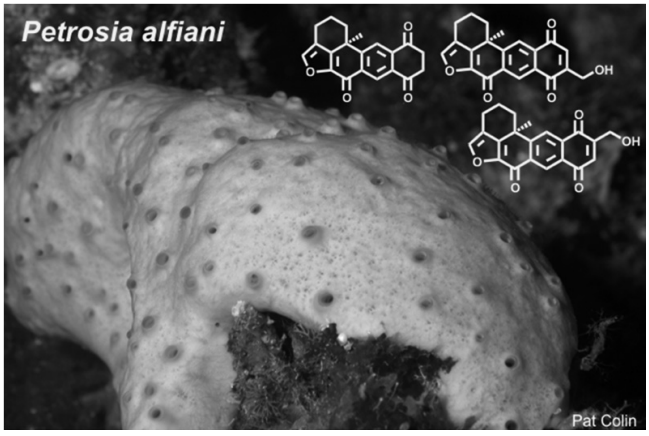
The organic extract of a marine sponge *Petrosia alfiani* selectively inhibited iron chelator-induced hypoxia-inducible factor-1 (HIF-1) activation in a human breast tumor T47D cell-based reporter assay. Bioassay-guided fractionation yielded seven xestoquinones (**1** - **7**) including three new compounds 14-hydroxymethylxestoquinone (**1**), 15-hydroxymethylxestoquinone (**2**), and 14,15-dihydroxestoquinone (**3**). Compounds **1** - **7** were evaluated for their effects on HIF-1 signaling, mitochondrial respiration, and tumor cell proliferation/viability. The known metabolites adociaquinones A (**5**) and B (**6**), that possess a 3,4-dihydro-2*H*-1,4-thiazine-1,1-dioxide moiety, potently and selectively inhibited iron chelator-induced HIF-1 activation in T47D cells, each with an IC_{50} value of 0.2 μM. Mechanistic studies revealed that adociaquinones promote oxygen consumption without affecting mitochondrial membrane potential. Compound **1** both enhances respiration and decreases mitochondrial membrane potential, suggesting that it acts as a protonophore that uncouples mitochondrial respiration.



PD91 Selective killing of leukemia cells by Cordiaquinone J targeting the stress response to ROS

Marinho-Filho JDB[1], Araújo AJ[1], Pessoa C[1], Diniz JC[2], Viana FA[2], Pessoa ODL[3], Silveira ER[3], da Costa MP[1], Moraes MO[1], Lotufo LVC[1]
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Cordiaquinone J (CLR) is a biological active meroterpenoid naphthoquinone isolated from the roots of *Cordia leucocephala*. This compound showed IC_{50} values ranging from 4.6 to 6.8 μM in leukemia cells and 33.6 to 37 μM in normal cells. CLR induced both necrosis and apoptosis and decreased C-Myc and GSH levels in HL-60 cells. CLR also promoted DNA damage activating protein kinases of the ATM/ATR pathway in tumor cells but not in normal cells in a mechanism dependent of the generation of ROS (Fig1). **Financial Support:** CNPq/CAPES/PRONEX.

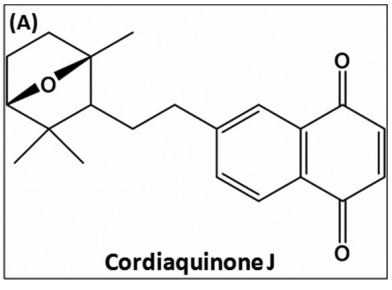

Fig. 1: (A) Molecular structure of cordiaquinone.

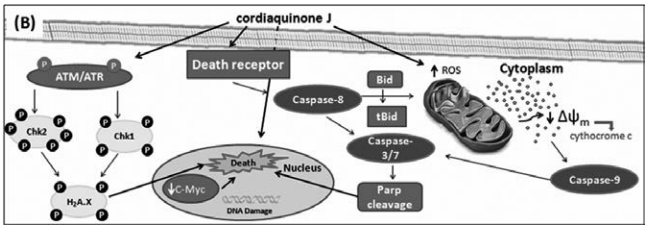

Fig. 2: (B) Mechanism of action of cordiaquinone.

PD92

Evidences for the involvement of HER on prodigiosin anticancer effects

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Prodigiosin (PG) is a well-know tripyrrole red pigment with immunosuppressive and anticancer activities. Previous studies demonstrated that PG recognizes selectively cells with different levels of ErbB2 expression [1]. In the present work, we evaluated PG activity in two different cell lines: SF-268, glioblastoma cells that present a mutation in the extracellular domain of EGFR, and Malme-3 M, metastatic melanoma cells, which expresses approximately equivalent levels of HER-2 and HER-3. PG showed cytotoxic effect in a time-dependent manner with IC_{50} values ranging from 15.7 to 2.7 μM after 24 and 72 h of incubation to Malme-3 M and 31 to 2.7 μM to SF-268, respectively. This effect was accentuated with the simultaneous use of erlotinib (25 μM). PG also reduced G_0/G_1 phase and induced DNA fragmentation in Malme-3 M cells after 48 h of treatment. Differenzial morphology staining indicated that PG induces several morphological alterations like apoptosis and necrosis. These results emphasize the involvement of HER kinases in PG anticancer activity. **Reference:** [1] Arthaud, IDB et al. (2012) Chemistry & biodiversity 9, p. 418 – 427. **Financial Support:** CNPq, CAPES, PRONEX.

PD93

Acetylcholinesterase and butyrylcholinesterase inhibition by *Phormopsis Sp* methanol extract and its isolated compounds

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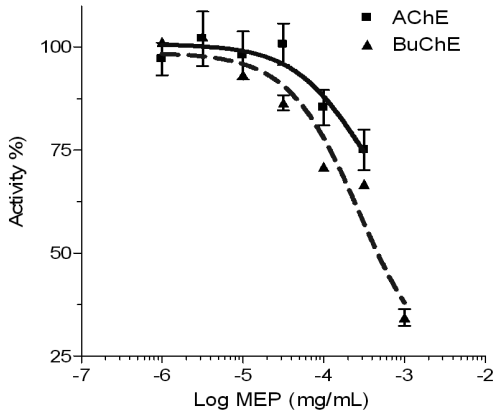
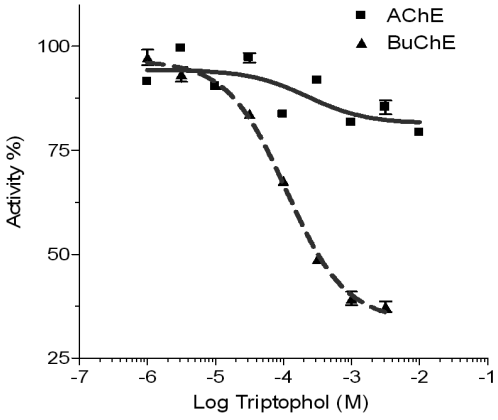
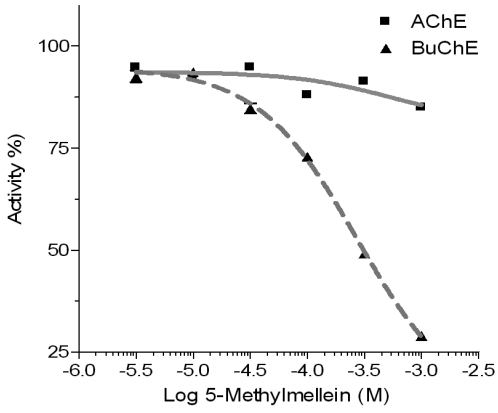
In the course of our continuous search for bioactive metabolites from endophytic fungi living in plants from the Brazilian flora, leaves of *Harconia speciosa* Gomes were submitted to isolation of endophytes. A *Phormopsis sp* was isolated from *H. speciosa* and its methanol extract (MEP) was evaluated as AChE and BuChE inhibitor. Triptophol and 5-methylmellein, isolated from MEP, have also been evaluated as AChE and BuChE inhibitor. Cholinesterase activity was evaluated by Elmman's method. Neostigmine was used as positive control. The results of the cholinesterase inhibition in the presence of MEP (A), triptophol (B), and 5-methylmellein (C) will be presented.







PD94

Ethyl acetate extract of *Baccharis dracunculifolia* DC inhibits TNF-α production in TNBS-induced colitis in rats

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Baccharis dracunculifolia (BD) is the main botanical source of Brazilian green propolis, a natural product used to improve health. The ethyl acetate extract of this plant is rich in caffeic acid, *p*-coumaric acid, aromadendrin-4-*O*-methyl ether, 3-prenyl-*p*-coumaric acid, 3,5-diprenyl-*p*-coumaric acid and baccharin. This extract was effective scavenger of DPPH with the EC50 value of 63.53 vs. 1.89 μg/ml of galic acid. Previous study in our research group showed that this extract presented improvement in experimental model of inflammatory bowel disease induced by trinitrobenzenesulfonic acid (TNBS) in rats. The aim of this study was to investigate by which means this extract acts. For this purpose, the rats were pre-treated for 5 days with 5 or 50 mg/Kg by BD extract or azathioprine (2 mg/Kg), the reference drug, before the colitis induction by TNBS. Macroscopic (score, extension of lesion, colonic weight/length) and biochemical parameters (gluthathione, myelo-

peroxidase, alkaline phosphatase, TNF-α and INF-γ) were evaluated. The doses of 5 and 50 mg/Kg reduced the colon weight/length and the myeloperoxidase activity. Additionally, the dose of 50 mg/Kg reduced colonic TNF-α levels. The reference drug only reduced the myeloperoxidase activity. The primary mechanism of BD anti-inflammatory actions appears to be immunomodulation via suppression of TNF-α production.

PD95 The alkaloid 1-hydroxy-rutaecarpine inhibits cathepsin activity

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Cathepsins B, L and K are cysteine proteases involved in various physiological and pathological processes. CatB and L are involved in tumoral processes while catK in cases of bone resorption. In a search for cathepsin inhibitors we have isolated from *Metrodorea stipullaris*, a number of compounds, among them the alkaloid 1-hydroxy-rutaecarpine which displayed moderate inhibitory activity on those enzymes. The alkaloid showed inhibition of 79% on cathepsin B, 100% on cathepsin L and 87.5% on cathepsin K, at the concentration of 125 μg/ml for the three cathepsins. IC_{50} experiments were run and the values obtained are described in the table below.

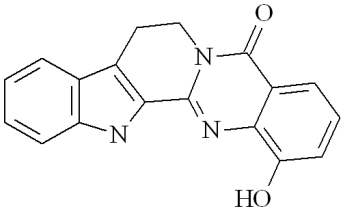

Fig. 1: 1-hydroxy-rutaecarpine

Tab. 1: IC_{50} values

Catepsins	IC_{50}
B	84.48 μM
L	28.83 μM
K	46.12 μM

PD96 A parthenolide-depleted tanacetum parthenium extract induces DNA repair process through the PI3K-NRF2-ARE pathway

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The NF-E2-related factor-2 (Nrf2) -antioxidant response element (ARE) pathway is one of the key cellular defense mechanisms that protect the cell from the effects of external aggressors by stimulating production of antioxidant and DNA repair genes. A Tanacetum parthenium (Feverfew) extract, which was depleted of parthenolide, induced nuclear translocation of Nrf2 and activated the ARE promoter in primary human keratinocytes. Blocking the *de novo* expression of Nrf2 by using specific siRNA diminished Feverfew-induced ARE promoter activity. Pre-treatment with LY294002, a selective pharmacological inhibitor of PI3 kinase led to a similar response suggesting that Feverfew activates the Nrf2-ARE pathway via PI3 kinase. In a comet assay, pretreatment with Feverfew enhanced the repair of DNA damage caused by UV in primary keratinocytes, and this effect was reversed by addition of the PI3 kinase inhibitor LY294002. Activation of DNA repair genes apurinic/apyrimidinic endonuclease-1 (APE-1) and DNA-damage binding protein-1 (DDB-1) by Feverfew was confirmed by western blot analysis of human skin equivalents topically treated with Feverfew. Through its ability to enhance endogenous antioxidant levels and induce DNA repair mechanisms in skin cells via activation of the Nrf2-ARE pathway, a purified Feverfew extract may protect skin from the harmful effects of numerous external aggressors, including UV radiation.

PD97 Pharmacological activity of Mexican basidiomycetes species cultivated *in vitro*

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A great variety of Basidiomycetes grow in Mexico, but most of their properties, includying pharmacological ones, have been scarcely investigated. In this study, the hypoglycemic, immunomodulating and antioxidant activity of aqueous extracts prepared from cultured mycelia of *Lentinus lepideus* (*Ll*), *Calvatia cyatiformis* (*Cc*) and *Ganoderma applanatum* (*Ga*) collected in Nuevo León, Mexico are presented. Hypoglycemic activity was evaluated in alloxan-induced diabetic rats which received a single dose (100 mg/kg po) of *Ll, Cc* or *Ga*, and the serum glucose was measured at 0, 3, and 6 h after administration, immunomodulating activity was evaluated in Balb/c mice according to the the Cunningham hemolytic plate assay. The antioxidant (DCFDA test) and cytotoxic (MTT test) effect were evaluated in Chang liver cells. None of the species tested had cytotoxic activity, *Ll, Cc, and Ga* lowered the serum glucose levels at 3 h and 6 h after administration *Ll* and *Cc* showed significant immunostimulation, *Ll, Cc and Ga* demonstrated relevant antioxidant activity (49%-75%); *Ll* was the species with major hypoglycemic, immunostimulating and antioxidant activity. A fractionation of *Ll* was carried out and four fractions were obtained (FI-FIV) and evaluated. Fractions II and III were the most active. Conclusion: Mexican Basidiomycetes are a potential source of compounds with hypoglycemic, immunomodulating and antioxidant activity.

PD98 Antibacterial effect of Romanian propolis on pseudomonas aeruginosa and staphylococcus intermidius

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The antimicrobial potential of ten propolis samples from Transylvania was investigated using a disk diffusion method and a broth microdilution assay. The three most active samples were tested for post antibiotic effect (PAE) against *Pseudomonas aeruginosa* and *Staphylococcus intermedius*. The results pointed out a complex antibacterial activity as indicated by the values obtained for the inhibition zones (10 – 23.5 mm) and for the minimal inhibitory (MIC) and bactericidal (MBC) concentrations (0.125%-1% v/v). The bactericidal efficacy was demonstrated against both Gram positive and Gram negative, but proved to be diminished in enrofloxacin-resistant bacterial strains. A positive correlation was found between these data and propolis composition in biologically active compounds – phenolics – determined by spectrophotometric and chromatographic methods (Folin Ciocalteu and HPLC-PDA). According to these results Transylvanian propolis presented typical poplar profile with high concentration in total phenolics 33,02 ± 9,04% and medium amount in total flavonoids 6,8 ± 1,48%.

PD99 In vivo anti-diabetic effect of *Aster koraiensis* extract

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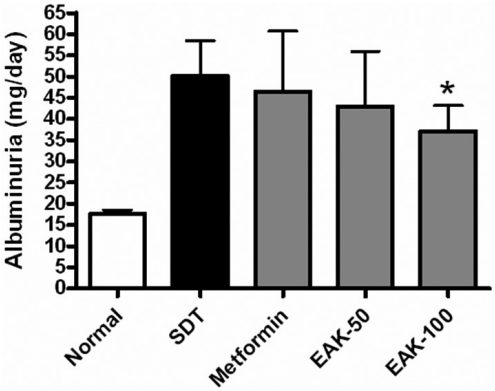
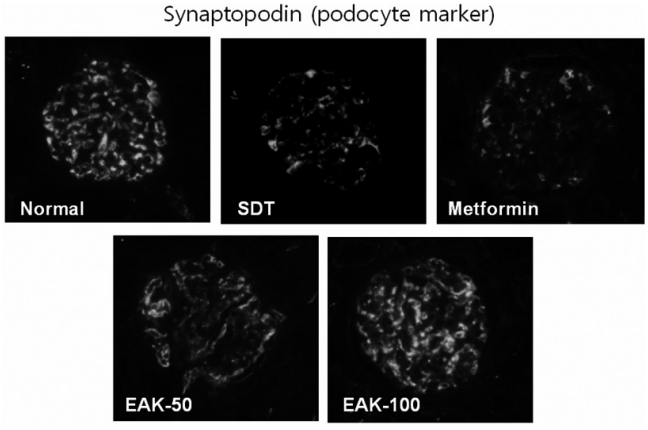
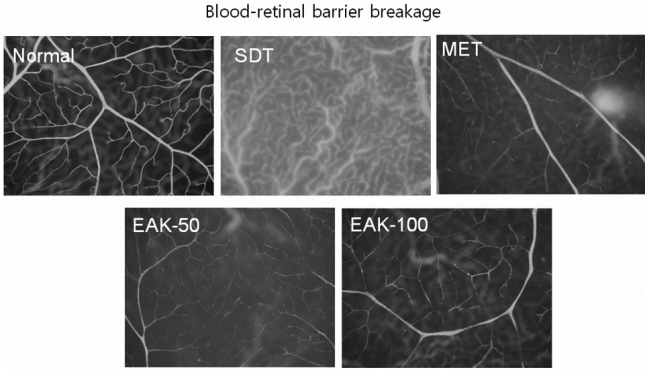
Korean Medicine Based Herbal Drug Research Group, Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon 305 – 811, South Korea

The extract of the aerial parts of *Aster koraiensis* (EAK) is a potential advanced glycation end products (AGEs) inhibitor with beneficial effects on diabetes. AGEs is one of the causative factors of diabetic complications. In this study, we investigated the effects of EAK on the pathogenesis of diabetic complications in spontaneous diabetic Torii (SDT) rats.

EAK (50 and 100 mg/kg) or metformin (350 mg/kg) as positive drug were treated once a day orally for 16 weeks. EAK reduced albuminuria and prevented renal AGEs deposition and podocyte apoptosis in SDT rats. The changes of retinal vasculature, such as retinal pericyte loss and blood-retinal barrier breakage were inhibited in EAK-treated SDT rats. Moreover, EAK showed significantly more benefit for diabetic nephropathy and retinopathy compared with metformin. Taken together, our results indicate that EGK could provide a valuable therapeutic approach against diabetic complications.







PD100 Microcystins as novel leads against OATP1B3-expressing tumors

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Microcystins are cyclic heptapeptides produced by cyanobacteria. They potently inhibit the eukaryotic protein phosphatase families PP1 and PP2A. They are able to display toxicity only after transporter-mediated uptake by the cell. This uptake is mediated by the organic anion transporting polypeptides OATP1B1, OATP1B3, and OATP1A2. Liver cells express OATP1B1 and OATP1B3 transporters, which results in a pronounced liver toxicity of microcystins. However, OATP1B3 is also expressed in a significant percentage of hepatocellular carcinoma cells as well as gastrointestinal, lung, breast and colon tumors. Interestingly, compared to OATP1B1, OATP1B3 is found only in low abundance in liver cells. Thus selectivity that favors OATP1B3 over OATP1B1 should lead to a decreased hepatic clearance and toxicity, and an increased uptake in OATP1B3-expressing tumors, thus creating a therapeutic window for the respective compound. Since many naturally occurring microcystin variants are known and many more have not been described yet, there is high potential for isolating variants with unique properties. In fact, in our initial screening of 20 microcystins, we have found four microcystin structural variants with an IC_{50} in the low nanomolecular range and with transporter selectivity that favors OATP1B3 over OATP1B1 by a factor of more than ten. Some of these microcystin variants have not yet been described in the literature.

PD101 *In vitro* anti-inflammatory and anti-microbial activity of cowslip flowers (*Primula veris* L.)

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Flowers of *Primula veris* L. are part of the medicinal product Sinupret®, an herbal remedy to treat acute and chronic inflammations of the paranasal sinuses. In this study, a Primulae flos dry extract (PFE) was tested *in vitro* for inhibition of prostaglandin biosynthesis by cyclooxygenases (COX-1 and -2) and for inhibition of leukotriene biosynthesis in human polymorphonuclear leukocytes. Furthermore, the T-cell mediated release of the pro-inflammatory cytokines IFN-γ and GM-CSF from human peripheral blood mononuclear cells was studied after incubation with PFE. In addition, *in vitro* anti-microbial activity of PFE was studied in virus plaque-reduction assays [human rhinovirus (HRV), respiratory syncytial virus (RSV)] and in microdilution assays for determination of minimum bactericidal concentration (MBC; targets: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*). PFE efficiently inhibited viral replication ($EC_{50,HRV}$= 58 µg/mL and $EC_{50,RSV}$= 62 µg/mL) and displayed anti-bacterial activity with MBC of 200 µg/mL. The targeted viruses and bacteria are involved in respiratory tract infection thus pointing to the therapeutic potential of PFE in this field. PFE significantly reduced IFN-γ and GM-CSF secretion in a dose-dependent manner and inhibited prostaglandin synthesis as well as leukotriene formation, thus showing a broad anti-inflammatory impact. Taken together, for the treatment of respiratory infections, PFE might act via a direct anti-microbial effect and also by inhibiting the inflammatory sequelae.

PD102 New phytochemical markers and pharmacological activity of dendrobium chrysotoxum flowers

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Some Dendrobium species are known to be used in Chinese materia and have pharmacological properties. Mitochondria regulate the energetic level of human cells via their respiratory chain. Aging and senescent cells allow a decreased energetic synthesis network. The phytochemical analysis of Dendrobium chrysotoxum (orchidaceae) flower ethanol-water 70/30 extract reveals the presence of mono, oligo, polysaccharides, terpenoids, lipids and phenolic compounds. A focus on flavonoid fraction was made using HPLC/LC-MS/RMN and HPTLC-AMD (automated multiple development) method with a methanol/diisopropylether/heptan gradient, isocratic elution and revelation using Neu + Peg reagents and detection at 254 and 366 nm. For the first time the presence of 4 different isorhamnetine3-O glycosides derivatives is described. A specifically designed human mitochondria PCR array centered on specific genes involved in energy synthesis was used on normal human skin cells cultures treated with Dendrobium chrysotoxum flavonoid extract. This identified extract at12.5 µg/ml stimulates (X 2.55) the gene SLC25A7 which manages the importation of H+ ions in the mitochondrial respiratory chain. Our study suggests that this Dendrobium flower

extract can be used to restore or boost senescent cell respiratory chain function.

PD103 Modulation of muscle mass and myogenic stem cells with natural products

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Muscle satellite cells are widely accepted as the resident stem cells of skeletal muscle, supplying myoblasts for growth, homeostasis and repair. Activation of these cells in response to muscle mechanical change or injury involves activation of the muscle-specific transcription networks. Previously we reported that brassinosteroids, a group of natural plant hormones that regulate growth and development, triggered an anabolic response when fed orally to rats. Here we show that this effect was associated with increased expression of the myogenic transcription factors and quiescent myogenic satellite cell Pax3/7 markers. In L6 rat myoblast lineage cells, brassinosteroid treatment accelerated differentiation, expression of structural proteins, and fusion into multinucleated myotubes. Collectively, these data indicate that activation, increased proliferation, and subsequent fusion of myogenic cells may be an important mechanism by which brassinosteroids enhance muscle hypertrophy. Thus, in appropriate doses, brassinosteroids may have therapeutic applications for the treatment of diseases associated with muscle loss.

PD104 Toxicity and apoptotic effects of selected compounds and extracts from edible plants

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Traditional medicinal and dietary plants consist of numerous bioactive compounds, which affect cell signaling and gene expression. Our study aims to clarify the mechanisms related to apoptosis and inflammation – the mechanisms behind e.g. cancer – of some selected compounds and extracts in cancerous and non-cancerous cells, focusing especially on plant extracts for their possible combinatorial effects of several compounds. Toxicity of plant-derived samples was determined by measuring the metabolic activity and membrane integrity of cells. Cell signaling processes were determined by detecting proteins p53, Bcl-2 and p65 by Western blotting and defining the caspase 3 activity in cells. Pilot study for differences in gene expression between treated and untreated cells was carried out by the cDNA-RDA method. We observed that quercetin and piceatannol – well-known compounds of plant origin – and juniper berry extract affected the caspase 3 activity and/or the amount and localization of p53 in cancerous cells. Further investigations are needed to define the potential of synergistic effects of multiple plant-derived compounds for their use as functional food products and/or drugs.

PD105 Effectiveness of xylose containing polysaccharides on human skin fibroblasts and keratinocytes

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Xyloses containing polysaccharides of Ispaghula seed husk (*Plantago ovata*, Forssk, Plantaginaceae, P1) and tamarind (*Tamarindus indica,* L., Fabaceae, TSw) significantly increase the proliferation of human skin keratinocytes (NHEK, HaCaT) and fibroblasts (NHDF) independent of the used concentration. To determine if these polysaccharides share the effectiveness gene expression studies as well as fluorescence based methods were carried out. As detected by confocal fluorescence laser scan microscopy both polysaccharides were internalized by NHDF and HaCaT-keratinocytes. Quantification of internalized polysaccharides by flow cytometry revealed that P1, an acidic arabinoxylan, was entirely up-taken already after 3 h by NHDF and HaCaTs while the xyloglycan TSw was completely internalized not before 12 h of incubation. Gene expression studies showed that these polysaccharides act upon different intra and extra cellular targets dependent of the used cells and incubation time. Within 6 h P1 significantly increased the gene expression of NHDF and NHEK whereas the gene expression was reduced after 24 h. While incubation with P1 mostly resulted in an up-regulation of genes TSw predominantly repressed the genes expression. Especially the genes referring to the extracellular matrix, cell cycle, metabolism, membrane proteins, intermediate filaments as well as DNA replication were differenzially regulated by P1 in NHEK and NHDF. So the results show that a different effectiveness results in a similar biologic activity.

PD106 Effects of a slight acidic arabinoxylan from *Plantago ovata* seed husk on human skin cells

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The *Plantago ovata*, Forssk, Plantaginaceae, seed husk has a high content of water-soluble polysaccharide identified as an arabinosyl (galactosyluronic acid) rhamnosylxylan further denominated as P1. The P1 activity on the cell proliferation, migration and cell cycle of keratinocytes (NHEK, HaCaT) and fibroblasts (NHDF) was analyzed by flow cytometry. Internalization studies were done to elucidate the effectiveness of P1. Enzymatic hydrolysis was done to investigate the necessary structure responsible for the biologic activity. Subfractions of P1 were obtained using α-L-arabinofuranosidase (P11-P18), endo-1,4-ß-xylanase (P21-P24) and both enzymes in combination (P31-P36). P1 significantly increased the proliferation rates of NHEK, HaCaT and NHDF independent of the used concentration. The scratch assay revealed that the migration of NHDF was also significantly enhanced while this effect was not observed using keratinocytes. Both skin cell types internalized P1 rapidly during 3 h of incubation and also triggered the NHDF and NHEK in the S and G2 phase after 6 h and 3 h of incubation. After enzymatic hydrolysis all the subfractions maintain the cell proliferation promoting effect on HaCaT whereas the subfractions P13-P18 and P31-P36 were more effective than P1. Subfractions P15-P16 and P31-P36 had no effect on NHDF but subfractions P11-P14 and P18 increased the effect.

PD107 The Novel Sinupret® dry extract BNO 1011 inhibits paw oedema development *in vivo* and inflammatory mediator release *in vitro*

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Inflammatory processes are important therapeutic targets in the treatment of rhinosinusitis. The novel dry extract BNO 1011 is based on the Sinupret® drug mixture (Gentianae radix, Primulae flos, Sambuci flos, Rumicis herba, and Verbenae herba), an herbal medicinal product to treat rhino-sinusitis. The anti-inflammatory activity of BNO 1011 was investigated in the rat paw oedema model of acute inflammation and potential underlying mechanisms were studied in vitro. In rats, carrageenan-induced oedema development was significantly reduced after oral application of BNO 1011. In vitro, BNO 1011 was able to reduce prostaglandin biosynthesis by COX-1 and COX-2 enzymes and inhibited leukotriene B_4 secretion, mediators known to contribute to inflammation of the upper and lower airways. The T-cell mediated release of pro-inflammatory IFN-γ was also reduced. Taken together, Sinupret® dry extract BNO 1011 was proven to reduce inflammation in vivo, most likely by targeting the release of pro-inflammatory mediators. The anti-inflammatory action of BNO 1011 supports its role in the treatment of rhinosinusitis, an infectious condition accompanied by inflammation of nasal and paranasal mucosal tissues.

PD108 Mucuna pruriens efficacy in Parkison disease: Systematic approach of clinically observed synergy

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Seeds of *Mucuna pruriens* (Fabaceae) are traditionally used in India against Parkinson's disease (PD), a nosologic entity defined as such in Ayurveda by Kampavata. The beans contain up to 5% of L-DOPA, explaining its relevance in PD treatment. A large variety of phytochemicals are also present along with L-DOPA in the seeds, including other amino-acids and alkaloids from the ß-carboline and tetrahydroisoquinoline types. Several pharmacological studies on crude extracts have shown significant effects supporting a role for other compounds in the pharmacological effect. A clinical study has shown that at equivalent doses of L-DOPA, *Mucuna* seed powder led to faster and longer onset of L-DOPA plasmatic levels, without the expected higher dyskinesias[3]. The isolation of *Mucuna* seed alkaloids, amino acids and other compounds, as well as the approach for their pharmacological assessment on different biological targets is described (MAO inhibition, DDC inhibition, dopaminergic and cholinergic receptors, neuroprotection, membrane crossing facilitation) is described. A LC-based online MAO and/or DDC inhibition test is designed. The obtention of an extract specifically devoided from L-DOPA is also described and proposed for the assessment of putative synergy phenomena.

PD109 Iberis amara extract shows anti-inflammatory activity and endothelium protective effect

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Iberis amara extract (STW 6, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany) has previously been shown to possess good anti-oxidant and anti-inflammatory activity *in vitro*. The present study deals with testing it *in vivo* in models of acute and chronic inflammation. The extract showed dose dependent anti-inflammatory activity in the rat paw carrageenan-induced edema model. In the six-day air pouch model the extract reduced markedly the rise in IL-1β, TNFα and PGE_2 as well as oxidative stress biomarkers (total nitrite, malondialdehyde and total anti-oxidant activity) in the exudate induced by injecting carrageenan into the pouch. In the adjuvant-induced arthritis model the extract effectively prevented the development of arthritis when given prophylactically and markedly reduced the inflammation in a curative setting. Furthermore, aortae isolated from rats with adjuvant induced arthritis showed a reduced response to endothelium dependent agonists like acetylcholine but little derangement in endothelium independent responses, such as those to norepinephrine and sodium nitroprusside. Treatment with the extract restored the normal responses to acetylcholine but did not influence those to nitroprusside, indicating a possible endothelium protective effect of the extract. The findings lend further evidence to the strong anti-inflammatory potency of *Iberis amara* and may prove beneficial in safeguarding the integrity of the endothelium in inflammatory conditions.

PD110 Effects of xyloglucans from *Tamarindus indica* seed on human skin cells

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Two xyloglucans were gained from *Tamarindus indica,* L., Fabaceae, seed using cold water extraction (TSw) and copper complex precipitation (TSc) respectively. TSw and TSc were investigated on human skin fibroblasts (NHDF) and keratinocytes (NHEK, HaCaT) concerning their effects on necrotic cytotoxicity, cell proliferation, cell cycle and migration. Furthermore internalization of TSw and TSc and enzymatic degradation of TSw and TSc using xyloglucanase were carried out. TSw and TSc exerted no necrotic cytotoxicity. Both were completely internalized after 12 h of incubation and significantly enhanced the cellular proliferation and migration of keratinocytes and fibroblasts. The effect on keratinocyte proliferation was shown to be independent of the used concentration. On the other hand 0.1 µg/mL and 1 µg/mL TSc significantly increased the proliferation rate of NHDF compared to 0.01 µg/mL and the effect of 10 µg/mL which were significantly different to 100 µg/mL. Both xyloglucans triggered the skin cells to move from G0/G1-phase into S and G2-phase already after 3 h incubation. Enzymatic hydrolysis revealed enhancement in activity on NHDF in comparison with those two xyloglucans but on HaCaT keratinocytes, the enzymatic hydrolyzed TSc lost its activity. On the other hand TSw maintained the cell proliferation promoting effect after enzymatic hydrolysis.

PD111 Influence of a slight acidic arabinoxylan and a xyloglucan on the gene expression of human skin keratinocytes and fibroblasts

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A slight acidic arabinoxylan of *Plantago ovata* seed husk, Forssk, Plantaginaceae (P1), and a watersoluble xyloglucan of *Tamarindus indica* seed, L., Fabaceae (TSw), significantly increase the proliferation and migration of human skin keratinocytes (NHEK) and fibroblasts (NHDF). In order to get a better knowledge of the underlying mechanism the influence of P1 and TSw on gene expression of these skin cells was investigated by PIQOR™ Skin Microarray and Real-Time PCR. Further the phosphorylation of different mitogen activated protein kinases was analyzed. Within 6 h of incubation P1 significantly increased the genes referring to calcium signaling, transcription and cytoskeleton and inhibited the expression of genes that concern to cell death, protein degradation and metabolism of NHEK. In case of NHDF the genes referring to cell cycle, cell adhesion, cytoskeleton and extracellular matrix (ECM) were up-regulated while the genes that belong to metabolism were down-regulated, whereas TSw up-regulated the genes in regard to ECM and down-regulated the genes referring to cell death and differentiation on NHEK. Further TSw up-regulated the genes concerning to cytoskeleton and response to toxins while the genes with respect to receptor signaling and metabolism were down-regulated in NHDF. Since both polysaccharides exerted similar effects on the human skin cell physiology the presented results show that the underlying mechanisms are different.

PD112 Evaluation of *Tagetes patula* L. (Asteraceae) against filamentous and entomopathogenic fungi

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Motivated by the search for a natural product for controlling pathogenic fungal species related to the habits of dogs and cats, without interfering in the growth of entomopathogenic fungi, responsible for the biological control of ticks, were tested extracts of *Tagetes patula* L. (Asteraceae) against strains of *Trichophyton rubrum*, *Microsporum canis*, *Trichophyton mentagrophytes* and entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. The minimum inhibitory concentrations (MIC) was determined by microdilution plate method for 70% ethanol extracts obtained by percolation of the aerial parts (PA_{EtOH70}), aerial parts without flowers ($PASF_{EtOH70}$) and flowers (Fl_{EtOH70}). Amphotericin B was a positive control. The results revealed that all extracts were ineffective against the entomopathogenic fungi, showing MIC values at the highest dilution tested (1250 ug/mL), except $PASF_{EtOH70}$ (650 ug/mL). Against dermatophytes were obtained significant activities of Fl_{EtOH70} against *M. canis* and *T. rubrum* (193.3 ug/mL and 253.9 ug/mL, respectively) and an interesting activity of PA_{EtOH70} against *T. rubrum* (312.5 ug/mL). These results suggest a selective action against pathogenic fungi, allowing future studies of the synergistic action of extracts of *T. patula* with entomopathogenic fungi. Support: FAPESP

PD113 Mechanisms of antitumor activity by melanoxoin on human non-small cell lung cancer cells

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Cancer is a leading cause of death worldwide. The standard treatments of cancer include surgery, chemotherapy, radiation therapy and the newer targeted therapy. Till now, there are various anticancer drugs, which derived from natural products. In this study, we screened a serious of compounds isolated from *Pterocarpus santalinus* to identify their cell cytotoxicity. We performed the MTT assay to evaluate the cytotoxic effects of test compounds against several human cancer cell lines. Among them, melanoxoin showed the highest cytotoxic effects with a IC_{50} of 1.98 μg/ml in the human non-small cell lung cancer H1299 cells. We next used Human OneArray® v5 to comprehensive analysis the DNA transcription of H1299 cells. It showed that melanoxoin regulated the transcription of several cell cycle regulators in the H1299 cells. Cell cycle analysis by flow cytometry revealed melanoxoin causes G2/M arrest and then increased tumor cell apoptosis. The neutral comet assay revealed that the treatment with melanoxoin induced significant DNA damage in H1299 cells. Therefore, we are interested in the development the molanoxoin as a new source of anti-cancer drug. .

PD114 Withaferin A activity against glioblastoma cells: Supression of microtubule dynamics

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Withaferin A (Wit A) is a natural product, isolated from the plant *Withania somnifera*, which is known to possess anti-cancer activity. The analysis of the cell cycle of glioblastoma cells (SF-268 cell line) treated with 1 or 2 μM of Wit A for 24 h showed an arrest at the G_2/M phase. Confocal microscopy images confirmed that cells were arrested in mitosis and that microtubule formation is altered. Furthermore a tubulin polymerization assay showed that Wit A acts in a similar way to paclitaxel by inducing and stabilizing the formation of microtubules (Figure 1).

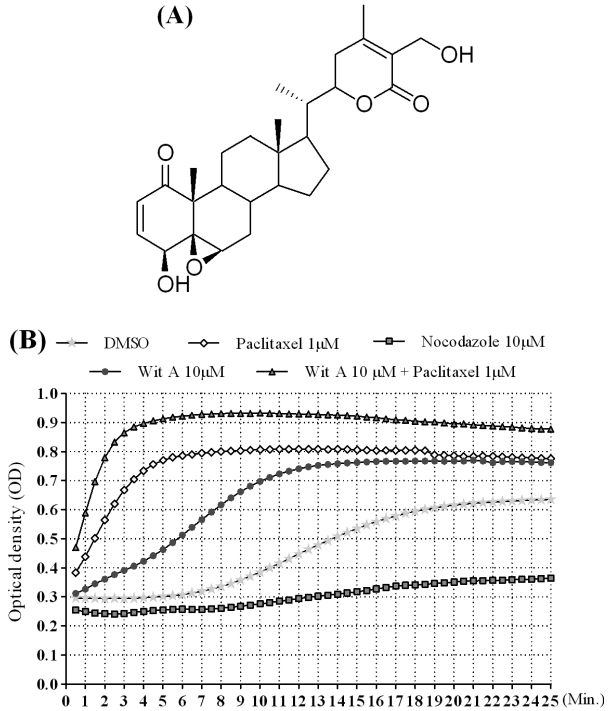

Fig. 1: (A) Molecular structure of withaferin A. (B) Tubulin polymerization assay.

PD115 Anti-inflammatory activity from new molecules derived from Convolutamydine A

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Convolutamydine A (4,6-dibromo-3hidroxyoxindole) is a substance isolated from the marine bryozoans *Amathia convoluta* and have several biological effects. The aim of this work was to evaluate the anti-inflammatory properties of three molecules synthesized from convolutamydine A in the carrageenan (Carr)-induced inflammation into the subcutaneous air pouch (SAP). Male mice (20 – 25 g) received oral administration of I-158, I-160 or I-161 (1 or 10 mg/kg) 1 h before Carr injection. After 24 h the mice were euthanized and the exsudate from SAP were collected to several measurements (listed below). Authorization for animals' assays was ICBDFBC015. Statistical analyses was performed by ANOVA and Bonferroni's test ($^*p < 0.05$). Results are:

			I-158		I-160		I-161	
	PBS	Carr	1	10	1	10	1	10
Cell	2.9 ± 1	63.1 ± 12	53 ± 9	44 ± 9	29 ± 8*	57 ± 9	33 ± 3*	48 ± 8
NO (μM)	20 ± 7	173 ± 14	79 ± 7*	112 ± 42*	86 ± 14*	28 ± 6*	148 ± 10	41 ± 13*
TNF-α (pg/mL)	61.1 ± 3	637 ± 53	340 ± 21*	281 ± 42*	391 ± 57*	190 ± 36*	240 ± 81*	382 ± 57*

Conclusions: All three substances demonstrated significant anti-inflammatory effect being I-160 the most promising to future assays. **Financial support:** CNPq, FAPERJ, Instituto Vital Brazil.

PD116 Evaluation of antinociceptive activity of *Pereskia bleo* Kunth

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Our aim was to identify the antinociceptive effect for *P. bleo* leaves as they are popularly used in Malaysia for the treatment of rheumatism, among other conditions. Plant material was collected in Malaysia (2009). An ethanol extract (E) was prepared from leaves (1.4 g) and submitted to liquid liquid partition with: hexane [H], ethyl acetate [EA], and butanol [B]. Antinociceptive activity was evaluated by the hot plate test (calculated as area under the curve, AUC) at the doses 30, 50, and 100 mg/kg. The mechanism of action was evaluated pretreating the animals with naloxone (1 mg/kg, N). The protocol for the use of animals received the number ICBDFBC015. Statistical analyses were performed by ANOVA and Bonferroni's test (* or $^\#$ $p < 0.05$ in relation to vehicle or fraction-treated group, respectively). **Results:**

mg/kg	Vehicle	Morphine	E	H	EA	B
30	1,425 ± 159	5,153 ± 581*	1,071 ± 113*	2,268 ± 233*	1,340 ± 141	4,341 ± 450*
50	1,425 ± 159	5,153 ± 581*	2,495 ± 244*	3,306 ± 344*	4,293 ± 401*	3,318 ± 365*
100	1,425 ± 159	5,153 ± 581*	2,826 ± 314*	2,415 ± 256*	3,153 ± 336*	7,292 ± 741*
100 + N	1,219 ± 113	133 ± 42#	1,808 ± 231#	256.5 ± 301#	172.5 ± 55#	1,146 ± 129#

Conclusions: Fractions from *P. bleo* demonstrated central antinociceptive effect mediated, at least in part, by opioid system. **Financial support:** CNPq, FAPERJ, and Instituto Vital Brazil.

PD117 Vasorelaxation induced by (-)-α-Bisabolol in rat aorta is preferential to voltage-operated contractile pathways

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(-)-α-Bisabolol (BIS) is a sesquiterpene alcohol found in the essential oil of *Matricaria chamomilla* L. Here, we investigated the underlying mechanism involved in its vasodilator effects. Ring-like preparations obtained from rat aorta were maintained in bath chambers for isometric trace recordings. BIS (1 – 1000 μM) more preferentially relaxed K^+ (60 mM)- than phenylephrine (PHE, 1 μM)-induced contractions, as evidenced by the IC_{50} values (24.9 [17.0 – 36.5] μM (n = 8) and 162.0 [91.0 – 288.2] (n = 7), respectively). Potency of BIS for relaxing PHE contractions was not changed by L-NAME, ODQ, TEA, glybenclamide or

indomethacin pretreatment. BIS did not reduce phasic contractions induced by caffeine or by PHE under Ca^{2+}-free conditions. It did not alter contractions induced by Ca^{2+} restoration outside the cell after cyclopiazonic acid treatment. In presence of BIS (100 μM), the contraction induced by Ca^{2+} (3 mM) corresponded to 73.3 ± 5.7% (expressed as % of an initial reference response induced by 60 mM K^+) when PHE was the contractile agent in presence of nifedipine, a value which was significantly higher ($p < 0.05$) than that (16.0 ± 2.1%) observed when the contractile agent was a depolarizing solution with a high K^+ (60 mM) content. Simultaneous measurement by confocal microscopy in Fura-4 AM-loaded vessels showed that BIS (100 μM) reduced intracellular Ca^{2+} levels in response to K^+. Thus, BIS has a vasodilator property in rat aorta acting preferentially against electromechanical pathways.

PD118 Relaxant effects of β-Citronellol in isolated aortic or tracheal rings of rats

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β-Citronellol (βCT) is a constituent found in the essential oil of *Cymbopogon citratus.* Here, we investigated the relaxant effects of βCT on vascular (aorta) or non-vascular (trachea) smooth muscle preparations obtained from rats. Trace recordings were obtained isometrically. βCT (1 – 1000 μM) had no measurable effects on trace recordings of aortic or tracheal preparations under resting tone conditions. βCT fully relaxed aortic rings contracted by 60 mM K^+ whereas it relaxed just partially (~70%) the phenylephrine (PHE, 1 μM)-induced contractions. Values of IC_{50} were 255.7 ± 55 (n = 6) and 356.15 ± 79.5 (n = 5) μM, respectively, which did not reach significant difference ($p > 0.05$) when compared one another. In tracheal preparations, βCT fully relaxed as 60 mM K^+-as carbachol (CCh)-induced contractions with a similar pharmacological potency (IC_{50} values were 111.3 ± 25.2 [n = 6] and 174.4 ± 32.0 [n = 6] μM, respectively). In tracheal tissues under Ca^{2+}-free conditions, βCT (200 μM, n = 6) impaired the contractions induced by exogenous addition of Ba^{2+} (0.1 – 50 mM), an exclusive voltage-operated Ca^{2+} channel (VOCC) permeable ion, in preparations depolarized by K^+ (60 mM). In aortic tissues, βCT (200 μM, n = 6) did not change Ba^{2+}-induced contractions under similar conditions. Thus, βCT has relaxant properties in aortic or tracheal smooth muscle preparations and it seems likely that βCT behaves as a VOCC blocker in tracheal tissues, but not in aortic rings.

PD119 Vasorelaxant effect of Dihydrospinochalcone A isolated from *Lonchocarpus xull*

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Lonchocarpus xuul is an endemic tree from the Yucatan Peninsula, Mexico.The present work research was conducted to evaluate the vasorelaxant effect of dihydrospinochalcone A (DECh A), a compound type chalcone, isolated from *Lonchocarpus xuul.* Activity of DECh A was determined *ex vivo* on aorta rat rings test with- and without endothelium, and *in vivo* on spontaneously hypertensive rats (SHR) model. DECh A showed a partially endothelium-dependent and concentration-dependent vasorelaxant effect (Emax = 79.67% and EC_{50} = 21.46 μM E+, Emax = 23.58% EC_{50} = 91.8 μM). The functional mechanism of action for DECh A was elucidated due to its significant activity. Pre-incubation with L-NAME, atropine, TEA and ODQ produced a significant change in the maximum vasorelaxant effect. Finally, orally DECh A administration (50 mg/kg) in rats SHR decreased diastolic and systolic blood pressure up to 30% from the first hour and the effect was sustained during all experimental time. The heart rate was not modified. In conclusion, DECh A functional vasorelaxant mechanism is linked to the NO/GMPc system derived-endothelium, due to the possible muscarinic receptor agonist and the increment in the production of nitric oxide. The release of NO increases cGMP production in smooth muscle, which mediates the opening of potassium channels, and in consequence, the vasorelaxant effect occurs. Finally, the *in vivo* antihypertensive effect induced by DECh A is directly related with its vasorelaxant activity.

PD120 Linalool-rich rosewood oil evoked a vago-vagal bradycardic and depressor effect in normotensive rats

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Cardiovascular effects of the linalool-rich essential oil of *Aniba rosaeodora* (EOAR or rosewood) in normotensive rats were investigated. Male Wistar rats were anesthetized and two catheters were implanted for blood pressure recording and drug injection. In anesthetized rats, intravenous (i.v.) EOAR elicited dose-dependent hypotensive and bradycardiac effects which were characterized in two periods (phases 1 and 2). The first rapid component (phase 1) evoked by EOAR was abolished by bilateral vagotomy, perineural treatment of both cervical vagus nerves with capsaicin and was absent after left ventricle injection. However, i.v. pretreatment with capsazepine, ondansetron or HC030031 did not alter phase 1 of the cardiovascular responses to EOAR. In conscious rats, EOAR evoked rapid hypotensive and bradycardiac (phase 1) effects that were abolished by i.v. methylatropine. EOAR induces a vago-vagal bradycardiac and depressor reflex (phase 1) that apparently results from the stimulation of vagal pulmonary rather than cardiac C-fiber afferents and does appear to involve activation of neither the $5HT_3$ receptors nor the two chemosensory ion channels $TRPV_1$ and $TRPA_1$ receptors. The phase 2 hypotensive response to EOAR seems to result from a direct vasodilatory effect since EOAR relaxed phenylephrine-induced contractions in rat isolated aortic rings.

PD121 Caffeic acid methyl and ethyl esters induce the translocation of glucose transporter GLUT4 in cultured skeletal muscle cells

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Derivatives of caffeic acid (CA) are widely distributed in the plant kingdom. In a previous study, CA methyl ester (CAME) and CA ethyl ester (CAEE) were reported to potently stimulate glucose uptake in cultured C2C12 skeletal muscle cells. In the present study, we investigated the effect of these compounds on the translocation of insulin-sensitive glucose transporter GLUT4 in cultured L6-GLUT4*myc* cells. Levels of GLUT4-*myc* at the cell surface were measured by O-phenylenediamine dihydrochloride (OPD) assay. The effects of CAME and CAEE on insulin and AMPK signaling pathways as well as on GLUT4 content were also assessed by western immunoblot. Both compounds significantly increased glucose uptake and GLUT4 translocation to the cell membrane of L6-GLUT4*myc* cells. Moreover, the two compounds increased phosphorylation of AMPK and increased GLUT4 content. CAME and CAEE may hence improve glucose uptake in L6 cells by promoting GLUT4 expression and translocation through an insulin-independent mechanism involving AMPK. The results of the present study suggest that CAME and CAEE may have therapeutic potential to counter the hyperglycemia of diabetes.

PD122 Antioxidant, anticancer and apoptotic inducer activities of piperaceae extracts on hela cells line

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Background: Our research is to explore the herbal medicine as an alternative therapy for obtaining chemotherapeutic drugs base on the antioxidant, cytotoxic and apoptotic inducer activities. *Piperaceae* including *Piper betle* L., *Piper cf. fragile* Benth, *Piper umbellatum* L., *Piper aduncum* L., *Piper pellucidum* L. are popularly used as herbal efficacy. **The objective:** this research was conducted to elucidate the cytotoxic and apop-

totic inducer activities on HeLa cell line and antioxidant of *Piperaceae* water extracts. **Method:** the cytotoxic activity was determined by inhibiting proliferations cells and apoptotic activity was determined with Sub-G1 flowcytometric, the antioxidant activity was determined by using Superoxide Dismutase (SOD) value and Inhibitory Concentration-median (IC-50) of *2,2-diphenyl-1-picrylhydrazyl* (DPPH) radical scavenging activity. **Results:** The IC-50 of cytotoxic for 24 hours incubation were 7.13; 2.93; 6.71; 3.91; 2.85 μg/ml respectively and Cisplatin 0.07 μg/ml; 48 hours incubation were lower than 24 hours incubation. The highest apoptotic inducer for 24 hours and 48 hours were *P. betle* 12.5 μg/ml, *P. cf. fragile* 50 μg/ml, *P. umbelatum* 25 μg/ml, *P. aduncum* 100 μg/ml, Cisplatin 8 μg/m. The highest SOD activity *was P. betle* extract 108.01 U/ml. The IC-50 of DPPH scavenging activity respectively were 5.56; 52.49; 15.36; 102.84; 9.00 μg/ml. **Conclusions:** *P. betle* extract has the highest antioxidant activities, all *Piperaceae* extracts has high cytotoxic. *P betle* extract exhibit the highest apoptotic inducer activity.

PD123 Behavioral effects of cannabinoids on zebrafish larvae

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Cannabinoids are natural or synthetic compounds related chemically to Δ^9-THC, the principle psychotropic constituent of the hemp plant, *Cannabis sativa* L. Here, we examine the effects of the cannabinoids Δ^9-THC, WIN 55,212 – 2 and CP 55,940, and the cannabinoid antagonist (Am 251). Exposures were either acute (1 – 12 h exposure at 108 hour post fertilization [hpf]); or chronic (96 h exposure starting at 24 hpf. Wild type zebrafish embryos (3,250, including controls), were cultured individually in 250 μl defined buffer in 96-well plates. Geometric range-finding was used to determine the experimental concentrations At day 5, behavioural analysis (visual motor response test) was carried out in which movement of individual larvae was analysed using automated video-tracking. With acute exposure, embryos showed a biphasic response to the dark challenge with Δ^9-THC, WIN55, 212 – 2 and CP55,940. This response consisted of stimulation of locomotor activity at low concentrations, suppression at high doses. With the antagonist AM251 alone, the locomotor activity was suppressed at high concentrations. With chronic exposure, embryos habituated to the effects of all three cannabinoids when assayed with the dark challenge phase. Furthermore, the excitation was ameliorated when antagonist was co-administered with the cannabinoid. We conclude that cannabinoids have similar effects in zebrafish and mammals. In particular, the acute exposure response resembles behavioural effects reported for adult rodents.

PD124 Screening of Venezuelan medicinal plant extracts for cytostatic and cytotoxic activity against tumour cell lines

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There are estimated to be more than 20,000 species of plants in Venezuela, of which more than 1,500 are used for medicinal purposes by indigenous and local communities. Only a relatively small proportion of these have been evaluated in terms of their potential as antitumour agents. In this study, we screened 308 extracts from 102 species for cytostatic and cytotoxic activity against a panel of 6 tumour cell lines using a 24 h sulphorhodamine B assay. Extracts from *Clavija lancifolia, Hamelia patens, Piper san-vicentense, Physalis cordata, Jacaranda copaia, Heliotropium indicum* and *Annona squamosa* were the most cytotoxic, whereas other extracts from *Calotropis gigantea, Hyptis dilatata, Chromolaena odorata, Siparuna guianensis, Jacaranda obtusifolia, Tapirira guianensis, Xylopia aromatica, Protium heptaphyllum* and *Piper arboreum* showed the greatest cytostatic activity. These results confirm previous reports on the cytotoxic activities of the above-mentioned plants as well as prompting further studies on others such as *Clavija lancifolia* and *Hyptis dilatata* that have not been so extensively studied.

PD125 Development and optimization of a metabolite extraction process for the high throughput screening of microalgal chimiodiversity

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Since a decade, using microalgae for biofuel has become a major challenge worldwide. Several studies have evidenced that potent bioactive molecules can be purified from microalgae for cosmetic or health applications (*e.g.* antioxidants, immunostimulants, anticancer and antiviral compounds) in order to upgrade biomass. In some species, extraction of bioactive metabolites is tricky for the presence of highly resistant cell walls (*Phaeodactylum tricornutum*) or exopolysaccharidic barriers surrounding the cell membrane (*Porphyridium purpureum*). It was therefore essential to develop a process that preserves sensitive molecules. We optimized an extraction grinding process, the Mix Mill process, based on the use of vibrating microbeads. The process efficiency was assessed in pigments extraction experiments. It gave excellent extraction yields, and combined fidelity (no modification of analytes), compatibility with HPLC and LC-MS analysis, accuracy, simplicity, rapidity and safety. Moreover, it allowed to simultaneously extract all pigments, whatever their polarity or the strength that linked them to the cell structures. As a conclusion, the Mix Mill process is a general process for the total extraction of microalgal metabolites thought for high throughput screening of the microalgal chimiodiversity.

PD126 Inhibition of leukemia-associated transcription factor c-Myb by sesquiterpene lactones and further natural products

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The transcription factor c-Myb plays an important role in haematopoiesis, particularly during expansion and self-renewal of immature myeloid and lymphoid progenitor cells. Acute and chronic myeloid leukemia cells depend on the presence of c-Myb for their proliferation and viability and are more sensitive to c-Myb inhibition than their normal counterparts [1]. We recently reported the potential of sesquiterpene lactones (STLs) as first cell-permeable, low-weight inhibitors of the transcription factor c-Myb [2]. Currently, more than 90 different natural substances have been tested as possible c-Myb inhibitors in our GFP-based reporter gene assay. The most active compounds identified so far (two STLs, three Naphthoquinones and two quinone methide triterpenoids) displayed IC_{50} values < 1 μM. MTS-Assays were conducted to exclude unspecific cytotoxic effects as mere cause for the observed activity. Analysis of structure-activity relationships revealed alkylation as likely mechanism of action. However, it was shown that the mere capability to react covalently with biomolecules alone is not sufficient for c-Myb inhibition. An ongoing investigation of quantitative structure-activity relationships (QSAR) for more than 50 tested STLs gives insights into structural requirements for strong c-Myb inhibition. References: [1] Ramsay, R.G. & Gonda, T.J. (2008), *Nature Rev. Cancer* 8: 523 – 534 [2] Bujnicki T, Wilczek C, Schomburg C et al. (2012) *Leukemia* 26, 615 – 622.

PD127 Optimization of the microplate resazurin assay as a screening tool for natural products with anti-mycobacterial activity

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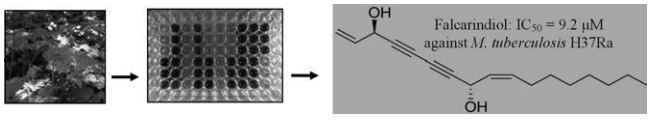
The microplate resazurin assay (MRA) is commonly used for the isolation of anti-mycobacterial compounds. However, we found the MRA to be problematic in that it provided inconsistent results when used with phytochemical extracts. In this study we optimized the MRA for both screening and bioassay-guided fractionation of phytochemical extracts to facilitate the discovery of potential anti-mycobacterial therapeutics. MRA aspects that were evaluated include: assay duration; optimization of resazurin indicator concentration; solvent (DMSO) effect on mycobacterial growth; and microtitre plate type used. The optimized MRA was validated through bioassay guided isolation of falcarindiol, the major anti-mycobacterial natural product in extracts of Heracleum maximum, a medicinal plant used by the Eastern Canadian First Nations to treat TB.



PD128 Tubulin ligands identified on screening natural products from nubbe database

Valli M[1], Santos RN[2], Figueira LD[1], Vieira Jr. GM[3], Funari CS[1], Regasini LO[1], Lopes MN[1], Cavalheiro AJ[1], Araújo AR[1], Furlan M[1], Silva DHS[1], Castro-Gamboa I[1], Andricopulo AD[2], Bolzani VS[1]
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One of the objectives of the research group Nucleus of Bioassays, Ecophysiology and Biosynthesis of Natural Products (NuBBE) is the search for biologically active compounds from plants, marine organisms, and endophytic fungi from Brazilian biodiversity. To date, 640 secondary metabolites and derivatives have been published by NuBBE in specialized literature. Considering the importance of this molecular information, a new computational system was developed for the creation of a virtual database of these compounds beholding chemical and biological properties, chemical structure and further pharmacological and toxicological information. This database was designed to be a useful tool to manage information on natural products from Brazilian biomes and semi-synthetic derivatives, for future studies on computational screening, dereplication, metabolomics and design of novel bioactive compounds. Furthermore, molecular docking studies were accomplished with these compounds with the protein tubulin, and 25 compounds were selected for tubulin *in vitro* screening. Six active compounds were identified, which will be presented and discussed along with the developed database.

PD129 Tetrahydroisoquinoline alkaloid derivatives Inhibit 7-Dehydrocholesterol reductase

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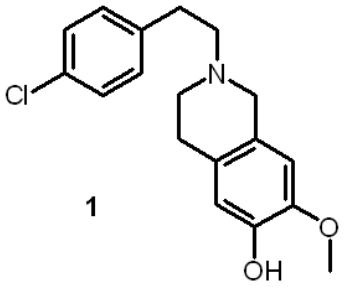
We prepared new phenethyltetrahydroisoquinolines, derivatives of plant isoquinoline alkaloids. Several of the compounds showed a strong and selective inhibition of 7-dehydrocholesterol reductase (7-DHCR), an enzyme responsible for the conversion of 7-DHC to cholesterol in the last step of cholesterol biosynthesis. A defect of 7-DHCR is associated with the Smith-Lemli-Opitz syndrome (SLOS), a known autosomal recessive trait accompanied by severe abnormalities. Our investigations led to the discovery of **1** as a selective and 200 times stronger inhibitor of 7-DHCR than the established less selective inhibitor BM 15.766. The isoquinoline **1** had no effect on ergosterol biosynthesis in fungi and displayed very low cytotoxicity. The new inhibitor of 7-DHCR will be a useful tool for studying molecular details of the pathogenesis of SLOS.



1

PD130 Antibacterial and anti-biofilm activities of cinchona alkaloid derivatives against *Staphylococcus aureus*

Skogman ME[1], Kujala J[1], Busygin I[2,3], Leino R[2], Vuorela P[1], Fallarero A[1]
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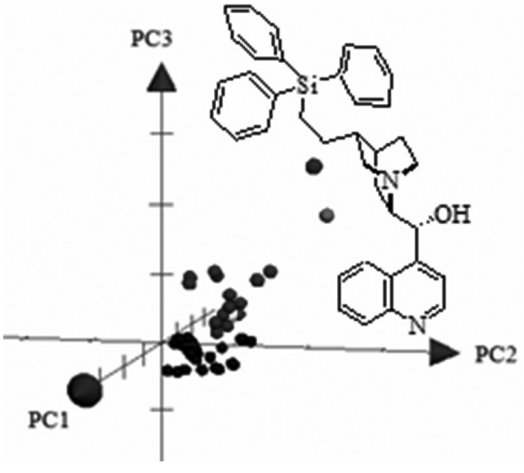
Biofilms is the predominant bacterial lifestyle in nature and they are resistant to commonly available antibacterials. Thus, an enormous need exists to find new effective anti-biofilm therapy. Here a library of cinchona alkaloids was screened for activity against *Staphylococcus aureus* biofilms. Crystal violet and resazurin assays were used to measure effects on biomass and viability, respectively. One derivative, 11-TPSCD (structure), was found effective against planktonic bacteria and in preventing biofilm formation (IC_{50} ~ 6 µM) but higher concentrations were required to eradicate mature biofilms. An exploration of the chemical space occupied by the active derivative (red dot) in comparison to the cinchona alkaloid library and the structurally related quinolone antibiotics was made using the principal component analysis (PCA)-based model ChemGPS-NP. The results suggest that another interesting region exists for antibacterial and anti-biofilm activity that can be populated by quinine-type antimicrobials, outside of the one defined by quinolone antibiotics.



PD131 Natural products as butyrylcholinesterase inhibitors – Screening of a small compound collection

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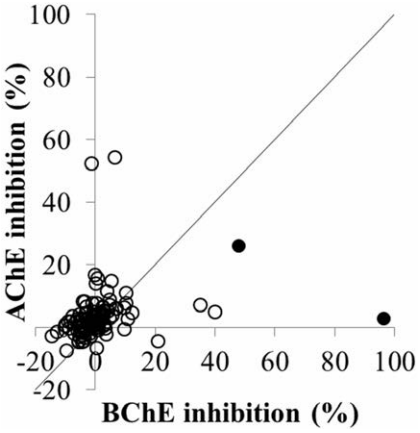
Investigation of the involvement of butyrylcholinesterase (BChE) in diseases such as Alzheimer's disease (AD), type 2 diabetes mellitus (T2DM) and multiple sclerosis (MS) makes it a valuable target for drug discovery. Cholinesterase inhibitors of natural origin have shown success in AD therapy and have encouraged further exploration of nature as a source of cholinesterase inhibitors. Using the Ellman's reaction a collection of 111 compounds containing commercially available compounds e.g. flavonoids and coumarins, as well as a subset of cinchona alkaloid derivatives synthesized by our group, were screened for anti-cholinesterase activity. Two cinchona alkaloids were found to inhibit the hydrolase activity of BChE in the micromolar range ($IC_{50} \leq 10\,\mu M$) and were further characterized. Considering their wide medical use (mostly as antimalarials) the cinchona alkaloid scaffold may serve as an advantage in drug development for various diseases.



PD132 The isoflavone biochanin a inhibits the growth of the intracellular bacteria *Chlamydia trachomatis* and *Chlamydia pneumoniae*

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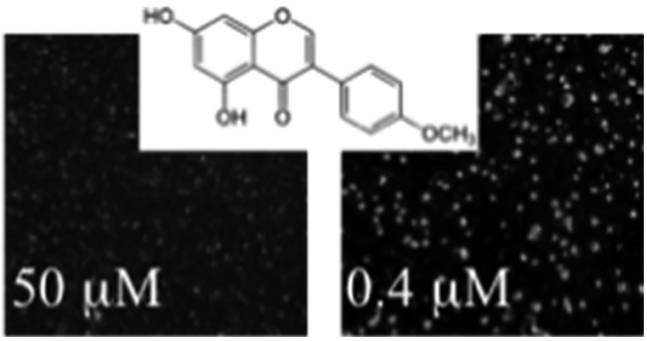
Epidemiology and physiological consequences of chlamydial infections show these intracellular bacteria to have maintained their prevalence. Especially *C. pneumoniae* are able to confer to a treatment refractory chronic state of infection that cannot be eradicated with currently available therapeutic options. Here we report the effects of biochanin A on the growth of intracellular *Chlamydia* spp. It is the main flavonoid component of red clover (*Trifolium pratense*) extracts, which besides its estrogenic and antioxidative properties is known to potentiate the antibacterial effects of other chemical agents by inhibiting bacterial efflux pumps. We identified biochanin A as a hit compound in a high-content screen of purified natural products for *C. trachomatis* growth inhibitors. It was found to inhibit the replication of *C. pneumoniae* clinical strain K7 (IC_{50}= 12 μM) and to prevent 100% of infectious progeny production at 50 μM. Thus, biochanin A is a more potent inhibitor of *C. pneumoniae* than the related isoflavone genistein, which we have earlier shown to be only moderately active against this bacterium. Further, this data suggests that biochanin A acts as a direct growth inhibitor rather than an antibacterial potentiator against these pathogens.



PD133 Antiulcer and *in vitro* antioxidant activities of *Brachystegia eurycoma*

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The study aimed at evaluating the antiulcer activities in rats and determines its antioxidant capacity *in vitro*. The antiulcer property was evaluated using absolute ethanol, indomethacin and pylorus ligation as necrotizing agents. Acute toxicity was also carried out. The extract was screened for its *in vitro* antioxidant capacity using various methods. Polyphenolic contents were also quantified. *B. eurycoma* extract blocks ethanol induced gastric lesions, causing 44.30%, 79.96% and 52.74% protection at 50, 100 and 200 mg/kg respectively. Significant ($p < 0.05$) antiulcerogenic activity was observed with 100 mg/kg dose of the extract and cimetidine (70.46%) when compared to negative control group. In indomethacin model, the extract decreased ulcer index in a dose dependent manner; the highest dose (200 mg/kg) and cimetidine (100 mg/kg) produced statistically significant protection index. The extract though produces a significant protective effect and lowered gastric secretion volume at 50 and 100 mg/kg, failed to significantly decrease the acid output compared to control group. The extract did not produce any toxicity up to a dose of 8000 mg/kg. Various degree of the antioxidant activity was recorded for *B. eurycoma*. The extract possesses considerable amount of total phenols (62.83 ± 0.02 mg GAE/g of extract), flavonoids (6.58 ± 0.001 mg QE/g of extract) and proanthocyanidins (12.58 ± 0.006 mg catechin E/g of extract). The results indicate that the ethanol extract of *B. eurycoma* has a protective effect in gastric ulcers, which can be attributed to its antioxidant potential.

PD134 Multiple enzyme inhibition potential of *Butea superba* for management of erectile dysfunction

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Inhibitors of Rho-kinase II (ROCK-II), soluble epoxide hydrolase (sEH) and Phosphodiesterase type 5 (PDE5) enzymes have been reported to relax corpus cavernosum smooth muscles and increase intracavernous pressure. Plant extract capable of inhibiting the enzymes, which might be useful in management of erectile dysfunction (ED) were screened using commercially available kits. Methanolic extract of *Butea superba* (MEBS) at 50 μg/mL was found to inhibit ROCK-II, sEH and PDE5 upto 51.8 ± 0.5, 24.73 ± 0.1 and 51.9 ± 0.3% respectively. Erectile function increasing potential of *Butea superba* extract is reported in rats as well as humans. Hence, erectile function increasing activity of MEBS might be due to multiple enzyme inhibition potential of the extract.

PD135 Antibacterial activity of the methanolic extract of *Hyptis atrorubens* (Lamiaceae)

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The leaves and stems of *Hyptis atrorubens* Poit. (Lamiaceae) were collected in Guadeloupe. The methanol extract was tested at six concentrations (10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.2 mg/mL, 0.6 mg/mL and 0.3 mg/mL) against a panel of pathogenic Gram-positive and Gram-negative bacteria *in vitro*. This methanolic extract demonstrated antibacterial activity against two Gram-positive bacteria (*Staphylococcus epidermidis* and *Enterococcus faecalis*), and two Gram-negative bacteria (*Burkholderia cepacia* and *Stenotrophomonas maltophilia*) at all of the concentrations. Bioautography enabled the obtention and identification of two antibacterial compounds from this plant: rosmarinic acid and methyl rosmarinate. This work supports the recognition of *H. atrorubens*, a traditional remedy in French West Indies, as a medicinal plant in France.

PD136 Antimicrobial screening of natural product extracts using a bioluminescent assay

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[1]Centre for Drug Research; [2]Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki, Finland; [3]Department of Chemistry and Bioengineering, Tampere University of Technology, Finland

We have developed a high throughput screening method for the discovery of new antimicrobial compounds, using a novel approach with recombinant bioluminescent bacterial strain *Escherichia coli* K-12/pTetLux. This recombinant strain can particularly be used to indicate compounds that affect microbial transcriptional and translational events [1]. The developed method improves the feasibility of using natural product extracts as a screening resource as the assay is less prone to interference from coloured or turbid samples. Compared to conventional antimicrobial testing, this biosensor-based method offers other important improvements such as simultaneous data on antimicrobial activity and mode of action and reduction of assay time from 24 h to 2 – 3 h. Results reported here include method validation and miniaturisation into 384-well plate format as well as implementation on 22 plant extracts from our in-house collection Based on the results, fractions of seven promising extracts that had been prepared by HPLC into 96 well-plates were further tested. The results show that the developed bioluminescent technology is well suitable for natural product screening and is sensitive enough to identify bioactive fractions.

PD137 Potential anxiolytics acting via the neuropeptide S-receptor

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[1]Dept. of Pharmacognosy; [2]Dept. of Pharmaceutical Chemistry; [3]Dept. of Pharmacology, Institute of Pharmacy and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 80 – 82, 6020 Innsbruck, Austria

Neuropeptide S (NPS) represents the latest identified neuropeptide. Animal studies in rodents showed intracerebrally administrated NPS leading to an anxiolytic effect with a simultaneous increase of wakefulness. This activity profile of NPS differs from classical anxiolytic drugs, which cause beside the desired effects also drowsiness. It is therefore of high interest to investigate ligands of the NPS-receptor (NPSR) for their anxiolytic properties and evaluate their potential use as anxiolytic drugs. One promising strategy in the search for novel bioactive compounds is the use of virtual screening tools, which can effectively reduce cost and time efforts. This approach resulted in the first pharmacophore modeling study on NPSR ligands. Since the 3D structure of the G-protein coupled NPSR is not known, ligand-based pharmacophore models were generated. The theoretical model evaluation by two test sets containing non-peptidic active compounds and presumably inactive decoys revealed enrichment factors and ROC curves, which indicate a good predictive power of the generated models and will contribute to the rationalized search for novel NPSR ligands. The virtual screening of natural product databases led to promising structures in regard to traditional used plants.

PD138 The NPDI and Fundacion MEDINA collections combined again for natural products discovery

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An exciting new opportunity for Natural Products drug discovery: the non-profit research organizations, NPDI (Natural Products Discovery Institute) and Fundación MEDINA (Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía) are jointly offering a vast and diverse collection of plant and microbial fermentation samples to the research community for purposes of screening for pharmaceutical, agricultural, cosmeceutical and nutritional agents. These collections were constructed initially within the same company with the purpose of providing diversity and complementarity. Brought together again, this collection amounts to over 200K samples which are backed up with reserve materials and producing cultures, as well as extensive expertise in chemistry and microbiology garnered from decades of experience in a pharmaceutical setting. This poster will describe these collections in detail, along with some of their history, and will discuss terms under which researchers can access this productive resource through collaborations or fee-for-service arrangements.

PD139 Antileishmanial activity of bisbenzylisoquinoline alkaloids from the genus *Thalictrum*

Naman CB[1], Gupta G[2], Lezama-Davila CM[2], Doskotch RW[1], Satoskar AR[2], Kinghorn AD[1]
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As part of ongoing efforts to discover natural product compounds with antileishmanial activity as potential new drug candidates, we have recently begun screening a compound library comprising natural products and precursors in their total synthesis against *Leishmania donovani* promastigotes. One such sample, tb-00069, exhibited activity in this *in vitro* assay. The identity of tb-00069 was confirmed by NMR and MS to be *N*-desmethylthalrugosidine [1], a bisbenzylisoquinoline alkaloid isolated earlier from the roots of *Thalictrum alpinum* that has not been reported to be active against *L. donovani*. This work further represents the first full spectroscopic characterization of **1** as its structure was originally determined by chemical degradation techniques. Another sample, tb-00078, was shown to also be active in the same bioassay. It was suggested to have a similar structure to **1** and was examined by NMR and MS for structural verification.

PD140 Nicotine analogues: Phenyl azetidines and azetidinones as potential insecticidal agents

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Nicotine shows insecticidal properties, acting as an agonist at the insect nicotinic acetylcholine receptor. Our work involves the investigation of the insecticidal properties of compounds based on the structure of nicotine. The important structural components of nicotine include the aromatic pyridine ring and the aliphatic pyrolidine ring. Our interest is in changing both of these ring types (to benzene and azetidine, respectively) and measuring the insecticidal properties of the intermediates and end products. Because the pyridine ring is deactivated compared to benzene, we have deactivated the benzene ring by adding a chloro group. The chloro group has been added in the ortho, meta, and para position. Suitably substituted vinyl chloro benzene has been used as a starting compound to ultimately generate the azetidine. Synthesis of these compounds involves a (2+2) addition of chloro sulfonyl isocyanate to vinyl benzene to give the azetidinone in two steps followed by a reduction to yield the azetidine ring. A biological assay for each of the

intermediate compounds was performed using the third instar larval stage of *Musca domestica*. This stage was chosen because it is physiologically similar to the adult and easier to handle, and also because the strongest activity of nicotine is observed on soft-bodied insects. Synthesis and bioassay results will be presented.

PD141 Natural products as inhibitors of purine nucleoside phosphorylase, a target against *Plasmodium spp*

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Malaria is a parasitic disease killing over one million people every year, mainly in poor regions of the globe where access to diagnostics and conventional therapy is restricted. The use of traditional medicines with proved clinical efficacy has recently been considered a strategy in the early treatment of uncomplicated *falciparum* malaria. Such a strategy encourages the clinical assessment of traditional plant preparations based on their ethnopharmacology and *in vitro* results. Indeed, a large number of plant extracts and isolated natural products have shown *in vitro* and *in vivo* antimalarial activity so far. However, their mechanisms of action have rarely been identified. This work presents the validation of an assay measuring the inhibition of purine nucleoside phosphorylase (PNP), a target against *Plasmodium falciparum*, for the screening of natural products. Enzyme kinetic parameters were determined in a 96-well plate spectrophotometric assay. Cladribine, a known PNP inhibitor, was selected as a positive control for its IC_{50} (154 μM), K_i (76 μM) and mode of inhibition (non-competitive mixed). The validated assay was then applied to a library of ca. 40 natural products of various classes, known or predicted to present an *in vitro* antiplasmodial activity. Two compounds, plumbagin and rhein, inhibited PNP with IC_{50} values of 114 and 158 μM, respectively, comparable to cladribine. Previous studies showed the *in vitro* activity of plumbagin against *P. falciparum* with an IC_{50} of 0.4 μM and with no effect on the inhibition of β-hematin. Our results suggest a novel mechanism of action for this compound, and show the application of the PNP screening in searching for natural antimalarials and in elucidating mechanisms of action.

PD142 Anti- bovine viral diarrhea virus activity of cucurbitacins as new potential antiviral agents

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Cucurbitacins are a group of tetracyclic triterpenoids found in plants of the family *Cucurbitaceae*. Cucurbitacins have a wide range of biological activities including anti-cancer, anti-inflammatory, and anti-viral activity. Bovine viral diarrhea virus (BVDV) is the prototype of *Pestivirus* genus of the *Flavivridae* family. BVDV is considered to be a surrogate for hepatitis C virus (HCV) in identifications and development of anti-HCV drugs. In this study, six cucurbitacins (B, D, E, E glucoside, Iso B, and Iso D) were isolated from *Cucurbita texana* (Cucurbitaceae), and characterized by spectroscopic methods (NMR, MS). The antiviral affect of cucurbitacin compounds against BVDV (NADL strain) was determined using colorimetric lactate dehydrogenase (LDH) assay. Four compounds (B, D, E, and E- glucoside) showed anti BVDV activity with a 50% effective concentration (EC_{50}) ranging between 0.025 and 2 μM. A study to explore the mechanism of action using real time reverse transcription - PCR and anti-HCV activity of these compounds will be presented. This is the first known report of anti BVDV activity of cucurbitacin compounds.

PD143 Harnessing virtual screening for the targeted discovery of cisplatin adjuvants from marine invertebrates

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Cisplatin is a widely used chemotherapeutic agent that acts by damaging DNA, ultimately leading to apoptosis. However, studies with patient tissues have shown positive correlation between overexpression of the DNA repair enzyme ERCC1 and resistance to cisplatin. In this study, we have used virtual screening (VS) of 3D libraries derived from MarinLit targeting a protein binding pocket critical for recruitment of ERCC1. VS results were used in the chemotaxonomic selection of "prioritized" organisms as a pre-filter to in vitro HTS of marine natural products disrupting this protein-protein interaction. This methodology has provided a validation of the predictive value of the VS, a six-fold reduction of screening burden and a near two-fold increase in validated hit rate relative to "non-prioritized" organisms. We have also identified several potential leads from known compounds to discover disruptors of this DNA repair mediated resistance in cancer.

PD144 Developing a natural products extract library based on the biodiversity of the Southern Appalachian Mountains

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Natural product and biotechnology research demands taxonomically verified plant material of known genetic origins to produce high quality, reproducible results. Additionally, there is a need for a source of plant material known to be adulterants in commercial botanical products to develop screening methods. Historically germplasm collections focused on the long-term conservation of major crop species and their wild relatives. As a result, medicinal plants are under-represented in many germplasm collections. The Bent Creek Germplasm Repository (BCRG) at The North Carolina Arboretum, UNC maintains an extensive collection of sustainably sourced, taxonomically verified medicinal plant germplasm of known genetic origin focused on the extensive botanical diversity of the Southern Appalachian Mountains. The extensive collections of the BCGR are currently being used to develop a research library of natural products extracts. The library consists of alcoholic and aqueous extracts of botanical material and organic and aqueous extracts of cultured endophtyic fungi isolated from plant material. The library will be screened for bioactivity to identify active compounds for botanically based products and potential drug leads. The inclusion of endophytes with the plants allows the investigation of natural products from fungal cultures. And the potential for rapid scale up using preserved endophytic material for structural determination and future research. The BCGR is currently seeking collaboration with laboratories and researchers interested in screening the natural products library.

PD145 An image-based 384-well high-throughput screening method for phenotypic discovery of biofilm inhibitors in *Pseudomonas aeruginosa*

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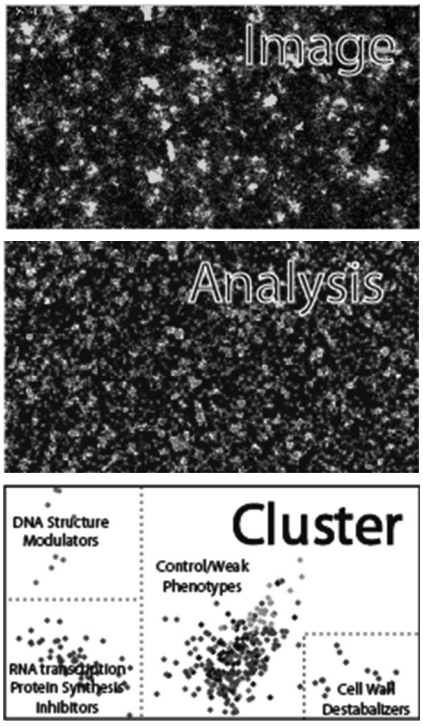
We developed a high-throughput high-content phenotypic screen for *Pseudomonas aeruginosa* to identify biofilm inhibitors and determine the mode of action of antibiotics in crude natural product extracts. The screen uses an image-based quantification of biofilm phenotypes to identify biofilm inhibitors and cluster natural product crude extracts by mode of action. Screening extracts of 300 unique marine microbes, we discovered 20 crude extract hits. From these 20 crude hits, we identified 2 unique biofilm inhibitors. Screen development and identification of small molecules will be presented.



PD146 Modulation of inflammation and apoptosis by FP7-MAREX marine natural products

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The European founded FP7-MAREX project (20011 – 14) is a joint effort of 19 academic, research institute, and industrial partners from 13 countries. Through close co-operation between industrial and academic partners, MAREX is collecting, isolating and classifying marine organisms, such as micro- and macroalgae, cyanobacteria, sea anemones, tunicates and fish from the Atlantic, Pacific and Indian Oceans as well as from the Mediterranean, Baltic and Arabian Seas. Extracts and purified compounds of these organisms are being studied for several therapeutically and industrially significant biological activities, specially by our group for anticancer anti anti-inflammatory activities by applying micro-capillary human cell based high content screening tools. New promising extracts subjected to chromatographic separation and showing bioactivities were found. New tools for a predictive model of idiosyncratic hepatotoxicity in which extracts are administered to human HepG2 cell line within an inflammatory context have been developed as hepatotoxicity commonly results from drug-induced hypersensitivity. Finally, MAREX is expected to offer novel marine-based lead compounds for European industries and strengthen their product portfolios related to pharmaceutical, nutraceutical, cosmetic, agrochemical, food processing, material and biosensor applications.

PD147 Screeninig for phytotoxicity crude exctracts from the flora of crete and phytochemical investigation of Bellis longifolia

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In continuation of our research for the discovery of bioactive compounds from plants of the flora of Crete [1] we have screened 65 plant species for their phytotoxic activity. Different plant parts have been extracted and 249 extracts have been evaluated for their phytotoxic activity in *Lactuca sativa* L. (Asteraceae) and *Agrostis stolonifera* L. (Graminae). Among the most potent extracts was the methanolic extract of *Bellis longifolia* which is an endemic plant of Crete that had never been investigated. The metahnolic extract of *B. longifolia* was elaborated by a very efficient technique, the countercurrent chromatography (CCC) using a step-gradient method. For this purpose a series of five biphasic systems consisting of the solvents n-Hex/BuOH/EtOH/H_2O were used and led to the isolation of ten compounds. Four flavonoids, one phenolic compound, two triterpenes and three triterpene saponins were isolated and structural elucidated using NMR and HRMS. It is interesting that the three saponins, 3-β-D-fucopyranosyl-bayogenin, 3-β-D-fucopyranosyl-polygalacic acid and 28-β-D-fucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl]-polygalacic acid are new natural products. References: 1. Fokialakis et al. *J. Nat. Med.* 2007, 61, 38 – 45.

PD148 Cellular assays for identification of inhibitors of specific breast cancer molecular phenotypes from Texas plants

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Breast cancer is the most common cancer and second leading cause of cancer death in women. New therapeutic strategies targeting the various molecular phenotypes of breast cancer are in crucial need to more effectively treat this heterogeneous disease. Natural products provide an excellent source for the identification of new probes and potential therapies for certain molecular subtypes of breast cancer. The harsh environment of Texas is likely to have a significant influence on the chemical composition of local plants, potentially producing a wide variety of biologically active compounds. A library of crude extracts from understudied Texas plants was screened for antiproliferative activity against a panel of six breast cancer cell lines with various molecular phenotypes, including estrogen receptor positive, *HER2* positive and triple-negative subtypes using the sulforhodamine B assay. We identified 12 extracts with potent and selective antiproliferative activity against a specific triple-negative breast cancer subtype; basal, mesenchymal or luminal androgen receptor and one extract with selectivity against a *HER2* positive breast cancer cell line. These extracts demonstrated a dose-dependent ability to selectively inhibit cells possessing each of these phenotypes. Our results demonstrate the potential to identify specific inhibitors of molecularly distinct breast cancer subtypes from Texas plants.

PD149 Phytochemical and biological investigation of *daniellia oliveri* leaves (Fabaceae)

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Daniellia oliveri (Rolfe).Hutch and Dalz. (Fabaceae),commonly known as Ilorin balsam or Copaihu Africaine is an indigenous African tree found extensively in Nigeria,Benin,Cameroon and Gambia.The leaves of this plant are being used traditionally in Nigeria to treat bacterial infections,-gastrointestinal disturbance, as diuretic and aphrodisiac. The aqueous ethanolic extract was suspended in water and successfully extracted with dichloromethane and n-butanol to give dichloromethane and n-butanol soluble extracts which were investigated for antibacterial activity. The n-butanol extract was found to be active against the test pathogens: *S.aureus*,*B.subtilis*,*E.coli*,*P.aeruginosa* and *S.typhi*, with zones of inhibition ranging from 16 – 27 mm using agar well diffusion assay method.The n-butanol extract also inhibited spasmogenes induced contractions of isolated guinea pig ileum and the rhythmic contractions of rabbit jejunum.The anti-diarrheal activity of the extract was also investigated using castor oil induced diarrhea in mice at doses of 50,100 and 200 mg/kg. The extract showed significant protection against mice-pretreated with castor oil in comparison with loperamide the standard drug used. Toxicity study of the extract intraperitoneally revealed that the extract was relatively safe.Fractionation of this extract over column chromatography, sephadex LH-20 and preparative tlc led to the isolation of five Flavonoid glycosides identified as isorhamnetin 3-O-rutinoside,-quercetin 3-O-rutinoside,quercetin 3-O-glucoside, quercetrin and quercemeritin the structures were elucidated by NMR spectroscopy and compared with literature.Biological studies of the isolated Flavonoids is in progress..

PD150 Promising natural preservatives from *Lippia origanoides* essential oils (Verbenaceae)

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As preservatives in food and medicines have been related to damages to human health, food and pharmaceutical industries have been suffering a broad pressure from consumers in order to reduce or even avoid the use of chemical preservatives in their products. An interesting alternative is the use of more effective and safer preservatives than the current ones. In light of these facts, the aim of this work is to evaluate the antimicrobial activity of essential oils of *Lippia origanoides* (Q1 and Q2) in combination for development of new natural preservatives. The essential oils were obtained by hidrodistillation of dried leaves, using a Clevenger apparatus, for 2 hours. Qualitative and quantitative analyses of the oils were carried out by gas chromatography and mass spectrometry (GC/MS). The identification of substances was performed by comparing their mass spectra with the database of the GC/MS (Nist 62 lib.) and Kovats retention index. Antimicrobial assays were conducted by using the microdilution method in 96- well-plates, according to the CLSI standards. Analysis of the essential oils showed presence of carvacrol (48%) and endo-fenchol (61%) the major constituents of *L. origanoides* Q1 and Q2 respectively. The synergistic combinations with the highest spectrum of antimicrobial activity were 0.312 μL.mL-1 for essential oil of Q1 and 0.625 $\mu L.mL^{-1}$ for Q2, reaching all microorganisms except for *C. albicans*, for which the former (0.312 $\mu L.mL^{-1}$) was one of the two oils promoting a synergistic effect. These results have shown that both quimiotypes of *L. origanoides* are promising new preservatives.

PD151 Selective antiproliferative activity of spinasterol from *Physospemum verticillatum* against A549 and COR-L23 cancer cells

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Several plants have been screened for their potential antitumor properties in order to identify putative compounds with novel structures and/or mechanism of action. Three triterpene saponins, saikosaponin a, buddlejasaponin IV, and songarosaponin D, were isolated from the roots of *Physospermum verticillatum* Waldst & Kit (Apiaceae) and exhibited a strong cytotoxic activity against COR-L23 cell line. In the present study spinasterol was isolated as main component from the ethyl acetate-soluble fraction of the methanol extract of *P. verticillatum* and was examined for its antiproliferative activity against a panel of human cancer cell lines including ACHN, C32, Caco-2, COR-L23, A375, A549, LNCaP, and Huh-7D12. The cytotoxicity was evaluated using the sulforhodamine B (SRB) assay. Ethyl acetate-soluble fraction was active against COR-L23 and Caco-2 cells (IC_{50} values of 74.2 and 84.6 μg/ml, respectively). Spinasterol exhibited an higher activity than the positive control vinblastine against COR-L23 and A549 cell lines with IC_{50} values of 16.2 and 36.6 μM, respectively. A selective activity against tumor cells was demonstrated since spinasterol not affect the proliferation of skin fibroblasts (142BR) used as control cell line.

PD152 Tyrosinase inhibition and free radical scavenging activities from Amazonian *Bauhinia coronata*

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Bauhinia species (Fabaceae) are a very common ethnopharmacological resource used in Brazil to treat diabetes. The hypoglicemiant activity of extracts containning kaempferitrin and other phenolic compounds is already confirmed and several cosmetic uses have been searched. Amazonian *Bauhinia* species are still poorly known and studied. The aim of this work was to verify the free radical scavenging (DPPH) and tyrosinase inhibition activities of methanolic extracts obtained from twigs (BCB) and leaves (BCL) from the Amazonian *Bauhinia coronata* Benth. The tyrosinase IC_{50} observed to BCB was 696.97 ± 1.27 ug/mL, but to BCL the IC_{50} was 209.65 ± 0.91 ug/mL a very interesting result compared to the positive control kojic acid 56.60 ± 0.16 ug/mL. The free radical scavenging activities using DPPH observed were 15.94 ± 1.97 ug/mL to BCB; and 14.01 ± 2.71 ug/mL to BCL, again, a promising result compare to the positive control, quercetin (2.57 ± 0.10 ug/mL). These results demonstrated a potential utilization of *B. coronata* extracts in cosmetic formulation aiming for anti-aging products.

PD153 Virtual screening for new lead compounds for Alzheimer's disease with dual mode of action

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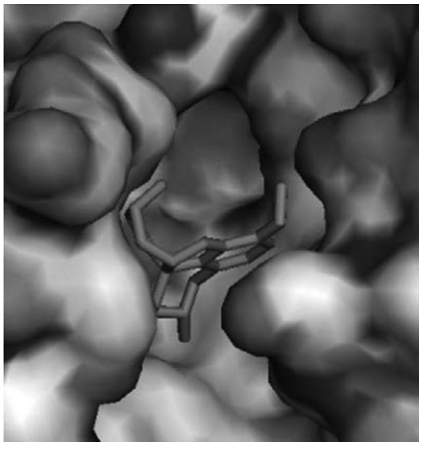
Galanthamine is a plant alkaloid isolated from Snowdrop (*Galanthus sp.*) and approved as a drug for the treatment of Alzheimer's disease. Galanthamine has a dual mode of action – it is an inhibitor of acetylcholinesterase (AChE) as well as an "allosterically potentiating ligand" at nicotinic acetylcholine receptors (nAChRs). Similar dual-action profiles are known for physiostigmine, codeine and the neurotransmitter serotonin and are of great interest in the search for new lead compounds for Alzheimer's and other neurodegenerative diseases. In the search for dual-action enhancers of ACh-mediated neurotransmission natural products including alkaloids isolated from Icelandic club mosses (Lycopodiaceae) were screened using high throughput virtual screening HTVS in an X-ray structure of the AChE and in a homology model at the α7-nAChR. Results of the homology modeling at the α7-nAChR along with results from virtual screening will be presented.



PD154 Investigation of the effects of Turkish folk medicine on *Caenorhabditis elegans* lifespan

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Research in the field of aging is getting important each day. The model organism *Caenorhabditis elegans* is a popular model for studying aging due to its short lifespan, rapid generation time and experimental flexibility. There is a high probability of developing a drug candidate molecule from plants used as a folk medicine. We therefore investigated the effects of Turkish folk medicines to aging and some age related diseases. 12 plant extracts (*Helichrysum plicatum* L., *H. stoechas* L., *Urtica dioica* L., *Mrytus communis* L., *Rubus sanctus* Schreber, *Salvia fruticosa* L., *S. verticillata* L., *S. tomentosa* Miller, *Hedera helix* L., *Paliurus spina christi* Miller, *Plantago major* L., *Vaccinium macrocarpon* Aiton) with antidiabetic and antibacterial activities, which are believed to be panacea, were chosen from folk medicine inventory studies. According to the toxicity assays,

aqueous extracts of *H. helix* (1000 µg/ml), *M. communis* (125 µg/ml) and *R. sanctus* (3.75 µg/ml) leaves prolonged mean lifespan of *C. elegans* by %46.42, %11.36 and %9.52, respectively. Additionally *sir-2.1* mutant strains were used to identify mechanism of action but none of three extracts showed longevity in lifespan. Further investigations should be done by using other mutants.

PD155 Trypanocidal activity of fruits extracts of *Solanum lycocarpum* St. Hill. on *Trypanosoma cruzi* and *Trypanosoma brucei* strains

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Solasonine (Sn) and solamargine (Sg) glycoalkaloids are the major compounds responsible for the biological effects of the fruits of *Solanum lycocarpum*. Chagas disease and sleeping sickness are caused by protozoan *T. cruzi* and *T. brucei*, respectively. The drugs used to both treatments presented hard side effects; so, the search for natural products is also interesting. The present investigation evaluated the possible trypanocidal activity. For this purpose, *S. lycocarpum* fruits powder were submitted to exhaustively extraction with 96% ethanol isolating the glycoalkaloids and also six hexanic fractions, which were analyzed *in vitro* by MTT assay in epimastigote forms of *T. cruzi* (Y strain) and procyclic forms of *T. brucei* (427 strain), using benznidazole and pentamidine, respectively, as positive control. The results showed that the ethanolic extract and two hexanic fractions presented activity in both trypanosomes. Sn did not show any activity in both parasites and Sg had activity only in *T. cruzi* strain. However, Sn+Sg (1:1) not improve the trypanocidal activity. *Acknowledgements*: FAPESP, CAPES, CNPq, UNIFEB by the financial support.

PD156 Evaluation of the antiangiogenic and anti-parasitic activities of flavonoids from gardenia species and their modified analogues

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Dedicated to the memory of Pr François Tillequin The structural similarity of methoxylated flavonoids with the antiangiogenic agent Combretastatin A4 makes them good candidates as potential inhibitors of tumour blood vessels. In addition, some of these secondary metabolites showed noticeable anti-parasite activities against *Plasmodium* and *Trypanosome*. In this context, six flavone derivatives, isolated from the glutinous exudate of flowering buds of two species from New-Caledonia *Gardenia urvillei* and *Gardenia oudiepe*, and their nine modified analogues were evaluated for antiangiogenic and anti-parasitic activities. Some structure-activity relationship are then proposed.

PD157 Evaluation of the cytotoxic potential of the medicinal species of *Croton antisyphiliticus*

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The species *Croton antisyphiliticus*, a native plant from the Brazilian Cerrado, has diterpenes as its chemical components. Diterpenes have numerous pharmacological properties and as such there is an economic interest in the *Croton* genus. The objective of the present work is to evaluate the cytotoxic activity of isolated diterpene and plant extracts obtained from roots and callus culture of *C. antisyphiliticus* against breast carcinoma cell lines (MCF7) and normal fibroblast cells (3T3). The cells were cultivated at 37°C in a humid atmosphere containing 5% CO_2 and DMEM culture medium supplemented with 10% foetal bovine serum. The cells were distributed into 96-well plates (5×10^3 cells/well) 24 hours before the assay. Next, the cells were incubated for 48 hours at different sample concentrations, that is, ranging from 100 to 4ug/mL and 1% DMSO (solvent control used for dilution). The cytotoxicity was evaluated by using the MTT colourimetric method. Doxorubicin was used as positive control. The results have revealed existence of cytotoxic activity in the assayed extracts as well as in the diterpene isolated from tumour line cells, thus demonstrating the potential of *Croton antisyphiliticus* diterpenes as a possible source of anti-cancer agents.

PD158 Characterization of plant polyphenols with dentin matrix activity

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Extracts of the polyphenol-rich plants, *Vitis vinifera* L. and *Theobroma cacao* L., exhibit a selective effect on the dentin collagen matrix of teeth by increasing of its stiffness and inhibiting its degradation by collagenase. This effect is of great value in terms of developing novel dental preventive and reparative therapies. Interestingly, the extract of another polyphenol-rich plant, *Euterpe precatoria* Mart. (Açaí), only inhibits collagenase activity, while not affecting dentin stiffness significantly. In order to map the active phytoconstituents underlying this activity, initial steps were performed to characterize both the extract and the primary active fractions. A new fractionation method was established based on vacuum liquid chromatography using Sephadex LH-20, with follow-up by TLC, HPLC and LC-MS. Biochromatograms were established and revealed that the relevant dentin matrix activity is concentrated in few fractions, which were shown to contain medium- to high-oligomeric proanthocyanidins. Further high-resolution preparative separation of these active fractions and characterization of the relatively high MW active components is under way.

PD159 Detection of antifungal compounds using lc microfractionation and bioautography with a hypersusceptible strain of *C. albicans0*

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There is an urgent need for new antifungal drugs due to the increase of invasive fungal infections and the appearance of multidrug-resistant strains. In this context, we have developed an efficient strategy for a rapid identification of potential antifungal agents in crude plant extract. The method combines HPLC-microfractionation in 96-well plates and subsequent bioautography for tracking the bioactive compounds with high resolution LC separation. In order to improve the sensitivity of the assay for the detection of potentially interesting minor bioactive natural products, a sensitive engineered strain of *C. albicans* (DSY2621) hypersusceptible to known drugs was used. This procedure enabled a precise localization of the antifungal compounds directly in the crude extracts

present at very low concentrations. In parallel an efficient chemical screening using LC-PDA-ELSD-MS and microflow NMR provided and efficient dereplication known compounds. This approach has been applied to a large series of plant extracts and allowed a rapid and efficient identification of potential antifungal compounds.

PD160 Larvicidal and deterrent activity of *Umbellularia californica* Nutt. essential oil against *Aedes aegypti*

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Aedes aegypti is a vector of dengue fever and yellow fever, and is the cause of high rates of human morbidity and mortality worldwide. There is a tremendous need for new efficacious compounds to protect humans from mosquito bites. Natural repellents from essential oils have the advantage of potential low mammalian and environmental toxicity. *Umbellularia californica* Nutt. was well known from the first European settlers as a spice and antibacterial, but also for its insecticide properties. Also known as "California bay laurel", it is a strong aromatic shrub belonging to the monotypic genus *Umbellularia*. The insecticidal activity of the essential oil against *Ae. Aegypti* was investigated by bio-guided assays. The essential oil of *U. californica* leaves was obtained by steam distillation and its chemical profile was determined using GC-FID and GC-MS. Umbellulone was isolated as one of the major constituents in very high yield (36.7%). Mosquito deterrent and larvicidal activities against *Aedes aegypti* L were evaluated. The oil showed higher activity against *Ae. aegypti* larvae than deterrent activity. Therefore, bioassay-guided isolation studies were performed on *U. californica* oil using larvicidal activity.

PD161 Study of cytotoxic compounds derived from natural products

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Natural products have been an important treasure house in new drug discovery, especially in anticancer drug development. This project is aimed at discovery of novel cytotoxic compounds that are able to kill cancer cells derived from organisms that have not been explored before. Active compounds will be identified using bioassay-guided fractionation, in which the brine shrimp lethality assay is used as the primary screening approach and mammalian cancer cell line assay as the secondary screening approach. In our primary screening, extracts from two marine sponges, *Darwinella mulleri* and *Dysidea camera* showed moderate activity with lethality of 50% and 80%, respectively. Following chromatography isolation, structural elucidation methods will be used to characterize the structures of compounds that responsible for the activity.

PD162 Analysis of thymol and carvacrol in bovine plasma and milk using SPME for sample preparation

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The carvacrol and thymol are phenolic monoterpenes found in essential oils, and are active in a formulation for intramammary treatment of mastitis bovine. The aim of this study was to develop an analytical method to identification and quantification thymol and carvacrol in milk and plasma of bovines treated with formulation.A method for the analysis was developed using gas chromatography-mass spectrometry (GC-MS) and solid-phase microextraction (SPME). The following chromatographic conditions were established: capillary column RTX®-5 ms with temperature starting at 100 °C (1 min) and programmed to increase 10 °C/min to 160 °C (1 min), helium flow rate of 1mL/min, injector and detector temperature set at 250 °C and ionization by electron impact. To obtain the optimum extraction conditions, the procedure based on headspace-SPME (HS-SPME) was optimized and the following conditions were established: sample volume; 10mL of milk or diluted plasma (1mL plasma and 9mL of water), fiber of polydimethylsiloxane-divinylbenzene (PMDS-DVB), 1.5 g NaCl, pre-heating the samples at 90 °C for 5 min with stirring speed of 540rpm, exposure of the SPME fiber in the headspace for 40 min and desorption in the injector at 250 °C for 5 min. The analytical method was validated showing quantification limits of 0.5 and 2.0 ng/mL for both analytes in plasma and milk respectively, and acceptable precision and accuracy.

PD163 Potential cytotoxic of extracts Maytenus sp. front of tumor strain MCF7 (breast cancer)

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The species of the genus *Maytenus* produce triterpenes quinonametideos. Generally, this class of metabolites has activity against ovarian, prostate and lung tumors. The objective of this study is to evaluate the cytotoxic activity of extracts rich in triterpenes quinonametideos from callus culture of *M. ilicifolia* and *M. aquifolium* characterized phenotypically against breast carcinoma cell lines (MCF7) and normal fibroblast cells (3T3). The cells were cultivated at 37ºC in a humid atmosphere containing 5% CO_2 and DMEM culture medium supplemented with 10% fetal bovine serum. The cells were distributed into 96-well plates (5×10^3 cells/well) 24 hours before the assay. Next, the cells were incubated for 48 hours at different sample concentrations, that is, ranging from 100 to 4ug/mL and 1% DMSO (solvent control used for dilution). The cytotoxicity was evaluated by using the MTT colourimetric method. Doxorubicin was used as positive control. The results have revealed that the strain known as *Maytenus qquifolium 1*, showed a better cytotoxic activity (IC50 20ug. mL^{-1}) than the other vegetal cell (IC50 100ug.mL^{-1}) also compared their cytotoxic activity against 3T3 normal strain, the same extract was less cytotoxic than doxorubicin positive control.

PD164 Pharmaceutical topical gel containing fraction of *Stryphnodendron adstringens*: validation by UV method

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Stem bark extracts from *Stryphnodendron adstringens* inhibits the growth of *Candida albicans* in vitro, and are popularly used in the treatment of vaginal candidiasis. However, no report on assay methods for validation for its quality control is available. This study validated a fraction with antifungal activity and vaginal gel containing it, for quantitative determination of phenolic compounds using spectrophotometry in the ultraviolet region. The method using the Folin-Ciocalteu reaction was analyzed for linearity, specificity, quantification and detection limit, precision, accuracy and robustness. The results of the pre-validation indicated the best conditions for the dosage setting parameters as read at 760 nm after 30 min of reaction, using pyrogallol as reference substance. Based on statistical analysis, the method satisfies the conditions, showing that the linear model does not present error due to lack of adjustment. Furthermore, variance analysis shows that the regression is significant and lower values of parameters like standard error of slope and intercept indicated high precision of the proposed methods. Goodness of fit of the regression equations was supported by high regression coefficient values and lower calculated *F*-values. The spectrophotometric UV-VIS method described was validated successfully for the determination of polyphenols in topical gel. This methodology complies with the analytical application demands and to secure the reliability of the results and proved to be precise, accurate, reproducible, robust, and easy to perform. *Financial support: CNPq, Fundação Araucária, FINEP, and CAPES.*

PD165 Synthesis and biological evaluation of chalcone derivatives as a novel class of anti-diabetic agents

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Diabetes and metabolic syndrome are growing health issues and life threatening events worldwide. Therefore, developing new substances for the treatment of diabetes and metabolic syndrome will be an urgent issue. Chalcone is a kind of well-known flavonoids with several bioactivities; however, its anti-diabetic effect has not been investigated yet. The Chalcone derivatives were synthesized by Claisen-Schmidt Condensation from acetophenones with aldehydes. For quick anti-diabetic effect screening, we established an *in vitro* model by measuring the 24-hour glucose consumption in the culture medium of 3T3-L1 adipocytes. Two halogen derivatives, AN7 – 13 and CHA79, showed the most potential anti-diabetic activity among the serial chalcone derivatives. From the high-fat diet fed mice model, AN7 – 13 and CHA-79 showed the effect to prevent the increase in body weight and improve the glucose intolerance. In addition, AN7 – 13 and CHA-79 showed no hepatic and renal toxicity. After completing this study, we should be able to obtain an optimized chalcone derivate for developing a new class of medication for type 2 diabetes treatment.

PD166 β-caryophyllene as a chemical marker for the Lantana (Verbenaceae) genus

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The Lantana genus consists of approximately 150 plant species, spanning from the tropics to the subtropics of the Americas, with a few members found in tropical Asia and Africa. A previous study of four genera from the Verbenaceae family (Lippia, Lantana, Aloysia and Phyla) proposed using iridoid glucosides as a taxonomic marker for this family. Unfortunately, the presence and type of iridoid glucosides in plants from the morphologically similar Lippia and Lantana genera are virtually indistinguishable, and so are not very helpful in differentiating between them. The current study aimed to provide further evidence to support the hypothesis of using the sesquiterpene β-caryophyllene as a unique chemical marker for plant species belonging to the Lantana genus. Two Brazilian Lantana species which had never been evaluated for their essential oil composition (L. lucida and L. salzmannii) were analyzed by GC and GC-MS. Results showed 17 predominant compounds for L. lucida, among which β-caryophyllene (19.0%) and α- caryophyllene (or humuleno, 33.0%) were the major components. L. salzmannii showed the presence of 58 compounds, the most abundant of which were β-caryophyllene (15.6%) and selin-11-en-4-ol (11.2%). In summary, the presence of β-caryophyllene in the two additional Lantana species evaluated here reinforced its use as a chemical marker for this genus.

PD167 Anti-*candida* potential of red fruits: Antifungal and anti-biofilm effects

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In the context of the prevention of dental caries, the cranberry fruit (*Vaccinium macrocarpon*), thanks to its richness in high molecular weight polyphenols, previously demonstrated anti-cariogenic properties, inhibiting biofilm formation of cariogenic bacteria. The antifungal activity of a dry extract of this fruit and three other red fruits (*Rubus idaeus*, *Vaccinium myrtillus* and *Malpighia punicifolia*) was assessed on *Candida albicans* and *C. glabrata* yeasts, which are oral cavity commensals, with a planktonic mode of growth. Moreover, the activity of these fruit extracts was studied against *Candida* spp. biofilms. A first approach was to evaluate the ability of yeasts to adhere to untreated polystyrene surfaces and to form biofilms in YNB medium supplemented with fruit extracts (0.02 – 40 mg/mL). A second approach was to pretreat the polystyrene surfaces by fruit extracts (2 – 40 mg/mL) and then to evaluate the ability of yeasts to both adhere to these surfaces and form biofilms. The MIC determination of the four extracts showed a lack of antifungal activity. Regarding the anti-adhesion and anti-biofilm effects, in the case of the first approach, a significant anti-adhesion activity was obtained with cranberry extract, using concentrations ≥ 1.25 mg/mL (*C. albicans)* and ≥ 2.5 mg/mL (*C. glabrata)*. Its activity was studied on five other strains of *C. albicans* and *C. glabrata* in the second approach. In conclusion, this preliminary work demonstrated the anti-adhesion potency of cranberry extract on *Candida* spp, but this activity was strain-dependent.

PD168 CSLNs with assembled LIPID layer mediated by hydrogen bonds for saquinavir release

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Application of cationic solid lipid nanoparticles (CSLNs), comprising complex internal matrix and lipid-regulated external surface, is an intriguing issue in current bionanotechnology. This study presents dissolution kinetics of saquinavir (SQV) from CSLNs with cholesterol-mediated esterquat 1 (EQ 1) and biocompatibility of SQV-loaded CSLNs with human brain-microvascular endothelial cells (HBMECs). CSLNs with SQV in lipid cores containing cholesterol were dissolved and incubated with HBMECs. The results revealed that an increase in the weight percentage of EQ 1 reduced the entrapment efficiency of SQV. In addition, the entrapment efficiency of SQV enhanced, when the weight percentage of cholesterol increased from 0% to 25% (w/w). The reverse was true when cholesterol increased from 0% to 75% (w/w). The dissolution profiles demonstrated that the mediation of cholesterol favored the sustained release of SQV. When the weight percentage of EQ 1 increased, the viability of HBMECs enhanced. An increase in the weight percentage of cholesterol, however, reduced the viability of HBMECs. The innovated CSLNs containing cholesterol can be effective in controlled release of SQV without inducing significant endothelial toxicity.

PD169 Novel berbamine derivatives block Jak2/Stat3 signaling, associated with induction of apoptosis on human melanoma cells

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Activated Jak/Stat3 signaling has been validated as a promising molecular target for cancer therapeutics discovery and development. Berbamine (BBM), a natural bis-benzylisoquinoline alkaloid, was identified from the traditional Chinese herbal medicine *Berberis amurensis* used for treatment of cancer patients. Here, we determine the antitumor activities of thirteen synthetic berbamine derivatives (BBMDs) against human solid tumor cells. BBMD 3, which is the most potent in this series of novel BBMDs, exhibits over 6-fold increase in biological activity compared to natural BBM. Moreover, BBMD 3, directly inhibits Jak2 autophosphorylation kinase activity *in vitro* with IC_{50} = 0.69 μM. Autophosphorylation of Jak2 kinase at Tyr1007/1008 sites also was strongly inhibited by BBMD 3 in human melanoma cells. Following inhibition of autophosphorylation of Jak2, BBMD 3 blocked constitutive activation of downstream Stat3 signaling in melanoma cells, associated with induction of apoptosis. In sum, our findings demonstrate that the novel BBMD 3 is an inhibitor of the Jak2/Stat3 signaling pathway, suggesting evidence for a molecular mechanism whereby BBMD 3 exerts at least part of apoptosis of human melanoma cells. In addition, BBMD 3 represents a promising lead compound for development of new therapeutics for cancer treatment.

PD170 Effects of resveratrol in a dopaminergic cell culture model for excitotoxicity

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The stilben resveratrol is a phytoalexin found in red wine grapes and is discussed as an antioxidative and anti-inflammatory neuroprotective compound. Resveratrol interacts with the complex III of the respiratory chain and is therefore not just a radical scavenger, but also a substance suppressing radical formation in the mitochondria. In this study, resveratrol was used to investigate its effects on glutamate damages. Excitotoxicity leads to increased formation of superoxide radicals. Therefore, in mesencephalic cell cultures of mice, we studied the influence of trans-resveratrol on the survival rate of dopaminergic neurons and propidium iodide uptake (uPI) after glutamate exposure. On the 10th DIV, glutamate (0, 0.5, or 5mM) was added to the cultures for 15 min, and then the cultures were incubated further (48 h) in resveratrol-containing (0, 0.01, 0.1, or 1 μM) medium. The number of dopaminergic cells remained unaltered when cultures were exposed to resveratrol alone. The compound did not alter the dopaminergic cell number in cultures that were treated with 0.5mM of glutamate, but after exposure to 5mM of the amino acid, resveratrol nearly doubled the number of surviving dopaminergic neurons. At lower concentrations of resveratrol (0.1 μM), the uPI was decreased by 28% (0.5mM glutamate) or by 16% (5mM glutamate), respectively. In this study, partial beneficial action of resveratrol (to a concentration up to 0.1 μM) in a mesencephalic culture system after glutamate affection could be shown.

PD171 Phytocannabinoids tetrahydrocannabinol and cannabidiol act against rotenone induced damages in murine cell cultures

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Phytocannabinoids (PCs) are terpenphenoles deriving from *Cannabis* species. In the brain, their activity is mediated by specific receptors such as the cannabinoid receptor 1 (CB1) or 2. These receptors are widely expressed in the brain. PCs as well have antioxidative capacities. Since one major event in PD is oxidative stress, cannabinoids are suggested as neuroprotective drugs. Oxidative stress can be induced by the complex I inhibitor rotenone. We investigated the effects of tetrahydrocannabinol (THC) and cannabidiol (CBD) on rotenone affected dissociated mesencephalic cultures and neuroblastoma cells (N18TG2) of mice. Cannabinoids (0.1 to 10 μM) were administered alone or concomitantly with rotenone (80nM) for 48 h. Rotenone treatment resulted in a degeneration of about 20% of the dopaminergic cells which was counteracted significantly by THC and CBD at 10 μM. A significant restoration of glutathione levels in rotenone treated cultures at 0.1 μM of THC and 10 μM of CBD was also found. Electron spin resonance spectroscopy in N18TG2 cells revealed that the amount of superoxide radicals in cells exposed to rotenone nearly doubled. Neither THC nor CBD counteracted this increase. Thus radical scavenging does not appear to be the main mechanism of PCs action. The mechanisms which lead to the observed effects remain to be investigated, but cannabinoids might be candidates for neuroprotective agents in disorders linked to oxidative stress.

PD172 Effects of phytocannabinoids CBD, CBDV, CBG, CBN, and THC on murine cell cultures

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Phytocannabinoids (PCs) affect cells by receptor binding and non-receptor mediated mechanisms. They are discussed as putative drugs in neurodegenerative diseases. In our study, we investigated the effects of cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabidivarine (CBDV), and tetrahydrocannabinol (THC) on murine dissociated mesencephalic cultures and neuroblastoma (N18TG2) cells. Cannabinoids (0.1 to 10 μM) were administered for 48 h to either cell culture. In mesencephalic cultures, dopaminergic neurons (DNs) were counted. Most PCs did not alter the number of DNs, only THC increased the count significantly (up to 118% at 10 μM), whereas CBD administration led to a decrease of cell number by 20% (10 μM). Glutathione levels were unchanged while all cannabinoids have similar radical scavenging characteristic (250 μg Trolox/mg substance) except THC which showed nearly doubled values in a DPPH assay (280 μg Trolox/mg substance). N18TG2 cells showed some vulnerability to most PCs. As shown by alamar blue assay and propidium iodide uptake measurements. A slight reduction of cell degeneration could be detected only at high doses of THC. Increased survival cells in THC treated cultures might be related to its radical scavenging abilities.

PD173 *In vitro* and *ex vivo* antitubercular activity of compounds from *Zanthoxylum capense*

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One approach to identify potential anti-tuberculosis drug leads is to screen medicinal plants traditionally used for tuberculosis treatment. By bioassay-guided fractionation, sixteen compounds, with various structural features, were isolated from the roots of the African medicinal plant *Zanthoxylum capense* (Thunb.) Harv., which is used by traditional healers in Mozambique to treat TB patients. The compounds were evaluated for antimycobacterial activity against different *M. tuberculosis* strains as well as cytotoxicity towards THP-1 macrophages. A benzophenanthridine type alkaloid and an *N*-isobutylamide were found to be promising antibactericidal for *M. tuberculosis* H37Rv with a found MIC of 1.6 μg/ml and a low cytotoxicity (IC_{50} > 60 μg/ml). In infected macrophages, the benzophenanthridine alkaloid was able to reduce the bacterial survival by almost two log units at a concentration of 6.2 μg/ml, 5 days post drug exposure. The promising anti-tubercular activity of the benzophenanthridine alkaloid, both *in vitro* and *ex vivo* against mycobacteria, and the low cytotoxicity towards human macrophages suggest its potential as an anti-TB drug scaffold. Acknowledgements: This study was supported by FCT, Portugal (SFRH/BPD/37179/2007; PEst-OE/SAU/UI4013/2011).

PD174 Inhibition of PGSH-1 (COX-1) by flavonoids isolated from a neotropical Lauraceae: A docking study

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Currently NSAIDs such as ibuprofen, naproxen, and celecoxib are clinical available for pain and inflammation treatment. However, NSAIDs have side effects including gastrointestinal irritation and renal damage, therefore development of new drugs for inhibiting COX-1 and COX-2 are still increasing. On this context, the *in vitro* ability of 12 flavonoids (five dihydrochalcone, two chalcone, three flavanone, one flavonol and one flavanonol) isolated from *Nectandra amazonum* for COX-1 inhibition was tested, which was found to be dose dependent. As part of the search of a structure-activity relationship, Autodock Vina was used to dock the compound structures with the active site of the COX-1(PDB: 3N8V). *In vitro* results showed that chalcone and dihydrochalcone compounds exhibited strong inhibitory properties (IC_{50}: 1.22 μM-1.56 μM), with reasonable correlations between docking and *in vitro* results. Some compounds do not correlate with the best poses obtained by Vina. It was considered either the possibility of presence of allosteric sites involved in observed *in vitro* results or other molecular mechanism not discovered yet. A brief discussion will be presented.

PD175 Docking analyses of aryltetralin lignans on Leishmania enzyme-based drug targets

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Leishmaniasis is a parasitic disease caused by protozoa of the genus Leishmania. Worldwide it is calculated that 12 million people are cur-

rently infected and each year there are ca 2 million infections, it being furthermore estimated that 350 million people in 88 countries are at risk of being infected. Therefore, as part of our research on antileishmanial agents, *in vitro* leishmanicidal effects of five lignans, a diterpene, and a dihydrochalcone obtained from *Pleurothyrium cinereum* were evaluated on promastigotes of *Leishmania panamensis* and *L. braziliensis*. All compounds showed activity against *Leishmania* parasites at different levels. Dihydrochalcone was found to be the most potent antileishmanial compound on both parasites, whilst alryltetralyn (+)-otobaphenol, was found to be the most selective compound on *L. panamensis*. In order to observe a structure-activity relationship, Autodock Vina was used to dock the most stable conformers from optimized structures at DFT level of test compounds within the active site of three enzyme-based drug targets of Leishmania such as cysteine synthase, *N*-myristoyltransferase, and UDP-glucose pyrophosphorylase. Good correlations were found between *in vitro* activities and docking. Most stable conformer of (+)-otobaphenol was found to exhibit the best correlation. Aryltetralin lignans might be considered as good candidates for structural optimization leading antileishmanial agents.

PD176 Anti-inflammatory sesquiterpenes from the fruits of *Pittosporum undulatum*

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Using a bioassay-guided fractionation approach, two unprecedented guaiane-type sesquiterpenes along with five known compounds were isolated from the fruits of *Pittosporum undulatum* (Pittosporacea), traditionally used in Azores archipelago due to their anti-inflammatory properties. The structures of compounds were established by 1D and 2D NMR spectroscopic techniques and MS analyses. The *in vitro* anti-inflammatory activity of compounds was evaluated by analyzing their inhibitory effects on chemical mediators released by the LPS activated RAW 264.7 murine macrophages cell line. The new compounds displayed anti-inflammatory activity comparable to that found for the positive control indomethacin and no cytotoxic effect in the mentioned cell line. Furthermore, some compounds were also assessed for their antiproliferative activity in lung large cell carcinoma COR-L23 and amelanotic melanoma C32 cells.

PD177 Cytotoxic alkaloids from the roots of *Tabernaemontan elegans*

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Tabernaemontana elegans is a traditional medicinal plant used in Mozambique to treat several diseases including cancer. The Dragendorff's reagent lead chromatographic fractionation of the methanolic extract of *Tabernaemontana elegans* yielded seven monoterpene indole and bisindole alkaloids. Their structures were established mainly by 1D and 2D NMR data. The cytotoxicity of alkaloids was evaluated in HCT116 colon carcinoma cells by the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay employing 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H-tetrazolium, inner salt (MTS), and phenazine methosulfate (PMS) as electron coupling reagent. Conversion of MTS into aqueous soluble formazan was accomplished by dehydrogenase enzymes in metabolically active cells, and absorbance was directly proportional to the number of viable cells in culture. After 48 h of compound incubation, the absorbance of soluble formazan product was measured directly from 96-well plates without additional processing, at 490 nm, using a microplate reader (Bio-Rad). Bisindole monoterpene indole alkaloids were cytotoxic to HCT cells, showing comparable results to positive control, 5-fluorouracil Acknowledgements: FCT Portugal (SFRH/BPD/30492/2006 & SFRH/BPD/70197/2010) and by projects PTDC/SAU-GMG/099162/2008 and PEst-OE/SAU/UI4013/2011).

PD178 Investigating the anti-tubercular mechanism of action of pseudopteroxazole: An activity-based photolabeling approach

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Mycobacterium tuberculosis (MTB), the etiologic agent of tuberculosis disease, causes nearly two million deaths annually and has established a latent infection in one third of the human population.[1] Pseudopteroxazole (Ptx), a diterpene isolated from the coral *Pseudopterogorgia elisabethae*,[2] exhibits promising activity in a model of non-replicating persistent MTB and against several drug-resistant MTB strains.[3,4] Considering the structural novelty of Ptx, as well as its efficacy against drug-resistant MTB strains, we hypothesize that Ptx may exert its activity through a unique mechanism of action (MoA). The aim of this research is to identify the MoA of Ptx by pursuing two complementary strategies. The mode of action of Ptx will be determined by measuring the incorporation of tritiated precursors into bacterial macromolecules. Meanwhile, the cellular target of Ptx will be identified using an activity-based photolabeling strategy by employing semisynthetic photoaffinity probes. 1) Barry, C.E.; et al. *Nat. Rev. Microbiol.* **2009**, *7*, 845. 2) Rodríguez, A.D.; et al. *Org. Lett.* **1999**, *1*, 527. 3) McCulloch, M.W.B.; et al., *J. Nat. Prod.* **2011**, *74*, 2250. 4) McCulloch, M.W.B.; Haltli, B.; Marchbank, D.H.; Kerr, R.G. *Mar. Drugs* **2012** [submitted].

PD179 Sesamin from the roots of YErba mansa (*Anemposis californica)*

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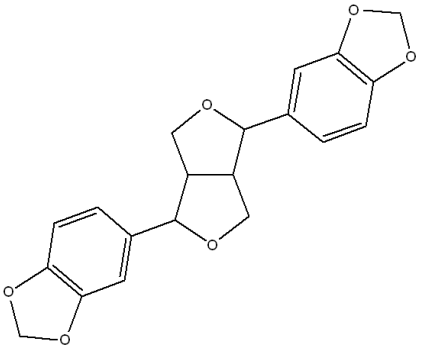
Anemopsis californica, a plant native to the southwestern United States and Northern Mexico, has a long history of use by Native Americans to treat infection. The goal of these studies was to identify chemical constituents of this sparsely studied plant. A crude extract of *A. californica* was shown to inhibit the growth of clinical isolates of *Mycobacterium avium* and *Mycobacterium marinum*. This extract underwent several steps of bioactivity-directed fractionation and purification with normal and reversed phase chromatography. Sesamin (1), a tetrahydrofuran derivative, was isolated from the active fractions, and characterized by NMR spectroscopy and X-ray crystallography. The compound demonstrated a MIC of 20 µg/mL against several mycobacterium species. This is the first report of sesamin produced by a plant in the Saururaceae plant family. Efforts to characterize additional bioactive compounds from *A. californica* are ongoing.



Topic E: Genomic Approaches

PE1 Transcriptome and microRNA profiling analyses reveal a stimulatory effect of phytochemical shikonin on the epithelial-mesenchymal transition (EMT) in mouse skin

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Although various pharmacological activities of the shikonins have been documented, understanding of the hierarchical regulation of these diverse and integrated bio-activities at the genome level is still lacking. In this study, through cross-examination between transcriptome and microRNA array analyses, we predicted that topical treatment of mouse skin tissues in vivo affects epithelial-mesenchymal transition (EMT) and the expression of related microRNAs, including 200a, 200b, 200c, 141, 205 and 429 microRNAs. In situ immunohistological analyses on test skin tissues demonstrated that specific EMT regulatory molecules are involved in the effect on shikonin-treated epidermal tissues. RT-PCR analyses subsequently confirmed the downregulating effects of shikonin on expression of microRNA 205 and members of the microRNA 200 family, which are known as key regulators of the EMT process. Expression of two RNA targets of the microRNA 200 family in EMT regulation, namely Sip1 (Zeb2) and Tcf8 (Zeb1), were consistently upregulated by shikonin treatment. These results suggest that topical treatment with shikonin can confer a potent stimulatory effect on EMT and suppress the expression of the associated microRNAs in vivo in skin tissues. The cellular and molecular evidence presented here supports our previous findings on the specific pharmacological effects of shikonin in wound-healing and immune-modulation.

PE2 The extract G115 of *Panax ginseng* C.A. Meyer enhance energy production in mammals

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A number of studies demonstrated that Ginseng increases endurance and vitality.The mechanism of action in these fields has not been clarified. We aimed to evaluate the ability of a standardized extract of Ginseng (G115) to improve energy production and metabolic activity in C_2C_{12} mouse skeletal muscle cell line and male C3 H/He mice. *In vitro*, digested G115 was used at concentration of 10 – 50 – 100 – 200 μg/ml for 24, 48 and 72 hours. Animals were treated with G115 at 25 mg/kg daily by oral administration. Gene expression of PGC-1a (gene involved in mitochondria biogenesis), AMPK and SIRT1 was evaluated by Real Time PCR. ATP production and O_2 consumption were evaluated. The same parameters were evaluated in the gastrocnemius muscle collected from mice after two weeks of treatment. A concentration/effect relationship was observed in in vitro experiments. At higher concentration the effects declined to basal values. Gene expression of PGC-1a increased by 40% after 72 hours of incubation with 50 mcg/ml of G115. Similar behaviour was observed of AMPK and SIRT1 gene expression. ATP production was 21.6 ± 2.3 nmol/mg proteins and 33.6 ± 5.8 nmol/mg proteins in the control and treated cells respectively and O_2 consumption was 11.3 ± 1.5 mmol/min/mg proteins and 27.3 ± 6.1 mmol/min/mg proteins in the control and treated cells respectively, after 72 hours incubation. The in vivo experiments confirmed the results observed in vitro. This study shows that the extract of Panax ginseng C.A. Meyer G115 is able to increase mitochondrial function and this may explain the its ability to increase endurance and vitality.

PE3 Identification of spontaneous Portuguese digitalis hybrids using RAPD markers

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Portuguese Flora includes the known species *Digitalis purpurea* L. and the endemism of Iberian Peninsula *Digitalis thapsi* L. with different morphological and chemical characteristics. Until now, and in spite of their economic importance, related to the cardenolides presence, little information is available concerning the genetic diversity of the natural populations of these species including the identification of spontaneous hybridization. Hereby results related to the characterization of genetic diversity of wild population of Digitalis in the Northeast region of Portugal by application of random amplified polymorphic DNA (RAPD) markers are presented and discussed. Hierarchical Cluster Analysis clearly allow the identification of 3 main *Digitalis* groups (*D. thapsi*, *D. purpurea* and *D. thapsi* x *D.purpurea*), fully consistent with those previously obtained using morphological affinities and confirms the need of the use of a minimum of 3 molecular markers to distinguish these populations.

PE4 DNA barcoding to characterize Brazilian species of *Senna, Cassia* and *Casearia*

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Brazil is the country which has the greatest biodiversity in the world. Nowadays DNA barcoding became an important tool to aid genetic approach as well as others biological information to the plants in different fields: forensic genetics, taxonomy and identification of cryptic species. In this work, DNA barcoding has been used to better identification the species of three genus of native plants, *Senna*, *Cassia* and *Casearia*, helping to classify some groups, specially *Senna* and *Cassia*, which has not been properly classified by traditional taxonomy. The results indicated differences in three *Senna* species: *S. spectabilis*, *S. multijuga* and *S. splendida* (psbA+trnH primers).

PE5 *De Novo* sequencing and characterization of developing seed transcriptome in two buckwheat species and metabolome profiling

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Common buckwheat (*Fagopyrum esculentum*) is an important food crop while Tartary buckwheat is prominent for high amount of secondary metabolites. Metabolomics allows the simultaneous detection of a wide range of compounds, providing an immediate information of the metabolome of a plant. The most universally used metabolomic approaches comprises NMR and MS. They have been applied as a proof of principle to show that metabolomics can constitute a major advancement in the study of plant. So, we use NMR and MS instruments to discuss the changes in relations between the buckwheat leaf, seed and flower of the secondary metabolite. Here we also present the results of transcriptome sequencing of developing seeds for two closely related plant species, with special emphasis on their secondary metabolites. Using ABI SOLiD 5500 sequencing technology, approximately 30 millions of reads with average length of 75 nucleotides were sequenced. *De novo* assembly of the reads produced about 20 thousands of contigs for each species with 100X coverage. Comparative analysis of two transcriptomes and metabolomes will be presented.

PE6 Effects of 14 – 3-3 λ on the biosynthesis of flavonoids in *Arabidopsis thaliana*

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Flavonoid biosynthetic pathways have been targeted for bio-engineering as flavonoids are known for their antioxidant, anti-inflammatory and vasodilatory activities. Our research interest is in developing plants that are an abundant source of flavonoids and are also tolerant to abiotic stress. The focus of our study is the family of proteins called 14 – 3-3 which are known signal transducers in eukaryotes. Our strategy is to map the nodes of the phenylpropanoid pathway affected by 14 – 3-3. This data will be used to manipulate the 14 – 3-3 genes that would result in increased production of flavonoids. Our laboratory has identified an isoform (14 – 3-3 λ) that demonstrates a role in drought tolerance and also affects the biosynthesis of flavonoids. To determine the role of the 14 – 3-3 λ in the phenylpropanoid pathway, we are using a reverse genetics approach, in which the amounts of secondary metabolites produced in a 14 – 3-3 λ knockout mutant are compared to the wild-type *Arabidopsis thaliana* (Columbia-0) throughout their development by LC-MS. Our results indicate the differenzial production of various kaempferol and quercetin glycosides in knockout compared to wild-type species, with kaempferitrin significantly increasing its production in week 4 of development, suggesting a role of 14 – 3-3 λ in flavonol biosynthesis during *A. thaliana* development.

PE7 Rosmaric acid modulates the kinetics of DNA repair in human fibroblast cell line submitted to H_2O_2-induced genotoxic stress

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Rosmarinic Acid (RA) is a natural phenolic compound found in *Lamiaceae* herbs and well known by its antioxidant and anti-inflammatory properties. RA has been also investigated as a chemopreventive agent; however, its ability to prevent or suppress DNA damage has not been investigated before in human cell lines. In the present study the effects of RA on DNA repair kinetics was investigated using the normal human fibroblast cell line (GM07492-A) submitted to *in vitro* genotoxic stress with H_2O_2. Cytotoxicity assay in a concentration range from 2 – 256 μM was performed using the XTT method. Alkaline version of comet assay was employed to assess the DNA repair kinetics with 2, 4, 8 μM of RA associated with hydrogen peroxide (H_2O_2) at 50 μM. Cells were harvested immediately after DNA damage induction with H_2O_2 (T0), and 1 h (T1), 2 h (T2), 4 h (T3) and 6 h (T2) after recovery with RA. Results demonstrated that RA was not cytotoxic in all tested concentrations. Comet assay revealed that RA exhibits antigenotoxic activity by the improvement of the DNA repair kinetics in H_2O_2 treated cells. The reduction in the extension of DNA damage was not concentration dependent, but cells submitted RA treatment were completely recovered at T3. These results demonstrate that RA can protect DNA against oxidative injury, representing a potent chemoprotective agent in normal cells. The next step of this study includes the elucidation of the molecular signaling pathways involved in the DNA repair process modulated by RA treatment in conditions of genotoxic stress.

PE8 Targeting of LOX-1 by willow bark, its etoh-fraction and by the antidepressant imipramine

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The NESDA-study showed a higher likelihood of atherosclerosis in depressive disorders[1], extending indications for anti-inflammatory drugs. The oxidized LDL receptor-1 (LOX-1) represents a key mo-lecule in atherosclerosis and a promising drug target, but appears different from "druggable" targets like G protein-coupled receptors[2]. We examined whether willow bark (WB), its salicin rich ethanol fraction and the tricyclic antidepressant imipramine modulate gene-expressions of LOX-1, MCP-1 and CRP in peripheral blood of rats in a model of depression. Male Sprague Dawley rats (n = 12 per group), treated for 14 days p.o. with WB extract STW 33-I (group A), its salicin rich fraction (group B) or imipramine (group C), showed a reduction of immobility time in the Porsolt Swimming Test[3]. Gene expressions of all groups (n = 4 per group) were analysed by Agilent whole genome microarray, validated by reverse transcriptase-PCR and compared to those of untreated controls (n = 4)[3]. Trans-cripts of LOX-1 were down regulated (-6.1 to -9.3 fold, $p < 0.01$) in all groups (Microarray, RT-PCR). CRP-transcripts were down regulated (-3.9 to -2.9, $p < 0.05$) whereas MCP-1 was not regulated (Microarray). Thus, the gene expression of LOX-1 is targeted by WB and imipramine. Transcript down regulation of LOX-1 may be easier than a receptor blockage. Further studies should elucidate WB's mode of action for a co-medication in indications with low grade inflammation. 1) J Psychosom Res. 2010 Aug;69(2):203 – 10. 2) Cardiovasc Drug Ther 2011; 25:379 – 391. 3) Phytomedicine 2012, 19(3 – 4):322 – 9.

PE9 Characterization of the AN6448 cluster in *Aspergillus nidulans*

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With the aim of mapping the polyketome of *A. nidulans* we have made a library of strains, which individually overexpress PKS genes from an ectopic locus. A screen of this collection on different media demonstrated that AN6448 leads to production of 3-MOA. An inspection of the DNA sequence surrounding this gene uncovered a putative gene cluster including a gene, AN6446, with homology to transcription factors. Based on this observation, we decided to overexpress AN6446. A qRT-PCR analysis of this strain was used to delineate the borders of the gene cluster as well as to stimulate formation of cichorine and a number of new products, from the gene cluster. Subsequent deletion of all genes allowed several steps in the biosynthetic pathway in this cluster to be clarified.

Topic F: Herbal Products/Botanicals: Ethnobotanical Approaches

PF1 Phytochemical analysis of Palauan traditional medicinal plant *Phaleria nisidai* to determine therapeutic dosing of Mangiferin, A C-glucosyl xanthone

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Obesity and ensuing diabetes mellitus type II are among the most prevalent NCDs in Palau and the Pacific as a whole. Local cultures have been utilizing plants for centuries performing clinical trials on themselves for centuries to find cures for common ailments. Prior phytochemical analysis of *Phaleria nisidai* (Kanehira) has revealed the presence of benzophenones, xanthones, acylglucosylsterols. Of these chemicals benzophenones and xanthones, specifically mangiferin, a C-glucosyl xanthone, has shown activity for metabolic disorder. To compare traditional preparations of Palauan medicinal to laboratory extraction techniques, we have quantified and compared a marker compound, mangiferin, in traditional, aqueous and whole methanol extracts of *Phaleria nisidai,* a plant used traditionally as an energy tonic and more currently as a treatment for diabetes. Xanthones previously isolated and indentified from *Phaleria nisidai* Kanehira were used as standards to perform quantitative analysis. Mangiferin standards were verified by NMR and HPLC-MS. Modifications of previously reported HPLC-PDA-ESIMS methods were used to quantify mangiferin. Mangiferin is isolated in higher percentage in traditional and whole aqueous extract than whole methanolic extracts. Understanding exact therapeutic dosing of mangiferin consumed by traditional usage will allow for future validation studies of *Phaleria nisidai.*

PF2 Mexican medicinal plants as source for new antiprotozoal agents

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Amoebiasis caused by *Entamoeba histolytica* is associated with high morbidity and mortality becoming a major public health problem, especially in developing countries. Because of the side-effects and the resistance that pathogenic protozoa build against the antiparasitic drugs, e. g. metronidazole, much recent attention has been paid to plants used in traditional medicine around the world in order to find new antiprotozoal agents. We collected 32 Mexican medicinal plants and the methanolic extracts of these species were screened for antiprotozoal activity against *E. histolytica* tropohzoites using *in vitro* tests. Each extract was dissolved in DMSO (150 μg/mL) and a 50 μl subsample was adjusted with 950 μL of *E. histolytica* at logarithmic phase suspension in peptone, pancreas and liver extract medium containing 10% of bovine serum. Vials were incubated for 72 h. After this time, the number of dead trophozoites per milliliter was determined by using a hematocytometer and inhibition percentage was estimated. Only 15 plant extracts showed more than 50% inhibition against *E. histolytica* and IC_{50} of these extracts were determined. We are presenting the results of this biological evaluation as well as the preliminary findings of the bioactivity guided isolation of natural products from *Lippia graveolens* Kunth and *Ruta chalepensis* Pers that showed the more significant antiprotozoal activity (91.50 and 90.50% growth inhibition with an IC_{50} of 59.14 and 60.07 μg/ml, respectively).

PF3 Theaflavin, chief flavanoid of black tea protect adjuvant induced rheumatoid arthritis in animal models

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Objectives: Aim of the study was to evaluate the anti arthritic activity of theaflavin (TF), chief flavonoid of black tea in rheumatoid arthritis animal model. Materials and Method: Rheumatoid arthritis (RA) was induced by Freund's complete adjuvant. Male albino Wistar rats (120 ± 10 g) were used and divided into following groups: Gr1- Sham control, Gr2 -Arthritis control, Gr 3- Standard drug, Gr 4- TF treated (0.01 mg $100gm^{-1}$ ip x14 days), Gr5- TF treated (0.05 mg $100gm^{-1}$ ip x14 days). Anti-arthritic activity of TF was examined through physical, urinary, serum, synovial fluid parameters, histological structure, X-ray of joints and bone minerals content. All animal experiments were approved by Institutional animal ethical committee. Results were expressed in terms of mean ± SEM (n = 6) and level of significance determined through one way ANOVA ($P < 0.05$). **Results:** Ankle and paw diameter significantly restored in TF treated group. Urinary markers hydroxyproline, glucosamine, pyridoline and deoxypridoline levels were significantly restored in TF treated groups. Serum enzymes (ACP/ALP), cytokines (osteocalcin, IL6, TNFα, IL 10, IL 12), synovial fluid cytokines, antioxidant markers, bone ash minerals (Ca^{++}, P and Na) were restored significantly in TF treated group. Histological and Xray studies showed partial restoration in TF treated groups. Conclusion: Findings showed that TF possess distinct anti arthritic activity in experimental arthritis models.

PF4 Comparative study of the alkaloids extracted from Vinca minor and those present in the homeopathic tincture 1X

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The major alkaloids of *Vinca minor* L. (Common Perriwinkle): Vincamine, Vincamone, Apovincamine, Vincaminol, Deoxyvincaminol and their derivatives were used in relieving certain cancers. Recent clinical and non-clinical trials have confirmed their beneficial cerebrovascular effect and neuroprotective action. Moreover, homeopathic tincture of Periwinkle was found useful in alleviating the itching, scaling and flaking caused by psoriasis, a matter which stimulated chemical investigation of the captioned tincture to support our intended petition to include it for external use in the Homeopathic Pharmacopoeia of the United States (HPUS). In this presentation, the homeopathic tincture 1X of the aerial parts of *V. minor* was prepared (following HPUS guidelines) and compared with a conventional total alkaloidal extract. The structures of the major constituents in these two extracts were investigated by using UV, IR, HPTLC, HPLC-PDA, and HPLC-ESI-MS. This helped to identify the main components present in the extracts. The type and relative abundance of the major constituents were compared. The total alkaloidal extract contained the major *Vinca* alkaloids such as Vincamine, Vincamone, 1,2-dehydroaspidosperimidine, Vincamidine, Vincadifformine, Vincareine, while the homeopathic tincture contained mainly Vincamine, Vincamone, Vincaminol, Vinderidine, and certain flavonoids such as Quercetin-3-gluco-7-rhamnoglucosde, Kaemferol-7-gluco-3-rhamnoglucoside, and Kaempferol-3-gluco-7-rhamnoglucoside. Procedure details and results are reported and discussed.

PF5 Screening and establishment of the high-glycyrrhizin strain of licorice, Glycyrrhiza uralensis

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Licorice, the root and stolon of *Glycyrrhiza uralensis*, is among the most important crude drugs in traditional oriental medicine. Glycyrrhizin, a triterpenoid saponin, is a major bioactive compound having several pharmacological activities. Here, we describe the screening of high-glycyrrhizin type strain from a same age population of *G. uralensis* cultivated under the same conditions in a field. Glycyrrhizin content of the selected strain was reached over 4% (average 2.11%, n = 100). We established clonal plants of the selected high-glycyrrhizin strain using *in virto* micro-propagation by plant tissue culture.

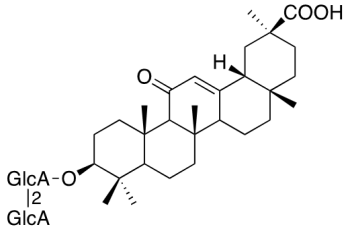

Fig. 1: glycyrrhizin

PF6 Isolation and characterization of the chemical constituents from *Gynura bicolor* and *G. divaricata*

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Two *Gynura* species, *G. bicolor* and *G. divaricata*, are used in folk recipes for the treatment of diabetes mellitus in south China. Our previous pharmacological study proved that both plants showed significant hypoglycemic activity on normal and alloxan-diabetic mice. Sixteen chemical constituents were isolated from the CH_2Cl_2 and EtOAc extracts of the aerial parts of *G. bicolor*. Their structures were determined spectroscopically as hydroxymethylfurfural, benzoic acid, 4-hydroxybenzoic acid, protocatechuic acid, vanillic acid, 4-hydroxybenzaldehyde, kaempferol, 3,3'-di-O-methylellagic acid-4-O-β-D-xylopyranoside, loliolide, ficusic acid, vomifoliol, dehydrovomifoliol, boscialin, (6S,9S)-roseoside, benzyl-β-D-glucopyranoside, and 2-phenylethyl-β-D-glucopyranoside. Fractionation and isolation of the EtOAc extract of aerial parts of *G. divaricata* yielded six compounds [succinic acid, ethyl methyl succinate, salicylic acid, isovanillic acid, 4-hydroxycinnamic acid, esculetin]. All compounds, except 4-hydroxybenzoic acid and kaempferol, were isolated for the first time from these two plant sources.

PF7 Synergistic cytoprotective effect of Crocin (CRN) and Resveratrol (RSV) on Primary Human Retinal Pigmentepithelium (RPE) cells

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CRN and RSV have shown potent cytoprotective effects against damage caused by intense light, a known risk factor for age-related macular degeneration (AMD). This study for the first time showed a synergistic effect on primary human RPE cells against light-induced oxidative damage when cells were pre-treated with a combination of CRN and RSV. Primary human RPE cell cultures were pre-treated for 48 hours with either CRN (100 μM), RSV (1 μM), or a combination of these two compounds. The pre-treated cell culture medium was then replaced with PBS and cells were illuminated (300 mW/cm2) for 60 min. Cells were re-incubated in serum-free cell culture medium for 24 hours, and cell viability was measured by methylthiotetrazole (MTT) assay. Each compound protected cells against phototoxic death (cell viability: CRN, 72%; RSV, 69%; control, 31%). The combination of CRN with RSV showed synergistic cytoprotective effect (78% cell viability), compared to individual treatments. Results also showed that each compound and their combination had no cell toxicity when tested at wide range of concentrations (CRN: 10 μM to 500 μM, RSV: 0.1 μM to 25 μM, CRN: 40 – 150 μM+RSV: 0.5 – 2.5 μM). This data in consideration with prior clinical observations supports a nutritional role for combination of CRN and RSV in protecting RPE cells from photooxidative damage. Experimental data on cell protection and safety will be presented.

PF8 Wood preservative potential of extracts of the leaves of *Morinda lucida* (Benth.) and *Datura stramonium* (L.)

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Morinda lucida and *Datura stramonium* are used medicinally for various purposes in Nigeria. Insecticidal activity of the fruit and antimicrobial activities of other parts of *M. lucida* had been reported. *D. stramonium* had been reported to be toxic to animals. Ethanol extracts of *M. lucida* and *D. stramonium* leaves were partitioned successively into petroleum ether, chloroform, ethyl acetate and methanol fractions. Each fraction was tested as wood preservative *in-vivo* at three different concentrations: 0.5%, 1.0% and 1.5% w/v. Basudin®, an organophosphate was used as standard. The extracts were applied on *Triplochiton schleroxylon,* a highly susceptible wood. The treated wood samples were exposed to termites for a period of twelve weeks. Observation was carried out weekly for signs of termite infestation. Weight loss in wood samples that were treated with fractions of *D. stramonium* extract ranged from 8.20% – 13.50% in petroleum ether fraction, 9.35%-19.99% in chloroform fraction, 6.15% and 10.89% in ethyl acetate fraction and 10.81%-20.27% in methanol fraction. Petroleum ether, chloroform, ethyl acetate and methanol soluble fractions of *M. lucida* gave a weight loss range of 5.25%- 9.50%, 6.28%-11.50%, 4.89%-6.70% and 8.50%-20.27% respectively. Ethyl acetate soluble fractions of the two plants are more potent and that of *M. lucida* was the most effective in damage reduction. The phytochemical screening, ^{1}H NMR and ^{13}C NMR suggest anthraquinone and scopoletin as the major components of ethyl acetate soluble fraction of *M. lucida.* This study reveals that anthraquinone and scopoletin are likely to be responsible for for the observed wood preservation.

PF9 *Schinus terebinthifolius* Raddi: A potential agent against HSV-1

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Herpes simplex virus type 1 (HSV-1) is normally associated with orofacial, pharyngeal, and ocular infections and transmitted by contact with contaminated secretions. Acyclovir is the most commonly used agent in the treatment of HSV infection, but high rates of resistance to this drug have been shown, mainly among immunocompromised patients. Because of this problem, several efforts have been made to discover anti-HSV-1 drugs. *Schinus terebinthifolius* Raddi, popularly known as Aroeira, is used in folk medicine to treat several illnesses, including in the skin and mucous membranes. Preliminary *in vitro* studies showed anti-HSV-1 activity of the crude hydroethanolic extract (CHE) of *S. terebinthifolius,* and bioguided fractionation demonstrated that the CHE has more effective antiviral effects than fractions of *S. terebinthifolius* and low cytotoxicity. To elucidate the mechanism of action of the effect of the CHE on HSV-1 replication in Vero cells, different experimental strategies were developed using the sulforhodamine B colorimetric method. The CHE was not able to inhibit the proliferation of HSV-1 in Vero cells when the cells were treated 1 and 24 h before and after virus entry. However,

cell culture protection was observed when the cells were treated during the early stages of HSV-1 entry into cells. Apparently, the CHE also exerts virucidal effects, but more accurate experiments are needed to confirm and elucidate these results. The evidence of the effectiveness and safety of the CHE from Aroeira could contribute to the validation of its use in the topical treatment of skin and mucous diseases caused by HSV-1.

PF10.1 Hepatoprotective effects of leaf extracts of *Azadirachta indica* and *Peristrophe bicalyculata* in alloxan induced diabetic rat models

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This study was designed to investigate the hepatoprotective effects of ethanolic leaf extracts of *Azadirachta indica* (A.I) and *Peristrophe bicalyculata* (P.B) on serum lipid profile and liver enzymes activity in diabetic and normal rat models after 14days administration at 500 mg/kg b.w. **Methods:** Forty two (42) albino Wistar rats of both sexes weighing 150 – 250 g were divided into 7 parallel groups of 6 rats each and treated viz: Groups 1 and 2 served as controls (Normal and diabetic) and received placebo, groups 3 and 4 (non diabetic rats) received A.I and P.B twice per day respectively,groups 5 and 6(diabetic rats) received A.I and P.B twice per day respectively while group 7 received 5 iu/kg b.w of insulin subcutaneously per day. There after the animals were sacrificed using choloroform vapour and sera collected and used to assay lipid profile and enzyme activity using standard kit methods of Agape diagnostics, Switzerland. **Results:** A non significant decrease ($P > 0.05$) was observed in LDL-c and HDL-c concentrations of the diabetic groups treated with combined extracts compared to diabetic control and diabetic groups treated with individual extracts, however, the serum ALT concentration in the diabetic groups treated with A.I and P.B individually and in combined administration showed a significant decrease ($P < 0.050$ compared to diabetic control. **Conclusion:** The result indicates that alloxan induced hyperlipidaemia may not be ameliorated by treatment with ethanolic leaf extracts of A.I and P.B. However,the two plants extracts individually and in combined administration were efficacious in hepatotoxicity protection associated with diabetes complications.

PF10.2 Antioxidant enzymes activity and hormonal changes following administration of ethanolic leaf extract of *Gongronema latifolium* and *Nauclea latifolia* in streptozotocin induced diabetic rats

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Aim of the work: The present study was carried out to evaluate the effects of ethanolic leaf extracts of *Gongronema latifolium* (G.L) and *Nauclea latifolia* (N.L) antioxidant enzymes activity and hormonal status in diabetic rats. **Methods:** Thirty six (36) albino Wistar rats of both sexes weighing 150 – 250 g were divided into 6 parallel groups of 6 rats each and treated viz: Groups 1 and 2 served as controls (Normal and diabetic) and received placebo, groups 3, 4 and 5 received 200 mg/kg b.w of G.L,N.L and 100 mg/kg b.w each of G.L and N.L respectively while the group 6 received 5 iu/kg b.w of insulin subcutaneously daily for 21 days. Fasting blood glucose was determined using glucometer at the start of the experiment, and thereafter at 72 hours interval and at the end of experimental period. The animals were sacrificed using choloroform vapours and whole blood collected for sera preparation that were used to assay antioxidant enzymes and some hormones using standard kit methods of Agape diagnostics, Switzerland. **Results:** A significant decrease ($P < 0.05$) was observed from initial concentration by 66.34%, 18.12% and 67.73% respectively in fasting blood glucose upon treatment with extracts compared to diabetic control and N.L. Also a significant decrease ($P < 0.05$) in insulin and T3 levels was observed in all treated groups compared to NC with an increase ($P < 0.05$) when compared to diabetic group. Whereas a significant decrease ($P < 0.05$) was observed in T4 level of all treated groups compared to the NC. A significant decrease ($P < 0.05$) was observed in insulin and T3 level compared to DC. However, a significant decrease ($P < 0.05$) was observed in T4 level of all treated groups compared to the NC.A significant decrease ($P < 0.05$) in antioxidant concentrations viz SOD and CAT were observed in all treated groups compared to normal control **Conclusion:** The result indicates that beside the antioxidant properties of these plants, they can also help in ameliorating the hormonal status of the diabetic rats. **Key words:** Antioxidant enzymes, diabetes mellitus, *Gongronema latifolium* and *Nauclea latifolia.*

PF11 Antioxidant enzymes effect and hormonal changes of leaf extracts of *Gongronema latifolium* and *Nauclea latifolia* in diabetic rats

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The present study was carried out to evaluate the effects of ethanolic leaf extracts of *Gongronema latifolium* (G.L) and *Nauclea latifolia* (N.L) antioxidant enzymes activity and hormonal status in diabetic rats. Thirty six (36) albino Wistar rats of both sexes weighing 150 – 250 g were divided into 6 parallel groups of 6 rats each and treated viz: Groups 1 and 2 served as controls (Normal and diabetic) and received placebo, groups 3, 4 and 5 received 200 mg/kg b.w of G.L,N.L and 100 mg/kg b.w each of G.L and N.L respectively while the group 6 received 5 iu/kg b.w of insulin subcutaneously daily for 21 days. Fasting blood glucose was determined using glucometer at the start of the experiment, and thereafter at 72 hours interval and at the end of experimental period. The animals were sacrificed using choloroform vapours and whole blood collected for sera preparation that were used to assay antioxidant enzymes and some hormones using standard kit methods of Agape diagnostics, Switzerland. A significant decrease ($P < 0.05$) was observed from initial concentration by 66.34%, 18.12% and 67.73% respectively in fasting blood glucose upon treatment with extracts compared to diabetic control and N.L. Also a significant decrease ($P < 0.05$) in insulin and T3 levels was observed in all treated groups compared to NC with an increase ($P < 0.05$) when compared to diabetic group. Whereas a significant decrease ($P < 0.05$) was observed in T4 level of all treated groups compared to the NC. A significant decrease ($P < 0.05$) was observed in insulin and T3 level compared to DC. However, a significant decrease ($P < 0.05$) was observed in T4 level of all treated groups compared to the NC.A significant decrease ($P < 0.05$) in antioxidant concentrations viz SOD and CAT were observed in all treated groups compared to normal control. The result indicates that beside the antioxidant properties of these plants, they can also help in ameliorating the hormonal status of the diabetic rats.

PF12 Antimicrobial activity of ethanol extract of *Thymus daenensis* Celak. under different water conditions

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Moisture deficiency induces various physiological and metabolic responses like stomatal closure and decline in growth rate and photosynthesis [1]. *Thymus daenensis* is an endemic subspecies of Iran and grows in high altitudes in Zagros mountains range [2]. An experiment was conducted in pot experimental of field, Shahrekord, Iran. Plants were exposed to three drought treatments at before of flowering: (1) 100% field capacity (control), (2) 20% field capacity, (3) 40% field capacity and (4) 60% field capacity until complete flowering. The antibacterial activity of the ethanol extract was tested assays against four pathogens by agar disc diffusion assay. The results of showed that the ethanol extracts from the different treatments studied showed antibacterial activities, with the diameters of the inhibition zone ranging from 8 to 31 mm. Increased drought levels not significant reduced antibacterial activity as compared with controls. In the present study we demonstrated, the potent antibacterial activity of *T. daenensis* extract against foodborn pathogens strains, which justifies the large use of this plant in traditional medicine. **Key words:** *Thymus daenensis*, water deficit, antimicrobial activity. [1] Flexas, J., Medrano, H. 2002. Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. *Ann. Bot* 89: 183 – 189. [2] Ghasemi Pirbalouti, A., Rahimmalek, M., Malekpoor, F., Karimi, A. 2011. Variation in antibacterial activity,

thymol and carvacrol contents of wild populations of *Thymus daenensis* subsp. *daenensis* Celak.. *Plant Omics*, 4: 209 – 214.

PF13 The diazepam-like sedative effects of vaporized essential oil from the Okinawan Kabuchii citrus fruit

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Kabuchii (*Citrus keraji* var. *kabuchii* hort. ex Tanaka, Rutaceae) is a citrus fruit peculiar to the Japanese island of Okinawa. Although local citrus fruit farmers report that its fragrance is highly relaxing, few studies have addressed either the chemical composition or the biological effects of its essential oil. In this study, the authors investigated the chemical composition and the sedative effects of kabuchii essential oil (KBEO). The effects of the oil and its components were determined via an open field test, a rotarod test and a pentobarbital sleep test using diazepam as a positive control. In the open field test, both volatilized KBEO (0.03 - 3 mg/cage, calculated conc. ca. 0.003 - 0.3 mg/L) and diazepam reduced spontaneous motor activity dose-dependently. The reduction in the 0.3 mg/cage KBEO group was greater than that in the 1 mg/kg (p.o.) diazepam group. In rotarod testing, KBEO did not affect motor performance even at the highest dosage applied (3 mg/cage), whereas diazepam reduced it dose-dependently. The effects of the major or characteristic components of kabuchii (*d*-limonen, γ-terpinene, thymol and *p*-cymene) were also evaluated via open field and rotarod testing. The results showed that γ-terpinene and thymol significantly reduced spontaneous motor activity at a dosage of 0.3 mg/cage without affecting motor performance. In pentobarbital sleep testing, both volatilized KBEO and diazepam reduced sleep latency and increased sleep duration. These effects for both treatments were similarly inhibited by pretreatment with flumazenil (a benzodiazepine receptor antagonist). γ-terpinene exhibited similar potentiating effects on pentobarbital-induced sleep. Thus, it was demonstrated that volatilized kabuchii essential oil (KBEO) have diazepam-like sedative effects and sleep improvement effects.

PF14 Shikonin induces apoptosis in stomach carcinoma cells through regulation of P53 and NF-E2-related factor 2 (NRF2)

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Shikonin, a naphthoquinone derivative found in the *Lithospermum erythrorhizon*, has known to inhibit various cancer cells proliferation. However its precise anti-cancer mechanisms of inducing cell death in stomach cancer. In this study, we investigated whether shikonin can induce to inhibit cell proliferation on human stomach carcinoma. Our results showed that shikonin was induced cell cycle arrest at the G2/M phase. Also, shikonin have induced activation of p53 which associated with oxidative stress. If the oxidative stress provokes DNA damage, cells respond to oxidative environment by activating the transcription of various antioxidant genes. NF-E2-related factor 2 (Nrf2) is doing pivotal role in against oxidative stress in cells. In case of this study, shikonin was increased activation of p53 but was decreased activation of Nrf2. Considering that p53 induced apoptosis requires an accumulation of reactive oxygen species, this negative control on the Nrf2 transactivation appears to be aimed to prevent the cytoprotection.

PF15 Anti-osteoporotic effects of multiple herbal components in Erxian decoction, a chinese medicinal formula

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Erxian Decoction (EXD) is an anti-menopausal Chinese medicinal formula with anti-osteoporotic effect. It has been clinically used for more than 60 years without adverse effects reported. In this study, the anti-osteoporotic mechanism of EXD and the drug compatibility according to Chinese medicine theory were investigated. Our results revealed that EXD inhibited the proliferation of RAW 264.7 osteoclast precursor cells mainly mediated by the Monarch herb (*Herba Epimedii*). The Monarch herb (*Herba Epimedii*) and Minister herb (*Radix Morindae officinalis*) also contribute to the inhibition of differentiation of RAW 264.7 into mature osteoclast cells. EXD also stimulates the proliferation of hFOB 1.19 osteoblast cells and the osteoprotegerin secretion. Immunoblotting analysis reveals that EXD down-regulates NFATc1, a key protein involved in osteoclastogenesis, through the action of the Assistant herb *Cortex Phellodendri Chinensis*. This study provides a scientific ground for EXD as an alternative approach coping with menopausal osteoporosis. The drug compatibility of EXD according to Chinese medicine theory has also been demonstrated.

PF16 Anti-diabetic properties of non-polar *Toona sinensis Roem* extract prepared by supercritical-CO_2 fluid

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Toona sinensis Roem (*T. sinensis*) leaves have been used as a nutritious vegetable and been suggested for medical applications; however, the reported bioactive compounds of *T. sinensis* leaves are, so far, from high to mid-high polar extracts. Our aims in this study were to reveal the non-polar constituents of the *T. sinensis* leave extract that were prepared by a method of using a supercritical-CO_2 fluid and to investigate the anti-diabetic potential of this extract. Through a GC/MS analysis, we revealed 24 major components of the non-polar *T. sinensis* leave extract. The non-polar *T. sinensis* leave extract showed to prevent the progression of diabetes and hepatosteatosis, the rise of triglycerol levels and the decrease of adiponectin levels in the type 2 diabetic mice. Our results suggest that the non-polar extract of *T. sinensis* leaves prepared using the supercritical-CO_2 fluid may contain effective constituents to prevent type 2 diabetes.

PF17 The effect of foliar application of jasmonic acid on hypercine of *Hypericum perfuratum* L.

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Hypericum perfuratum L. (St. John's wort) is one of medicinal plants belonging to Hypericaceae family [1], a perennial flowering plant, has nearly a 200-year history of use in traditional folk medicine for the treatment of various ailments [2]. The Pot experiments were carried out at the Researches Centre of Medicinal Plants & Ethno-veterinary, Shahrekord, Iran in 2011, to investigate the effect of the foliar application of jasmonic acid at the rates of 0.0 as a control, aceton as a solvent, 50, 100, 200 and 400 μL on hypercin content of *H. perfuratum* L. The hypercin of the tetra-hydrofuran extract obtained from the areal parts of *H. perfuratum* by British pharmacopea. The result of analysis of variance of the experiment showed that different levels of the foliar application of jasmonic acid do have significant impacts on hypercin content. The, mean comparison by Duncan test showed that highest amount of hypercin was 200 μL (0.8% hypericin/extract), and lowest amount of hypercin was aceton (0.8% hypericin/extract).

PF18 Wound healing modulation by a biomembrane of laticifers proteins from *Calotropis procera* (AIT.) R. BR.

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The latex of the medicinal plant *Calotropis procera* (Apocynaceae) has been widely used in folk medicine on dermatological disorders. Laticifer proteins (LP) obtained from natural latex was used to prepare a biomembrane associated with polyvinyl alcohol (PVA) in order to investigate their effects at wound healing. Experimental protocols were registered on the Institutional Ethics Committee under number 24/09. Swiss male mice (10 weeks) were subjected to surgery in the dorsal region to induce excisional wound (1 cm^2) followed by implantation of Biomembranes containing 1% PVA or 0.2% LP associated with PVA. On 2nd, 7th and 14th days after surgery each wound were measured by pachometer, colleted for microscopic analysis and used for determination of myeloperoxidase (MPO), nitrite and TNF-α levels. On the 2nd day was observed that the biomembrane containing LP stimulated mast cell degranulation, infiltrate of polymorfonuclear cells, as indicated by MPO, as well as increased nitrite and TNF-α levels. On the 7nd day, LP promoted reduction in wound area and a higher infiltrate of mononuclear cells. On the 14th day was observed that LP increased the population of fibroblasts and collagenesis. Integrated analysis of all results suggest that LP act significantly in the inflammatory phase of healing, which seems to directly influence the subsequent phases of the healing process.

PF19 Phenolic compounds analysis by capillary electrophoresis (CE) of Chilean bee pollen with high metal content

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Bee pollen is a conglomerate of vegetable pollen that bees bring to the beehive from flowers, where it is possible to find several molecules with interesting biological properties such as antioxidant capability. Chile has a native flora with great variety of species, the growth of which occurs even in the zones polluted with metals. Samples of bee pollen were obtained from potentially metal-polluted zones of Chile. Metal content was obtained by wet digestion of samples followed by ICP-OES analysis. Capillary electrophoresis (CE) was used for detecting and comparing the profiles of the phenolic compounds. The samples showed increased levels of Pb(II) (0.70 ± 0.03 mg/Kg) and Cd(II) (0.08 ± 0.01 mg/Kg), as compared with controls. Furthermore, 8 phenolic compounds were identified by CE, and shifts in migration times were detected for naringenine, rutine and caffeic acid due to the presence of the metals. Also, FRAP and DPPH activities in these bee pollens were lower than those in controls according to the results obtained by CE analysis. In this work, the relationship between metal content in bee pollens and the decreased antioxidant property is discussed.

PF20 Chemical markers of Chilean monofloral honey bee identified by capillary electrophoresis (CE)

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Chile produces several kinds of honey owing to the presence of a great endemic native flora. These bee products have important biological properties inherited from specific floral sources. In the last years, the detection of certain chemical compounds has allowed to certify the origin of honey and other beehive products. Among those chemicals is the family of phenolic compounds. These molecules are involved in honey natural capabilities and are useful as bio markers. Ethanolic extracts of unifloral honey and nectar of Quillaja saponaria and honey sac from bees were analyzed by capillary electrophoresis (CE), in order to identify any phenolic compound with potential use as chemical marker. The analyses showed that ferulic and p-coumaric acids may be used as markers due to their presence in all the extracts studied by CE. A third compound identified as caffeic acid, was detected only in two analyzed extracts (honey and honey sac bee). This result suggests that bee by itself is capable to modify the initial content of nectar and therefore the final composition of phenols in honey. In this work, the phenolic profiles obtained for each extract were discussed for establishing a "fingerprint" of those compounds in these beehive products. Acknowledgments: FONDEF D0811080 and FONDECYT 1110808 Grants to Professor Gloria Montenegro and FONDECYT POSTDOCTORAL GRANT 3110070 to Enrique Mejías.

PF21 Medicinal and aromatic plants: Sustainable use, conservation and rural economy

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In order to preserve the priceless natural resources of medicinal and aromatic plants (MAPs) in the plains and hilly tract of West Bengal, India along with their sustainable use and commercial exploitation for the welfare of the rural people, the authors attempted to carry out: survey and conservation strategies of widely growing MAPs, their traditional use by the local people, probable causes of depletion, scientific growing of few potential MAPs and finally commercial exploitation of aromatic grasses for the upliftment of rural economy. The studies identified the widely growing MAPs in the area, their traditional use by the local people and depletion by biotic and abiotic factors. Successful conservation of some potential MAPs including anti- diabetic plants have been done. Studies on growth, development and economics of cultivation of two potential aromatic grasses subjected to various experimental conditions clearly revealed the significant role of fertilizer, photoperiod and growth hormone for augmentation of growth and development associated with the increased yield and quality of essential oils and also the possibilities of commercial exploitation of the aromatic grasses as non- traditional cash crops for the welfare of rural people uplifting rural economy.

PF22 Relative ethnomedical used of *Oryza sativa* and their chemical ingradients

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The ethnomedical used of rice *(Orysa sativa* L.) in Thailand and its chemical ingredients were investigated. The method of ethnomedical study was the in-depth interview of 10 folk doctors from all parts of Thailand and using documentary research method from Thai Traditional books. Two parts of rice (rice bran and rice) were extracted following ethnomedical used and was investigated for chemical components by GC-MS. The ethnomedical results showed that the water extract from washing white rice exhibited the highest regarded ranking as antiallergy, blood tonic and antidiarrhea. It exhibited diethyl phthalate, methyl glycol phathalate and alpha hexyl cinnamaldehyde (HCA) (17.74, 10.32 and 9.72% respectively). The boiled rice was the second ranking and used to treat inflammation condition. The oleic acid, palmitic acid and metyl linoleate (44.89, 22.38 and 12.65%) were found in this extract. The relative of chemical ingredients and ethnomedical used were also regarded. This results was concluded that almost chemical components related with ethnomedical used.

PF23 Anti-inflammatory effect of a Thai traditional drug for muscle pain treatment via nitric oxide and COX-II inhibitor

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A Thai Traditional remedy called Sahasthara (SHT) is normally used to treat muscles pain and arthritis in the list of herbal medicinal products A.D. 2011 of Thailand. Its formula consists of twenty one plants. The objective of this investigation is to study on anti-inflammatory activity of SHT extract and its plant components on lipopolysaccharide (LPS) induced production in RAW 264.7 cells via nitric oxide (NO) and COX-II inhibitor. The ethanolic extracts of SHT, *Atractylodes lancea*, *Baliospermum montanum*, showed higher inhibition of NO production (IC_{50}= 2.81, 9.70 and 12.55 μg/ml respectively) than indomethacin (IC_{50}= 56.78 μM or 20.32 μg/ml), but SHT extract exhibited less anti-inflammatory activity via COX-II inhibitory on PGE_2 release than indomethacin [IC_{50}= 16.97and 1.00 μg/ml or 2.80 μM respectively]. The extract of plant ingredient such as *Myristica fragrans* (seed), *Piper nigrum and Piper retrofractum* showed good inhibitory activity on PGE_2 release with IC_{50} values as 16.99, 17.70 and 23.08 μg/ml respectively. It concluded that SHT extract had a potential as anti-inflammatory drug for pain treatment by possessing on NO and COX-II inhibition.

PF24 UHPLC/HRMS analysis of African mango (*Irvingia gabonensis*) seeds, seed extracts, and African mango based dietary supplements

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Dietary Supplements based on extract from *Irvingia gabonensis* (African Mango, or AM) seeds are one of the popular herbal weight loss dietary supplements in the US market. The extract is believed to be a natural and healthy way to lose weight and improve overall health. However, the chemical composition of African mango based-dietary supplements (AMDS) has never been reported. In this study, the chemical constituents of African mango seeds, African mango seeds extract (AMSE), and different kinds of commercially available AMDS have been investigated using an ultra high-performance liquid chromatography with high resolution mass spectrometry (UHPLC-HRMS) method. Ellagic acid derivatives were found as the major components in African Mango seeds. A wide variation in the constituents was found among AMSE products from China and AMDS products in the U.S. It appears that some manufacturers used regular mango seeds (*Mangifera indica* L) extract or unknown extracts instead of extract from AM seeds for the production of AMSE and AMDS products.

PF25 Anti-diabetic effect and mechanism of action of MaEsil (*Prunus mume)* extract

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Maesil (*Prunus mume*), is believed to be health food in oriental countries including Korea, and is presently reported to exert various physiological activities. However, anti-diabetic effect and underlying molecular mechanisms of Maesil are not reported. In the present study, we examined the anti-diabetic effect of Maesil, provided by Gwangyang City in C2C12 myotubes. A 70% ethanol extract of Maesil (EM) significantly stimulated the glucose uptake. In addition, we examined the activity of peroxisome proliferator-activated receptor gamma (PPAR-γ), a crucial target for the anti-diabetic action. EM significantly stimulated PPAR-γ transactivation. Moreover, we also investigated whether the 5′ AMP-activated protein kinase (AMPK), an insulin-independent anti-diabetic target, and Akt, an insulin-dependent anti-diabetic target, were involved in the stimulation of glucose uptake by EM. We found that EM did not stimulate the activations of AMPK and Akt. Finally, we screened 1318 differently expressed proteins by global protein analysis in the treatment of EM. We found that EM alters multiple protein expressions and signaling pathways. Taken together, these results suggest that EM exert anti-diabetic effect, at least in part via activation of PPAR-γ in cell culture systems. Therefore Maesil may be useful for the prevention of diabetes.

PF26 Medicinal plants of northern Thailand used by traditional doctors to treat mild cognitive impairment in the elderly

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Mild Cognitive Impairment (MCI) is the transition stage between normal aging and dementia. Memory impairment, in excess of what is considered normal for age, is the most common symptom of MCI. Some forms of dementia can be treated by increasing the amount of acetylcholine in the synapses of neurons by preventing its breakdown by the enzyme acetylcholinesterase. By inhibiting this enzyme more acetlycholine is available to the brain. The objective of this project is to investigate Thai medicinal plants with potential to slow the progression of MCI to dementia, and treat the memory loss that accompanies this disorder. Interviews were conducting with traditional doctors in Northern Thailand to identify plants with cognitive enhancing and neuroprotective activity. *In vitro* bioassays measuring total phenolic content, anti-oxidant and acetylcholinesterase activity were performed. One plant was selected for *in vivo* behavioral and enzymatic testing in normal, female rats. Behavioral models were used to measure spatial and non-spatial memory, and retention. Enzymatic tests of three different portions of the brain, the hippocampus, striatum and cortex were used to determine the level of acetylcholine and enzymes marking free radical oxidation. Many of the selected plants and plant formulas demonstrated anti-oxidant and acetylcholinesterase inhibition activity. My research successfully identified a single plant, which enhanced spatial memory, possessed strong acetylcholinesterase activity and offered protection against free radical oxidation. This research demonstrates the ability of traditional medical systems to provide solutions to modern problems.

PF27 Phytochemical analysis and antioxidant activity of wild and *ex situ* cultivated *Harpagophytum procumbens* from Botswana

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Comparative phytochemical analysis [TLC method] and antioxidant activity of wild and *ex situ* cultivated shoots and tubers of *Harpagophytum procumbens* were done. Total phenolic content [Folin-Ciocalteu method] and free radical scavenging activity[1,1-diphenyl-2-picryl-hydrazyl assay] of both chloroform and methanol extracts were determined. Analysis of *ex situ* cultivated plant material showed presence of iridiods harpagosides comparable with those found in the wild plants. The total phenolic contents in wild plants were comparable to the *ex situ* cultivated plants. The free radical scavenging activities also correlated well with the total phenolic contents of both wild and *ex situ* cultivated plants. The consistency in presence of phenolic compounds in these plant materials is of interest from both the pharmacological and conservation point of view given the role played by these compounds in oxidative stress. These results indicate that *ex situ* cultivation can be both a conservation strategy and can provide an alternative and sustainable source of therapeutically active compounds. Keywords: *Harpagophytum procumbens*; Phenolic compounds; free radical scavenging activity; wild shoots and tubers; *ex situ* cultivation

PF28 Chemical stability evaluation of *Cassia fistula* pod pulp extract by HPLC

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Cassia fistula Linn. (Fabaceae) is an ornamental plant widely grown in tropical and subtropical area. The ripe pod pulp has long been used as a traditional laxative drug due to anthraquinone glycosides and rhein is a major active constituent. This study was aimed to evaluate chemical stability of *C. fistula* pod pulp extracts which were storaged under the condition described in ASEAN guideline on stability of drug products[1].

The ripe pod pulp extract was prepared by decoction method. A validated HPLC was used for quantitative analysis of rhein content in the extracts. Three batches of the crude extract were stored for 6 months under accelerated (at 40 °C) and real time storage conditions. The proposed HPLC method showed acceptable validation parameters and the content of rhein in the decoction extract was remained more than 96% (96.88% – 99.62%) of the initial amount for all storage conditions. From the results, there was no significant change of the extracts and the acceptance criteria were met. Thus, the extract from *C. fistula* pod pulp had good stability and suitable to be further developed as an alternative laxative product. References: 1. ASEAN Countries. ASEAN Guideline on stability study of drug product. 9th ACCSQ-PPWG Meeting, the Philippines; 2005.

PF29 Gastroprotective effect of latex extract from *Plumeria rubra* in models of acute gastric lesion

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Plumeria rubra (Apocynaceae), a laticifer plant, has been widely used in traditional folk medicine to treat various diseases. This study was performed to investigate the gastroprotective activity of a non-dialyzable protein fraction (PrLP) of *P. rubra* latex in gastric ulcer model. Experimental protocols were registered on the Institutional Ethics Committee under number 57/2010. Swiss male mice were treated with PrLP in doses 0.5; 5 and 50 mg/kg (i.v.; n = 8). After 30 min they received 0.2 ml of absolute ethanol per oral and after 60 min, the animals were sacrificed and stomachs removed and analyzed the lesion index and dosage of GSH (reduced glutathione) and Nitrite. To study the gastroprotective mechanism(s), its relations to capsaicin-sensitive fibers, endogenous prostaglandins, nitric oxide, ATP-sensitive potassium channels and GMPc were analyzed. PrLP all doses prevented gastric injury ($p < 0.05$). PrLP also restored the GSH levels and Nitrite in mucosa ($p < 0.05$). Capsazepine, Indometacin, L-NAME, Glibenclamide and ODQ and were able to reverse the protective effect of PrLP ($p < 0.05$). We can conclude that the PrLP has protective activity in the gastric mucosa, this protection appears to be mediated in part by modulation of prostaglandin/NO/Katp/GMPc/TRPV1 receptors and by antioxidant mechanisms.

PF30 Variation of alkaloids content in *Stemona curtisii* roots in Thailand

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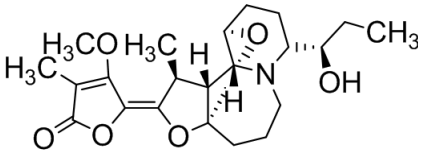
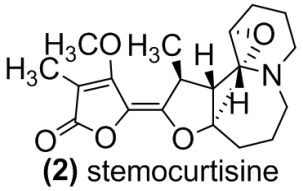
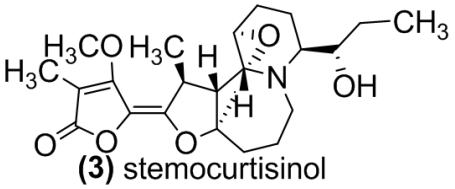
Stemona plants (Stemonaceae) have been traditionally used as natural pesticides and medicinal plants. *S. curtisii*, the dominant species distributed in the South and Southwest of Thailand, has been of interest for its insecticidal properties. Variation of active components affects their promised biological effects. Therefore, the study of variability of the contents of its bioactive components was done. Ten samples of *S. curtisii* were collected from various locations of Thailand. Each dried sample was exhaustively extracted with methanol by sonication and the extract was analyzed by the validated HPLC method. The contents of oxystemokerrine (**1**), stemocurtisine (**2**), stemocurtisinol (**3**), and stemofoline (**4**) in dried powder of *S. curtisii* roots were calculated. A remarkable variation of alkaloids profile was observed in different geographical provenances of *S. curtisii* in Thailand. This study would provide a basis for standardization of *S. curtisii* raw materials for a better source and for further insecticidal development.



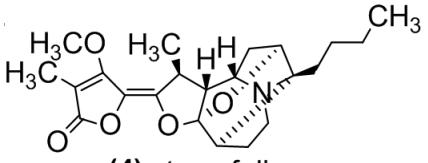
(1) oxystemokerrine



(2) stemocurtisine



(3) stemocurtisinol



(4) stemofoline

PF31 Antibacterial activity of Thai medicinal plants against five *Salmonella* species isolated from diarrheal broilers

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The ethanolic extracts of forty Thai medicinal plants and three preparations used for antidiarrheal treatment in Thai traditional medicine were determined antibacterial activity against five *Salmonella* species isolated from diarrheal broilers such as *S. tyhimirium, S. hadar, S. virchow, S. infantis* and *S. enteritidis*. Disc diffusion and broth dilution method were used for testing. Surprisingly, only six plants showed antibacterial activity against these five species. *Caesalpinia sappan* wood extract exhibited the highest antibacterial activity against all isolated *Salmonella* strains with diameter of clear zone as 10.33 – 16.33 mm and MIC values between 0.3125 – 1.25 mg/ml following by *Strephania venosa* root and *Tinospora crispa* leaves extracts (diameter of clear zone as 8.67 – 11.00 and 9.00 – 10.33 mm, MIC values of all strains as 5 mg/ml on both extracts). The ethnomedical used of the positive plant results were discussed and continued the studying for product development as antidiarrheal drugs for broilers.

PF32 Extracts, anthocyanins and procyanidins from *Aronia melanocarpa* as radical scavengers and enzyme inhibitors

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Aronia berries, [*Aronia melanocarpa* (Michx.) Elliott var. Moscow (Rosaceae)], originate from North America and have been traditionally used in Native American medicine. Extracts, subfractions, isolated anthocyanins and isolated procyanidins B2, B5 and C1 from berries and bark of *A. melanocarpa* were investigated for their antioxidant and enzyme inhibitory activities. Four different bioassays were used, namely scavenging of the diphenylpicrylhydrazyl (DPPH) radical, inhibition of 15-lipoxygenase (15-LO), inhibition of xanthine oxidase (XO) and inhibition of α-glucosidase. Among the anthocyanins, cyanidin 3-arabinoside possessed the strongest and cyanidin 3-xyloside the weakest radical scavenging and enzyme inhibitory activity. These effects seem to be influenced by the sugar units linked to the anthocyanidin. Subfractions enriched in procyanidins were found to be potent α-glucosidase inhibitors, they pos-

sessed high radical scavenging properties, strong inhibitory activity towards 15-LO and moderate inhibitory activity towards XO. Trimeric procyanidin C1 showed higher activity in the biological assays compared to the dimeric procyanidins B2 and B5. This study suggests that different polyphenolic compounds of Aronia may have beneficial effects in reducing blood glucose levels due to inhibition of α-glucosidase and, provided sufficient bioavailability, may have a potential to alleviate oxidative stress.

PF33 Effects of bio-stimulators on morphological and phytochemical parameters of *Calendula officinalis* L

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Calendula officinalis L. from Compositae family as an important medicinal plant is used in Homeopathic methods and treatment of scalds and skin illnesses. This investigation is planned for the desirable effect of bio-stimulators foliar application on morphological and phytochemical parameters of plants. The experiment with completely randomized blocks design was conducted in 10 treatments with 3 replicates in 1390. Treatments of experiment included bio-stimulators with commercial formulations of Aminol forte, Kadostim, Fosnutren, Humiforte (0.75 and 1.5 $L.ha^{-1}$) and chemical fertilizer N, P, K (70 $kg.ha^{-1}$ before sowing) and control treatment (without foliar application). Results showed that effect of these treatments was significant ($P < 0.01$) on 6 parameters (except for chlorophyll) in a way that the most effect on leaf fresh weight (215 g/m^2), leaf dry weight (54.24 g/m^2), relative water content (RWC) (88.58 g), leaf area (492.33 mm^2) and total flavonoides in leaves (18.76 mg/g DW) with Humiforte 1.5 $L.ha^{-1}$, total flavonoides in capitols with Aminolforte 1.5 $L.ha^{-1}$ (50.02 mg/g DW) and the least effect on all of parameters was obtained with control. According to these results application of Humiforte and Aminolforte is recommended because of existence of three macro elements Nitrogen, Phosphorous, Potassium and amino acid compounds.

PF34 Preliminary ethnopharmacological survey of plants used in Mexico for the treatment of hypertension

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Traditional Mexican medicine is one of the most important health systems in the world, among Chinese and Indian systems. Furthermore, medicinal plants play an important role in these systems. Investigation of medicinal plants allowed the isolation of several active compounds that have been used as leads for the developing of several therapeutic agents. In this context, from our continuous effort for the investigation of Mexican medicinal plants from different point of views, in this opportunity we are reporting a preliminary ethnopharmacologic, chemical and pharmacological survey of 186 plant species used in México for the treatment of hypertension. From these, it was registered a total of 163 genera and 76 families and is important to mention that the most abundant were Asteraceae (17), Lamiaceae (12), Solanaceae (11), Fabaceae (10) and Rutaceae (8). Furthermore, 85 were wild type. To the best of our knowledge, 47% of the total was studied at least once from phytochemical view and 74% were subjected to investigation of *in vitro* and *in vivo* pharmacological assays. These last investigations were carried out in order to validate their medicinal uses as antihypertensive agents in the Mexican traditional medicine.

PF35 The effect of foliar application of jasmonic acid on *Thymus daenensis* Celak

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Thymus daenensis subsp. *daenensis* is an endemic aromatic and medicinal plant of Iran; it's belonging to Lamiaceae family [1]. This subspecies generally grows in high altitudes in Zagros mountains range [2]. The Pot experiments were carried out at the Researches Centre of Medicinal Plants & Ethno-veterinary, Shahrekord, Iran in 2011, to investigate the effect of the foliar application of jasmonic acid (JA) at the rates of 0.0 (JA_0) as a control, aceton (JA_a) as a solvent, 50 (JA_1), 100 (JA_2), 200 (JA_3) and 400 (JA_4) μL on phytochemical essential oil of *T. daenensis*. The result of analysis of variance of the experiment showed that different levels of the foliar application of jasmonic acid do have significant impacts on thymol and carvacrol content. The highest amount of thymol was 100 (JA_2) μL (70% essential oil), and highest amount of carvacrol was100 (JA_2) μL (7% essential oil). **Key words:** *Thymus daenensis*, Jasmonic acid, thymol, carvacrol. **References** [1] Mozaffarian, V.1996. A dictionary of Iranian plant names, Farahang Moaser, Tehran, pp. 522. [2] Ghasemi Pirbalouti, A., Jahanbazi, P., Enteshari, S., Malekpoor, F., Hamedi, B., 2010c. Antimicrobial activity of some of the Iranian medicinal plants. *Arch Bio Sci* 62, 633 – 642. .

PF36 Quantitative analysis of anti-inflammatory activity of a kampo fomula, orengedokuto: importance of combination of flavonoids

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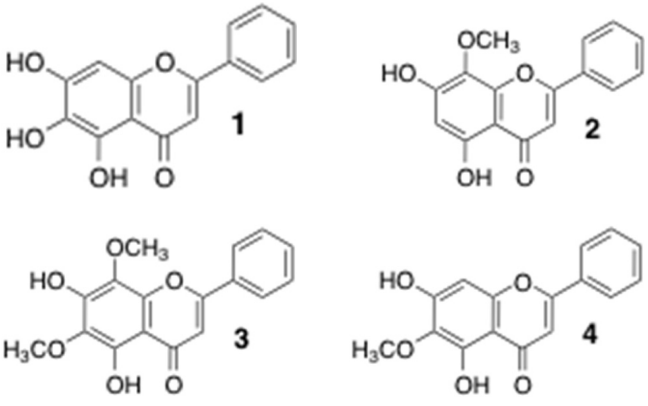
The role of component crude drugs and their constituents of orengedokuto, one of the Kampo (the Japanese traditional Medicine) formulae for treatment of inflammatory diseases, was quantitatively analyzed using inhibition of PGE_2 production in J774.1 cells. Comparisons of PGE_2 production inhibitory activities of the component crude drugs showed that the activity could be ascribed to one of the component crude drugs, Scutellaria Root. Although activity guided isolation of the extract of Scutellaria Root gave four flavonoids **1**-**4** as active constituents, none of them alone could explain the activity of the extract. However, combinations of these compounds showed stronger activity than each compound at their concentrations in the extract, and the combination of the four compounds could account for the activity of the extract of Scutellaria Root, and orengedokuto.



PF37 Anti-mycobacterial natural products from *Aralia nudicaulis*

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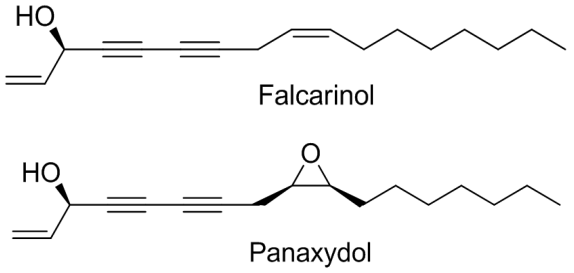
Infusions of the rhizomes of *Aralia nudicaulis* (wild sarsaparilla) are widely used as a traditional medicine to treat respiratory ailments by various First Nation communities in Canada. Methanolic extracts of *A. nudicaulis* rhizomes were found to have significant antimycobacterial activity when screened in the microplate resazurin assay against *Mycobacterium tuberculosis* (H37Ra). Bioassay guided fractionation of the rhizome extract resulted in the isolation of two C17 diynes identified as falcarinol and panaxydol by NMR and MS that exhibited MICs of 25.6 μM and 36.0 μM and IC_{50}s of 15.3 μM and 23.5 μM against *M. tuberculosis* (H37Ra), respectively. Additionally, seven endophytic fungi were isolated from the leaves of *A. nudicaulis*, and extracts of two of these have also exhibited significant anti-mycobacterial activity. The endophyte extracts will be screened using LC-MS to investigate whether falcarinol and panaxydol could be responsible for the anti-mycobacterial activity of both plant and endophyte extracts.



PF38 Quality evaluation of turmeric capsules prepared in Thai hospitals

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Turmeric rhizome capsules are popularly used for treatment of flatulent and dyspepsia.Standardized turmeric is recommended by Thai Herbal Pharmacopoeia (THP). This study evaluated physical and chemical properties, i.e. weight variation, disintegration time, contents of moisture, volatile oil, total curcuminoid and microbial contamination, of turmeric capsules prepared by 10 different hospitals in Thailand. The results revealed that weight variation, disintegration time and moisture content of all samples were conformed with the THP standard while 90% of the samples contained volatile oil and total curcuminoids within the recommended amounts. For microbial contamination, 70% of samples contained exceeded amounts of total aerobic bacteria, yeasts and molds. However, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Clostridium* spp. were not found in all samples. The results showed that most of turmeric capsules prepared in hospitals in Thailand have high standards in terms of physical and chemical qualities. However, a sanitary in manufacturing process of turmeric capsules has to be more concern.

PF39 Effect of aqueous extract from *Ligustrum vulgare* L. on ROS production and other functions of human neutrophils

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Ligustrum vulgare L. (common privet, *Oleaceae*) leaves have been used for disease prevention or treatment of oropharyngeal inflammations or as anti-rheumatic, diuretic and hypotensive agent in folk medicine in southern Europe [1]. The aim of the study was to determine the effect of aqueous extract from *Ligustrum vulgare* leaves on the functions of human neutrophils. The main compounds detected (HPLC-DAD-MS/MS) in aqueous extract from *Ligustrum vulgare* were flavonoids, phenylpropanoids and iridoids. The inhibition of neutrophil oxidative burst by the extract was comparable in both stimuli models (f-MLP: IC_{50}= 18 ± 4 μg/ml; PMA: IC_{50}= 19.8 ± 3 μg/ml). The extract acted as scavenger of superoxide anion (SC_{50}= 16 ± 1 μg/ml) and hydrogen peroxide (SC_{50}= 19.5 ± 1.5 μg/ml), but it did not scavenge hypochlorous acid in cell free systems. The extract at concentration range of 5 – 50 μg/ml inhibited neutrophil elastase release by 24 – 34% and myeloperoxidase release by 24 – 37.5%. The effect on MMP-9 and IL-8 production was around 20%. Our results partially support the traditional use of common privet leaves as anti-inflammatory agent. References: 1. Pieroni, A. et al. (2000) Fitoterapia 71: S89-S94.

PF40 Potential herbal preparations for the prevention of the metabolic syndrome in rats

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Metabolic syndrome (MetS) describes a cluster of different metabolic risk factors including obesity, hyperglycaemia, insulin resistance and dyslipidemia. Nutritional supplementation with botanicals that effectively address pathogenic mechanisms, combined with the acceptance and widespread use of botanical supplements by the general public, represents an attractive, novel, and potentially effective approach to the problem. It was the objective of this study to investigate the preventive effects of aqueous extracts *Ficus carica* L. (FC), *Artemisia dracunculus* L. (AD), and *Hibiscus sabdariffa* L. (HS) on risk factors of MetS in rats since these plants have traditionally been used for the treatment of obesity or hyperglycemia. Male Sprague-Dawley rats were fed with a low fat diet, high fat diet (HFD) or HFD + oral treatment of either 50 mg/kg or 100 mg/kg of each extract or 30 mg/kg pioglitazone for six weeks. A range of parameters was evaluated including body weight development, plasma levels of total cholesterol (TC), triglycerides (TG), low-density-lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), adiponectin, leptin, glucose, insulin, atherogenic index (AI) and the coronary risk index (CRI). It was demonstrated that the aqueous extracts of FC, AD and HS have potential positive effects on the lipid profile, most effectively FC and HS.

PF41 Urban ethnobotany: open-air fairs in the State of Rio de Janeiro, Brazil, as a potential source of new antitubercular plants

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Ethnobotany and Ethnopharmacology have been used as a strategy to select plants that can result in the development of new drugs. This study aims to relate the medicinal plants traded in open-air fairs in Rio de Janeiro State, popularly used to treat tuberculosis. Ethnobotanical techniques were used among the sellers and Salience Index was calculated. As a result from the interviews, 36 plant species were listed belonging to 12 families. Plants indicated for tuberculosis with a higher Salience value were Erva-de-passarinho (*Struthanthus marginatus* and *S. concinnus*) and Assapeixe (*Vernonia* spp.). The ethanol extracts of these three species were assayed *in vitro* against susceptible (H_{37}Rv) and resistant (35338) *M. tuberculosis* with Minimum Inhibitory Concentration (MIC) values ranging from 25 to 200 μg/ml. From the active fractions, phytol, lupeol, 3-O-n-acil-lup-20(29)-en-3β,7β,15α-triol (*Struthanthus marginatus*) and taraxerol, obtusifoliol and phytol (*S. concinnus*) were isolated. This study demonstrated the importance of ethnobotanical surveys in open-air markets as a source for the research of new drugs and scientific validation of popular use.

PF42 Antiinflammatory effect of Spirodela polyrhiza in RAW264.7 cells

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Spirodela polyrhiza is widely used in Korean traditional medicine. We assessed the effects of *S. polyrhiza* extract (SPE) on the production of inflammatory mediators in lipopolysaccharide (LPS)-stimulated RAW264.7 cells and investigated some potential underlying mechanisms. RAW264.7 cells were subjected to 5, 10, 20, and 50 μg/mL of SPE for 1 h then treated with LPS for 24 h. Production of namely nitric oxide (NO), prostaglandin E_2 (PGE_2) and cytokine levels were measured by the Griess reagent and ELISA, respectively. To investigate the underlying mechanisms of the anti-inflammatory activities of SPE, expression of nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and nuclear factor-kappa B (NF-κB) proteins were evaluated by western blot analysis. SPE treatment significantly inhibited the LPS-induced production of NO, PGE_2, interleukin (IL)-6 and tumor necrosis factor (TNF)-α and inhibited the expression of iNOS and COX-2 via attenuation of NF-κB p65 expression. These results indicate that the anti-inflammatory activity of SPE may be NF-κB p65 signaling.

PF43 Chemical characterization and antimicrobial property of essential oils of *Juniperus macrocarpa* Sibth. ET SM. leaves and cones

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All over the world plants from the *Juniperus* genus have been regarded as a well-known traditional remedy and spice. However, there are only few literature data about their chemical composition and pharmaceutical activity. In this study chemical characterization and antimicrobial property of essential oil of leaves and cones of poorly investigated species *Juniperus macrocarpa* Sibth. et Sm. were studied. GC-MS analysis showed a simple and similar terpene composition between plant organs, with monoterpenes dominant and α-pinene as the most abundant compound, 49.4% in leaves and 47.8% in cones. Interestingly, sesquiterpenes were presented in great amounts, especially Germacren D. Antimicrobial activity of essential oils was determined using agar dilution method, and they exhibited the highest activity against Gram positive bacteria – *C. perfingens* and *S. aureus*. Essential oil from leaves was more active (MICs 2 and 4 μl/ml, respectively) than cones (MICs 4 and 16 μl/ml, respectively). To sum up, examined *J. macrocarpa* could be regarded as a promising source of bioactive natural compounds, which can be used as a food supplement, remedy and preserving agent.

PF44 Effect of different pharmaceutical vehicles on antimicrobial action of essential oils

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The aim of present work was to investigate the effect of different pharmaceutical vehicles on antimicrobial action of essential oils. Based on preliminary antimicrobial studies of essential oils from Patchouli (P), Geranium (G) and Coriander (R) in pure form as well as in mixture, three combinations (1:1) of oils were prepared and designated as P+G, G+C and C+P. Incorporating these combination of oils as active pharmaceutical ingredient (3% w/w), a variety of bases viz. base 1 and base 2 (hydrophilic ointment), base 3 (hydrophobic ointment), base 4 and base 5 (O/W emulsion), base 6 (light-liquid paraffin) and base 7 (Tween 80) were formulated and subjected to agar diffusion assay against a wide spectrum of gram positive and gram negative bacteria. For comparison a popular marketed antiseptic cream was also evaluated for antimicrobial activity. It was observed that base 1 showed promising bactericidal action (4 – 13 mm) followed by marketed cream (2 – 8 mm) and base 2 (1 – 6 mm). Base 5 and base 6 exhibited bacteriostatic action whereas base 3, base 4 and base 7 did not show any antimicrobial activity. Divergence in antimicrobial action of essential oils into different pharmaceutical vehicles may be attributed to the release characteristic of oil to the outer environment (agar medium). Hydrophilic ointment facilitates release of oils in comparison to hydrophobic ointment base resulting into bactericidal action, whereas emulsification and solubilisation of essential oil caused diminished antimicrobial activity. Chemical binding of non-ionic surfactant Tween 80 with active ingredient may be responsible for loss of antimicrobial activity.

PF45 Quality control of the contents of major compound in *Cissus quadrangularis*

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The *Cissus quadrangularis* stem or Thai name as Phet sang-khaat (Vitaceae) has been traditionally used for the treatment of hemorrhoid and their products were also widely marketed. The mixture of Phet sang-khaat capsule is also recommended to used for the hemorrhoid in The National List of Essential Herbal Medicines (2011). This formula consists of 3 medicinal plants including *C. quadrangularis*, *Clerodendrum serratum* (L.) Moon. var. *wallichii* C.B.Clarke (Lamiaceae) and *Rheum officinale* Baillon (Polygonaceae). The flavonoids are the major compounds present in the aerial part of *C. quadrangularis*. Quercetin is used to the marker compound. The aim of the study is the quality control of the contents of quercetin in *C. quadrangularis*. HPLC was used to determine the content of quercetin from 5 different regions. The samples were extracted with 50% ethanol. The optimal conditions of determination were achieve on an Shimadzu Phenomenex column (250 mm x 4.6 mm, 20 μl) with a gradient of acetonitrile and water at a flow rate of 0.8 ml/min, detection at 254 nm. The results showed that the amount of quercetin in *C. quadrangularis* from Khon Khen, Nakornratchasima, Maha Sarakham, Phetchabun and Roied provinces were obtained 0.14, 0.12, 0.42, 0.09 and 0.39 (%w/w), respectively. Thus, the high contents of quercetin in *C. quadrangularis* from Maha Sarakham was suggested to used for preparation of capsule which have been studied. This result is lead to preliminary development for quality control of active compound in Phet sang-khaat capsule or Phet sang-khaat formula.

PF46 Ethnobotanical clues for the discovery of natural product mediators of microbial pathogenesis

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Ethnobotanical research offers unique insight into traditional pharmacopeias, which often integrate both psychological and pharmacological aspects to improving human health. The holistic nature of ethnomedicine is frequently poorly understood -and consequentially often dismissed as placebo when viewed through the lens of biomedicine. For example, anti-infective remedies that do not exhibit microbial killing or growth inhibitory activity in laboratory studies have commonly been abandoned for their "lack of efficacy". More recently, new approaches that take into account pathogenesis in pharmaceutical R&D have also been applied to ethnopharmacological studies, resulting in the discovery of new anti-pathogenesis leads. Importantly, incorporation of this approach offers an alternative explanation as to how and why some traditional remedies can still be effective without actually killing or diminishing growth of the pathogen. In this talk, I will discuss some of the exciting discoveries resulting from more than a decade of ethnobotanical research undertaken in the Mediterranean. In particular, I will highlight studies evaluating medicinal plants used in the treatment of skin and soft tissue infection that have led to the isolation of extracts with potent biofilm inhibitors (i.e. ellagic acid derivatives isolated from *Rubus ulmifolius* that inhibit biofilm formation and improve antibiotic efficacy) and quorum sensing inhibitors that target and disrupt the *agr* signaling pathways in multidrug-resistant isolates of *Staphylococcus aureus*. Lastly, I will discuss how in addition to validating the efficacy of these age-old remedies, such studies can also contribute to the greatly diminished pipeline of new antibiotics and lead to the improvement of human health.

PF47 The determination of phenolic and flavonoid contents in *Thunbergia laurifolia* extracts and their DPPH radical scavenging activities

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Phenolic and flavonoid contents as well as 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging effects of *Thunbergia laurifolia* leaf extracts obtained from different extraction conditions were determined. The leaves were extracted using decoction with various temperatures and extraction period. Total phenolic and total flavonoid contents of the extracts were quantitatively analyzed using UV Folin-Ciocalteu and aluminium chloride spectrophotometric methods, respectively. The extracts were investigated for free-radical-scavenging activity using the DPPH scavenging assay. Total phenolic and flavonoid contents of the extracts ranged from 1.80 to 2.85 g. gallic acid equivalent per 100 g. dried powder and from 0.76 to 0.96 g. rutin equivalent per 100 g. dried powder, respectively. The leaves extracted at 100 °C for 4 hrs. showed the strongest free radical scavenging activity with the highest contents of phenolic and flavonoid. Phytochemical analysis of the extracts by thin-layer chromatography revealed some phenolic compounds with the major constituent corresponding to caffeic acid.

PF48 PDE inhibition by *Gloriosa superba* and its main alkaloid colchicine

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The use of *Gloriosa superba* against a variety of degenerative and inflammatory diseases in Ethiopi- an traditional medicine is mainly attributed to its alkaloid colchicine [1], which reduces neutrophil migration, causing an anti-inflammatory effect. However, the traditional indications cannot be ex- plained by this single pharmacological action alone. It seems to have an additional phosphodiesterase (PDE) inhibiting activity. *G. superba* rhizomes from north-western Ethiopia were mashed and extracted with 50% MeOH. The resulting raw extract was partitioned between water and CH_2Cl_2 by serial liquid-liquid extraction, removing Dragendorff-positive components from the aqueous phase. The remaining plant material was boiled in MeOH for 30 min. The PDE inhibiting potential of all four extracts was tested in an *in vitro* system on equine bronchial fibroblasts according to [2] with pure colchicine and 3-isobutyl-1-methylxanthine as positive controls. Already the *G. superba* raw extract exhibited a significant PDE inhibiting activity that was enhanced more than twofold in the CH_2Cl_2 alkaloid phase. Conversely, the aqueous phase and the final boiling MeOH extract were largely inactive. A remarkable effect surpassing that of the raw extract but not on the same level as the CH_2Cl_2 phase could be observed for isolated colchicine. The PDE-inhibiting activity of *G. su- perba* cannot thus be explained with this single constituent alone, indicating the presence of further natural compounds with PDE inhibiting activity in this traditional Ethiopian medicinal plant. **References:** 1. Neuwinger HD (1996) African Ethnobotany. Chapman and Hall. London. 2. Werner, C. et al. (1997) J Auton Pharmacol 17: 237 – 242.

PF49 The Global Institute for BioExploration (GIBEX): Building research and development capacity worldwide

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Established in 2004, the Global Institute for BioExploration (GIBEX – www.gibex.org) is a research and development network that promotes ethical, natural product-based pharmacological bioexploration to benefit human health and the environment in developing countries by employing "Screens-to-Nature" (STN) technologies. STN uses rapid, cost-effective, non-hazardous, field deployable pharmacological assays to screen plants for biotherapeutic potential. The STN technology has proved to be a viable tool for early stage discovery of bioactive substances in plants, thereby facilitating the research and product development capacity of institutions in developing countries with limited resources. Building R&D collaborations between developed and developing nations also enhances the ability to discover and preserve the health-promoting potential of global natural resources before those resources, or the traditional knowledge associated with their use, is lost or destroyed.

PF50 Immunostimulatory effects of the traditional palauan adaptogen *Phaleria nisidai* Kaneh

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Phaleria nisidai Kaneh., locally known as *delalakar* or "the Mother of medicines," is a tree in the Thymeleaeaceae family with modern and historical use in the Micronesian country of Palau as an invigorating, strengthening, and healing prophylactic beverage. In an attempt to evaluate its traditional use as an adaptogen, extracts of *P. nisidai* were tested on human peripheral blood mononuclear cells (PBMC) to determine if there are immunostimulatory effects. Methanol (PNM) and water (PNW) extracts of *P. nisidai* were prepared by refluxing for two hours. PBMCs were isolated from healthy donor blood and cultured in 10% HS/RPMI media supplemented with low concentrations of PNM and PNW for up to 72 hours. Cytotoxicity and cell proliferation were measured by using the MTT assay on cells in culture. Cell-free supernatant was used to determine secretion of IFNγ by PBMCs in the ELISA assay. Both organic and traditional water extracts of *P. nisidai* were able to stimulate proliferation and IFNγ-secretion of PBMCs dose- and time-dependently. The superantigen *Staphyloccus* enterotoxin B (SEB) showed IFNγ-releasing activity but did not cause an increase in PBMC proliferation. *Phaleria nisidai* was able to stimulate both cell proliferation and IFNγ, unlike the nonspecific T-cell activating SEB, which was only able to induce cytokine release. These results demonstrate that *P. nisidai* may have cellular immunity-enhancing effects, supporting its traditional use as a healing adaptogenic beverage.

PF51 ID 1201, chinaberry extract, decreases β-amyloid and attenuates memory deficit

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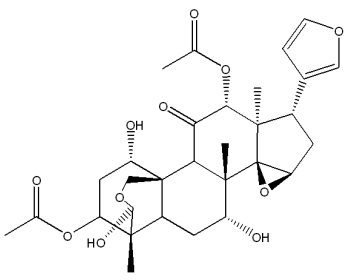
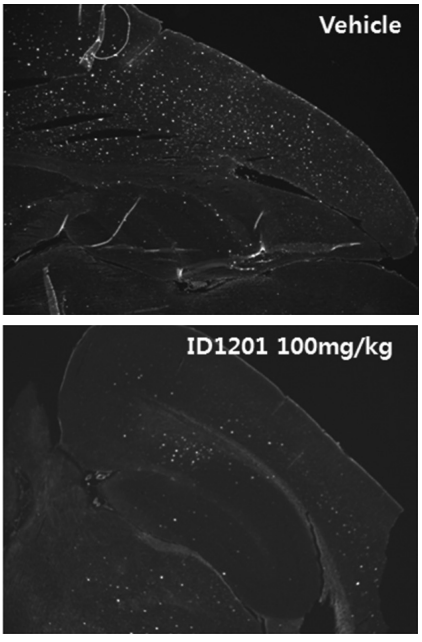
We newly found that chinaberry (fruits of *Melia toosendan*) extracts potently block production of β-amyloid, which is implicated as a cause of Alzheimer's disease, through PI3-kinase mediated α-secretase activation *in vitro*. Toosendanin, a major triterpenoid in chinaberry, is isolated as an active compound. In consideration of safety and efficacy, we finally choose ID 1201, 30% ethanol extract of chinaberry. In studies *in vivo*, we identified that the treatment with 100 mg/kg ID 1201 not only significantly reduced β-amyloid in the brains of 5X FAD mice but also ameliorated scopolamine induced amnesia. Taken together, our results present the possibility that ID 1201 may have potential for the treatment of Alzheimer's disease. Currently, ID 1201 is under preclinical trial.



Toosendanin



Inhibition of β-amyloid plaques in the brain tissue of 5X FAD mice

PF52 Effects of extracts and compounds from *Cudrania tricuspidata* leaves on alpha-glucosidase activity

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To clarify the postprandial glucose suppression effect of the leaves of *Cudrania. Tricuspidata* Bureau (Moraceae; CT), the extracts of CT were evaluated for the inhibition on α-glucosidase *in vitro*. The results indicated that the concentrations which gave 50% inhibition (IC_{50}) of the aqueous extract and the ethanolic extract were 3.14 and 0.0345 mg/mL compared to 0.419 mM of acarbose (a positive control). To identify the active compounds which are responsible for the inhibition on α-glucosidase, seventeen compounds (caffeic acid, p-coumaric acid, chlorogenic acid, cryptochlorogenic acid, protocatechuic acid, catechin, epicatechin, kaempferol, kaempferol 3-β-D-glucopyranoside, quercetin, quercitrin, isoquercitrin, rutin, resveratrol, oxyresveratrol, umbelliferone and isobavachalcone) were quantified by HPLC in CT extract, and were examined for the inhibition on α-glucosidase. The compounds in high contents were chlorogenic acid, 0.726, catechin 0.229, rutin 0.152, isobavachalcone 0.041, and caffeic acid 0.034% (w/w) in the ethanolic extract. The IC_{50} values on α-glucosidase were kaemferol 0.0001, kaempferol 3-β-D-glucopyranoside 0.002, quercitrin 0.002, p-coumaric acid 0.004, and oxyresveratrol 0.005 mM. The result indicated that the compounds tested were responsible for the inhibitory activity of CT, although the content and the inhibitory potency was not linear. The study suggests that CT might suppress increase in postprandial glucose via the inhibitory effect on α-glucosidase. Acknowledged for KFRI and KRF (NRF-2010 – 0024475).

PF53 The ethanol extract from *Artemisia princeps* Pampanini induces apoptosis by inhibition of NF-κB signaling in colon cancer cells

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Artemisia princeps Pampanini is widely used in Eastern traditional medicine for the treatment of circulatory disorders, such as, dysmenorrhea, hematuria, hemorrhoids, and inflammation. In recent years, it has been proposed that *A. princeps* may possess anti-tumorigenic potential in certain cancer cell type. In this study, the anti-carcinogenic effects of ethanol extract of *A. princeps* Pampanini (EAPP) were investigated in HT-29 and HCT-116 human colon cancer cells and AOM/DSS-induced colitis-associated cancer (CAC) mouse models. We found that EAPP treatment of cells resulted in induction of apoptosis involving the cleavages of caspase family proteins and PARP, while decreased expression of anti-apoptotic proteins. In addition, we observed that EAPP treatment inhibited NF-κB transcriptional activity and decreased translocation of p65 subunit into nuclear fractions in colon cancer cells. In an AOM/DSS-induced CAC models, administration with EAPP significantly attenuated shortening of the colon and decreased the number of colon tumors. Furthermore, EAPP treatment decreased expression of anti-apoptotic proteins and translocation of p65 subunit in nuclear fractions, whereas EAPP markedly induced the cleavage of PARP in colon tissue of an AOM/DSS-induced CAC models. Taken together, our data indicated that EAPP could be a useful agent for prevention and treatment of colon cancer.

PF54 Quality assessment of tulbaghia bulbs

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Bulbs of *Tulbaghia* species (Alliaceae) are used in traditional medicine in Southern Africa for anti-fungal purposes due to their content of sulfur-containing compounds. The main compound is marasmin which is enzymatical converted to marasmecin, which undergoes further chemical degradation. A number of species, *T. acutiloba, T. alliacea, T. cominsii, T. galpinii, T. montana, T. natalensis, T. simmlerii* and *T. violacea,* were included in the study. Crushing of fresh bulb material with liquid nitrogen followed by extraction with ethanol yielded good extraction of the sulfur compounds. Sulfur compounds were evaluated by TLC on silica plates eluted with toluene:ethyl acetate 10:3, detected with palladium-II-chloride. All species contained the same sulfur compounds, but in varying concentrations, except *T. simmelerii*, which had very low levels of sulfur compounds. A simple, quantitative TLC dilution method was developed. Extracts were applied in decreasing amounts until the main sulfur compound at R_f 0.5 no longer was visible. Setting the minimum detection limit to 1 µl of test extract, three of the species tested, *T. alliaceae*, *T. violaceae* and *T. galpinii* would be included in the drug Tulbaghia bulbus. Possible adulterants could be *Allium sativa* and *Agapanthus campanulatus*. *A. sativa* contains sulfur compounds indistinguishable from those in *Tulbaghia*. *A. campanulatus* does not contain sulfur compounds, and the ethanol extract has characteristic bands on TLC when treated with anisaldehyde-R, which allows detection of adulteration with 10% *A. campanulatus*.

PF55 Investigation of soy based botanical supplements by FT-IR spectroscopy for a fast and non-destructive quality control

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Nutritional preparations of soy (*Glycine max* (L.) Merr.) have gained interest in western countries as an alternative to hormonal therapy in the treatment of menopausal problems such as hot flushes. The active compounds responsible for this are the present isoflavones, mainly genistin, daidzin and glycitin which show a weak estrogenic activity and are therefore called phytoestrogens. In the present study, FTIR-ATR spectroscopy has been applied for the characterization and identification of the main isoflavones in soy based nutritional preparations available in German pharmacies. Informations about the functional groups and the chemical composition could be obtained which makes FTIR analysis expedient for a simple and non-destructive quality control. Moreover, using the partial least square (PLS) analysis multivariate calibration models were employed. This made estimations about the amount of the individual isoflavones and the total isoflavone content possible. The results of these infrared spectroscopy studies in combination with multivariate calibration showed the potential for production monitoring by pharmaceutical manufacturers and public supervisory authorities.

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

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

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

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

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

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

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

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

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

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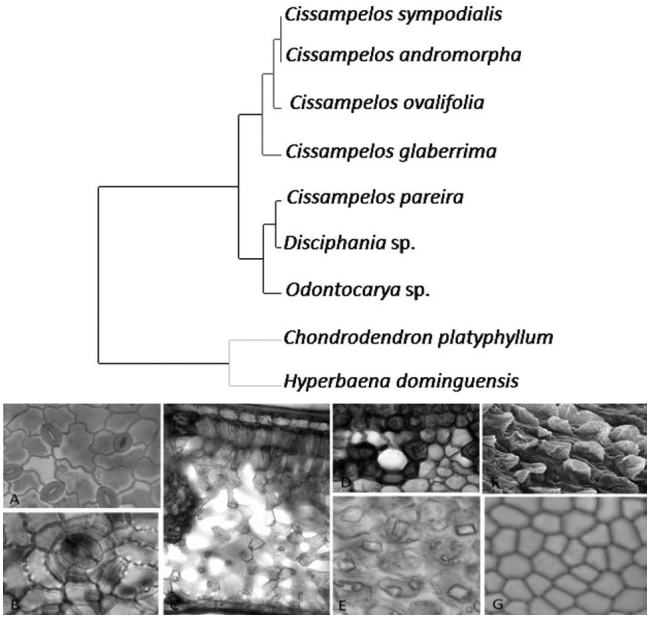
We carried out a multivariate analysis of leaf anatomy of five genera and nine species of Menispermaceae known to be toxic or used as medicinal in Brazil. The dendrogram obtained by Ward's method showed two groups (A and B), with cophenetic correlation coefficient of 0.9178. The group A with six species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



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

PF61 Effect of essential oils on the activity of human neutrophil myeloperoxidase *in vitro*

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Myeloperoxidase (MPO) is a key component of innate immune defence, linked to inflammatory diseases. In the present work, the effect on MPO activity has been studied for 15 essential oils (EO) (bay laurel, cajeput, clove, coriander, ginger, juniper, lemon, lemon grass, niaouli, nutmeg, rosemary, red thyme, Spanish oregano, tarragon and tea tree) and 4 pure EO constituents (bornyl acetate, carvacrol, eugenol and thymol). MPO activity was assessed by oxidation of O-dianisidine dihydrochloride by H_2O_2 in A) absence of cells (MPO inhibition) and B) a human neutrophil preparation (extracellular release and inhibition of MPO) [1 – 3]. Quercetin was used as positive control. Inhibitory activity was mainly detected in the phenol rich EO (clove, red thyme and Spanish oregano) and the corresponding main constituents (eugenol, thymol and carvacrol). Clove EO, with IC_{50} of 37.2 ± 1.0 μg/mL (test A) and 16.3 ± 1.3 μg/mL (test B), and eugenol, with IC_{50} of 35.9 ± 2.4 μg/mL (test A) and 19.2 ± 2.0 μg/mL (test B), showed the highest activity. Results suggest that the inhibition mechanism of clove oil and eugenol involves direct enzyme inhibition and that it could also be linked to a modification in the extracellular release of MPO. *Acknowledgenments:* Authors are grateful to Lidervet S.L. (Tarragona, Spain) for financial support. R. Pérez-Rosés was supported by the Generalitat de Catalunya and the European Social Fund. **References: 1.** Bradley PP, *et al. Blood* 60(3): 618 – 622, 1982. **2.** The Local Food-Nutraceuticals Consortium *Pharmacol Res* 52: 353 – 366, 2005. **3.** Speyer CL, *et al. Am J Physiol Cell Physiol* 288: 881 – 890, 2005.

PF62 Antiarthritic effects of Ethyl acetate fraction from *Brassica rapa* (EABR) through suppression of NF-κB

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In an attempt to identify bioactive natural products with anti-inflammatory activity, we evaluated the anti-inflammatory and antiarthritic potential of *Brassica rapa* (Brassicaceae) in activated macrophages and an experimental arthritis rats model. Ethyl acetate fraction from *B. rapa* (EABR) was found to reduce the productions of PGE_2, NO, TNF-α, and IL-6, and expressions of COX-2, iNOS, TNF-α, and IL-6 in LPS-induced macrophages. EABR attenuated LPS-induced DNA-binding, transcriptional activities, nuclear translocation, and phosphorylation of NF-κB. These effects are paralleled with reduction in the degradation and phosphorylation of IκBα and IKK activation. In rats with acute inflammation, oral administration of EABR reduced paw swelling and release of PGE_2 and MPO in carrageenan-injected tissue. In rat with adjuvant-induced arthritis, ERBR significantly reduced the paw swelling and arthritic index compared with the vehicle group. Moreover, anti-arthritic effects of EABR correlated with significant decrease of inflammatory mediators and inhibition of NF-κB activation in paw homogenates. Hence, these results clearly indicate that EABR is a potential therapeutic agent for arthritis and inflammatory-associated disorders.

PF63 Botanical characterization to authenticate sophorae tonkinensis radix et rhizoma based on anatomy and molecular analysis

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Sophora tonkinensis Gapnep. (Leguminosae) is distributed in Vietnam and southwest China, and its roots and rhizomes have been used to reduce a fever, to treat inflammation, or to relieve pains as a traditional medicine in East Asia. It was once reported that the original plant of this traditional medicine was *S. subprostrata* Chun & T.C. Chen, which is currently treated as the same species with *S. tonkinensis*. Even though roots and rhizomes of *S. tonkinensis* are commonly used as 'Shandougen', which is the local name for the herbal medicine, those of different plants are frequently sold in the markets with the same name. We carried out anatomical examination as well as molecular analysis to set up the criteria to authenticate roots and rhizomes of *S. tonkinensis*. In the root, vascular cambium is well developed with cortex, phloem, and xylem. In xylem, vessel elements are roundish polygonal in shape and alternated with xylem fibers in cluster. Xylem parenchyma rows in 2 – 3 cells in parallel with rays. Parenchyma cells contain starch grains, which are also found in ones of cortex. Periderm, over 20 cell thick, is easily taken apart. In parenchyma, oil cells are often observed. Sequence analysis of psbA-trnH Intergenic Spacer region of cpDNA could discriminate varieties recognized in *S. tonkinensis*.

PF64 Composition of the essential oils from *Ziziphora aragonensis* and *Micromeria fruticosa* from Spain

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Ziziphora aragonensis Pau and *Micromeria fruticosa* (L.) Ducre (Lamiaceae) are usually grown in Spanish home gardens for medicinal purposes. They are commonly known as "poleos" and traditionally used as teas for gastrointestinal disorders and colds. While the chemical composition of the essential oil of *M. fruticosa* has been widely analyzed, there are few reports on the oil of *Z. aragonensis*. The study of the volatile oils of these old-grown plants is important to typify the cultured material, in most cases selected from wild. With this aim, the hydrodistilled essential oils from the aerial parts of both species were analyzed by GC-FID and GC-MS. Identification of the constituents was achieved from their retention indices in two columns of different polarity, and by comparison of their mass spectral fragmentation patterns with those stored in our own database and with literature data. More than 80% of each oil was identified. The major constituents of *Z. aragonensis* oil were pulegone (59.4%), α-copaene (4.4%), estragole (4.1%), and 8-hydroxy-*p*-menthan-3-one (4.0%), whereas in the oil of *M. fruticosa* isomenthone (44.5%), pulegone (17.1%), menthol (14.6%), and isomenthol (3.5%) were found to be the major compounds.

PF65 Water extract of *Artemisia asiatica* protects ethanol-induced gastric injury

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The leaves of *Artemisia asiatica* are used clinically in Traditional Korean Medicine as the treatment of digestive disorder, abdominal pain, and anorexia. To treat the diseases, *A. asiatica* is extracted with water at boiling point and administered orally. However, the water extract of *A. asiatica* was yet to be studied to have the gastroprotective effects, although the ethanol extract was already known. The aim of the present study is to investigate the effects of the clinical remedy, which is water extract, as well as ethanol extract. Gastrohemorrhagic lesion was experimentally created by oral intubation of 80% ethanol containing 0.15mol/L HCl to the rats. The rats were divided into six groups consisting normal group, the water extract treating (100 and 300 mg/kg, p.o.) groups, the ethanol extract treating (100 and 300 mg/kg, p.o.) groups, and positive control group using cimetidine (100 mg/kg, p.o.). Samples were administered 30 minutes before EtOH-HCl. The rats were sacrificed an hour later and the gastrohemorrhagic lesion was measured. The treatment with water extract 300 mg/kg significantly reduced the gastrohemorrhagic lesion by 86.0% ($p < 0.001$) as compared to the control group. These results suggest that *A. asiatica* extracted with water, clinical remedy, is available to treat gastritis as the ethanol extract.

PF66 The inhibiotry effect of honokiol, a natural plant product, on vestibular schwannoma cells

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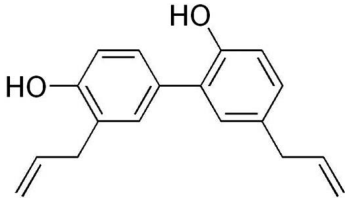
As the molecular biology of vestibular schwannoma (VS) is better understood, new means of targeting the pathways involved for intervention in schwannoma cells are being developed. Honokiol, a bioactive constituent of *Magnolia officinalis*, has attracted attention due to its diverse biological effects. Honokiol exhibited significant anti-proliferative activity in a dose-dependent manner on HEI 193 cells. Significant apoptosis was detected on schwannoma cells with 7 μg/mL (IC_{50}) honokiol. Western blot analysis showed significant inhibition of ERK phosphorylation.



PF67 Anti-osteoporotic effect of the dried root of *Rehmannia glutinosa* in ovariectomized rats

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Dried root of *Rehmannia glutinosa* is a kidney-tonifying herbal medicine with a long history of safe use for treatment of joint diseases in traditional Korean medicine. However, the anti-osteoporotic effect of dried root of *R. glutinosa* has not yet been reported *in vivo* model. In the present study was conducted to investigate anti-osteoporotic effect of dried root of *R. glutinosa* in ovariectomized (OVX) rat model of postmenopausal bone loss. Dried root of *R. glutinosa* was extracted with 70% ethanol for 3 h at 80 °C in a reflux apparatus. The OVX rats were divided into four groups, which treated by vehicle, 17β-estradiol (10 ug/kg, i.p) and *R. glutinosa* (100 and 300 mg/kg, p.o). All groups were treated for 8 weeks. Body weight and femur and lumbar vertebrae bone mineral density (BMD) were determined weekly. Serum calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP) concentrations were measured by biochemistry analyzer. The treatment with *R. glutinosa* at 300 mg/kg was significantly inhibited BMD decrease in the femur and lumbar by OVX without affecting body and organ weights. Also, *R. glutinosa* at 300 mg/kg for 8 weeks significantly decreased ALP levels compared to OVX control group. These results suggest that dried root of *R. glutinosa* is effective at preventing bone loss in OVX rats without the influence of hormones such as estrogen.

PF68 Studies on morphology and anatomy of Acanthopanax gracilistylus, a. Koreanum and A. sieboldianus

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Acanthopanax species are well known medicinal plants in Korea for their adaptogenic efficacy. With regard to the botanical classification of *Acanthopanax koreanum*, an indigenous species in Jeju island of Korea, it has been classified as a different species of *Acanthopanax* genus. However, the morphological characteristics of *A. koreanum* are very similar with other *Acanthopanax* species, especially with *A. gracilistylus* and the profile of chemical constituents showed no significant difference among *Acanthopanax* species. In order to provide further classification information among these botanically related species, anatomical studies on these Acanthopanax species were performed with microscope. It has been found that *A. koreanum* is similar to *A. gracilistylus* in terms of histological characteristics and was distinguished from *A. sieboldianus* by their morphological and anatomical differences. From this result, *A. koreanum* could be classified as a variety species of *A. gracilistylus* such as *A. gracilistylus* var. *koreanum*.

PF69 Effects of *Amomum villosum* on longitudinal bone growth in adolescent rats

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The fruit of *Amomum villosum* has been used for an improvement of gastrointestinal motility in traditional Korean medicine. In *Dongeuibogam*, the traditional book of Korean medicine, there was mentioned that an herbal mixture containing *A. villosum* used as medicine for malnutrition associated with growth retardation. Many functional studies of *A. villosum* have been performed, but its effect on the bone growth has not yet reported. This study was aimed to investigate the effect of *A. villosum* on longitudinal bone growth in adolescent rats. *A. villosum* was extracted with water for 3 h at 100 °C in a reflux apparatus. The *A. villosum* treated group (500 mg/kg) and the control group (vehicle) were administered orally twice daily for 4 days. On day 3, tetracycline (20 mg/kg) was injected intraperitoneally to form a fluorescent band on the growth plates. On days 2 – 4, bromodeoxyuridine (BrdU) (50 mg/kg) was injected intraperitoneally for labeling proliferating cells. *A. villosum* caused a significant acceleration of longitudinal bone growth, compared to control group. BrdU-positive cells were increased in the chondrocytes of the *A. villosum* group. The growth plate width was significantly increased, compared to control group. BMP-2 and IGF-1 were highly expressed in the hypertrophic and proliferative zone, respectively. These results suggest that *A. villosum* increase the longitudinal bone growth by stimulation of the chondrocyte hypertrophy and chondrogenesis, through regulation of IGF-1 and BMP signaling in the growth plate.

PF70 Gastroprotective effect of *Atractylodes japonica* and *Atractylodes macrocephala* on ethanol-HCl induced gastric ulcer in rats

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The rhizome of *Atractylodes japonica* and *Atractylodes macrocephala* are popularly used in traditional Korean medicine to treat gastric ulcer. Both of these plants are used combinely disregard of their origin for the treatment of gastric ulcer. Consequently, comparative effect of the rhizome of *A. japonica* and *A. macrocephala* with antiulcerogenic agent has not been reported yet. We compared the antiulcerogenic effect of 70% EtOH extract of *A. japonica* and *A. macrocephala* on EtOH-HCl induced gastric lesions in rats. Gastric lesions were experimentally created by oral administration of 80% ethanol containing 0.15 mol/L HCl. Rats were divided into six groups, and they were treated with vehicle, cimetidine (100 mg/kg, p.o), *A. japonica* and *A. macrocephala* (100 and 300 mg/kg, p.o). Samples were administered 30 minute before the administration of EtOH-HCl orally. Rats were sacrificed an hour later and stomach was washed out. Photograph of ulcerated region was taken and lesion area was measured by ImageJ software. Treatment with *A. japonica* 300 mg/kg significantly reduced the gastric lesion by 90.8% ($p < 0.001$) as compared to the control group whereas *A. macrocephala*, 300 mg/kg reduced the gastric lesion by 81.9% ($p < 0.001$). These results suggest that *A. japonica* is more effective than that of *A. macrocephala* on EtOH-HCl induced gastric ulcer in rats. *A. japonica* can be the alternative therapy for the treatment of gastric ulcer.

PF71 Neuroprotective effect of four flavonoids in the root of *Scutellaria baicalensis* Georgi

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Scutellaria baicalensis Georgi has been used for the treatment of chronic inflammatory syndromes including respiratory disease, fever and gastric ulcer in traditional Eastern medicine and its major components; baicalin, baicalein and wogonin; were reported to have various biological effects. In this study, we compared the neuroprotective effect of four

isolated flavones form the root of *S. baicalensis* on global ischemic model in rat by 4-vessel occlusion method. *S. baicalensis* methanol extract was administered at a dose of 100 mg/kg whereas four flavones namely, wogonin, baicalein, wogonoside and baicalin were orally administered at a dose of 10 mg/kg. The viable pyramidal neurons were counted in the CA1 portion of hippocampus approximately +3.3, 3.5 and 3.7 mm caudal to the bregma. At a dose of 10 mg/kg, isolated compounds, wogonin, baicalein, wogonoside and baicalin inhibited the hippocampal neuronal cell death by 78.6%, 91.0%, 81.0% and 41.0% respectively whereas *S. baicalensis* methanol extract inhibited the neuronal cell death by 92% ($p < 0.001$). There was no significant difference in the neuroprotective effect of wogonin, wogonoside and baicalein with that of *S. baicalensis* methanol extract. Our study suggested that *S. baicalensis* and its isolated flavones have potential neuroprotective effect and these findings may be one of the alternative therapies for the management of stroke.

PF72 Phytogenics to prevent chicken coccidiosis

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Coccidiosis of farm animals is mainly caused by the apicomplexan parasite *Eimeria*. The disease has substantial impact on animal production economy, health and welfare. Anticoccidial drugs are preventively used in poultry feed, with the associated problems of emerging parasite resistance and residues in animal products. As an alternative, plant-derived extracts and pure compounds were studied *in vitro* for parasite inhibition. Among several active samples, thyme extracts and thymol had specific anti-eimerial effects, but carvacrol did not. Challenge trials using monospecific or multi-species infections of broiler chicken showed slight improvement of coccidiosis symptoms when pure thymol was added to the diet. Two types of encapsulated thymol however protected effectively against disease-associated weight loss, feed malabsorption and mortality. Parasite shedding and intestinal lesions were also reduced, though not to the same extent as by the anticoccidial drug lasalocid. Functional feed additives of plant origin (phytogenics) show potential to alleviate coccidiosis. Antiparasitic effects of thymol were not as strong as those exerted by lasalocid, but efficacy may be increased by improving product formulation.

PF73 Antimicrobial interest of essential oils extracted from Tunisian plants

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The aim of this study was to evaluate the antimicrobial activities of some essential oils (EOs) of Tunisian plants that were extracted by hydro-distillation and analyzed by GC and GC-MS. EOs (from *e.g. Thymus vulgaris*, *Ruta graveolens*, *Artemesia herba alba)* were tested individually and in combinations against various pathogenic bacteria and fungi using the 96-well microplates method. Minimal inhibitory concentrations (MIC) were determined. The results showed that the EOs of *Thymus vulgaris* has a high activity against all the tested species: *Legionella pneumophila* (MIC = 0.06 µL/mL), *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (MIC = 0.31 µL/mL), *Trichophyton mentagrophytes* (MIC = 0,007 µL/mL), *Cryptococcus neoformans* (MIC = 0.015 µL/mL). *Ruta graveolens*'EOs has a lower activity: *L. pneumophila* (MIC = 0.12 µL/ml), *T. mentagrophytes* and *C. neoformans* (MIC = 4 µL/mL). Analysis of *Thymus vulgaris*'EOs showed that thymol is the major component. The various EOs combinations showed synergic activities in some associations, such as EOs from *Thymus vulgaris* and *Ruta graveolens.* Our results suggest a potential interest of these EOs in water and food protections (spas, hammams, food packaging, etc.).

PF74 Memory improving effect of mixed herbal extract, HX106 in Alzheimer's disease model

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Although the mechanisms of Alzheimer's disease (AD) pathology have not been proved exactly yet, it is well known that aggregation of amyloid-β and abnormalities of cholinergic neurotransmission induce neurotoxicity, which is closely related to memory impairment in the disease. HX106 is a mixed extract of four kinds of herbs which are known to have memory improving effects in traditional oriental medicine. We investigated memory improving effect of HX106 in terms of neuroprotective effect and acetylcholine esterase inhibitory effect. HX106 prevented apoptosis of the HT22, hippocampal neuronal cells induced by high dose of glutamate. We showed that oral administration of HX106 improved the cognitive function in AD rat model induced by amyloid-β i.c.v injection through behavior tests. Moreover, oral administration of HX106 inhibited the activity of acetylcholine esterase in a mouse hippocampus up to 60% compared to non-treated group. We also found that the mice received HX106 recovered the cognitive function in scopolamine (acetylcholine receptor antagonist)-induced short term memory defective model. These results demonstrate that HX106 has a possibility of an effective treatment for AD.

PF75 *In vitro* anti-eimerial activity of several phytogenics

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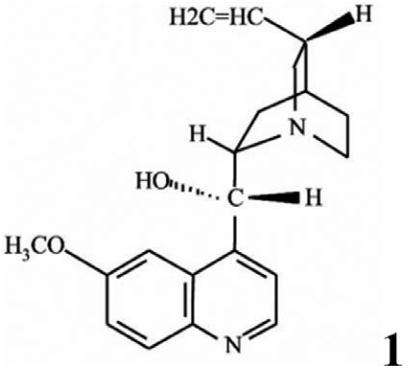
The control of coccidiosis, an infection of the intestinal tract, is a fundamental concern in poultry farming. In this study an *in vitro* model was used to simulate the first stage of an infection, the invasion of epithelial cells in the cecum by *Eimeria tenella* sporozoites. This model was applied to screen various phytogenic samples for their ability to inhibit cell invasion. Fluorescence-labeled sporozoites were co-cultured with Madin-Darby bovine kidney (MDBK) cells in the presence of samples, cell culture medium (negative control) or monensin sodium (positive control). After incubation fluorescent intracellular sporozoites were counted either by microscope or flow cytometer and relative inhibition rates were calculated. Monensin sodium showed a MIC_{50} of 180 nM. Among the tested phytogenics quinine (**1**) (MIC_{50}= 2.5 – 19.3 µM) and cinchonidine (**2**) (MIC_{50}= 2.7 – 21.2 µM) performed best. Other *Cinchona* alkaloids showed less effect. A link between molecular configuration and anti-eimerial activity is assumed.



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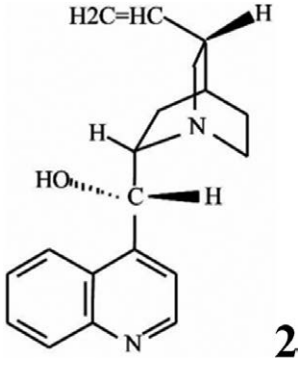

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PF76 Anti-osteoporotic effect of Eucommia ulmoides cortex in ovariectomized rats

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Eucommia ulmoides Oliver, rich in polyphenolic compounds such as lignans, phenolic acid, and flavonoids, is a herbal medicine of tonifying kidney with a long history of safe use for treatment of bone fractures and joint diseases in Korea. This study was conducted to investigate anti-osteoporotic effect of *E. ulmoides* in ovariectomized (OVX) rat model of postmenopausal bone loss. *E. ulmoides* was extracted with 70% ethanol for 3 h at 80 °C in a reflux apparatus. The Sprague-Dawley rats were anesthetized with 2% of isoflurane and ovaries were removed bilaterally. Rats were divided into four groups, which treated by vehicle, 17β-estradiol (10 μg/kg, i.p) and *E. ulmoides* (100 and 300 mg/kg, p.o), daily for 8 weeks. Bone mineral density (BMD) was significantly increased in the *E. ulmoides* 300 mg/kg treatment group without affecting body weight. Also, *E. ulmoides* at 300 mg/kg for 8 weeks significantly decreased ALP levels compared to OVX control group. These results suggest that *E. ulmoides* is effective at preventing bone loss in OVX rats.

PF77 Evaluation of the biochemical and anti-snake venom effects of *Calliandra portoricensis* extract fractions in wistar rat models

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Aim of the work: The present study was designed to evaluate the anti-snake venom effects of flavonoid fraction, polyphenolic fraction and whole ethanolic fraction of the leaves of *Calliandra portoricensis* on some biochemical indices in Wistar rats. **Methods:** Phytochemical screening was carried out using bioactivity guided fractionation and standard methods. Subsequently,thirty (30) rats of both sexes weighing between 100 – 150 g were divided into five (5) groups of 6 rats each. Groups 1 and 2 served as normal control and viperian venom control respectively, groups 3,4 and 5 were injected intramuscularly with 0.2 ml of 1 mg/ml equivalent of 0.2 mg of viperian venom and subsequently injected with 0.5 ml of 100 mg/100 g body weight antidote fractions (flavonoid rich, polyphenolic and whole ethanolic) extracts of *Calliandra portoricensis* respectively. The animals were sacrificed using chloroform as anaesthesia and whole blood collected for haematological indices using haem analyzer and sera for the estimation of some biochemical indices using standard kit methods of Agape diagnostics, Switzerland. **Results:** Phytochemical screening revealed the presence of flavonoids and polyphenols and more specifically 2 hydroxy-4-methoxy benzoic acid.AST and ALT activity showed a significant increase ($P < 0.05$) in all treated groups compared to the normal control. A significant decrease ($P < 0.05$) in creatine kinase activity was observed in group 5 compared to groups1 and 2. The Hb level, RBC and WBC count showed a significant increase ($P < 0.05$) in all treated groups compared to normal control.The LDL:HDL ratio was found to be 9.47,0.44,0.11,0.37 and 1.44 respectively for groups 2,3,4,5 and 1. A marked increased ($P < 0.05$) in SOD,CAT and Gpx activity was observed in groups 3,4 and 5 when compared to normal controls. **Conclusion:** Results showed that the phytochemical constituents inherent in the plant extract may significantly lower the high lipid peroxidation and also ameliorate hematotoxic effects induced by viperian venom, hence a protection against cardiotoxicity and the shock that normally accompanies carpet viper envenomation.

PF78 Ethnobotanical study on the area of the G.E. Ghirardi botanical garden (Brescia, Italy)

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The Botanical Garden G.E. Ghirardi in Toscolano Maderno (Brescia, Italy) was founded in 1964 as an experimental botanical station of the SIMES pharmaceutical company. It was donated to the University of Milan in 1991. The last work of taxonomic revision of the species has been carried out in 2000. In this context, the project 'GE Ghirardi Botanical Garden: taxonomic revision for the proper conservation and enhancement of natural heritage', is in progress. It provides, in addition to the other objectives, an ethnobotanical study on the species present in the Garden, traditionally used by local people. Inhabitants (50 interviews) of this area still collect plants to employ as cure for several ailments, in cookery and rituals. Information concerning 5 species (*Sambucus racemosa* L., *Sempervivum montanum* L., *Equisetum arvense* L., *Rhododendron ferrugineum* L., *Laurus nobilis* L. and *Panicum miliaceum* L.) was never ghatered by other ethnobotanical investigations realised in the Italian alpine area. Nevertheless, the value of the ethnobotanicity index was 5.2%. This suggests that the species known and used in the popular tradition are not many, in accordance with data reported for other Italian areas (Guarrera et al., 2008). The Botanical Garden also has many species with a wide geographical distribution (for example, *Zingiber officinale* Roscoe and *Coffea arabica* L.), which allow to highlight the traditions of populations away from local reality and the acquired uses in our territory.

PF79 Phytochemical and pharmacological studies on commercial teas

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In the present study an attempt has been made to evaluate phytochemical and pharmacological aspects of 25 different commercial tea products available in Warangal district of Andhra Pradesh, India. The studies revealed that pH of the all the products varied from 3.58 – 6.40 and the caffeine content varied from 1 mg – 20 mg. The loss on drying in various the tea products was found to be 3.91 – 7.61% and ash values varied from 8 – 28.6%. The *in – vitro* cytotoxicity studies on Human Red Blood Cells revealed that the cytotoxic potential varied from 30.5 – 79.8%. The AVT premium tea has lowest cytotoxicity when compared to others. The antioxidant capacity measured by β- carotene bleaching assay varied from 55.9 – 84.5%. G – tee with mint has highest antioxidant potential amongst all the tea products. The *in – vitro* anti-inflammatory activity measured by membrane stabilization method revealed that tea products have anti-inflammatory activity ranging from 20.40 – 95.84. Tringrai Assam Dust tea has highest anti-inflammatory activity than other tea products. The results of our studies suggest that there is a need to assess the therapeutic claims and standardize the tea products using the modern phytochemical and pharmacological methods.

PF80 Effects of pressed juices of common ice plant and nopal on human skin cells as a function of incubation time and filter pore size

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Pressed juices from the common ice plant (*Mesembryanthemum crystallinum* L., Aizoaceae) are part of cosmetic formulations but little is known with respect to their bioactivity. In the present study pressed juices were passed through 1.2 μm (McPI) or 0.2 μm (McPII) filters prior to monitoring their effects on the proliferation and metabolic activity of human skin keratinocytes (HaCaT) and fibroblasts (NHDF). Incubation was carried out over 48 h and 72 h, respectively. Independent of the pressed juice and the experimental conditions applied, the metabolic activity determined by MTT reduction was not changed. While during 48 h the proliferation of both skin cell types was not altered, a significant increase of proliferation rates was observed if NHDF and HaCaTs were incubated with McPI. On the other hand McPII selectively increased the proliferation of HaCaT keratinocytes. To conclude the effect of pressed juices of common ice plant predominantly influenced the cell proliferation of NHDF and HaCaTs in a time-dependent manner. In the case of keratinocytes, the biological activity also depended on the specific composition that was altered by pretreatment using different filter pore sizes.

PF81 Efficacy of a novel multi component TCM therapy in AD patients

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TCM offers interesting novel treatment options for Atopic Dermatitis (AD). In the present open-label clinical trial, the efficacy and safety of the multi component therapy approach Kujin-Plus was inves- tigated. 94 AD patients received the formula Kujin-Plus-I orally, combined with both the skin lotion Kujin-Plus-II, and the ointment Kujin-Plus-III. All raw drugs were extracted with boiling wa- ter for 5 h, concentrated and reworked into the formulations. Standardized scores were used for evaluating the severity of the disease (clinical score; 0 – 4) and the severity of pruritus (pruritus score; 0 – 4) with both scores having significantly improved at the end of treatment ($P < 0.01$; nonparametric test) after 12 months. Blood eosinophil-ratio and serum IgE-levels, elevated in AD patients, were significantly reduced at the end of therapy ($P < 0.001$). Of 94 AD patients, 32 were markedly im- proved, 59 were improved, 3 were slightly improved with no case of ineffective treatment. There was no hint of renal or hepatic toxicity or other adverse effects. Thus, the present study confirms that Kujin-Plus therapy is clinically efficacious on otherwise intractable AD, accomplishing a significant reduction in blood eosinophil-ratio and serum IgE-level.

PF82 Effects of cactus pear polysacchairdes on human skin cells as a function of incubation time and monosaccharide composition

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Cactus pear (*Opuntia ficus-indica* L., Cactaceae) is a famous food plant originating from Mexico characcterizted by high amounts of polysaccharides. While these polysaccharides are well investigated concerning their physico-chemical properties less is known about their biologic activity. To obtain a more complete insight into the dermopharmaceutical properties, a pectic polysaccharide (NPec) and a water soluble polysaccharide (NwPS) from cactus pear mainly differing in their glucose and glucuronic acid amounts were chosen for the present investigation. To achive this, human skin fibroblasts (NHDF) and keratinocytes (HaCaT) were incubated with different concentrations of the cactus pear polysaccharides for 48 h and 72 h, respectively. Afterwards their effect on cell proliferation and metabolic activity was determined by BrdU incorporation and MTT reduction assays. While the metabolic activity of NHDF was neither affected by NPec nor by NwPS, independent of incubation time or concentration, the metabolic activity of HaCats was increased after incubation with NwPS for 48 h. The HaCaT proliferation was not significantly influenced by low levels of cactus pear polysaccharides; however, it was inhibited by 100 µg/mL NPec. 100 µg/mL of NwPS and1 µg/mL NPec stimulated the proliferation of NHDF. In conclusion, cactus pear polysaccharides affected skin cell metabolism depending on their specific composition, concentration and incubation time.

PF83 Standardized plant extracts in the treatment of cancer

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In this project three plant extracts are evaluated for their potential use in the treatment of cancer: *Chelidonium majus* (Papaveraceae), containing benzophenanthridine alkaloids; *Steganotaenia araliacea* (Apiaceae), known in African traditional medicine for its antitumoral activity and containing lignans such as steganacine; and *Gloriosa superba* (Liliaceae), traditionally used in India and containing colchicine and related alkaloids. The extracts are analyzed and standardized for these constituents. The hypothesis is that a combination of various active principles in an extract may have more beneficial effects than the pure substances, due to synergism and the presence of prodrugs such as glycosides. Cytotoxicity (IC_{50}, µg/mL) of 80% EtOH extracts was determined against MDA-MB-231 WT (breast cancer), PANC-1 (pancreatic carcinoma) and HT-29 (colon adenocarcinoma) using the sulforhodamine B assay. After 24 h of incubation, IC_{50} values of 73.8 ± 11.5 µg/mL, 20.7 ± 1.0 µg/mL, and 20.6 ± 2.5 µg/mL, respectively, were observed for *C. majus*; 165.5 ± 8.0 µg/mL, 64.0 ± 3.3 µg/mL, and 68.7 ± 3.9 µg/mL for *S. araliacea*; and 0.33 ± 0.05 µg/mL, 0.13 µg/mL and 0.12 µg/mL for *G. superba*.

PF84 Year-round yield of essential oil of *Vitex negundo* and its chemical constituent analysis

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Leaves of 3 years old *Vitex negundo* L., were harvested and subjected to steam distillation to extract essential oil. Both leaves biomass and yield of essential oils were the highest during May to September. The extraction rate ranged from 0.5 – 0.6%, higher than the reported 0.35% by Mainland China. Consistent with previous reports, Caryophyllene is one major constituent occupy approximately 30 – 38% of total essential oil. In contrast, Guaiene is a minor constituent in previous reports but accounts for 50% of the total essential oil in our study. The results reveal that major constituents of essential oils of *Vitex negundo* L. can vary substantially among different months and countries of origin. The quality and functions of essential oil of *Vitex negundo* L. are worth of further investigation.

PF85 High-throughput screening program for botanical extracts

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The chemistry and activity of botanical extracts used in Ayurveda are well studied, however opportunities exist to develop high-throughput screening programs to better understand the botanical fractions in commercial extracts responsible for observed anti-inflammatory, antioxidant and anti-diabetic activities. Botanical extracts using various processing methods, including those from *Eugenia jambolana, Withania somnifera, Punica granatum, Curcuma longa, Momordica charantia* and *Cinnamomum zeylanicum* were used to develop the model. Inhibition of nitric oxide in activated RAW 264.7 macrophages, 2,2-diphenyl-1-picrahydrazyl (DPPH) antioxidant, and alpha-glucosidase inhibition assays were used as primary activity screening methods alongside chemical screening and chromatographic methods to determine correlation, if any, between concentration of various botanical extracts and their *in vitro* activity. This screening program showed a positive relationship between concentration and activity, and can be used to select ideal candidates for further preclinical and clinical studies on botanical-based therapeutic interventions.

PF86 Monofloral pollens: Chemical analysis and quality control

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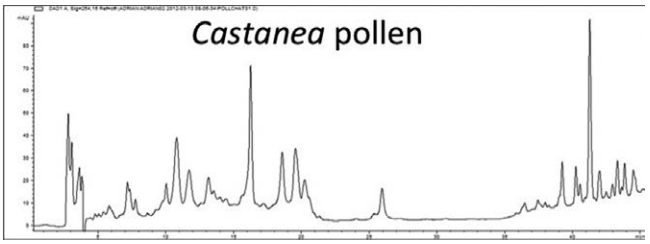
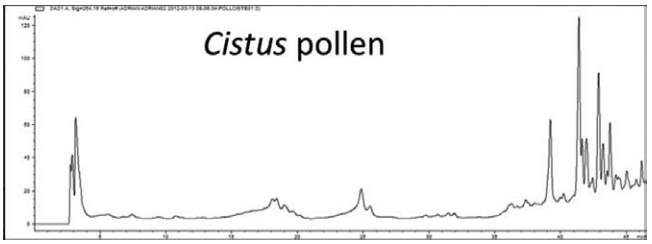
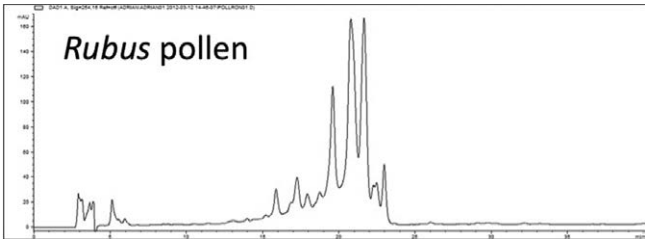
Monofloral pollens may have nutritional and medicinal potential, either by themselves or by their function of substrate for probiotic bacterias. Relationships between pollens chemical composition and their use by the bees has not been studied. Pollens are poorly studied in terms of chemical composition, and are subject to great variability. Chemical profiling and fingerprinting is necessary to allow the elaboration of identity checking, quality control and metabonomics. LC-HRMS analysis from methanolic extracts of pollens of *Castanea sativa*, *Rubus fruticosus* and *Cistus* spp. show different specific patterns. Major flavonoids have been identified in each species. An alternative, original method using

phenolic acids sublimation has also been used as a complementary method to assess pollen chemical composition.







PF87 Seeking novel Leishmanicidal natural products from common medicinal plants, the example of *Eryngium foetidum* L

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Leishmaniasis is a human parasitic tropical disease that urgently requires new chemotherapeutic agents. *Eryngium foetidum* L. (Apiaceae), known as "culantro", is a popular culinary herb and a medicinal plant used in Latin America for its anti-inflammatory and anti-parasitic properties. A defatted methanolic extract was partitioned with organic solvents and water. The n-hexane fraction inhibited the Leishmania growth by 41.4% at 20 µg/ml. Bioassay-guided fractionation of the n-hexane fraction yielded two compounds: **(1)** lasidiol p-methoxybenzoate, a daucane sesquiterpene and **(2)** a terpene aldehyde ester. Compound **1** showed an IC_{50} of 5.34 µg/ml in the Leishmania assay and no cytotoxicity in L6 cells (IC_{50} > 20 µg/ml), while compound **2** was inactive in the Leishmania assay (IC_{50} > 20 µg/ml). These compounds were previously isolated from the medicinal plants *Xanthium catharticum* Kunth (**1**) and *Ferula hispanica* Rouy (**2**). However, this is the first report of their Leishmanicidal activity, cytotoxicity, and their isolation from a *Eryngium* species. In conclusion, there is potential to find new Leishmanicidal natural products from common medicinal plants.

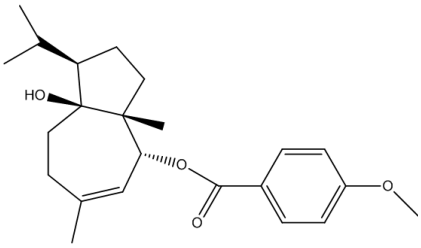

Fig. 1: lasidiol p-methoxybenzoate (MW = 372.50)

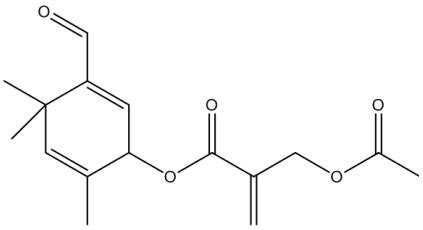

Fig. 2: terpene aldehyde ester (MW = 306.35)

PF88 Exploration of bioactivity in traditionally used medicinal plants

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Arachidonic acid (AA) is metabolized in the body through two main metabolic pathways with the enzymes: cyclooxygenases (COX) and lipoxygenases (LOX). Elevated levels of prostanoids and leukotrienes, products of the two respective pathways, have been linked to inflammatory diseases as well as to early stages of carcinogenesis. Finding a dual inhibitor of COX and LOX is promising in preventing the inflammation and diseases that are linked to the overproduction of both pathways while minimizing the side effects associated with inhibition of individual pathways. The purpose of this research project is to find a dual inhibitor of COX and LOX by examining plants used traditionally as anti-inflammatory medicines: *Tussilago farfara, Grindelia squarrosa, Uritca dioica,* and *Trigonella foenum-graecum. G. squarrosa* has been traditionally used in treatment of catarrhs, associated with the inflammation of the respiratory tract. *T. farfara* has been used in traditional Chinese medicines for treatment of asthmatic and bronchial infections as well as rheumatism. *U. dioica* has been used for treatment of rheumatism, bleeding, prostate hyperplasia, and urinary tract infections. *Trigonella foenum-graecum* has been used to treat gastric inflammation, menstrual pain, and respiratory conditions, such as bronchitis. Tests have been performed on crude extracts of these plants for the following: total phenolics, free radical scavenging activity, LOX inhibition, and COX inhibition. The results of these bioactivity assays will be presented.

PF89 Ethnomedical knowledge among the "Quilombolas" from Oriximiná, Brazil, with a special focus on plants used as nerve tonics

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By definition, the "remnants of quilombos" or "quilombola" communities are ethnic groups with a historical background and presumption of black ancestry related to resistance to oppression suffered historically. Their close contact with nature over centuries and their geographic isolation has given these community members a vast knowledge of medicinal plants. This work focused on plants with indications to "rejuvenating" or used as "neurotonic", employed by the quilombola communities of Oriximina to prevent forgetfulness and improve memory. The methodology used was semistructured interviews, participating observation, walk-in-the-woods, as well as quantitative techniques of data analysis such as salience index (S), relative importance of species (RI), and the corrected major use agreement (MUAc). Thirty four interviewed people related 254 ethnospecies totalizing 2508 use indications. Among these, 227 species were identified, belonging to 211 genus and to 77 botanic families. The ethnodirected method of free-list focusing on forgetfulness, youthfulness, elderness, "head weakness", "weakness of mind", "weakness of men", "nerve tonic", aphrodisiac, among other terms, was applied to the informants, leading to 36 ethnospecies. This diversity identifies a clear relationship between their traditional knowledge and the mega biodiversity of the region.

PF90 Differentiation of Flos Chrysanthemi cultivars by NMR fingerprinting and chemometric analysis

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Flos Chrysanthemi (Ju Hua), the flowerhead of the daisy plant *Chrysanthemum morifolium* Ramat., has been used as an herbal medicine in TCM for centuries. Recent studies showed that Ju Hua possesses antibacterial, antifungal, antispirochetal, anti-inflammatory, and anti-HIV activities. *C. morifolium* is native to China. Varieties of *C. morifolium* plants have been cultivated in China with a long history. In the Chinese Pharmacopeia, four kinds of Ju Hua cultivars, namely "Bo Ju", "Gong Ju", "Hang Ju", "Chu Ju", are officially recorded under the same title as Ju Hua. Due to the differences in their growing locations, varieties and processing methods, it is believed that the qualities and pharmacologic properties of the different Ju Hua groups may vary, thus their values differ. NMR-based Metabolomics is a potential technology to provide better insight into the qualitative and quantitative shifts in metabolite patterns of the organism. In the present study, we reported the investigation for the differentiation of Flos Chrysanthemi cultivars by using NMR fingerprinting and chemometric analysis.

PF91 Quality control of herbal medicines containing *Sennae*

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The aim of this study is to evaluate the quality of the most representative products used in weight-loss schemes, containing *Sennae folium*, *Sennae fructus acutifoliae* and/or *Sennae fructus angustifoliae* sold in community pharmacies and parapharmacies in the Lisbon region, containing 8 herbal medicine formulations including different pharmaceutical dosages forms. The results based on the botanical and chemical tests showed that sennosides A and B are present in all samples (100%) according to requisites described in the Portuguese Pharmacopeia, but 62.5% of the total samples do not show the proper identification (binomial name and part of the plant used), 25% of the total samples analyzed revealed the presence of phenolphthalein and diazepam as adulterants and these substances were not mentioned in the label content. In conclusion 37.5% of all the products analyzed are not in conditions to be in the market. This study revealed a lack of quality control in some of these products, which poses a concern related with public health. These findings showed that much concerns have to be taken by the rule authorities on this kind of products in order to avoid harmful misuse.

PF92 Chemotaxonomic authentication: importance and implications in herbal medicine practice

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The use of herbs and herbal products is on the rise and becoming more popular and acceptable to the populace Worldwide, this is as a result of the numerous biochemical evaluations of some of these plant materials supporting their efficacy and safety. Nevertheless the inconsistence and non reproducible results obtained in practice is a great challenge to the Traditional Medical practitioners, especially in Africa, this is due to the non availability of instruments and competent hands for constituents standardization of their raw materials. The correct identification or authentication of the plant materials, time of harvest, location of plantation and processing are all factors that affect the reproducibility of the efficacy of these herbal medicines. In this report we discuss some practical challenges encountered with the *hypoestes spp,* regarding establishing plantations and the effects of seasonal variation on its bioactive components.

PF93 Medicinal plants used by traditional community in Mato Grosso state – Brazil

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Brazil stands out for being the country with the highest biodiversity on the planet. The tool which facilitates the study area for medicinal plants bioprospecting for development of natural products is ethnobotany. The objective of this research was to understand the diversity of medicinal plants used to cure diseases through local knowledge, in one traditional community in Nossa Senhora do Livramento municipality, Mato Grosso State. The study consisted in qualitative and quantitative research, through ethnobotanical studies, according to the knowledge of traditional farmers. For the field research we used camera and semi-structured interview. The selected study area was an old community that has experienced farmers in the use of medicinal plants. 22 botanical families and 34 genera were identified in this study. The botanical families with the highest numbers of species were Asteraceae, Lamiaceae (12%) and Liliaceae (9%). The diseases with highest citation index were: cold/cough, sore stomach and uterine infections. It was observed in some cases that the same plant is used to cure various diseases. The knowledge of these plants in the community is passed on orally from generation to generation.

PF94 Anti-inflammatory effect of *Uncaria tomentosa* in an experimental model of cyclophosphamide-induced hemorrhagic cystitis

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Hemorrhagic cystitis (HC) is an adverse effect resulting from the use of cyclophosphamide (CFS). Previous studies has demonstrated the anti-inflammatory activities of the plant *Uncaria tomentosa* (Rubiaceae). The aim of this work is investigate the effect of aqueous extract of *Uncaria tomentosa* (UT) in an experimental model of CH induced with CFS. Experimental protocols were registered on the Institutional Ethics Committee under number 10724196 – 0/04. Swiss female mice (n = 10) were treated with aqueous extract of UT 2% *ad libitum* for 9 days. On the 10th day, the animals were treated again with UT (200 mg/kg by gavage) 1 h before, 3, 6 and 9 h after the administration of CFS (400 mg/kg i. p.). The control group received only the aqueous vehicle, orally. Animals were sacrificed 12 h after the administration of CFS. The bladders of animals were removed to determine their wet weight (PUV) and scored macroscopically according to Gray's criteria. CFS was able to induce hemorrhagic cystitis. However, the treatment with UT significantly reduced the PUV in 30.5% compared to control group (130,1%) ($p < 0.05$). Moreover, bladders from UT group showed less edema (0 [0 – 1]) compared to control group (1 [1 – 2]) ($p < 0.05$). These findings demonstrated the anti-inflammatory activity of aqueous extract from *Uncaria tomentosa* in the model of cyclophosphamide-induced hemorrhagic cystitis. New approaches are being taken to elucidate the possible mechanisms involved.

PF95 Composition of the essential oils from rhizome and aerial parts of *Dictamnus albus* from Spain

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Dictamnus albus L. (Rutaceae) is a European medicinal plant which use dates back to the classical Greco-Roman period. It is found in the Center and North-East of Spain. In the present work, the composition of the essential oils from the rhizome and the aerial parts of *D. albus* from Spain is reported for the first time. The oils were obtained by hidrodis-

tillation from dried plant material collected from wild. They were analyzed by GC-FID and GC-MS using two columns of different polarity and their constituents were identified by comparison of their mass spectra and two different types of retention indices (determined in relation to homologous series of fatty acid methyl esters and *n*-alkanes, respectively) with those of literature data and our own data bases. The main constituents of the oil from aerial parts were estragole (23%), germacrene D (22%), bicyclogermacrene (8%), spathulenol (5%), γ-palmitolactone (4%), phytol (3%) and palmitic acid (3%). In the essential oil from the rhizome, estragole (22%), γ-palmitolactone (8%), geijerene (6%) and thymyl methyl ether (3%) were the major constituents identified. In addition, both oils contain an unidentified angelicine type furanocoumarin which accounts for 18% of the rhizome oil and 2% of the aerial parts oil.

PF96 A new method for quantifying ginsenosides in American ginseng (*Panax quinquefolius*) roots and leaves

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Ginsenosides from American ginseng have shown a wide range of biological effects, including antioxidant and anti-cancer properties. The goals of this project were to develop a new method using electrospray ionization-mass spectrometry (ESI-MS) to identify and quantify seven ginsenosides in ginseng (*Panax quinquefolius* L., Araliaceae). An acetonitrile/5% aqueous acetic acid gradient was used, the ginsenosides were detected as negatively charged acetate clusters. Using the new method, sixteen leaf and their corresponding root extracts were analyzed for ginsenoside content. It was determined that for ginsenosides Rb2, Rd, Re, and Rf, there was a higher content in the leaves as opposed to the roots. For ginsenosides Rb1, Rc, and Rg1, there was not a significant difference between leaf and root contents. These findings indicate that ginseng leaves are a viable source of ginsenosides, which is significant given that native ginseng populations are threatened by overharvesting.

Topic G: Innovative Approaches – Misc.

PG1 Electrochemistry as an adjunct to mass spectrometry in drug development

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Oxidation plays a number of critical roles in biology and drug metabolism. In addition to being the major process for generating energy in a living cell, it also is the major mechanism by which drugs are metabolized. Oxidation is a contributor to drug bioactivation and idiosyncratic toxicity, and is also involved in drug stability and degradation. Electrochemistry (EC) when used with HPLC has long been recognized as a sensitive and selective detection technique. Indeed more than 90% of pharmaceuticals in the market place are easily oxidized and can be detected using this technique. However, the inherent electrochemical nature of many drugs goes beyond simple analysis. This commonality of oxidation makes EC particularly well suited to studying the oxidative processes in pharmaceuticals – both in the formulation and by the organism. Presented here are techniques that combine EC and MS in a number of formats that can be used: a) to enhance ionization thereby extending the range of compounds measured by LC-MS; b) for the micro-synthesis and identification of drug metabolites; c) for the detection of reactive intermediates and their conjugates; d) identification of potentially problematic molecular "soft spots"; and e) to rapidly study drug stability and predict which antioxidant(s) should be included in formulations.

PG2 Cannabinoid analysis of laser-microdissected trichomes of *Cannabis sativa* L. BY LC-MS and cryogenic NMR

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Trichomes of *Cannabis sativa* have been reported as the main site of cannabinoids production. A comprehensive study of cannabinoids was performed on capitate-stalked and capitate-sessile trichomes, and on capitate-stalked glands and stems harvested by laser microdissection (LMD) during flowering time (week 4 – 8). LC-MS and cryogenic NMR analysis were used for qualitative and quantitative assessment of cannabinoids in the collected cells. Δ^9-tetrahydrocannabinolic acid, cannabidiolic acid and cannabigerolic acid were identified as the major constituents in the all tested samples, while Δ^9-tetrahydrocannabinol, cannabidiol and cannabigerol were present in less quantity. Cannabichromene and cannabinol were detected as minor compounds only in intact capitate-stalked trichomes in week 8. Based on the cannabinoid levels, discrimination of capitate-stalked and capitate-sessile trichomes at flowering time was possible. The study demonstrated the possibility of other spots for cannabinoids production besides the gland of the capitate-stalked trichomes. In particular the presence of cannabinoids in the stem of capitate-stalked trichomes is reported for the first time. The combined use of LMD, LC-MS and cryogenic NMR constitutes a valuable method for the comprehensive assessment of plant metabolites at the cellular level.

PG3 Phytochemical and growth responses of purple coneflower (*Echinacea purpurea* L.) to hydroalcoholic solutions

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This study was conducted at the Institute of Medicinal Plants, in Karaj to determine the effects of foliar applications of hydroalcoholic solutions on purple coneflower. *Echinacea purpurea* L. is an herbaceous perennial plant that is native to eastern North America and is beneficial to the immune system. This experiment was conducted in randomized complete block design with eleven treatments and three replications. The treatments of this study include: control (without distilled water), ethanol and methanol aqueous solutions with 10, 20, 30, 40 and 50% (v/v). The maximums for plant height, stem diameter, dry stem weight, dry leaf weight, dry capitulum weight, capitulum per plant, dry root weight, root diameter and length were obtained using a solution of 40% methanol. In addition, the maximums for leaf area and chlorophyll content (SPAD) were obtained by using a solution of 30% methanol. High amount of seed weight per plant was obtained by using a solution of 50% methanol. Also, the maximums for capitulum diameter, content and yield of chicoric acid of shoot, total flavonoid yield of root were found at 40% ethanol solutions. The high amount of total flavonoid of root, content and yield of chicoric acid in the root, total flavonoid and yield of root were observed at 50% ethanol solutions. In general, the results indicated that foliar application of hydroalcoholic solutions such as methanol and ethanol can be used as a carbon source and a bio-stimulator to improve quantity and quality of phytochemicals and growth response in purple coneflower.

PG4 The chalcone-flavanone isomerization affects in vitro bio-assays as exemplified with isoliquiritigenin from licorice

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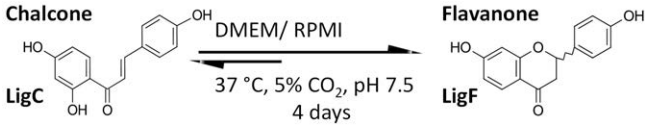
Although well-studied from a chemical perspective, the real-time biological impact of the chalcone-flavanone isomerization, e.g., under cell-based bioassay conditions is not fully understood yet. Among the principal polyphenols of licorice roots (*Glycyrrhiza sp.* Fabaceae) are glycosides of the *f*lavanone, *li*quiritigenin (LigF), and the chalcone, iso*li*quiritigenin (LigC). Their isomerization in two widely used culture media, DMEM and RPMI, was analyzed over 4 days by LC-UV-MS. In both media, 50% of LigC were converted into LigF within 24 hrs. A steady state with 80/20% of LigF/LigC was reached at 72 hrs. The influence of the media on chalcone glycoside isomerization and flavanone racemization will be discussed. The findings indicate that flavanone rearrangement products play a role in observed *in vitro* and possibly *in vivo* biological activities of LigC and related plant chalcones.



PG5 Shikonin induces immunogenic cell death in tumor cells and enhances dendritic cell-based cancer vaccine

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Novel strategies/approaches to are being actively explored to overcome the relapse of cancers, resulting in tumor cells escaping from traditional therapies and/or host immune-surveillance. This study exploited the feasibility of improving the efficacy of therapeutic dendritic cell (DC)-based cancer vaccines with a combined use of phytochemical shikonin as an adjuvant. Shikonin, a phytochemical purified from *Lithospermum erythrorhizon*, has been shown to confer diverse pharmacological activities, including anti-inflammation, anti-tumor and others. Immunogenic cell death is characterized by damage-associated molecular patterns (DAMPs), which can enhance the maturation and antigen uptake of DCs. The anti-tumor effect of shikonin can effectively activate both receptor- and mitochondria-mediated apoptosis and increase the expression of all five tested DAMPs, including HSP70, HSP90, GRP78, CRT and HMGB1, in the resultant tumor cell lysate (TCL), termed as SK-TCL. The combination treatment with DAMPs and LPS activates DCs to a high maturation status and enhances the priming of Th1/Th17 effector cells. SK-TCL-loaded mature DCs exhibit a high level of CD86 and histocompartibility complex class II and activate Th1 cells. The shikonin-TCL-loaded DC-based vaccines result in a strong induction of CTL activity of splenocytes, a retardation in tumor growth, and an increase in the survival time of test mice. Together, our findings suggest that the much enhanced immunogenicity and efficacy of the cancer vaccine formulation, i.e, the use of shikonin-derived tumor cell lysates for pulse of DCs, may suggest a new *ex vivo* approach for developing individualized, dendritic cell-based cancer vaccines.

PG6 Categorization of traditional medicinal plants on inflammation-regulatory and anti-tumor activities by differenzial cytokine expression in dendritic cells

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Traditional medicinal plants (TMP) are increasingly recognized for use in public health care throughout the world. However, systematic investigation of TMPs on specific and heath care-applicable immuno-regulatory activities is limited. Dendritic cells (DCs), a key type of professional antigen presenting cells are key mediators in human's immune systems. Therefore, DCs are considered by many as a viable pharmacological platform or target for evaluating TMP's immuno-regulatory activities. The objective of this study is to evaluate the regulatory activities of specific TMPs on cytokine regulation in DCs and anti-tumor activities in orthotopic mammary tumor model. Our results show that a number of extracts of test Taiwan specialty medicinal herbs exhibited an inhibitory activity on LPS-induced expression of IL-6 and IL-12 in mouse DCs. In addition, these herbal extracts were categorized into several functional sub-groups based on their capacities to regulate cytokine expression. In an orthotopic mammary tumor model, the extract of TMP1 effectively suppressed primary tumor growth. The extracts of both TMP1 and TMP2 suppressed mammary tumor metastasis, and the results were further confirmed by a mammary tumor resection model. Taken together, our results suggest that TMP1 and TMP2 may warrant further investigation for potential application as anti-tumor natural product agents.

PG7 Evaluation of antioxidant and DNA protection activities in the extracts of *Oncidium* flower

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Oncidium is a tropical species of the family Orchidaceae. The *Oncidium*, also named as the Dancing Lady Orchids, is commercialized as cut flowers in Taiwan. The aim of the present study was to examine the antioxidant and DNA protection activities which could be applied for the anti-ageing cosmetic products.The aqueous extract from the *Oncidium* flower was used in this study. Antioxidant activities were measured by both 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH·) scavenging and 2, 2-azinobis (3-ethylbenz- thiazoline-6-sulfonic acid) (ABTS·+) decolourisation methods. To further evaluate the effect of *Oncidium* flower extracts on UV induced DNA damages, the DNA protection assay was employed. *Oncidium* flower extracts had effective DPPH bleaching activity and ABTS·+ radical scavenging activity in a concentration dependent manner. Furthermore, the *Oncidium* flower extracts to inhibit the oxidative DNA damages were assessed by measuring the conversion of supercoiled pUC119 plasmid DNA to the linear forms. UV irradiation of DNA with hydrogen peroxide resulted in the formation of linear forms of DNA, indicating double-strand DNA breaks. Addition of the *Oncidium* flower extracts to DNA resulted in a partial inhibition of the conversion of supercoiled DNA to linear forms, indicating that the *Oncidium* flower extract was able to protect plasmid DNA against hydroxyl radical induced oxidative damage. The inhibition of hydroxyl radical induced DNA strand breaks by *Oncidium* flower extracts exhibited a concentration dependent relationship.

PG8 A prescreening system for enriched selection of secondary metabolite-producing unicellular bacteria

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Many inventive culture-based antibiotic screening methods have been described, yet the availability of sensitive automated methods to identi-

fy functional molecules directly from microbial cells still limits the search for new biologically active compounds. Recently we described a semi-automated system for detecting diffusible functional molecules, e.g. antibiotics, from bacterial colonies employing two opposed agar layers sequentially formed in prototype Society for Biomolecular Screening plates, named *Janus* plates. Bacteria can be factorially replicated onto *Janus* plates with different media and growth parameters in the growth layer. Once grown, replicated colony arrays can be assayed against multiple target cells in the opposed assay layer. The prescreening system has enabled the construction of a strain library enriched in metabolite-producing unicellular bacteria and a corresponding extract library enriched in bioactive metabolites with up to 80% of the extracts of the resulting library exhibiting activity in phenotypic assays with pathogenic bacteria, yeasts, fungi, *Plasmodium falciparum* and human cell lines. Among the first metabolites we have detected include the infrequently reported antibiotics, e.g. katanosins, and the recently discovered cyclic imine, koranimine. The ability to reveal new metabolites will be illustrated with the structural characterization of a series of new peptides from *Pseudomonas* spp.

PG9 Innate defense response stimulated by ADAPT-232 in nueroglia cells augments tolerance and adaptation to stress

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We demonstrated that ADAPT-232, a fixed combination of adaptogens *Eleutherococcus senticosus* root extract, *Schisandra chinensis* berry extract, *Rhodiola rosea* root extract SHR-5, and its active constituent salidroside stimulated the expression of NPY and Hsp72 in isolated human neurolgia cells. We further validated the central role of NPY in experiments in which pre-treatment of human neuroglia cells with NPY-siRNA and HSF1-siRNA resulted in the significant suppression of ADAPT-232-induced NPY and Hsp72 release. Our studies suggest that the stimulation and release of the stress hormones, NPY and Hsp72, into systemic circulation is an innate defense response against mild stressors (ADAPT-232), which increase tolerance and adaptation to stress.

PG10 Systematic isolation and metabolomic analysis of uterine active compounds from the leaf extracts of *Ficus exasperata* (Moraceae)

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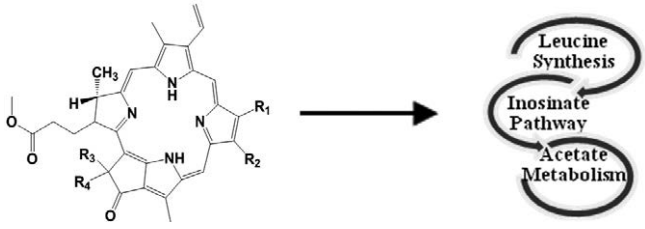
New potential lead uterine active agents for the safe and effective management and/or therapy of dysfunctional uterine activities were isolated from the leaves of the tropical plant *F. exasperata* via *in vitro* bioassay-guided functional uterine assays as well as several spectroscopic procedures which included 1D, 2D NMR and LC-HRMS. A total of 17 active compounds comprising four fatty acids, one triglyceride, six pheophorbide related compounds, four flavonoids, one pyrimidine, and an inorganic salt were identified from the ethylacetate and methanol extracts. Metabolomic tools were used to further investigate pathways that were down and/or up regulated during uterine activities.



(1) R_1= CH_3, R_2= CH_3, R_3= OH, R_4= COOH, R_5= Phythyl group; **(2)** R_1= CH_3, R_2= CH_3, R_3= OH, R_4= COOH, R_5= CH_3; **(3)** R_1= CH_3, R_2= C_2H_5, R_3= $COOCH_3$, R_4= H, R_5 = H; **(4)** R_1= H, R_2= H, R_3= H, R_4= CH_3; **(5)** R_1= CHO, R_2= CH_3, R_3= OH, R_4= COOH, R_5= CH_3; **(6)** R_1= CHO, R_2= CH_3, R_3= OH, R_4= COOH, R_5= Phythyl group.

PG11 Tailored beads made of dissolved cellulose and printed drug delivery systems to improve release of isoflavone biochanin a

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Most of the beneficial health effects linked to isoflavones such as biochanin A are believed to be mediated by the estrogenic and antioxidative properties of these compounds. Also antimicrobial effects have been reported for biochanin A. However, majority of biochanin A is converted to genistein prior to its absorption via demethylation reactions and the bioavailability of biochanin A is also limited by its poor water-solubility. Thus less than 5% of biochanin A is found in the bloodstream in its parent form after ingestion of the pure isoflavone. To facilitate the stability and release of biochanin A we evaluated the use of beads of dissolved cellulose and printed systems for drug loading and delivery in a controlled and tailored manner. Surface and internal structure of empty and biochanin A -loaded formulations were examined using a scanning electron microscope (SEM). Drug releases in appropriate solutions mimicking GI-conditions were determined in an automated dissolution testing system (USP paddle method). Bioactivities of the formulations were assayed against the intracellular bacteria *Chlamydia pneumoniae* and its host cells of human origin. In conclusion, these new delivery systems show great promise in controlling delivery of biochanin A without interfering with its bioactivity.

PG12 The phytochemical response of lemon balm (*Melissa officinalis* L.) to methanol and ethanol hydroalcoholic solutions

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The purpose of this study was evaluating effect of methanol and ethanol foliar application as a new and safe technology in plant production on lemon balm's volatile oil components. This experiment was based on a randomized complete block design with 11 treatments and 3 replications. The treatments of this study include: control (without distilled water), ethanol and methanol aqueous solutions with 10, 20, 30, 40 and 50% (v/v). phytochemical result of treatment indicated that the most of biosynthesis of Beta Caryophyllene, Neral, Geranial, Caryophyllene Oxide, Delta Cadinene, Delta Cadinol, Citronellal, Germacrene B, Geranyl Acetate were affected by methanol 50% solution and the most of biosynthesis of Caryophyllene and Alpha Cadinol were obtained by ethanol 50% solution. In general, the results indicated that foliar application of hydro alcoholic solutions such as methanol and ethanol on Lemon Balm can increase the biosynthesis of Lemon Balm's Volatile oil components. It seems that foliar application of hydro alcoholic solutions can be as a new method that is useful for increasing performance of medicinal plant especially in Arid and semiarid. In production of medicinal plant particularly in Sustainable agriculture in order to produce medicine, It is recommended that foliar application of hydro alcoholic solution (50% ethanol and methanol) in climates the same of study area can increase the quantity of essential oil on lemon balm.

PG13 Natural products biotransformation and its application

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Saponins are one sort of active natural product from herbs. Modification of saponins by biotransformation is regarded as high selectivity, high yield, mild reaction and friendly to environment. The glycosyl hydrolysis, glycosylation, hydroxylation and isomerization of saponins were carried out by using 1,4-alpha-D-glucan glucohydrolase (EC 3.2.1.3, GA) from *Curvularia lunata*, commercial enzyme Klerzyme-150, Cyclodextrin

Glucanotransferase and so on. Diosgenin, presently produced by acidic hydrolysis of *Dioscorea zingiberensis*, is a main initiative material of lots of steroidal drugs. Two enzymes were found to hydrolyze main saponins of *D. zingiberensis* to sapogenin completely. We also constructed and expressed a glucuronidase which could hydrolyze glycurrhizin to glycyrrhetic acid with a high efficiency to solve the pollution and safety problems caused by strong acid hydrolysis.

PG14 A polyphasic approach (metabolomics, morphological and molecular analyses) in the systematics of *Cladobotryum* species in Greece

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The secondary metabolism of mycophilic fungi is a source of bioactive natural products. In continuation of our research on the investigation of fungi, *Cladobotryum* species from Greece were studied. Due to the taxonomic complexity of the genus, a polyphasic approach for species identification and characterization was employed. The genus presents complex and diverse metabolic profile. Therefore, an experimental design was developed combining conventional methods of morphological and molecular analyses together with NMR based metabolomics. The multivariate analysis of the complex spectroscopic data resulted in unique metabolic profiles of *Cladobotryum* strains that could be used for discriminating the species within *Cladobotryum*. The combination of all data has shown that these strains could be placed well within the known species of the genus. It also showed a diversity which could offer the potential of isolating different and specific metabolites of medicinal or biotechnological interest. To the best of our knowledge, this is the first successful attempt to use NMR based metabolomics in combination with morphological and molecular characteristics for the study of mycophilic fungi.

PG15 Mutual information analysis of salvia exudates

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As *Salvia* exudates in a screening work have shown to possess inhibitory activity on the germination of *Papaver rhoeas* L. and *Avena sativa* L., a database for fast and efficient data collection among the various collaborating groups to be used in the following research steps to define the allelopathic potential of these species has been developed. Data about the effect of 13 species of *Salvia* (13 species and a control species) on *Avena* and *Papaver* has been extracted from the database and some statistical analysis have been done. First, an Artificial Neural Network (ANN) clustering algorithm has been developed in order to group data in at most five clusters. 100 trials have been done to cluster data, in order to have unbiased results. Then, the clustering results have been ranked by the following innovative index, to give a ranking of the effectiveness of every *Salvia* species:

$$BGP_i = \sum_t \sum_e \frac{N_t - c_{te}}{N_t - 1} \cdot \frac{NC_{it}}{NT_{it}}$$

N_t is the number of clusters in the trial t; c_{te} is the cluster in which the element e is present in the trial t; NC_{it} is the number of neurons of the cluster in which the species i is present in the trial t; NT_{it} is the total number of neurons where the species i is present in the trial t. Finally, a Mutual Information analysis has been done in order to decide what number of clusters could be the one that leads to the best classification. The best one has found to be 3.

PG16 HT-MALDI-MS: A keystone for Brazilian biodiversity conservation

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In this work we provide a HT-MALDI-MS forest screening method to provide added value to local specimens of plants. Ionic liquid was used to get around known ionization problems. Our strategy is based on the ability of MALDI to quickly screen a very large number of samples using an ionic liquid as matrix. Using this approach, we can screen up to 400 samples within a 6 hour time-frame. Finding plants with the correct signatures, indicating candidate molecules that have commercial value, are then prioritized for the lower throughput but quantitative LC-MS studies where the retention time, UV and tandem MS signatures are used to confirm the identity of the molecule. Thus, the screening of an entire forest can be accomplished in a short time, which is currently not feasible with other methodologies. Our current forest dereplication revealed high levels of alkaloids on leaves sampled at SMSP, with emphasis to strictosidine (> 5%) in *Simira sampaioana* (Rubiaceae). This is the starter of indolic alkaloid synthesis, such as Vincristine. The occurrence of such high levels of its key biosynthesizer represents a big opportunity for forest inhabitants to profit on yielding leave's compounds by single solvent-recrystallization methods, and making money furnishing this compound for a spin of company.

Topic H: Metabolism, PK and Safety

PH1 Biotransformation of vulgarin

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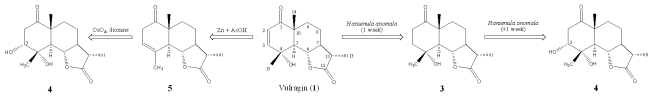
Microbial transformation studies of the eudesmanolide vulgarin (**1**) have revealed that it was metabolized by a number of microorganisms. Using a standard two-stage fermentation technique, *Beauvaria bassiana* (ATCC 7159) produced one more polar metabolite **2**. Moreover, *Hansenula anomala* (ATCC 20170) partially converted vulgarin into another less polar metabolite **3** and a more polar one **4**. These metabolites were characterized on the basis of their spectral data as 1-*epi*-tetrahydrovulgarin (1α,4α-dihydroxy-5αH,6,11βH-eudesman-6,12-olide, **2**), dihydrovulgarin (4α-hydroxy-1-oxo-5αH,6,11βH-eudesman-6,12-olide, **3**), and 3α-hydroxydihydrovulgarin (3α,4α-dihydroxy-1-oxo-5αH,6,11βH-eudesman-6,12-olide, **4**). Metabolite **4** identity was further confirmed through chemical synthesis.



PH2 The molecular basis of the antidiabetic activity of quercetin in skeletal muscle cells and hepatocytes in culture

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Quercetin is most abundant flavonoid in the human diet. In a previous study, we have reported that quercetin stimulated glucose uptake in cultured C2C12 skeletal muscle through an insulin-independent mechanism involving adenosine monophosphate-activated protein kinase (AMPK). In skeletal muscle, the activation of AMPK increases glucose uptake through the stimulation of glucose transporter GLUT4 translocation to the plasma membrane. In liver, AMPK decreases hepatic glucose production mainly through the downregulation of the key gluconeogenesis enzymes such as phosphoenolpyruvate carboxylase and Glucose 6-

phosphate (G6Pase). In the present study, an 18 h treatment with quercetin (50 μM) was reported to stimulate AMPK and to increase GLUT4 translocation and expression in cultured rat L6 skeletal muscle cells. On the other hand, we reported that quercetin induced hepatic AMPK activation and inhibited G6pase in murine H4IIE hepatocytes. Finally, we have observed that querectin exhibited a mild tendency to increase the activity of glycogen synthase (GS), the rate-limiting enzyme of glycogen synthesis, in the human hepatoma cell line HepG2. Overall, these data demonstrate that quercetin positively influences glucose metabolism in both liver and skeletal muscle, and therefore appear to be a promising therapeutic candidate for the treatment of type 2 diabetes mellitus.

PH3 Pro-inflammatory capacity of sesquiterpene lactones from asteraceae – their impact on allergic contact dermatitis

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Medicinal plants of the Asteraceae family, such as *Arnica montana*, *Tanacetum parthenium* and others, are commonly used to soothe inflammatory conditions. Nonetheless these plants are known to exhibit allergenic potential after skin exposure. For both effects, sesquiterpene lactones (STLs) are held responsible. Their well known role in allergic contact dermatitis (ACD) is formation of full antigens with proteins of the skin. One of the early steps in sensitization as well as in elicitation of an ACD is the production of pro-inflammatory signals by residing keratinocytes (KC). It was an open question, whether STLs, generally considered anti-inflammatory molecules, can directly induce such signals. The effects of selected STLs and allergy-relevant plant extracts on HaCaT KC were therefore examined in comparison to the known strong sensitizer dinitrochlorobenzene. Time-resolved mRNA microarray experiments showed that various STLs indeed induce a diverse arsenal of inflammation-associated mechanisms. Furthermore we could demonstrate that these compounds, in a concentration-dependent manner, elicit considerable changes in the levels of the pro-inflammatory cytokines IL-6, IL-8 and IFNγ in culture supernatants. Although the regulation was not consistently up- or downwards for all tested compounds, an overall increase in inflammatory mediators could be confirmed. Therefore we conclude that STLs can directly contribute to the onset of ACD by provoking keratinocytes to emit "danger signals" in form of pro-inflammatory mediators.

PH4 Blood alcohol concentrations after oral administration of Thyme herb and Primrose root fluid extract (T+P) to children

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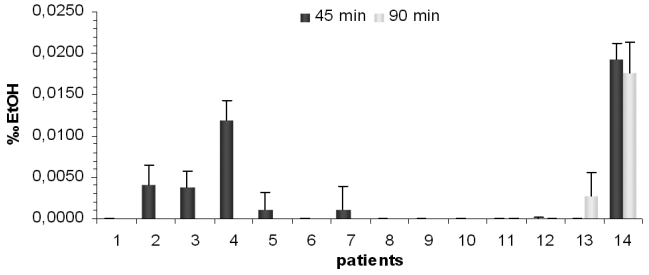
The aim of this open clinical study (indication: acute bronchitis) was to prove that after the oral administration of a single dose of T+P (containing 4.9 % EtOH) the blood EtOH concentrations in children (1 – 4y n = 12, 5 – 12y n = 4) rest below a safety threshold of 0.125 ‰. T+P was administered over a period of 7 – 9 days (6 x 2.5 ml or 4 x 7.5 ml/d). During visit 2 (d 3 – 5) blood samples were taken 45 and 90 min (only 5 – 12-y) after the second application and the EtOH concentrations were measured with a validated dehydrogenase assay. In addition the clinical symptoms (Bronchitis Severity Score, BSS) and the tolerability were assessed. All measured blood EtOH concentrations stayed far below the safety threshold of 0.125‰ and BSS decreased significantly:



PH5 Acute human pharmacokinetics of a lipid-dissolved turmeric extract

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Curcumin is a lipophilic compound found in the rhizome of turmeric (*Curcuma longa* Linn.) that targets several inflammatory and neurological pathways. Dosing of turmeric extract is associated with safety and tolerability in humans, yet literature on its efficacy is limited, possibly due to its low bioavailability in plasma and high rate of metabolism, mainly to glucuronide conjugate. The primary aim of this study was to quantify plasma and red blood cell levels of curcumin by HPLC after ingestion of 40 mg curcumin from a lipid-dissolved turmeric extract formulation (SLCP-1) at a dosage of 200 mg in healthy human volunteers. The secondary aim of this study was to determine the relationship between plasma and red blood cell (RBC) curcumin in blood samples treated with and without glucuronidase enzyme. An HPLC system (Shimadzu 1100) with PDA detector and C 18 column using mobile phase gradient of 0.1 % phosphoric acid and acetonitrile was used, as previously published. The mean peak concentration (Cmax) of free curcumin in blood prepared with and without glucuronidase was 48.3 and 39.2 ng/mL, respectively, with time of maximum concentration (Tmax) occurring at 6 hours. Most of the curcumin from dosing of the formulation was detected in blood in free form in plasma and RBC fractions. The concentrations of curcumin detected are in the range offering therapeutic impact in various models.

PH6 Hypolipidemic and weight reducing activity of two *Hypericum* species extracts in cafeteria fed overweight rats

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This study examined the anti-overweight effect of *Hypericum silenoides* Juss and *Hypericum philonotis* Cham. & Schlecht in male Wistar rats fed with a cafeteria diet. Rats were fed with cafeteria diet (human snacks and Mexican foods) for 77 days. *Hypericum* species were daily administrated orally at the doses 10, 30 and 100 mg/kg of body weight during 35 days. The effect of extracts of both plants on body weight, food intake, anorexic effect and various biochemical parameters like serum glucose, lipid profile, alanine transaminase (ALT), aspartate transaminase (AST) and atherogenic index (A.I.) was measured. Additionally, inhibitory lipase activity assay and forced swimming test were performed. *H. silenoides* and *H. philonotis* extracts significantly reduced serum glucose levels and body weight gain induced by cafeteria diet in rats. Treatment with aqueous extract of *H. silenoides* showed anorexic, antidepressant effects and also significantly ($p < 0.05$) decreased total-cholesterol, triglycerides and HDL-C while LDL-C, A.I., AST and ALT were not altered. On the other hand, dichloromethane extract of *H. silenoides* (IC_{50}= 262.79 ± 0.09 μg/mL) and hexane extract of *H. philonotis* (IC_{50}= 162.60 ± 0.02 μg/mL) showed the most active lipase inhibition. These results demonstrate that some *H. silenoides* and *H. philonotis* extracts decrease body weight gain and serum parameter (glucose, triglycerides), produced anorexic and antidepressant effect in cafeteria diet-fed rats. *Acknowledgements*: This work was supported by grant of "Dirección General de Asuntos del Personal Académico" DGAPA-UNAM IN 210112

PH7 Valerian: No evidence for Clinically Relevant interactions

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In recent popular publications directed to cancer patients as well as in widely-used patient information websites (e.g. www.cancer.org or www.mskcc.org) valerian is claimed to have a potential of adverse inter-

actions with anti-cancer drugs, thereby questioning its use (1) as a safe replacement for benzodiazepines. Therefore a review on the interaction potential of valerian preparations was conducted. Literature was retrieved by systematic data base search and by search in a clinical drug interaction data base (www.mediq.ch). Thereafter a systematic assessment of publications was performed. Several *in vitro* studies on four CYP 450 isoenzymes, p-glycoprotein and two UGT isoenzymes could be identified. However, the methodological assessment of these studies did not support their suitability for the prediction of clinically relevant interactions. In addition, clinical studies on CYP 450 1A2, 2D6, 2E1 and 3A4 did not show any relevant interaction potential. We therefore conclude that the interaction potential of valerian preparations, if any, is low and unlikely to be clinically relevant, suggesting that its use is safe also in cancer patients. Literature: 1. Fernández-San-Martin et al., Sleep Med. 2010; 11: 505 .

PH8 The pharmacokinetic properties of pure γ-mangostin in rats in comparison to mangosteen extract

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α- and γ-mangostin are the major bioactive compounds in *Garcinia mangostana* L. (mangosteen) extracts. Previously, we reported the pharmacokinetic (PK) properties of α-mangostin in rats. The purpose of this study was to compare the PK properties of γ-mangostin in rats if administered as pure compound or as mangosteen extract. The absolute bioavailability of γ-mangostin was determined by giving male Sprague Dawley rats 2 mg/kg γ-mangostin intravenously (i.v.) or 20 mg/kg orally. 160 mg/kg of the mangosteen extract were administered which contained a α- and γ-mangostin dose equal to 20 mg/kg and 4.5 mg/kg of pure the compound. Plasma samples were collected in both PK studies and compound concentrations were measured by LC-MS/MS. The PK of γ-mangostin after i.v. administration followed a two-compartment body model. The half-life of the distribution phase was 2.40 min and that of the elimination phase was 1.52 hr. After oral administration, both α- and γ-mangostin underwent an intensive first-pass metabolism and both compounds were conjugated rapidly after oral administration. When given as extract the absorption of α- and γ-mangostin was not increased, but the metabolism was affected through changes in the elimination of the conjugates from two phases to one phase. In conclusion, since food supplements contain mangosteen extracts, further investigations are necessary to link the pharmacokinetics of free active compounds with the *in vivo* activity of mangosteen extracts.

PH9 From *in vitro* to *in vivo*: Pre-clinical data on the oxidative metabolism and pharmacokinetics of lapachol

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The naturally occurring 1,4-naphtoquinone, lapachol, has been widely studied due to its diverse biological activities, of which the anti-cancer activity has taken a prominent position. Even though lapachol and other reported quinone derivatives are known as constituents in extracts/products used in traditional medicine, only few data are available on their metabolism, absorption and pharmaco-kinetics. Regarding this need, we here present an original study that aims a pre-clinical strategy to generate putative phase I metabolites of lapachol and with that further enable *in vivo* metabolism and pharmacokinetic studies. Upon its isolation and synthesis, the quinone was studied by biomimetic *in vitro* models. Based on the identification of more than ten putative oxidation metabolites and correspondent ESI-MS/MS fragmentation studies, an UPLC-MS/MS method was developed and validated to monitor the respective naphtoquinones in rat plasma. Finally, *in vivo* experiments in a rat model delivered data on the metabolism and pharmacokinetic parameters of lapachol. Such strategy was successfully demonstrated for a quinone, but may also be applied for the study of several other kinds of natural compounds.

PH10 Herb-Drug Interactions: Effects of *Echinacea* preparations on cytochrome P450 activities in rats

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Different preparations of *Echinacea angustifolia* DC., *Echinacea purpurea* (L.) Moench and *Echinacea pallida* (Nutt.) Nutt. were investigated for its cytochrome P450 (CYP) interaction potential in rats. Rats were assigned to the different study groups with various dosages, positive controls (ketoconazole, quinidine), or pure compounds (dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides; tetraenes). After pretreatment with the different *Echinacea* preparations for 14 days, a cocktail of probe drugs for CYP enzymes (theophylline [CYP1A2], tolbutamide [CYP2C9], dextromethorphan [CYP2D6] and midazolam [CYP3A4]) was orally administered before blood sampling. Plasma levels of probe drugs and their metabolites were quantified using a validated LC-MS/MS method before and 0.25, 0.5, 1, 2, 4, 6, 10 and 24 h after a single dose of the probe cocktail. Pharmacokinetic parameters (C_{max}, AUC_{last}) were calculated and compared with the control group using geometric mean ratio (GMR) and its 90% confidence interval (CI). Some *E. purpurea* preparations showed significant inhibitions in CYP1A2 activities. In addition, the tetraenes inhibited CYP1A2 with a GMR of 8.65 (7.72 – 9.68) for the AUC_{last} and 2.96 (2.59 – 3.39) for C_{max}. *Echinacea* preparations showed no inhibition in CYP3A4 and CYP2C9, and only a moderate or weak inhibition in CYP2D6 activities.

PH11 *In vitro* metabolism study of the bioactive lignan (-)-Grandisin

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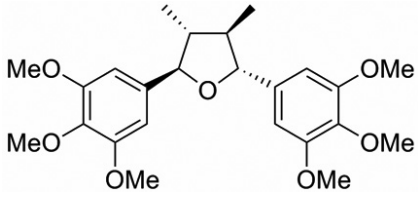
The lignan (-)-grandisin has shown important pharmacological activities, such as citotoxicity and antiangiogenic, antibacterial and trypanocidal activities. So, it has been considered as a potential drug candidate. In the early drug development process, drug metabolism is one of the main parameters that should be evaluated; therefore, the biotransformation of this lignan by rat liver microsomes was investigated for the first time. In order to perform the biotransformation study and to determine the kinetic parameters, a simple, sensitive and selective HPLC method was developed and fully validated. After method validation, the biotransformation study was accomplished and the kinetic parameters were determined. The biotransformation study obeyed the Michaelis-Menten kinetics. The V_{max} and K_m were 1.46 ± 0.034 µmol/mg protein/h and 8.99 ± 0.488 µM, respectively. In addition, the formation of dihydrograndisin, characterized by GC-MS, by mammalian systems indicated the involvement of a CYP450 enzyme type.



PH12 Fatty acid derivatives of phenethylamine and glycerol as endocannabinoids and endovanilloids

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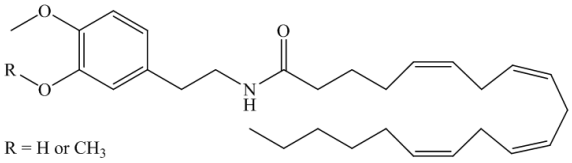
The endocannabinoid system (ECS) was discovered to be the biological target for the psychoactive components from hemp. It consists of two GPC receptors (CB1 and CB2), a few endocannabinoids, their degradative enzymes FAAH and MAGL and the connected signaling pathways. The ECS also shares some endogenous ligands with the TRPV1 ion channels of the endovanilloid system. Several putative fatty acid metabolites of biogenic amines and glycerol were prepared and their activity was tested in vitro on components of the endocannabinoid and endovanilloid system. The arachidonic acid amides of 2-(3,4-dimethoxyphenyl)ethanamine and 3-hydroxy-4-methoxyphenyl-ethanamine, both derivatives of the natural alkaloid phenethylamine, showed very good replacement rates in both CB1 and CB2 binding assays, and the latter was an active agonist on the TRPV1 receptor.



R = H or CH_3

The 2-acylglycerolesters of different C16 fatty acids were less active in the receptor assays but showed better values in the enzyme inhibition assays. These potential metabolites could take part in endocannabinoid signaling, contributing to the overall activity of biogenic amines and fatty acids.

PH13 Modulation of P-Glycoprotein mediated efflux by Nigerian plant extracts used in the management of diabetes

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The use of herbal medicines alongside conventional drugs for the therapeutic management of diseases is common practice particularly in chronic conditions. A recent field study carried out in Nigeria showed that more than 50% of diabetic patients co-administer herbal preparations with their prescription medicines, thereby raising the risks of herb-drug interactions. Thirty medicinal plants traditionally used for the management of diabetes in different areas of Nigeria were collected during the field work for *in-vitro* pharmacokinetic investigations to identify potential herb-drug interactions. The ability of these plants to modulate the effect of the intestinal efflux transporter P-glycoprotein (P-gp) was determined in Caco-2 cells using the fluorescent P-gp substrate, rhodamine-123. At concentrations ≤ 100 µg/ml, aqueous extracts of *Annona senegalensis, Bridelia ferruginea* and *Khaya senegalensis* significantly inhibited the efflux of accumulated rhodamine-123 from the cells. The inhibition was either comparable or much higher than that produced by 20 µM verapamil, a known P-gp inhibitor. Aqueous extracts of *Syzygium guineense* and *Isoberlinia doka* on the other hand enhanced the efflux of rhodamine-123 from the cells. These results suggest the possibility of interactions when these extracts are co-administered with prescription medicines which are also P-gp substrates such as glibenclamide.

PH14 Pivotal role of Indole-3-Acetic acid in the senescence and flower absicission of *Lupinus mutabilis L. Sweet*

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The role of the most common auxin Indole-3-Acetic Acid (IAA) levels was determined all along the inflorescence of *Lupinus mutabilis (L.sweet)* during the senescence and flower abscission stage. *Lupinus mutabilis* Sweet is apparently unknown in the wild but is cultivated as an important food crop throughout the Andes and is also the focus of crop breeding and development elsewhere in Australia and Europe. Concentrations of indole-3-acetic acid were identified and quantified by CG-SIM-MS in different regions of the inflorescence and at different stages in the development. IAA levels were steady at first stages of the plant development. However, the IAA levels increased as the Senescence and Abscission Syndrome (SAS) of flowers and young pods starts. IAA concentrations were different depending on the stage of development of the process but it was detected an acropetal increase of the phytohormone levels. The results here presented strongly support the hypothesis that IAA plays a fundamental role in flower abscission in Lupinus Mutabilis. Moreover the region and staged analysis suggests the existence of a spatio-temporal gradient of IAA related to the Senescence and Abscission process. This data could clearly explain the sequential pattern of the plant senescence and pose new questions about the timing of IAA synthesis.

PH15 Human metabolites of natural products generated by bacterial CYP102A1 mutants

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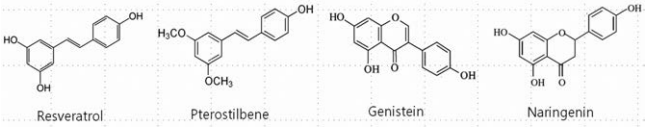
Cytochrome P450 BM3 (CYP102A1) from *Bacillus megaterium* is a soluble, catalytically self-sufficient monooxygenase which contains a heme monooxygenase domain and a diflavin reductase domain on a single polypeptide chain. In recent studies, several CYP102A1 mutants were found to generate human metabolites by oxidizing various kinds of drugs. CYP102A1 has therefore become the prime candidate monooxygenase for directed evolution and rational design towards biocatalytic applications. In this study, a set of CYP102A1 mutants were found to oxidize several natural products with chemopreventive potential such as stilbenoids (resveratrol, pterostilbene) and flavonoids (naringenin, genistein), to make human metabolites, mainly monohydroxylated metabolites. These results demonstrate that CYP102A1 mutants can be used to produce human metabolites of natural products, which can be used in several industrial fields including drug development. [This research was supported by Bio-industry Technology Development Program (No. 111052 – 04 – 1-SB010), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.].



PH16 Hypoglycemic and hypolipidemic activities of *Myrcia bella* cambess. in streptozotocin – diabetic mice

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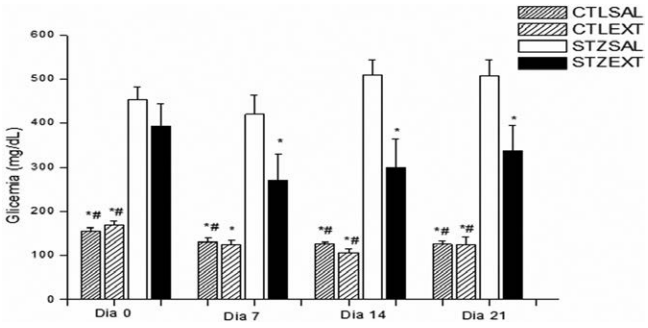
The aim of this study was to access the effects of *M. bella* on streptozotocin- diabetic mice. Male albino Swiss mice (90 days, 40 g) were divided into 4 groups treated by gavage for 21 consecutive days: CTL SAL (control mice treated with saline), CTL EXT (control mice treated with *M.*

bella extract, 600 mg/Kg.day), STZ SAL (diabetic mice treated with saline) and STZ EXT (diabetic mice treated with *M. bella* extract, 600 mg/Kg.day). STZ EXT had decreased glucose and lipid levels and also food and water intake compared with STZ SAL ($P < 0.05$). The crude extract seems to be a valuable source of natural products that can act as a hypoglycemic and hypolipidemic agent.



	CTLSAL	CTLEXT	STZSAL	STZEXT
Fasting blood glucose (mg/dL)	126,4±6,8 *#	125,5±16,7 *#	507±37,3	338,1±56,6*
Triglycerides (mg/dL)	105,1±12,5	79±17,88*	128,9±11,9	70,2±8,18*
Cholesterol (mg/dL)	142,9±12#	83,6±14,8*&	172,4±16,1	88,5±11,3*
Food intake (g/Kg)	22,6±0.94 *#	19,66±1,2*#	51,1±3,3	31,45±2,0*
Water intake (mL/animal)	12,1±1,1*#	10.5±0.7*#	45,2±2,4	26,2±0,9*

* vs. STZ SAL, # vs. STZ EXT, & vs. CTL SAL

PH17

Hypoglycemic effect of *Bauhinia holophylla* Steud. in streptozotocin diabetic mice

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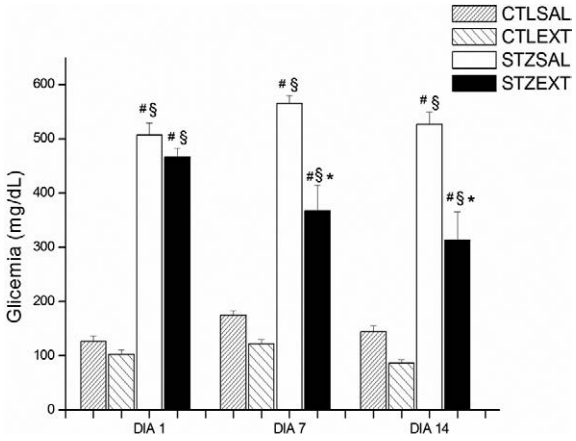
B. holophylla is widely used in folk medicine. We investigate hypoglycemic properties of crude extract of *B. holophylla* in streptozotocin-diabetic mice. Male Swiss mice (90-days old, 40 g) were divided in 4 groups: CTLSAL (normal mice treated with saline), CTLEXT (normal mice treated with *B. holophylla*, 15 days, 400 mg/kg.day), STZSAL (diabetic mice treated with saline) and STZEXT (diabetic mice treated with *B. holophylla*, 15 days, 400 mg/kg.day). STZEXT had decreased glucose and lipid levels, hydric and food ingestion and increased hepatic glycogen compared to STZSAL ($P < 0.05$), indicating beneficial effects in the treatment of streptozotocin diabetic mice.



	CTLSAL	CTLEXT	STZSAL	STZEXT
Cholesterol (mg/dL)	97.2±8.4	102.5±9.5	86.2±9.5	72.7±7.4 §*
Triglycerides (mg/dL)	114.8±6.3	111.0±6.3	138.9±13.7	94.5±5.1 #*
Body weight (%) day 1	100±0	100±0	100±0	100±0
Body weight (%) day 14	101.7±1.6	107.6±5.4	93.5±1.6	99.1±3.3
Food intake (g/100g b.w)	18.9±0.6	16.7±0.3	35.7±1.3 #§	32.1±0.4 #§*
Hydric intake (mL/animal)	11.8±1.4	8.7±1.3	60.7±1.5 #§	47±2.5 #§*
Hepatic Glycogen content (mg/%)	1.8±0.26	1.6±0.3	1.16±0.19 #§*	1.85±0.27

vs. CTLSAL; § vs. CTLEXT; * vs. STZSAL

PH18

Assessment of intestinal absorption of O-glycoside flavonoid avicularin using *in situ* single-pass intestinal perfusion technique

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For a drug to become a product it is required to elucidate its behavior in the organism, and one of the steps is to characterize the intestinal absorption, including the transport mechanisms and the transport rate of the drug. The aim of this study was to investigate the absorption mechanism of Quercetin 3-*O*-α-L-arabinofuranoside, avicularin, isolated from the aerial parts of *Bidens sulphurea,* in the rat intestine, using the *in situ* single-pass intestinal perfusion technique. The influence of the P-glycoprotein inhibitor aerapamil in the absorption of avicularin was studied as well. Because water absorption and secretion during perfusion may cause errors, phenolred, a non-absorbable marker, was co-perfused to correct water flux. A fast, sensitive, and specific UPLC-MS-MS method for determination of avicularin and phenolred in the intestinal samples has been developed and validated. Avicularin, phenolred and coumarin (internal standard) were extracted from the intestinal perfusion samples by solid phase extraction. The proposed method was successfully applied to the determination of AV and PR in intestinal perfusion samples and pharmacokinetics parameters were calculated.

PH19

Ethanol in herbal medicinal products for children: Study data from 50.425 children support safety

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Many herbal medicinal products for use in children contain ethanol. For answering the question, whether this is safe, a systematic evaluation of pro- and retrospective studies as well as a broad survey of market figures and pharmacovigilance data was conducted. Therefore, 17 studies covering 50.425 children aged 0 – 12 years were evaluated. None of the 15 adverse events reported were related to the ethanol content of the products. During the past few years more than 764 million daily doses have been sold. In terms of packages, 10.8 millions sold in Germany were reimbursed by the health insurance between 2005 and 2009, which indicates that they were prescribed to children. In parallel, no adverse effects attributable to the ethanol content have been reported, showing that the ethanol content of herbal medicinal products does not give causes for concerns, even regarding their safety in children. Dedication to: † Prof. Dr. Hilke Winterhoff, Institute for Pharmacology and Toxicology, Münster

PH20 Hydroxytyrosol: A compound from nature's arsenal against metabolic syndrome

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Metabolic syndrome is a clustering of interrelated risk factors for cardiovascular disease and diabetes. Hydroxytyrosol (HT) is a single phenol and it is present in large amount in byproducts from olives. An experimental protocol has been setup encompassing isolated HT administration in a diet induced rat model of metabolic syndrome. The first step was the investigation of the PK/PD of HT in plasma and tissues. As HT does not circulate anymore 30 min after the administration, it was pertinent to investigate its metabolic fate. Thus, *targeted* identification of circulating in plasma HT metabolites was performed using the mass defect filter technique. Additionally, an *untargeted* approach has been attempted on the PK plasma samples and new biomarkers related to HT administration have been identified. The aforementioned metabonomic methodology has been applied to the samples from the metabolic syndrome model in order to discern differences in the biochemistry within groups. HT supplementation for 8 weeks reduced visceral obesity and was associated with improved left ventricular structure and function, reduced blood pressure, improved glucose disposal and reduced hepatic steatosis and infiltration of inflammatory cells into the liver. Data of the targeted and untargeted approaches will be presented.

PH21 Characterization of *in vitro* metabolites of evocarpine in rat liver microsomes and their influence on antibacterial activity

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After incubation of evocarpine, the major bioactive compound of the *n*-hexane extract of the fruits of *Evodiae fructus*, with rat liver microsomes (S9 mix) nine metabolites were identified by their characteristic product ions using LC-PDA-ESI-MS analysis. The main biotransformation reactions observed were hydroxylation, hydration, dehydrogenation and N-demethylation. Comparison of incubation times between 1 and 72 hours showed no qualitative difference in biotransformation. In order to assess the influence of metabolism on the antibacterial activity of evocarpine and the crude extract, the test solutions were pre-incubated with the S9 mix prior the determination of the minimum inhibitory concentration (MIC), which revealed a four-fold increase of the MIC against *Mycobacterium smegmatis* ATCC 14468 for pre-incubation times of 1, 24 and 72 hours for the crude extract and a sixteen-fold increase for evocarpine for a pre-incubation time of 1 hour.

PH22 Sustained cognitive effects and safety of HPLC-standardized *Bacopa Monnieri* extract: A randomized, placebo controlled clinical trial

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Bacopa monnieri is an adaptogenic herb used since time immemorial as an Ayurvedic *rasayana.* While several clinical trials have investigated the cognitive effects of *Bacopa* extract, few have been well characterized for the *Bacopa* saponin glycosides associated with pharmacological activity at neuroreceptors. The current study examined effects of an ethanolic extract from aerial parts of an HPLC-standardized *Bacopa monnieri* extract (BACOGNIZE®) on elements of cognitive function in humans. Twenty healthy older adults between the ages of 60 and 75 were administered either a single 300 mg capsule of the *Bacopa* extract or a placebo capsule once daily for 12 weeks. The extract contained 11% of *Bacopa* glycosides, including Bacoside A3, Bacopaside X, Bacopasaponin C, and Bacopaside II. Standardized cognitive tests were conducted at baseline, 12 and 16 weeks. Statistical measures included two-tailed t-test compared to baseline and to placebo group. The characterized *Bacopa* extract showed significant improvement ($p < 0.05$) over baseline in short-term memory, processing speed, attention and depression, an effect which was also observed at 16 weeks, four weeks after dosing stopped. No adverse events were reported in either group. Studies in a larger number of subjects are needed to support this conclusion.

PH23 Investigation of cytochrome P450-mediated toxicity of extracts of *Polygonum multiflorum* and *Chelidonium majus*

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Botanical dietary supplements are commonly used throughout the world, and adverse hepatic reactions have been reported following their intake. In several cases, hepatic toxicity of botanical dietary supplements is the result of cytochrome P450 (CYP450) – mediated mechanisms leading to the formation of reactive metabolites (herbal bioactivation). These reactive metabolites covalently bind to cellular macromolecules such as DNA and protein, leading to toxicity via multiple mechanisms. In our investigation, metabolism-mediated toxicity of extracts of *Polygonum multiflorum*, *Chelidonium majus* and *Symphytum spp*. was studied using an *in vitro* assay. Human hepatocarcinoma (HepG2) cells were incubated with plant extracts in the presence and absence of an external metabolizing system (rat liver S9 fraction and NADPH) for 6 h and the cytotoxicity was assessed as lowered mitochondrial activity (reduction of MTT). The potential reactive metabolites formed during the incubation will be identified by using mass spectrometric methods.

PH24 Genotoxicity evaluation of a traditional herbal formulation with *Anacardium occidentale* bark

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The medicinal plant *Anacardium occidentale* L. stem bark, commonly known as cashew bark, is traditionally used in the Community of Portuguese Language Speaking Countries (CPLP) due to its anti-inflammatory, antidiarrheal and antidiabetic activities. The genotoxicity of (hydro)-ethanolic or methanolic extracts of different parts of this medicinal plant has been evaluated. Yet, no study has evaluated the genotoxicity of a traditional recipe obtained from the cashew stem bark. In this study the genotoxic potential of a cashew stem bark decoction was evaluated *in vivo* by the Comet assay. Mice were orally administered with three doses (40.2, 127, 402 mg/kg/day) of the traditional recipe of two types of *A. occidentale* (red and white) and with water as control. At the end of the study period, 14 days, the animals were sacrificed and collected blood/PBS samples were used in the assay. The nucleoids were immediately evaluated after staining. The analysis of the results obtained with the *in vivo* Comet assay revealed that tested concentrations were genotoxic ($P < 0.001$). The anacardic acids, the compounds claimed to be responsible for the antidiabetic traditional therapeutic use, have been shown to not produce any mutagenic effects. Yet, in our traditional recipe of both types of *A. occidentale* (red and white) these compounds are scarce.

PH25 Annonaceous acetogenins as environmental neurotoxins: Human exposure from edible *Annona* fruits

Bonneau N[1], Le Ven J[1], Schmitz-Afonso I[2], Guérineau V[2], Bajin ba Ndob I[1], Baloul L[1], Lewin G[1], Laprévote O[2,3], Brunelle A[2], Touboul D[2], Champy P[1]
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Annonaceous acetogenins (AAGs) are inhibitors of the first complex in mitochondrial respiratory chain. They are proposed as environmental neurotoxins responsible for Guadeloupean atypical Parkinsonism. The edible fruits of *Annona muricata* L. (soursop) and *Asimina triloba* Dunal (pawpaw) were previously shown to constitute major sources of exposure to these compounds. We performed study of total extracts using MALDI-TOF MS and dereplicative methods, and here propose quantitative data showing homogeneity between fruits of *A. muricata* from diverse origins, in which annonacin is the major AAG. The fruits of *A. squamosa* also constitute a source of exposure, with squamocin as the major representative of AAGs. The fruit of *A. reticulata* and edible products derived from *A. cherimolia* were similarly studied. Our results are significant in regard to public health and support the inclusion of *Annona* spp. exposure/consumption in studies investigating potential risk factors for neurodegeneration.

PH26 *in vitro* phase I metabolism and approach to the distribution of annonacin, a neurotoxic annonaceous acetogenin

Le Ven J[1], Schmitz-Afonso I[2], Bonneau N[1], Cresteil T[3], Akagah B[4], Lewin G[1], Brunelle A[2], Touboul D[2], Champy P[1]
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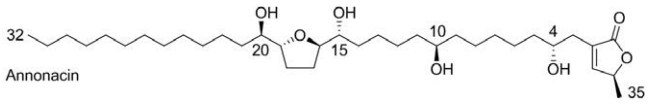
Annonacin is a prototypical Annonaceous acetogenin and is proposed as an environmental neurotoxin responsible for Guadeloupean atypical Parkinsonism, *via* consumption of fruits of *Annona muricata* L. (soursop). The molecule induces neurodegeneration in several rodent models. Phase 1 metabolism of annonacin was determined *in vitro*, using Rat and Human microsomes. Three metabolites were only identified in the latter, and were semisynthetized by porphyrin-catalyzed oxygenation, so as to allow their structural identification. A method for purification of annonacin from plasma, and subsequent determination using LC-ESI-Q^3 is also proposed.



PH27 Comparison of pharmacokinetic parameters of α-mangostin after administration of α-mangostin and mangosteen extract in mouse

Han SY, Chin YW, Choi YH
College of Pharmacy, Dongguk University-Seoul, 814 – 9, Siksa-dong, Goyang si, Ilsandong-gu, Gyeonggi-Do, South Korea

The pericarp of the mangosteen (MG) has a variety of pharmacological properties such as antioxidant, cytotoxic, anti-inflammatory, antibacterial, antifungal, antiviral and chemopreventive effects. Currently, these mangosteen products are being widely used for the perceived or purported health benefits and the main secondary metabolites xanthones including α-mangostin, are assumed to be responsible for these health benefits. Hence, the pharmacokinetic properties of xanthones in the mangosteen extracts may provide some clues how manogsteen extract exert its biological activity. In the present study, the pharmacokinetics of the α-MG after administration of α-MG and MG extract. α-MG and MG extract intravenously (containing 5 mg of α-MG) and orally (containing 20 mg of α-MG) to mice were evaluated. After intravenous administration of α-MG and MG extract at a dose of 5 mg/kg as α-MG, the pharmacokinetic parameters were comparable in mice, suggesting that the metabolism of α-MG after α-MG and MG extract administration. However, after oral administration of MG extract at a dose of 20 mg/kg as α-MG, the area under the plasma concentration-time (AUC) and unabsorbed fraction of α-MG were significantly greater and smaller, respectively, than that of α-MG administration. This could be due to the increased absorption of α-MG after α-MG compared to that of MG extract. Thus, these results suggest that oral absorption of MG extract containing α-MG is more efficient than α-MG administration.

PH28 Pharmacokinetics of isoliquiritigenin and its metabolites in rats

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The importance of pharmacokinetic studies as well as *in vitro* studies in herbal components is increasing because a large number of herbal products have been used as adjuvant or alternative medicines. Isoliquiritigenin (isoLQ) has shown a variety of biological activity including antioxidative, anti-inflammatory, estrogenic, chemopreventive and antitumor effects. In this study, we were evaluated the pharmacokinetics of isoLQ and its metabolites after its intravenous (10, 20 and 50 mg/kg) and oral (20, 50 and 100 mg/kg) administration in rats. After the intravenous administration and oral administration of isoLQ, the AUCs of isoLQ and its metabolites show dose-independent manners. The oral bioavailability of isoLQ was approximately 10% in rats, possibly due to the metabolism of isoLQ. Also the studies for tissue distribution, plasma protein binding and *in vitro* metabolism of isoLQ were conducted in rats.

Topic I: Natural Products Discovery: Marine, Microbes, Plants and other

PI1 Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae

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Green synthesis of nanoparticles that have environmentally acceptable solvent systems and eco-friendly reducing agents is of great importance. The aim of the work was to synthesis of silver nanoparticles (AgNPs) using polysaccharides extracted from four marine macro-algae, namely, *Pterocladia capillacae (Pc) Jania rubins (Jr) Ulva faciata (Uf)* and *Colpmenia sinusa* (Cs) as reducing agents for silver ions as well as stabilizing agents for the synthesized AgNPs. Hot water extracts of the *algae Pc, Jr, Uf,* and Cs were studied for their polysaccharides (ps) contents and were found to be 6.46, 5.63, 8.84 and 4.33% respectively. The GLC analysis of these extracted ps revealed that rhamnose (46.88% in Pc), galactose (30.2% in Uv; 22.23% in Jr), and fucose (10% in Cs) constitute the major sugars comprising a part of structural polysaccharide. The formed AgNPs have been confirmed by UV-Vis spectroscopy, FTIR analysis and TEM. The resultant AgNPs colloidal solutions were applied to cotton fabrics with/without binder. The antimicrobial activity of the treated fabrics loaded with AgNPs was evaluated.

PI2 Biologically active meroterpenoids from *Erythrobacter* SP

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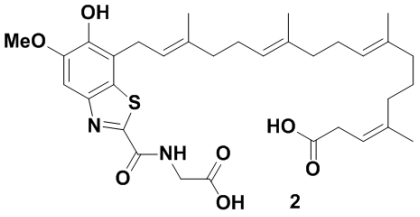
Erythrobacter are Gram-negative bacteria that are ubiquitous in the marine environment. Through high-throughput screening efforts, we identified the extract from marine derived *Erythrobacter* strain SNB-035 to possess activity in the Locus Derepression Assay (LDR), which identifies molecules that modulate epigenetic regulation. Bioassay guided fractionation led to the isolation of erythrazoles A-B (**1 – 2**) and erythrolic acids A-E (**3 – 7**). Structurally, **1** and **2** possess a benzothiazole-diterpene moiety, which is rare in natural products. While the erythrolic acids contain a 4-hydroxybenzoic acid appended with a modified terpene side chain. Furthermore, **2** arises from four biosynthetic pathways; NRPS, terpene, shikimate, and polyketide. Among these compounds, erythrazole B (**2**) is cytotoxic to a panel NSCLC cell lines, with IC_{50} values of 1.5, 2.5, and 6.8 μM against H1325, H2122, and HCC366, respectively, while erythrolic acid D (**6**) shown modest activity against HCC44 cancer cell line with IC_{50} of 2.5 μM. Both **2** and **6** acted as epigenetic modulator in the Locus Derepression Assay. The discovery of these unusual bioactive meroterpenes demonstrates the natural product potential of a previously unstudied group of bacteria, *Erythrobacter* species, for drug discovery.



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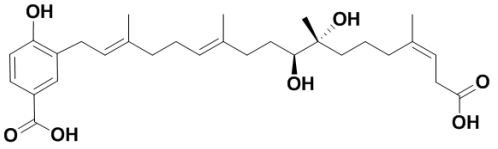

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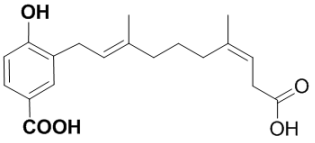

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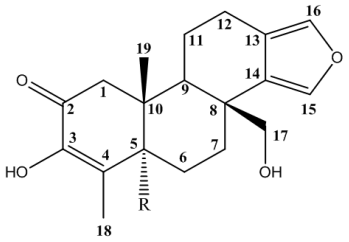
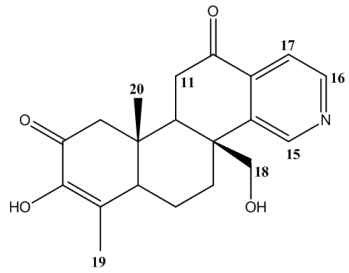
PI3 New spongians isolated from a species of *Cacospongia* SP

Parrish SM[1], Yoshida WY[1], Kelly M[2], Williams P[1]
[1]Department of Chemistry, University of Hawaii at Manoa, Honolulu Hawaii, 96822; [2]National Centre for Aquatic Biodiversity and Biosecurity, National Institute of Water and Atmospheric Research, Ltd, 41 Market Place, Auckland Central 1010, New Zealand

Two norditerpenes (**1 – 2**) and one diterpene (**3**) were isolated from a tropical Suluwesi sponge in the genus *Cacospongia* sp. (Order Dictyoceratida, Family Thorectidae). Diterpene **3** contains a unique pyridine moiety as the D ring. The structures of **1 – 3** were deduced by analysis of the spectrometric and spectroscopic data. The structure elucidation of **3** was complicated by the rapid exchange of the axial proton at the C-11 position with deuterium from methanol.



1 R=H
2 R=OH



3

PI4 Isolation and structure elucidation of five new cytotoxic chlorinated lipopetides from *Moorea bouillonii*

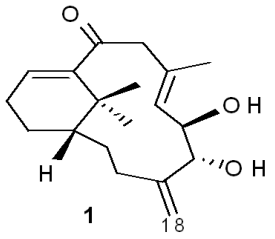
Mevers E[1,2], Choi H[1], Byrum T[1], Gerwick WH[1,2]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography University of California San Diego, La Jolla, California 92093, United States; [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California 92093, United States

Marine cyanobacteria have been widely studied and proven to be prolific sources of structurally intriguing natural products possessing interesting pharmacological activities. A subset of these natural products are highly halogenated, such as the crossbyanols, lyngbyabellins, dysidenins, jamaicamide A and taveuniamide, and represent both structural diversity and a broad range of biological activity. A recent investigation into two collections of *Moorea bouillonii* from Palmyra Atoll yielded five new lipopetides, all exhibiting isotopic patterns consistent with the presence of mono- or di- chlorination, similar to the lyngbyabellin family of compounds. The planar structures and absolute configurations were elucidated by the combination of various techniques in spectroscopy, chromatography and semi-synthesis. These new metabolites possess not only the characteristic structural features of known lyngbyabellins such as two thiazole rings and a 2-methylocatanoate residue with halogenations and acylations, but they also possess unique structural features such as mono-chlorination on the 3-acyloxy-2-methyloctanoate residue and a terminal *N,N*-dimethylvaline residue, that have never been observed in this structure class before. Lyngbyabellin N, possessing *N,N*-dimehthylvaline and leucine statine, showed moderate anti-cancer activity against H460 human lung cancer cells with the IC_{50} of 3.3 μM.

PI5 New verticillane diterpenoids from *Cespitularia taeniata*

Chang JY[1], Lin YC[1], Hwang TL[2], Shen YC[1]
[1]School of Pharmacy, College of Medicine, National Taiwan University, Jen-Ai Rd. Sec. 1, Taipei 100, Taiwan; [2]Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan

Chemical investigation of *Cespitularia taeniata* has led to the isolation of three new verticillanes, cespitulins E-G (**1 – 3**). Compound **1** possesses a rare nor-verticillane skeleton with two adjacent hydroxyl groups at C-5 and C-6, while seco-compound **2** with an aldehyde group at C-9 results from an unusual bond cleavage between C-9 and C-10. Pharmacological studies revealed that compound **3** exhibited significant activities on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB.



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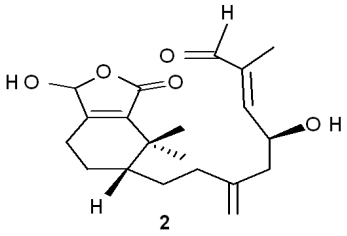

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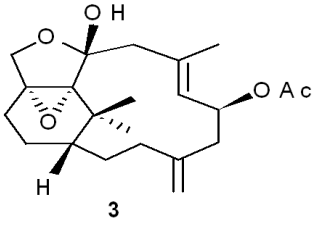

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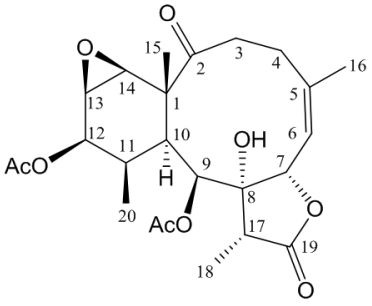
PI6

Briarenolides E-J, new Briarane diterpenoids from an octocoral *Briareum* sp

Su YD[1,2], Hong PH[2,3], Chen YH[2], Sheu JH[1], Sung PJ[1,2,3]
[1]Department of Marine Biotechnology and Resources and Division of Marine Biotechnology, Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan

[2] National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan, [3] Graduate Institute of Marine Biotechnology, National Dong Hwa University, Pingtung 944, Taiwan Six new briarane-type diterpenoids, briarenolides E-J (**1**-**6**), were isolated from an octocoral *Briareum* sp. The structures of briaranes **1**-**6** were established by spectroscopic methods and by comparison the spectral data with those of known briarane analogues. It is worth noting that briarenolide E (**1**) is the first 2-ketobriarane, briarenolide F (**2**) is the first 6-hydroperoxybriarane, and briarenolide I (**5**) is the first 6-oxymethylbriarane analogues. Briarenolide E (**1**) displayed modestly inhibitory effects on the generation of superoxide anion and the release of elastase and briarenolide F (**2**) displayed a significant inhibitory effect on the generation of superoxide anion by human neutrophils.



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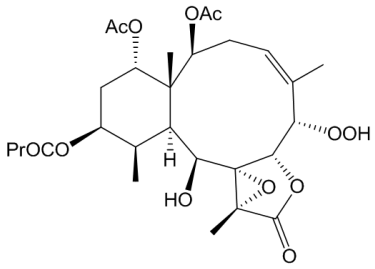

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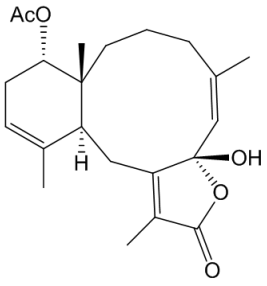

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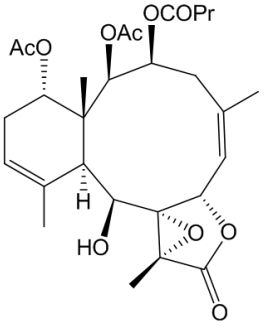

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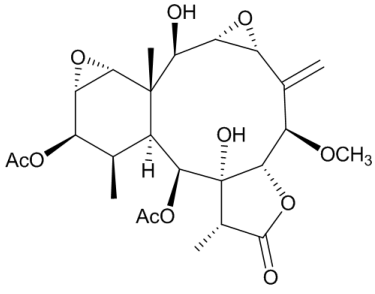

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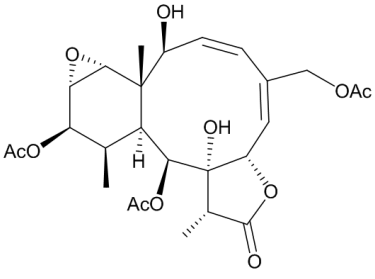

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PI7 Brown macroalgae produce anti-leukemia compounds

Vizetto-Duarte C[1], Santos D[1], Custódio L[1], Barreira L[1], Pereira H[1], Rauter AP[2], Alberício F[3], Varela J[1]
[1]Centre of Marine Sciences, Marine Biotechnology Laboratory, University of Algarve, Campus de Gambelas, 8005 – 139 Faro, Portugal; [2]Faculty of Sciences, University of Lisbon, Lisbon, Portugal; [3]Barcelona Science Park, University of Barcelona, Barcelona, Spain

Acute lymphoblastic leukemia (ALL) is a fast-growing cancer in which the body produces a large number of immature white blood cells (lymphocytes). Although treatable in children with fairly good results, in adults complete remission rates are low and unlikely to improve by any significant degree. The challenge is to maintain the remissions and, for those who develop recurrent disease, to provide effective therapy. To this end, it is imperative to identify new and active leukemia-specific drugs. In this sense, we evaluated the *in vitro* anti-proliferative effect of extracts from brown algae, namely *Halopteris scoparia*, *Cladostephus spongiosus*, *Cystoseira tamariscifolia* and *Cystoseira nodicaulis*. MTT assays, DAPI staining and flow cytometry led us to conclude that *Cystoseira* extracts selectively inhibited proliferation and induced apoptosis in several human leukemia cell lines (SUP-T1, PF-382 and THP-1), but not in non-tumoral S 17 cells. Further studies have demonstrated the anti-proliferative activity of *Cystoseira* associated with a clear arrest in the G0/G1 phase, indicating that the cell cycle status of leukemic cells plays an important role in the response to treatment. Though the active compounds remain to be identified, *Cystoseira* extracts proved to be a promising source of anti-leukemia compounds.

PI8 Phospholipids and amino-acid composition of eggs of sea urchin from Barents Sea

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Green sea urchins *Strongylocentrotus droebachiensis* (Müller) are major indweller in the shallow waters of Barents Sea. In continuation of our studies on sea urchin [1], phospholipids and amino acid composition of eggs were analyzed. Sea urchin was harvested by divers in Barents Sea close to Murmansk (Russia). The eggs were extracted with chloroform/methanol (2/0.5 v/v) and with 95% EtOH, concentrated *in vacuo* and lyophilized. Phospholipids were separated by HPLC with evaporative light scattering detection after separation on a Waters Spherisorb® 5 µm Silica 4.6 x 150 mm column. Amino acids were analyzed by GC-MS after separation on an Rxi®-5MS capillary column (15 m; 0.25 mm ID). The main phospholipids were phosphatidylcholine followed with phosphatidylethanolamine, and phosphatidylinositol. The protein content was 39.7%. Higher amounts of glycine (11.6%), alanine (7.8%), leucine (3.3%), and valine (3.0%) were found in sea urchin eggs. Other amino acids such as methionine, isoleucine, lysine, serine, threonine, phenylalanine, proline, histidine, tyrosine, and tryptophan were also identified. The ratio of essential to non-essential amino acids was found to be 0.75. Sea urchin eggs are a reach source of phospholipids and amino acids. References: 1. Shikov et al. (2011) Planta Med., 77, 1357 – 1358.

PI9 Developmental toxicity of one marine dinoflagellate toxicant – Saxitoxin

Tian L, Wang M
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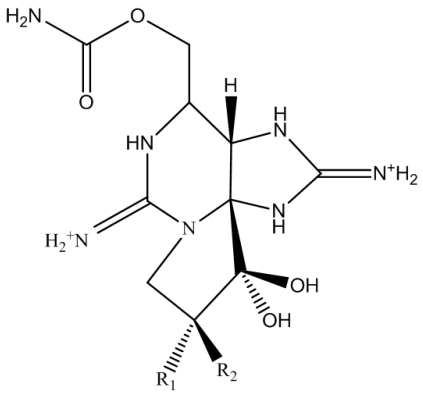
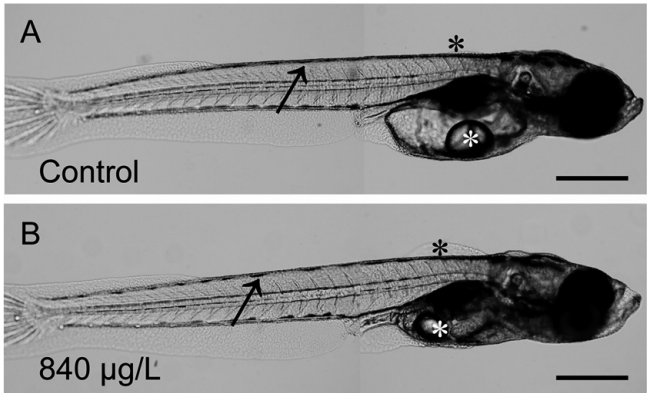
Saxitoxin (STX), found mainly in marine dinoflagellates, is one water-soluble neurotoxin. When some fishes accumulate STX and are consumed by humans, it would cause a series of diseases, especially to children. STX was known to possess high binding affinity to the soluble sites of sodium channels and interrupts the propagation of neuronal impulses by blocking the sodium channel in excitable cells, however, it still remain unknown whether STX can contribute to other toxic effects or not. In our current study, we adopted medaka fish (*Oryzias melastigma*) embryos as model animals to study its developmental toxicity to children. By detecting the protein markers using 2-D electrophoresis combined with MALDI TOF/TOF MS, we found that STX could accelerate the embryonic development by enhancing the expression of stress related proteins and metabolism proteins, and by regulating phosphorylation processes.



Saxitoxin (STX): R_1,R_2=H



PI10 Antioxidant effect of four bromophenols from the red algae *Vertebrata lanosa*

Olsen EK, Hansen E, Isaksson J, Andersen JH
University of Tromso, Science Park, Tromso, Norway 9037

One new bromophenol, 5,5'-oxybis(methylene)bis(3-bromo-4-(2,3-dibromo-4,5-dihydroxylbenzyl)benzene-1,2-diol) (**4**) together with three known bromophenols 2,3-dibromo-4,5-dihydroxybenzylaldehyde (**1**), 2,2',3-tribromo-3',4,4',5-tetrahydroxy-6'-hydroxymethyl-diphenylmethane (**2**) and bis(2,3-dibromo-4,5-dihydroxylbenzyl) ether (**3**) were isolated from the red algae *Vertebrata lanosa*. The antioxidant activity was examined using oxygen radical absorbance capacity (ORAC), cellular antioxidant activity (CAA) and cellular lipid peroxidation antioxidant activity (CLPAA) assays. All four compounds were tested in the concentration range from 1 – 50 µg/mL. Compound **2** exerted antioxidant activity in the biochemical assay ORAC. More interestingly it also showed good activity in CAA, which means that the compound is able to pass the cell membrane and act as an antioxidant intracellularly. The effect was dose-dependent and significant at 10 µg/mL. In addition compound **2** exerted activity in CLPAA, which is used to measure the ability to inhibit lipid peroxidation in the cell membrane. Although several bromophenols are known to be potent antioxidants in biochemical assays, this is the first time a bromophenol has shown activity in cellular assays.

PI11 DNA damage initiated by merosesquiterpenes from the sponge *Spongia* sp

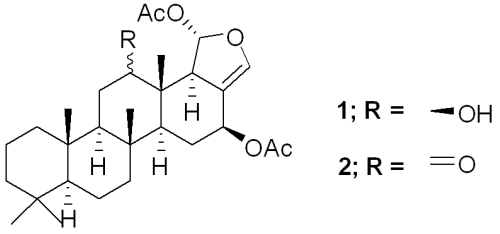
Kittiwisut S[1], Yuenyongsawad S[1], Mooberry SL[2], Plubrukarn A[1]
[1]Marine Natural Products Research Unit, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand; [2]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

Five merosesquiterpenes from the sponge *Spongia* sp., ilimaquinone, smenodiol, dactylospontriol, hydroxymethoxyfarnesylquinone and (-)-cyclospongiaquinone1 were investigated for antiproliferative activity using SRB assay. In HeLa cells, ilimaquinone, smenodiol, dactylospontriol, and (-)-cyclospongiaquinone1 had IC_{50}s of 7.6, 4.3, 3.5, and 27.9 μM, respectively, and they all initiated cytotoxicity. On the other hand, hydroxymethoxyfarnesylquinone had an IC_{50} of 29.2 μM and was cytostatic but did not initiate cytotoxicity. Flow cytometric analysis revealed that dactylospontriol and hydroxymethoxyfarnesylquinone caused G_0/G_1 arrest, suggesting the antiproliferative mechanism relating to DNA damage. The immunofluorescence staining of γH2AX, showed that showed that dactylospontriol and hydroxymethoxyfarnesylquinone initiated DNA double strand breaks. To investigate whether there was an interaction of compound and DNA, the DNA band shift assay was performed; however the retardation of DNA-drug adducts was not observed.

PI12 Initiation of autophagy by marine-derived scalarane sesterterpenes

Kittiwisut S[1], Yuenyongsawad S[1], Mooberry SL[2], Plubrukarn A[1]
[1]Marine Natural Products Research Unit, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand; [2]Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX, 78229, USA

Heteronemin (**1**) and oxoheteronemin (**2**) were evaluated for the antiproliferative effects using SRB assay. Both showed good potency against HeLa cervical cancer cells, MCF-7, MDA-MB-231, and Hs578T breast cancer cells, and PC3 prostate cancer cells with IC_{50}s of 0.6 – 1.2 μM. Both compounds increased the expression of LC3-II protein, a marker protein present on membranes of the autophagosome at the beginning of cellular degradation process in HeLa cells. The mechanism of cytotoxicity of both **1** and **2** therefore is proposed to relate closely to the initiation of autophagy.



PI13 LC-MS-based evaluation of *Mycobacterium tuberculosis* shikimate kinase inhibitory activity of marine natural compounds

Simithy J[1], Hamann MT[2], Calderón AI[1]
[1]Department of Pharmacal Sciences, Auburn University, Auburn, AL 36849, Department of Pharmacognosy, University of Mississippi, 407 Faser Hall, University, MS 38677

Shikimate kinase, an enzyme that catalyzes the 5th reaction of the shikimate pathway in bacteria, has been proven to be vital for the survival of *Mycobacterium tuberculosis*. In this study, twenty six purified natural marine compounds were screened using a mass spectrometry-based functional assay to measure their *Mt*SK inhibitory activity. Four compounds exhibited more than 50% inhibition of the enzyme catalytic activity at 1 μM: ircinol A (**1**), manzamine A (**2**), 6-nitroharmane (**3**) and aaptamine (**4**). This is the first report on the *Mt*SK inhibitory activity for these natural compounds.

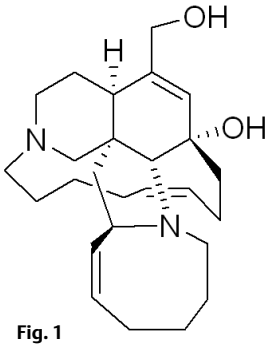


Fig. 1

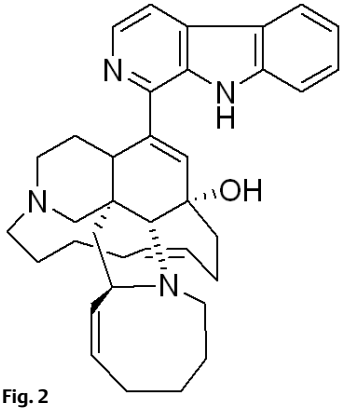


Fig. 2

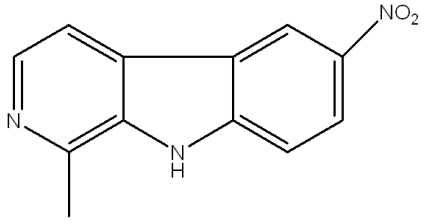


Fig. 3

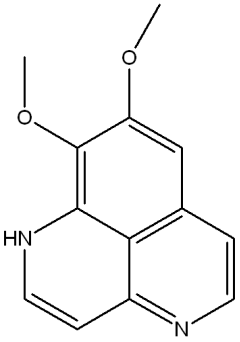
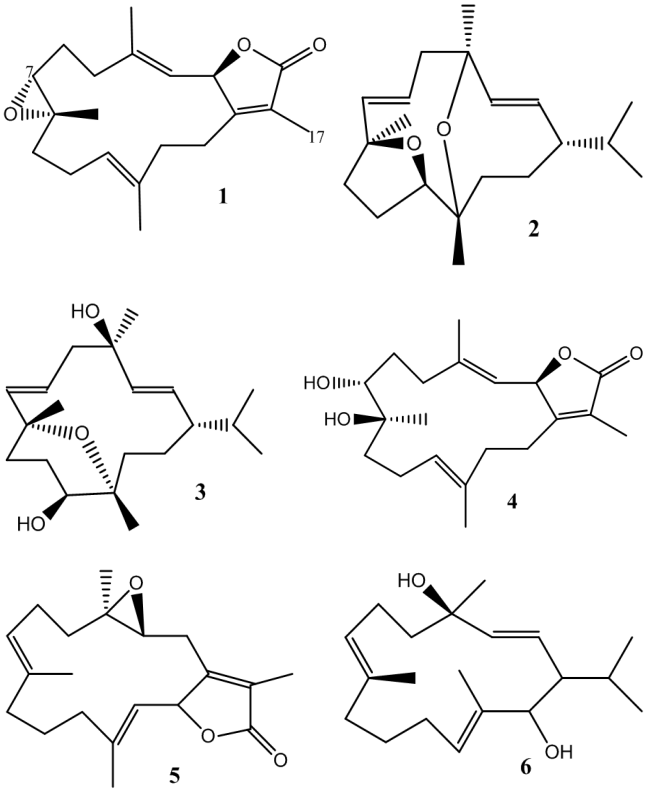


Fig. 4

PI14 Bioactive cembranoids from the Red Sea soft coral Sarcophyton glaucum

Ross SA[1,2], Abou El-Ezz RF[3], Ahmed SA[4], Radwan MM[1], Ayoub NA[5], Afifi MS[3,5], Khalifa SI[6]
[1]National Center for Natural Products Research, [2]Department of Pharmacognosy, School of Pharmacy, University of University of Mississippi, University, MS 38677, USA, [3]Department of Pharmacognosy, Faculty of Pharmacy, Misr International University, Cairo, Egypt, [4]Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt, [5]Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, [6]College of Pharmacy, Qatar University, Doha 02713, Qatar

A chemical investigation of the Red Sea soft coral *Sarcophyton glaucum* has led to the isolation of two new cembranoid diterpenes, **(2, 6)** and, one new natural cembranoid **(3)**, as well as three known compounds, Sarcophine **(1)**, (+)-7α,8β-dihydroxydeepoxy sarcophine **(4)** and Sarcophytolide **(5)**. Structure elucidation was achieved using spectroscopic techniques, including 1D and 2D NMR and HRMS. The antimicrobial and cytotoxic activities of the isolates were evaluated.

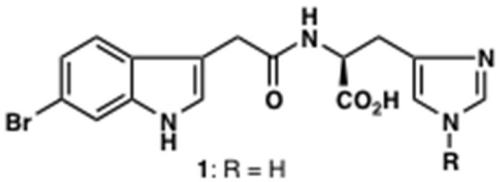


PI15 Bunodosines: novel analgesic acylamino acids from the venom of the sea anemone Bunodosoma cangicum

Zaharenko AJ[1], Picolo G[2], Ferreira Jr. WA[2], Murakami T[3], Kazuma K[4], Hashimoto M[3], Cury Y[2], de Freitas JC[5], Konno K[4]

[1]Laboratory of Genetics; [2]Laboratory of Pain and Signaling, Butantan Institute, Av. Vital Brasil 1500, São Paulo, 05503 – 900, Brazil; [3]Department of Agriculture and Bioscience, Hirosaki University, 3-Bunkyo-cho, Hirosaki, 036 – 8561, Japan; [4]Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930 – 0194, Japan; [5]Institute of Biosciences, University of São Paulo, Rua do Matão 321, São Paulo 05508 – 090, Brazil

Sea anemones are known as a rich source of protein and peptide toxins. In contrast, however, only little is known about the non-peptidic, small molecules in the sea anemones. We have been searching for small molecule toxins in the venom of the Brazilian sea anemone, *Bunodosoma cangicum*, which has led to the isolation of new acylamino acids, bunodosine 391 (BDS 391, **1**) and bunodosine 405 (BDS 405, **2**), showing a potent analgesic activity mediated by serotonin receptors.



PI16 Chemistry and biology of novel cyanobacterial secondary metabolites from Guam

Montaser R[1], Paul VJ[2], Luesch H[1]

[1]Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610; [2]Smithsonian Marine Station, Fort Pierce, Florida 34949

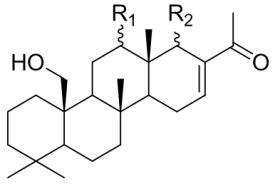
Marine cyanobacteria represent one of the most prolific sources of bioactive secondary metabolites with promising biomedical potential. Among the marine cyanobacteria, *Lyngbya* spp. are well known for their enormous potential to mix nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) pathways for the production of peptide-polyketide hybrids with a wide range of biological activities. In our continuous quest for novel drug leads from marine cyanobacteria, Guamanian varieties of the marine cyanobacteria *Lyngbya* spp. from Piti Bay were explored. This work led to the identification of a group of bioactive compounds which contain structural moieties with characteristic biosynthetic signatures of this organism. We will present the structure elucidation and bioactivity assessment of some of those compounds. Structural characterization was done using several techniques including nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS) and chemical modifications followed by enantioselective LC/MS. Biological characterization involved cytotoxicity screening in cancer cell lines, antibacterial assays and cannabimimetic activity evaluation.

PI17 Antiproliferative-assay directed isolation of scalarane sesterterpenes from an unidentified sponge from Madagascar

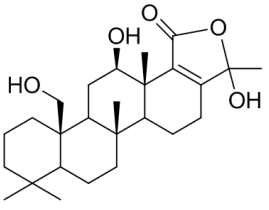
Harinantenaina L[1], Brodie PJ[1], Maharavo J[2], Bakary G[2], Kingston DGI[1]

[1]Department of Chemistry, Virginia Tech, Blacksburg, Virginia 24061, USA, [2]Centre National de Recherches Océanographiques, Nosy-Bé, Madagascar

The Madagascar International Cooperative Biodiversity Group (ICBG) program aims to integrate the improvement of human health through anticancer drug discovery from natural products with biodiversity conservation and economic development in Madagascar. An extract of a marine organism with moderate activity against the A2780 ovarian cancer cell line (IC_{50} 3.4 µg/mL) was selected for study. Bioassay-directed fractionation afforded four new type-II scalarane sesterterpenes: 12α-hydroxy-22-hydroxy-24-methyl-24-oxoscalar-16-en-25α-al **(1)**, 12β-hydroxy-22-hydroxy-24-methyl-24-oxoscalar-16-en-25β-al (**2**), 22-hydroxy-12β,24-dihydroxy-24-methylscalaran-25,24-olactone **(3)**, and 22-hydroxy-24-oxo-24-homoscalara-16,25(26)-dien-12β-ol **(4)**. The structures of all compounds were determined by physical, chemical and spectroscopic evidence including 1D and 2D-NMR. Compounds **1** and **2** displayed strong antiproliferative activity against the A2780 human ovarian cancer cell line (IC_{50} values of 0.28 µM and 0.26 µM, respectively) while compounds **3** and **4** exhibited modest activity (IC_{50} values of 9 µM and 4.5 µM, respectively).



1: R_1 = αOH, R_2 = αCHO
2: R_1 = βOH, R_2 = βCHO



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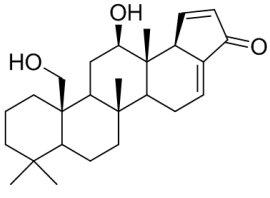


4

PI18

Pentacyclic ingamine-type alkaloids, a new antiplasmodial pharmacophore from the marine sponge *Petrosid Ng5 Sp5*

Muhammad I[1], Ibrahim MA[1], Khan S[1,2], Jacob M[1], Tekwani BL[1,3], Walker LA[1,3], Sameylenko V[1]
[1]National Center for Natural Products Research; [2]Department of Pharmacognosy; [3]Department of Pharmacology, Research Institute of Pharmaceutical Sciences School of Pharmacy, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, MS 38677

Two new pentacyclic ingamine alkaloids, 22(*S*)-hydroxyingamine A (**2**) and dihydroingenamine D (**3**), together with the known ingamine A (**1**) have been isolated from marine sponge *Petrosid Ng5 Sp5* (Petrosiidae) obtained from the open repository of NCI, USA. The structures of **1-3** were determined using NMR and MS techniques. The absolute configuration of OH-groups at C9 and C22 of **2** was determined as (*S*) using a modified Mosher esterification method. **1** and **3** showed strong antiplasmodial activity against CQ-sensitive (D6) and -resistant (W2) strains of *P. falciparum* with IC_{50} values of 90 and 78 ng/mL, and 72 and 57 ng/mL, respectively, while **2** was found to be less active. The compounds were found to be devoid of *in vitro* cytotoxicity against tumor cells of ductal (BT-549), ovary (SK-OV-3), epidermoid (KB) carcinomas and skin melanoma (SK-MEL), and non-cancerous monkey kidney fibroblasts (VERO) and pig kidney epithelial (LLC-PK_{11}) cells, up to a maximum concentration of 10 μg/mL. These polycyclic ingamine alkaloids represents the first example of antiplasmodial leads without a β-carboline ring, which is responsible for the cytotoxicity of the antiplasmodial manzamine class of marine alkaloids related to **1-3**.

PI19

Comparison of polar metabolites in Australian deep-sea sponges using HPLC-ESI-HRMS in combination with multivariate analysis

Wei L[1], Skropeta D[1]
[1]School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia

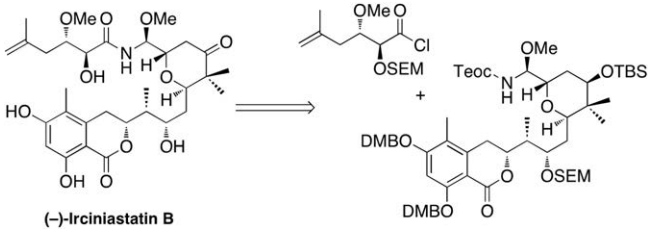
More than 70% of the earth's surface is covered by oceans. Yet, of the 30,000 marine natural products reported, less than 2% derive from deep-sea organisms. There is a paucity of research on deep-sea natural products due to the prohibitively expensive nature and logistical difficulty of conducting deep-sea research. Using remotely operated vehicles, a variety of deep-sea sponges have been collected off the shelves of Australia at depths of 82 – 235 m. These organisms were extracted with solvents of varying polarity and their polar extracts investigated using HPLC-ESI-HRMS in combination with principal components analysis. A total of 76 metabolites were identified from five deep-sea demosponges belonging to the genera *Hyatella, Haliclona, Neopetrosia, Spongia* and *Pachastrella.* These metabolites were used to determine similarities and differences between the different deep-sea species along with examining the influence of environmental parameters.. The outcome shows that sponges collected in similar locations produce similar polar metabolites, but at different geographic conditions such as location and depth, sponges produce different polar metabolites. Additionally, the isolation and characterisation of novel metabolites from the deep-sea sponge, *Pachypellina* sp. is also currently underway.

PI20

Total synthesis of (-)-Irciniastatin B

An C, Hoye AT, Smith III AB
Department of Chemistry, Laboratory for the Research on the Structure of Matter, and Monell Chemical Senses Center. University of Pennsylvania, Philadelphia, Pennsylvania 19104

(-)-Irciniastatin B, a novel cytotoxin with potent anticancer activity, was isolated from marine sponge *Ircinia ramosa*. Studies towards the total synthesis were initially based on our previously published synthesis of (+)-irciniastatin A [Smith, A. B.; *et al. Org. Lett.* **2008,** 10, 5625]. The earlier route, employing phenolic SEM-ether as a protecting group proved too labile during the new multiple step synthetic sequence. A revised strategy employing the more robust 3,4-dimethoxybenzyl ethers now permits access to advanced intermediates. Studies directed towards the completion of (-)-irciniastatin B will be presented.



PI21

Secondary metabolites from Panamanian mat forming cyanobacteria

Tidgewell K[1,2], Moy J[2], Herrera L[3], Spadafora C[3], Gerwick WH[1]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093; [2]Smithsonian Tropical Research Institute, Ancón, Panama City, Panamá; [3]INDICASAT, Ciudad del Saber, Clayton, Panama City, Panamá

Marine cyanobacteria are prolific producers of highly active secondary metabolites. While the majority of secondary metabolites from marine cyanobacteria are modified peptides, a variety of structural classes have been isolated and interesting structural features are often integrated into these compounds which are produced by unique biosynthetic pathways. Cyanobacterial secondary metabolites often show cytotoxic activity, however a number have been shown to be highly selective against parasites responsible for tropical diseases. The Panama ICBG has focused on discovery of natural products with activities against malaria, leishmaniasis, Chagas' disease and cancer. Many filamentous cyanobacteria grow in mats which cover rock, reef, sponge and other surfaces. A number of mats have been collected in different locations across Panama with varied morphology yet they produce similar secondary metabolites. Additionally there have been some mats found at distant sites which have nearly identical morphology that produce very different secondary metabolites with different activities in our bioassays. These cyanobacteria have shown activity against the malaria parasite as well as activity against cancer cells in vitro. Secondary metabolites and activity will be presented from a variety of mat forming cyanobacteria collected across different sites in Panama.

PI22

Ceramides and steroids of the zoanthides *Palythoa caribaeorum* and *Protopalythoa variabilis*

Pessoa ODL[1], Almeida JGL[1], Maia IV[1], Silveira ER[1], Wilke D[2], Costa-Lotufo LV[2]
[1]Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Fortaleza, CE, Brazil; [2]Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, Fortaleza, CE, Brazil

The chemical investigation of the marine species *Palythoa caribaeorum* and *Protopalythoa variabilis,* both collected at Paracurú beach, state of Ceará – Brazil, resulted in the isolation of tetracyclic sterols possessing the ergostan skeleton: 24(*R*)-ergost-5-en-3β-ol; 5α,8α-epidioxy-24(*R*)-ergost-6-en-3β-ol; 24(*R*)-ergost-5-en-3β,7α-diol; 24(*R*)-7α-hydroperoxy-ergost-5-en-3β-ol; 24(*R*)-ergost-7-en-3β,5α,6β-triol and 24(*R*)-B-norergostan-3β-5β-diol-6β-carboxylic acid. In addition, four ceramides: *N*-(2*S*,3*R*,4*E*,8*E*,1,3-dihydroxy-4,8-octadecadienyl)hexadecanamide; *N*-(2*S*,3*R*,4*E*,1,3-dihydroxy-4-octadecenyl)hexadecanamide (*N*-[2*S*,3*R*,4*E*,8*E*,1-(2"-methylamino-ethanosulfonyl)-3-hydroxy-4,8-octadecaenyl]hexadecanamide and *N*-[2*S*,3*R*,4*E*,1-(2"-methylaminoethano-sulfonyl)-3-hydroxy-4-octadecenyl]hexadeca-namide, were also isolated. The cytotoxic and antifungal properties of all ceramides were evaluated, nevertheless none of them showed any activity. The structures of the isolated compounds were elucidated using spectrometric techniques, such as: GC/MS, HRESIMS, IR and NMR (^{1}H, ^{13}C and ^{15}N) through 1D and 2D pulse sequences and, whenever the case, comparison with literature data

PI23 Isolation and identification of natural products as possible inhibitors of NF1/TP53-Null astrocytoma cell proliferation

Chan STS[1], Wilson JA[1], Henrich CJ[2], Reilly KM[3], Gustafson KR[1], McMahon JB[1], McKee TC[1]
[1]Molecular Targets Laboratory, Frederick National Laboratory for Cancer Research, Bldg 562, Rm 101, Frederick, MD 21702; [2]SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702; [3]Mouse Cancer Genetics Program, Frederick National Laboratory for Cancer Research, Frederick, MD 21702

One of the most common and lethal forms of brain cancer is astrocytoma, which does not respond well to any of the current cancer treatments available. Patients with the automsomal dominant syndrome neurofibromatosis type 1 (NF1) carry a mutation in the NF1 gene, and are at greater risk of developing cancers such as astrocytoma. In our search for natural products active against NF1, a new acetylene compound, dihydropetrosynone, along with the closely related known petrosynol, were isolated from the marine sponge *Niphates* sp. using bioassay-guided fractionation. Both compounds showed modest activity against NF1.

PI24 Inhibition of hypoxia inducible Factor-2 transcription: Isolation of active modulators from marine sponges

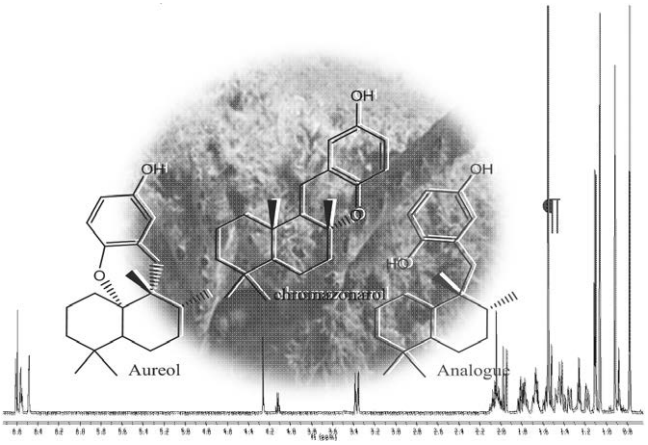
McKee TC[1], Rabe D[2], Bokesch HR[3], Grkovic T[1], Whitson EL[1], Diyabalanage T[1], Van Wyk AWW[1], Marcum SR[1], Gardella RS[3], Gustafson KR[1], Linehan WM[2], McMahon JB[2], Bottaro DP[2]
Molecular Targets Laboratory, Molecular Discovery Program, Center for Cancer Research, Frederick National Laboratory for Cancer Research, Frederick, MD 21702; [2]Urologic OncologyBranch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892 – 1107; [3]SAIC-Frederick, Inc, Frederick, MD 21702

Renal or kidney cancer accounts for about 3% of all cancer cases reported each year in the US. Molecular signatures that define the cancer, such as the loss of functional VHL, are found in both sporadic and familial cases of cancer. In clear cell renal cancer, the transcription factor HIF-2a has been shown to have a distinct role in tumorigenesis. Our laboratories developed a cell-based screen to identify modulators of HIF-2a. Screening of the NCI's Natural Product Extract Repository resulted in the identification of ten sponge extracts from which 12 compounds were isolated. The biological evaluation of these compounds will be discussed including preliminary evaluation of HIF-1a *vs.* HIF-2a selectively and the isolated compounds' effects on mRNA from several pathways regulated by HIF.

PI25 Bioactive terpenoids from the Bahaman sponge *Smenospongia aurea*

Fairman K, Huang S, MacMillan J
Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas

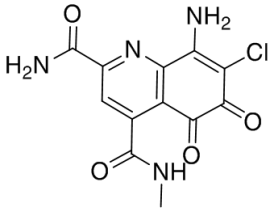
Sixty percent of colon cancers exhibit PTEN loop mutations which makes this mutation a valuable target for cancer chemotherapeutics. Recently, ENTPD5, a regulator of protein N-glycosylation and of proper protein folding in the ER of cells exhibiting mutations in the AKT/PTEN pathway, was suggested to provide a therapeutic intervention in PTEN driven colon tumors. In a high throughput screen to look for small molecule inhibitors of ENTPD5, we identified extracts from the marine sponge *Smenospongia aurea* that had potent inhibitory activity. Using bioassay guided fractionation we isolated and characterized a family of diterpene natural products that inhibit ENTPD5, the most potent, chromozonarol, has a 10uM IC_{50} (figure below). This poster will describe the isolation, characterization, and biological activity of this class of compounds.



PI26 Ammosamide d from a marine-derived *Streptomyces variabilis*

Pan E[1], Jamison M[1], Youssufuddin M[2], MacMillan J[1]
[1]Department of Biochemistry, Division of Chemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas, TX 75390; [2]Center for Nanostructured Materials, University of Texas at Arlington, Arlington, TX

In our continuing efforts to search for natural products from marine bacteria with selective cytotoxicity against cancer cell lines, we obtained a series of fractions from the marine-derived *Streptomyces variabilis* (strain SNA-020) that exhibited modest selectivity and potency for the MiaPaca-2 pancreatic cancer cell line. Analysis of the active fractions by LC-UV-MS showed the presence of chlorine bearing molecules to ammosamide A and B, leading to the isolation of ammosamide D (**1**). It is the first example of an oxidized analog resulting in a 5,6-dioxo-5,6-dihydroquinoline ring system. Ammosamide D has modest cytotoxicity to the Mia-Paca2 pancreatic cancer cell line (IC_{50}= 3.2 μM).

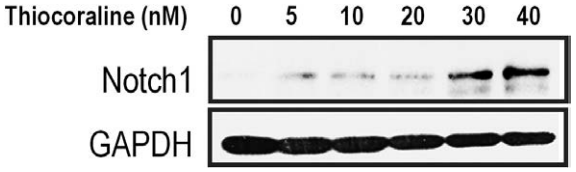


1

PI27 Thiocoraline activates transcription of notch and inhibits the proliferation of carcinoid tumor cells

Wyche TP[1], Jaskula-Sztul R[2], Dammalapati A[2], Cho H[1], Kwon G[1], Chen H[2], Bugni TS[1]
[1]Division of Pharmaceutical Sciences, University of Wisconsin-Madison, 777 Highland Avenue, Madison, WI 53705, USA; [2]Department of Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792

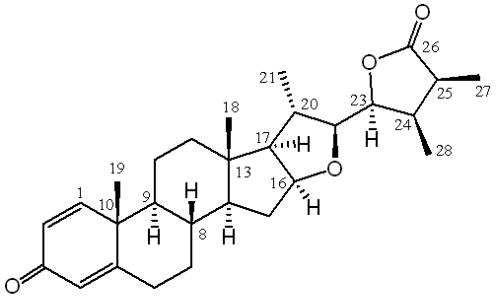
Carcinoids, slow-growing neuroendocrine tumors (NETs) that are characterized by hormone overproduction, have demonstrated resistance to chemotherapeutics, and consequently, surgery remains as the only effective method of treatment. The marine-derived thiodepsipeptide, thiocoraline, was found to activate the Notch pathway in pancreatic carcinoid (BON) cells. Carcinoid cells treated with thiocoraline resulted in an alteration of malignant phenotype evidenced by decrease of NET markers, ASCL-1, CgA, and NSE. Thiocoraline effectively suppressed carcinoid cell growth by promoting cell cycle arrest. Formulation of thiocoraline in a polymeric micelle increased the aqueous solubility and allowed for *in vivo* studies. Therefore, our work demonstrates the therapeutic potential for thiocoraline against carcinoids.



PI28 Withanolide-based steroids from a cultured soft coral *Sinularia brassica*

Huang CY[1], Su JH[1,2], Sung PJ[1,2], Sheu JH[1]
[1]Department of Marine Biotechnology and Resources and Division of Marine Biotechnology, National Sun Yat-sen University, Kaohsiung 804, Taiwan; [2]National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan

Six new withanolides, sinubrasolide A-F (**1-6**), were isolated from a cultured soft coral *Sinularia brassica*. The molecular structures of **1-6** were established by detailed spectroscopic analysis, including extensive examination of 1D NMR spectroscopic data and 2D NMR (^{1}H-^{1}H COSY, HMQC, HMBC and NOESY) correlations. The structure of **1** was further confirmed by a single-crystal X-ray diffraction analysis. Furthermore, sinubrasolide A (**1**) was found to exhibit significant cytotoxicity against a limited panel of cancer cell lines.



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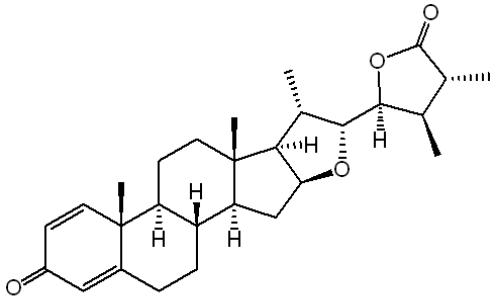

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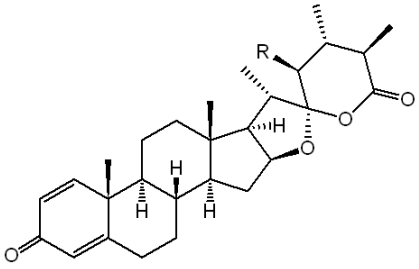
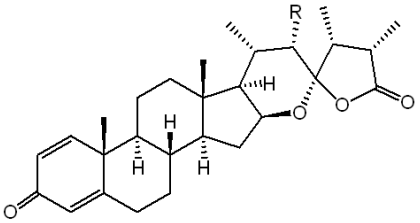


3 R=H
4 R=OH



5 R=OH
6 R=OAc

PI29 Secondary metabolism variation in endophytic marine fungi by chemical epigenetic elicitation approaches

de Felício R[1], Almeida TL[1], Soares Cunha ÉF[1], Tomaz JC[1], Debonsi HM[1]
[1]Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, CEP 14040903, Universidade de São Paulo, Ribeirão Preto-SP, Brazil

Chemical epigenetic elicitation is a new approach to access unknown and/or remarkable natural products that have been used with success in last years. Three strains of marine endophytic fungi, isolated from red algae, were submitted to chemical elicitation and they showed changes in their secondary metabolism. Some major substances of *Penicillium decaturense* were inhibited when sodium butyrate (100 μM) was used. Moreover, new peaks were observed by means of HPLC analysis, indicating the presence of new metabolites biosynthesized through elicitation experiments. The Xylariaceae sp. strain had its metabolism increased speed using procaine as elicitor while valproic acid (both 100 μM) was responsible to promote the production of some metabolites in 7 days experiment, metabolites which usually appeared in 14 days without any elicitated conditions. In *Phomopsis longicolla* strain, the production of bioactive dicerandrol C (earlier isolated just in solid rice medium) was stimulated in liquid medium (PDB) in 7 days by sodium butyrate (100 μM) modulation. In the same experiment, after 21 days growth, new chromatogram peaks pointed out the new halogenated metabolites (isotopic abundance observed by mass spectrometry) production. More detailed chemical investigations are being carried out to determine the new secondary metabolites produced. Therefore, we can corroborate that chemical epigenetic elicitation is an important approach to validate the chemical profile variation in fungi, besides to promote new metabolites biosyntheses.

PI30 Immunomodulating extracts from Icelandic marine invertebrates

Gudmundsdottir AB[1,2,3], Freysdottir J[2,3,4], Omarsdottir S[1]
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Iceland has a 200 nautical miles exclusive economic zone that is largely unexplored with respect to chemical constituents of the marine biota. In particular, the confluence of cold and warm water masses and geothermal activity creates a unique marine environment that has never been evaluated for the potential of marine natural product diversity. The aim of this study was to prepare extracts from Icelandic marine invertebrates and screen for their immunomodulating activity in an *in vitro* dendritic cell (DC) model, followed by bioassay-guided isolation. The effects of the extracts and fractions were assessed by their ability to alter the maturation of monocyte-derived human DCs, assessed by measuring their cytokine secretion and expression of surface molecules. Extracts of ninety marine invertebrates, collected in Icelandic waters have been tested and ten extracts have been discovered that affect the maturation of the DCs without being cytotoxic. Two active extracts, from the marine sponges *Clathria barleei* and *Isodictya palmata,* were selected for further fractionation. These extracts had similar effects; reduction in IL-12 and IL-10 secretion of DCs and down-regulation of CD86 and HLA-DR surface expression. We conclude that the active fractions from these marine sponges have inhibitory effects on the maturation of dendritic cells,

and isolated compound(s) could prove to be useful in the treatment of autoimmune diseases.

PI31 MAREX: Exploring marine natural products for novel bioactive compounds

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Biodiversity defines the variability among all living organisms, including marine ecosystems and their ecological complexes. Oceans are considered a rich source of biological and chemical diversity. Through co-operation between 19 industrial and academic partners, EU FP7-funded project MAREX focuses on collecting and classifying marine organisms, such as micro- and macroalgae, sea anemones, tunicates and fish from Atlantic, Pacific and Indian Oceans and the Mediterranean, Baltic and Arabian Seas. Extracts and purified compounds of these organisms are studied for biological activities with potential therapeutic and industrial use, including anticancer, anti-inflammatory and antiviral. Chromatographic isolation of bioactive compounds will be followed by structural determination. Sustainable cultivation methods for promising organisms and biotechnological processes for selected compounds will be developed. So far, our laboratory has screened over 500 samples against diverse microbial strains and 17 of them showed potential antimicrobial activity. MAREX also aims at better understanding of environmentally conscious sourcing of marine natural products and increased public awareness of marine biodiversity and potential. Finally, MAREX is expected to offer novel marine-based lead compounds for industries related to pharmaceutical, nutraceutical, agrochemical, food processing and biosensor applications.

PI32 Antiastrocytoma natural products that target tumor cells with defects in the tumor suppressor neurofibromin

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Astrocytic glyomas, the most common malignant form of brain cancer, are often unresponsive to the current treatment options. Patient prognosis is generally quite poor, with the 5-year survival rate for patients with glioblastoma multiforme (GMB) below 5%. People with neurofibromatosis type 1 syndrome (NF1) have a high predisposition to develop tumors, with benign gliomas and neurofibromas being the most frequent type, but malignancies such as astrocytomas and GMB are also common. The *NF1* gene codes for neurofibromin, a rasGAP tumor suppressor protein that downregulates the RAS signaling pathway. Lack of functional neurofibromin leads to overactivation of the oncogenic RAS pathway, a critical step in the development of many tumors. A recently developed assay for materials that selectively target cells expressing mutant neurofibromin was used to screen extracts from the NCI Natural Products Repository. A number of extracts were active in this screen and subjected to detailed chemical study. Bioassay-guided fraction of these extracts, looking for compounds with antiproliferative activity targeting astrocytoma cells and not healthy astrocytes, will be described.

PI33 Antifungal compounds from four marine sponges

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Screening a large number of organic extracts of marine sponges from the National Cancer Institute Open Repository for antifungal drug discovery produced potent hits for bioassay-guided fractionation. We have shown that the phloeodictines from the marine sponge *Pellina eusiphonia* and the microsclerodermins from *Microscleroderma herdmani* exhibited broad spectrum antifungal activities against fungal pathogens including drug-resistant strains. Our continued efforts in this regard have led to the identification of the cyclic peptide aciculitin B from *Aciculites ciliate* that showed remarkable in vitro antifungal activity against the clinically important fungal pathogens *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*. Its potency against *C. neoformans* is 60-fold stronger than the antifungal drug amphotericin B. We have also isolated the antifungal compounds including the tetramic acid glycoside aurantoside E from *Plakinolopha mirabilis*, 4α-isocyanogorgon-11-ene from *Axinyssa terpinis*, and the biphenyl ether 3,5-dibromo-2-(3,5-dibromo-2-methoxyphenoxy)phenol from an unidentified sponge. These compounds may be used as molecular probes to uncover novel antifungal drug targets or as leads for preparation of new compounds with improved antifungal properties.

PI34 Diterpenes from the French marine alga *Bifurcaria bifurcata* (Sargassaceae) inhibit growth of the human pathogen *P. Falciparum*

Galle JB[1], Kaiser M[2], Rusig AM[3], Vonthron-Sénécheau C[1]
[1]UMR CNRS 7200 Laboratoire d'Innovation Thérapeutique, Faculté de Pharmacie, Université de Strasbourg, 64701 Illkirch, France; [2]Swiss Tropical and Public Health Institute, University of Basel, 4002 Basel, Switzerland; [3]CNRS INEE – FRE3484 Biologie des Mollusques Marins et des Ecosystèmes Associés, **Université de Caen Basse-Normandie, 14032 Caen Cedex, France**

Eleganolone has been isolated by a bioassay-guided approach from a crude ethyl acetate extract of *Bifurcaria bifurcata*, as a putative active principle responsible of the strong trypanocidal activity previously found in this extract. The *in vitro* activity of eleganolone against *Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi* trypomastigotes will be presented together with its *in vitro* antiprotozoal activity against other protozoa. Cytotoxicity against mammalian cultured cells and selectivity indexes will also be presented.

PI35 Antimitotic activity of a dithiolpyrrolone from Streptomyces sp. recovered from the Brazilian tunicate Eudistoma vannamei

Jiménez P[1,2], Abreu PA[1,2], Sousa TS[3], Maia AIV[3], Pessoa ODL[3], Costa-Lotufo LV[1,2]
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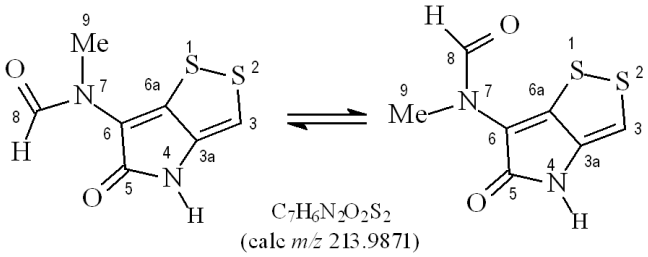
Eudistoma vannamei is an endemic tunicate from the northeastern Brazilian coast and, yet, the most abundant species in the state of Ceará. Previous studies have shown the hydromethanolic extract to be highly active against cultured tumor cells. Bioassay-guided fractionation yielded novel highly cytotoxic staurosporines. Natural staurosporines

have frequently been obtained from actinomycetes, and so, the microbiota associated to this tunicate was investigated for their biomedical potential. Bacterial strains isolate from crushed specimens of the tunicate were elected based on phenotypical characteristics, up-scale grown and extracted with ethyl acetate. Extracts obtained from microorganisms were screened for cytotoxicity in tumor cell lines and one, identified as *Streptomyces* sp., presented the highest cytotoxicity. This extract was then grown in large quantities, and fractionated using a cytotoxicity-guided approach to yield the dithiolopyrrolone *N*-(4,5-dihydro-5-oxo-1,2-dithiolo[4,3-*b*]pyrrol-6-yl)-*N*-methyl-formamide. The compound presented IC_{50} ranging from 1.68 to 3.17 μM in various cell lines. Moreover, it induced cell cycle arrest in mitosis, as suggested by flow cytometry and western blot analyses and observation under confocal microscope.



$C_7H_6N_2O_2S_2$
(calc *m/z* 213.9871)

PI36 Laboratory-cultured Red Sea cyanobacteria as a source of biologically active natural products

Thornburg C[1], Youssef DT[2], Shaala LA[2], McPhail K[1]
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The Red Sea represents an unexplored repository of diverse cyanobacteria, although in low abundance. This may result from the low annual rainfall, minimal freshwater input and high evaporation rate that make the Red Sea one of the most saline and pristine water bodies in the world. Despite these conditions, we have collected specimens from a range of cyanobacterial genera that have been maintained in laboratory culture and produce several biosynthetically distinct metabolites to date. Notably, a cultured black *Lyngbya* sp. collected from the Nabq mangroves near Sharm El-Sheikh, Egypt produces two new apratoxin analogues along with several known apratoxin and lyngbyabellin analogues. Additionally, cultures of a phormidolide-producing Red Sea *Leptolyngbya* sp. have led to the isolation of three new macrolides. Finally, cultures of a newly identified *Symploca* sp. collected from the *Excalibur* shipwreck have yielded a series of uncharacterized metabolites that show nanomolar toxicity to NCI-H460 lung cancer cells. Molecular characterization of the component metabolites has been achieved using a combination of NMR spectroscopy and mass spectrometry. Although the organisms in this study were collected from a relatively isolated habitat, the compounds reported here show biosynthetic capabilities comparable to cyanobacteria collected pantropically, which poses additional questions as to the biogenetic origin of these metabolites.

PI37 Production of unique natural products through micro-scale co-cultivation of marine bacteria

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Nature continues to supply chemically diverse and biologically active compounds, or natural products, with historically successful potential as therapeutic leads. Unfortunately, many of the biosynthetic gene clusters remain silent, or cryptic, under laboratory culture conditions. To coax the expression of cryptic biosynthetic gene clusters in marine invertebrate-associated bacteria, the bacteria were co-cultured to facilitate interspecies interactions and metabolite production. In this study, we utilize LC/MS followed by principal component analysis (PCA) for rapid and sensitive analysis, resulting in a graphical representation of metabolic differences between co-cultures and mono-culture controls. Furthermore, culture volumes were reduced to 500 μL and grown in 96-well low-evaporation plates. This system allows for the simultaneous co-cultivation and chemical profiling of hundreds of strains, greatly improving throughput. Previous studies suggested natural product biosynthesis within *Streptomyces* sp. was induced when co-cultured them with mycolic acid-containing bacteria[1]. As a preliminary study, we selected *Streptomyces* sp. strains with no antibiotic activity and have shown the induction of antibiotic production upon co-culturing with mycolic acid-containing bacteria. [1]Onaka, H.; Mori, Y.; Igarashi, Y.; Furumai, T. *Appl. Environ. Microbiol.* **2011**, *77*, 400 – 406

PI38 New depsipeptides and brominated macrolides from cultured marine cyanobacteria

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Laboratory culture of two cyanobacteria isolated from the Panamanian mixed cyanobacterial assemblage that yielded the potent cancer cell toxin coibamide A has lead to the characterization of seven new glycosidic macrolides and seven depsipeptides. The production of these metabolites varies under different culture conditions, and when the cyanobacteria are co-cultured versus grown separately. Comprehensive NMR spectroscopy and HR-TOF-MS established that the glycosidic macrolides are related to the known cytotoxins lyngbouilloside and lyngbyalosides A-C, and are thus named lyngbyalosides D-J. The biosynthetic source of these macrolides is proposed to be the minor cyanobacterial partner, phylogenetic analysis of which suggests that it is a *Phormidium* species. The relative configuration of lyngbyalosides D-J was determined using *J*-based analysis and ROESY correlations, as well as comparison with the literature. Five new depsipeptides have also been identified from culture and closely resemble two unreported depsipeptides, named symtropamides A and B, that were previously isolated from the field-collected cyanobacterial assemblage. Symtropamides A and B display preliminary antimalarial activity, which will be investigated further using all seven analogs. The biosynthetic source of these depsipeptides is likely the coibamide-producing cyanobacterium, which is related to the genus *Symploca*, but may warrant assignment to a separate new genus.

PI39 Principle component analysis of the Antarctic sponge *Dendrilla membranosa* secondary metabolome

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The sponge *Dendrilla membranosa* is common on the benthos around the Western Antarctic Peninsula (WAP) and is easily recognized by its yellow cactus-like bulbs. Chemical defenses are employed by *D. membranosa* and many other sessile Antarctic marine invertebrates to deter predation and fouling. Diterpenoids, like aplysulphurin and membranolide, have been reported as feeding deterrents against known sponge predators. *D. membranosa* samples have been collected at various depths from Alan's Wall, Bonaparte, Gamage Point and North Laggard around Palmer Station, Antarctica. Crude extracts were analyzed via Q-TOF-LC-MS and subjected to Principle Component Analysis (PCA) to determine metabolic variations between location and depth.

PI40 Bioprospecting anticancer compounds in marine bacteria recovered from sediments in the northeast of Brazil

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The diversity of organisms in the marine environment has inspired researchers for many years to identify novel marine natural products with therapeutical potential. The coast of Ceará (Brazil) has been prospected for the pharmacological potential housed in sponges, tunicates and corals, but little data are available regarding the microorganisms. This study focused on the identification of potential anticancer compounds derived from actinomycetes isolated from sediment samples collected at different locations in the Ceará coast. Twenty strains were selected for cytotoxicity evaluation of their crude extract using adenocarcinoma cells through the MTT assay. Among them, eight were considered active (BRA-028, BRA-029, BRA-031, BRA-068, BRA-073, BRA-090, BRA-093 and BRA-098), with IC_{50} values ranging from 0.02 to 46.77 µg/mL. The LC-MS analysis of active extracts showed the occurrence of compounds related to antimycin A in BRA-093 and related to chromomycins in the BRA-090, while the other extracts are currently under chemical characterization.

PI41 Immunomodulating polysaccharides from North Atlantic Sea cucumber *(Cucumaria frondosa)*

Kale VA[1,2,3,4], Fridjonsson OH[2], Hreggvidsson GO[2], Freysdottir J[3,4,5], Omarsdottir S[1]
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Chondroitin sulfate (CS) is a glycosaminoglycan composed of a chain of alternating sugars (N- acetyl galactosamine and glucuronic acid) and present in cartilage tissue of most animals. CS is mostly sulfated at C-6 and/or C-4 position of N- acetyl galactosamine. The type and amount of sulfo groups on CS varies with source organism, tissue, location with in the tissue and age. Novel type of fucose branched CS has been isolated from sea cucumbers. The aim of the study was to investigate whether chondroitin sulfate isolated from the cartilage of the North Atlantic sea cucumber (Cucumaria frondosa) affected maturation of human dendritic cells and their ability to activate allogeneic $CD4^+$ T cells in vitro. The CS polysaccharides were separated into three different fractions on an anion-exchange column and named CS-1A, CS-1B and CS-1C. The fraction CS-1B suppressed the secretion of IL-10 and IL-12p40 from human monocyte-derived dendritic cells and allogeneic $CD4^+$ T cells co-cultured with dendritic cells that had been matured in the presence of CS-1B secreted reduced levels of IFN-γ and increased levels of IL-17 than allogeneic $CD4^+$ T cells co-cultured with dendritic cells that had been matured without any CS. These data suggest that saccharide composition of CS-1B can affect the maturation of dendritic cells and their ability to activate T cells. The effects on T cells are suggestive of increased Th 17 and reduced Th 1 activity.

PI42 Cupriachelin, a photoreactive siderophore from the freshwater bacterium *Cupriavidus necator* H16

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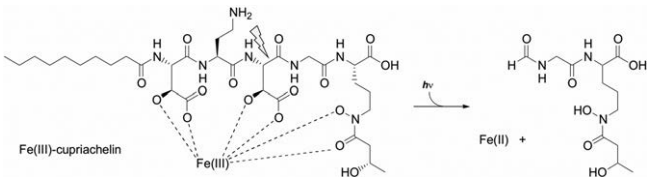
The bacterium *Cupriavidus necator* H16 is known to accumulate organic carbon in the form of polyhydroxyalkanoates, which are used as raw material for the production of medical devices. Analysis of the H16 genome sequence now led to the identification of a previously unrecognized biosynthesis gene cluster. Insertional mutagenesis confirmed this locus to be operational under iron-deficient conditions and spurred the isolation of the associated metabolite. The structure of this natural product is reminiscent of siderophores that are excreted by marine bacteria. Comparable to marine siderophores, the ferric form of the isolated molecule exhibits photoreactive properties. Exposure to UV light induces an oxidation of its peptidic backbone and a reduction of the coordinated Fe(III).



PI43 Endophytic diversity of pahrmaceutically important *Cannabis sativa*

Kusari P[1], Kusari S[2], Spiteller M[2], Kayser O[1]
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[2]Institute of Environmental Research (INFU) of the Faculty of Chemistry, Chair of Environmental Chemistry and Analytical Chemistry, TU Dortmund, Otto-Hahn-Straße 6, D-44221 Dortmund, Germany *Cannabis sativa* is an annual herbaceous plant of the Cannabaceae family from central Asia. Cannabinoids like Tetrahydrocannabinol (THC), cannabinol, cannabidiol, cannabigerol are one of the major secondary metabolites of this plant, which are known to have important pharmaceutical benefits like analgesic, anti-inflammatory, neuro-protective, appetite-stimulant and many more. Endophytic microorganisms (endophytes) still remain an unexplored group of very promising organism with diverse potential for exploitation, that are capable of producing bioactive secondary metabolites, sometimes even those natural products considered exclusive to their host plants. Thus, these microorganisms are important not only from molecular and biochemical standpoint but also from the ecological perspectives. We have isolated a plethora of endophytes, both bacteria and fungi, from various tissues of *Cannabis* plant like the leaf, stem, and roots. We are evaluating the ecological significance of these endophytes by taxonomically characterizing these microfloras using several microbiological, molecular and bioinformatics tools and techniques. Such a comprehensive phylogenetic approach will enable us in understanding the spatial distribution and species diversity of endophytes of this important plant. Despite the legal problems associated with this plant the better understanding of the plant-endophyte relationship will provide a potential of exploring the pharmaceutical benefits of the plant and thus a proper difference between drug of abuse and medicine can be made with proper research. Consequently, the synergy between compounds and potential for interaction within the plant may provide successful and better therapeutic potential of *Cannabis* as a medicine.

PI44 Termination of the structural confusion between Plipastatin A1 and Fengycin IX

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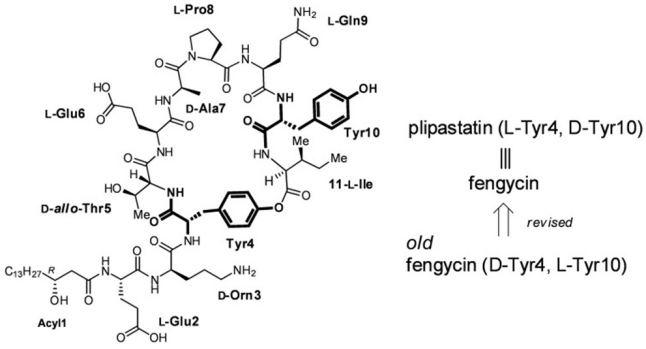
Plipastatin A1 and fengycin IX had been considered as diastereomers due to the permutation of the enantiomeric pair of Tyr in most papers. We succeeded in proving experimentally that these are identical compounds, although their ^1H NMR spectral data were reported to be considerably different each other. The ^1H NMR spectrum of a depsipeptide from *Bacillus subtilis* H336B initially showed nice accordance with that of fengycin. However, the spectrum changed to become quite similar to that of plipastatin, when the sample was converted into the potassium salt. Our structural investigations disclosed that the structures of these molecules should be settled into that of plipastatin A1 by Umezawa (L-Tyr4 and D-Tyr10) in spite of more than 200 scientific papers about

fengycins. These results consisted with the biogenesis of fengycin rather than old structure.



PI45

withdrawn

PI46

Bioactive natural products from North Atlantic algal endophytes

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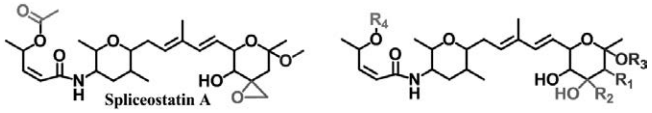
Research into the natural products chemistry of marine endophytic fungi is an area where few studies have been undertaken. The discovery that terrestrial endophytes can be an important source of bioactive compounds coupled with the constant stresses imposed on algae by the marine environment, suggests that algal endophytic microorganisms could represent an important source of biologically active secondary metabolites. Fungal endophytes were isolated from nine algal species (four brown, three red and two green), collected from the Shetland Islands, United Kingdom in October 2010. Endophyte isolates were identified both morphologically and through DNA sequencing resulting in 64 distinct endophyte isolates covering 47 species being isolated from the nine algal host species. Extracts of the endophytes were screened in common laboratory bioassays with 16 fungi showing cytotoxicity, 25 fungi showing antibacterial activity and 24 fungi showing antifungal activity. An extract from a *Penicillium* species was subjected to bioassay-guided fractionation resulting in the isolation of (±)-patulin (IC_{50} 42 μg/mL *Staphylococcus aureus*, 139 μg/mL *Pseudomonas aeruginosa*) as the major bioactive constituent and three biosynthetically related compounds that exhibited weak bioactivity.

PI47

Potent spliceostatin analogs isolated from *Pseudomonas* by mixed fermentation and modifications of culture conditions

Zuck KM[1], Shipley S[1], Giddings LA[2], DeLloyd T[1], He M[2], Newman DJ[2]
[1]Natural Products Support Group, SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702; [2]Natural Products Branch, Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702

Recently, the SF3b subunit of the spliceosome was identified as a target for the natural products pladienolide, herboxidiene and spliceostatin A. These metabolites show in-vitro activity in tumor cell lines with IC_{50} values in the low nanomolar range, induce cell cycle arrest at the G1 and G2/M phases and block angiogenesis. Spliceostatin analogs have been reported from Pseudomonas sp. and Burkholderia sp. Several reports suggest that the epoxide group of spliceostatin A is indispensable for the antitumor activity. However, by co-culturing Pseudomonas with other bacteria as well as varying fermentation time and media composition, we were able to isolate several new analogs of spliceostatin A without the epoxide functionality. Notably, some of these metabolites exhibit GI_{50} values between < 0.1 nM – 25 nM on the NCI60 Tumor Cell Line screen. In addition to analyze the SAR, we also explored the biosynthetic origin of these novel inhibitors in order to understand and improve their production. Funded by NCI Contract No. HHSN261200800001E.



PI48

Norspermidine – A self-produced trigger for biofilm disassembly that targets exopolysaccharide

Cao S[1], Kolodkin-Gal I[2], Boettcher T[1], Chai L[3], Kolter R[3], Losick R[2], Clardy J[1]
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Biofilms are structured communities of bacteria that are held together by an extracellular matrix consisting of protein, exopolysaccharide and DNA etc. Biofilms often have a limited lifespan, disassembling as nutrients become exhausted and waste products accumulate. D-amino acids were previously identified as a self-produced factor that mediates biofilm disassembly by causing the release of the protein component of the matrix in *Bacillus subtilis*. Here we report that *B. subtilis* produces an additional biofilm disassembly factor, norspermidine (H_2N-CH_2-CH_2-CH_2-NH- CH_2-CH_2-CH_2-NH_2). Dynamic light scattering and scanning electron microscopy experiments indicated that norspermidine interacts directly and specifically with exopolysaccharide. D-amino acids and norspermidine acted together to break down existing biofilms and mutants blocked in the production of both factors formed long-lived biofilms. Norspermidine, but not closely related polyamines, prevented biofilm formation by B. *subtilis*, *Escherichia coli*, and *Staphylococcus aureus*.

PI49

Novel purification protocol for *in vivo* produced analogues of Amphotericin B

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Amphotericin B (1), produced by *Streptomyces nodosus*, is a medically important antifungal that is also active against *Leishmania* parasites. However, its use is severely limited by its toxicity. Genetic disruption of one of the post PKS genes encoding a cytochrome P450 produces **2**, which has been shown to be less toxic and retains antifungal activity. We report here improved production and purification protocols involving the intermediacy of a less polar Fmoc derivative. Development of this methodology will enable the production of gram quantities of these metabolites for extensive biological assays and assist with investigations into the mode of action of these antibiotics.

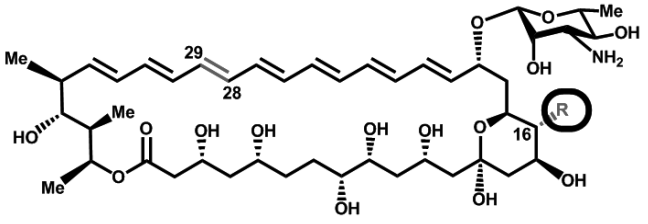

Fig. 1: R = COOH Amphotericin B

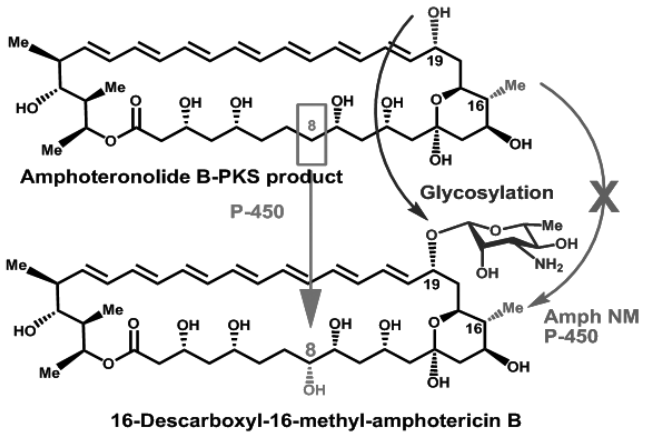

Fig. 2: R = Me 16-Descarboxyl-16-methyl-amphotericin B

PI50 New Natural products from a myxobacterium of the recently discovered genus "*Aetherobacter*"

Etzbach L, Plaza A, Garcia R, Müller R
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Myxobacteria are a rich source of chemically diverse and bioactive natural products. In particular new myxobacterial genera or families hold great promise as a source of currently unknown secondary metabolites. Recently, we discovered the new myxobacterial genus "*Aetherobacter*" which is phylogenetically related to *Sorangium*, one of the most prolific producers of active natural products among myxobacteria. The first *Aetherobacter* strain that was chemically studied, *A. rufus* (SBSr003^T), was isolated in 2007 from a soil sample containing decaying plant material and the strain also became interesting as source of commercially valuable omega-3 polyunsaturated fatty acids (PUFAs). Further investigations on the secondary metabolites produced by *A. rufus* led to the discovery of the aetheramides A and B, potent HIV-1-inhibitory and cytotoxic depsipeptides. The second studied *Aetherobacter* strain SBSr001 was found to exhibit cytotoxicity against a colon carcinoma cell line (HCT-116) and also antibacterial activity against the Gram-positive bacterium *Bacillus subtilis*. Fractionation based on biological activity and structural features from hyphenated LC-SPE-NMR/-MS yielded the isolation of new compounds including two highly cytotoxic spirangien derivatives. The new structures were established using spectroscopic techniques including 1D and 2D NMR experiments, HR-ESIMS, and chemical derivatization. The new *"Aetherobacter"* genus will be presented including the isolation and phylogeny of the strain and the structure elucidation of the isolated secondary metabolites together with their biological data.

PI51 Antagonistic effects of honeybee microflora metabolites against *Paenibacillus larvae*, The cause of American foulbrood disease

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American foulbrood disease (AFB) is one of the most serious honeybee diseases. AFB is capable to destroy the entire colony. Without appropriate management and therapy, AFB would spread quickly to whole farm by beekeepers and robber bees. AFB presents a worldwide distribution and causes severe economic losses to the beekeeping industry. So far, burning contaminated hives and treatment of colonies with antibiotics have been applied on controlling AFB. However, it is become more difficult to control AFB because the antibiotics resistant strains have spread worldwide. The overall goal of this study is to apply biological control strategy or biopesticides on AFB control. We isolate the microflora of honeybee guts and screen their antagonistic effects against *Paenibacillus larvae*. Multiple active metabolites have been identified from the strain BE74 via *in vitro* anti-AFB activity-guided fractionation and isolation. The results suggest the strain BE74 is a potential biopesticide on AFB control.

PI52 Identification of polyether antibiotics produced by the endophyte Streptomyces platensis RTd22

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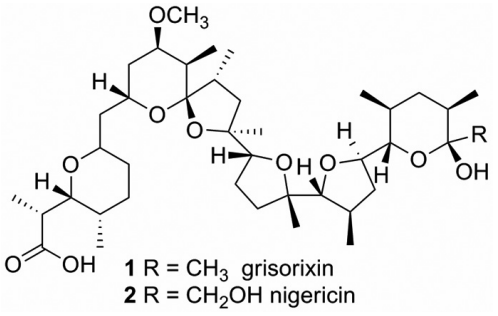
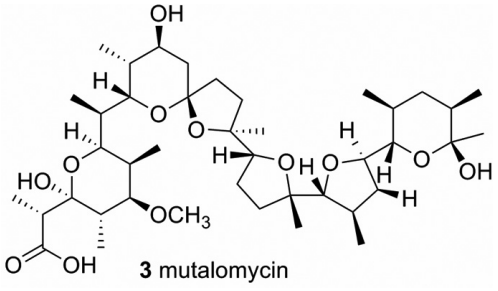
The endophytic actimomycete *S. platensis* RTd22 was cultured in rice solid medium and shaken ISP-2 liquid medium for different periods of time. Extracts and sub-fractions obtained after the chromatographic procedures showed remarkable activity against *Staphylococcus aureus* ATCC 6538, *S. saprophyticus* ATCC 15305 and *S. aureus* ATCC 29213. Some samples from both culture media also showed high activity against the antibiotic-resistant bacteria: vancomycin-intermediate *S. aureus* Mu50 (VISA), methicillin-resistant *S. aureus* SA16 (MRSA) and vancomycin-resistant *Enterococcus faecium* VRE16. Analyses by ESI-MS (AcquityTM UPLC/Waters) of the active fractions allowed the rapid identification of the ionophore antibiotics grisorixin (**1**), nigericin (**2**), and mutalomycin (**3**), structurally related polyether polyketides. The structural identification of **1 – 3** together with the antibiotic activities will be presented.





PI53 Effect of lactic acid bacterial extract on the elimination of antibiotic resistance of some clinical bacterial isolates

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Background & objectives: Multiple drug resistance (MDR) is a serious health problem and major challenge to global drug discovery programmes. Most of the genetic determinants that confer resistance to antibiotics are located on plasmids in bacteria. The present investigation was undertaken to investigate the ability of the extra- and intra-cellular extract of lactic acid bacteria (LAB) to cure plasmid acquiring resistance in certain clinical antibiotic- resistant bacterial isolates (*pseudomonas aeruginosa, staphylococcus areus, klebsiella penuomonia and shigella sp*). *Methods:* Transformation experiments were carried out using clinical isolates as plasmid donor and *Escherichia coli* strain HB101 (sensitive to the tested antibiotic), as recipient. Minimal inhibitory concentration (MIC) of LAB extracts was determined using the microtiter plate method. Plasmid curing activity of LAB extracts was determined by evaluating

the inability of bacterial colonies (pre treated with LAB extract for 18 h) to grow in the presence of antibiotics. The physical loss of plasmid DNA in the cured derivatives was further confirmed by agarose gel electrophoresis. *Results*: The presence of plasmid in transformants was confirmed through electrophoresis and the transformants were also tested for each antibiotic resistance already recorded for the donor isolates. Both extracts (extra – & intra-cellular extracts) inhibited the growth of the clinical isolates. Extracellular extracts exceeded 90% inhibition on some isolates. The LAB extract mediated plasmid curing resulted in the subsequent loss of antibiotic (Chl, Dox, Ery, Gm, Kaf, Lin, and Pen) resistance encoded in the plasmids as revealed by antibiotic resistance profile of cured strains. *Conclusions*: The extracellular extract of LAB may be a source of anti-plasmid (plasmid borne multiple antibiotic resistance) agents of natural origin.

PI54 Bioactivity of endophytes from traditionally used medicinal plants in New Brunswick, Canada

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Fungal endophytes have been found to contain bioactive metabolites and are an important yet largely overlooked source of new chemical entities with therapeutic potential. Our current knowledge of endophyte biology is very limited, and only a fraction of the total number of estimated fungal endophyte species have been isolated, cultured, identified and tested for bioactivity in the laboratory. This research project was focused on the isolation, identification and evaluation of the bioactivity of culturable endophytes from traditionally used Canadian medicinal plants. A total of 97 distinct endophytic strains were isolated from twelve traditionally used medicinal plants from southern New Brunswick, Canada. Morphological examination and DNA sequencing allowed the identification of 55 fungal species from 35 genera. The endophytes were evaluated for their ability to produce bioactive natural products in common laboratory bioassays. Extracts of 18 endophytes displayed cytotoxicity (from seven host plants), 11 showed bioactivity against *Candida albicans* (from five host plants), and eight displayed bioactivity against *Staphylococcus aureus* (from seven host plants). The extracts are currently being evaluated for anti-tuberculosis activity. Future work will focus on the bioassay guided fractionation of the bioactive extracts to isolate the active compounds.

PI55 Homesteadamides A – H, antiproliferative cyclic lipodecapeptides from the cultured freshwater cyanobacterium cf. *Anabaena* sp. (UIC 10035)

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Cyanobacteria are one of the well-known producers of lipopeptides. The UIC 10035 strain was obtained from the sample collected near the town of Homestead, south Florida, and designated as Cf. *Anabaena* sp. based on morphological and 16S rRNA gene sequence analyses. The extract of the cultured strain UIC 10035 showed an antiproliferative activity against MDA-MB-435 cells. Bioassay-guided fractionation of the extract led to the isolation of a series of lipopeptides, named homesteadamides A – H. The planar structures were determined by analysis of HRESIMS, tandem MS, and 1D and 2D NMR data. The stereoconfigurations were assigned by LC-MS analysis of the Marfey's derivatives after acid hydrolysis. Homesteadamides A – H exhibited antiproliferative activity against MDA-MB-435 cells with low micromolar IC_{50} values ranging between 1 and 10 μM. The structures of homestedamides A – H are characterized by the presence of a lipophilic β-amino acid and three non-standard amino acids *N*MeAsn, *O*MeThr and *D*hb (α,β-dehydro-α-aminobutyric acid).

PI56 9*S*,11*R*-(+)-Ascosalitoxin from an endophytic fungus isolated from Hintonia latiflora

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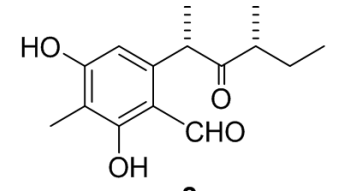
A new fungal endophytic species (*39140 – 2*) was isolated from the medicinal plant *Hintonia latiflora*, a Rubiaceae widely used in Mexico as antidiabetic agent. Endophyte *39140 – 2* was cultured at room temperature for 30 days in a Fernbach flask containing 200 g of moist rice. Chromatographic separation (Sephadex, MeOH) of an extract (1.49 g) of the culture medium yielded four major primary fractions (F_I-F_{IV}). Fraction F_{III} (372 mg) yield the known polyketide vermelhotin (**1**); TLC separation (CH_2Cl_2-MeOH 99:1) of fraction F_{II} (357.3 mg) afforded 84.9 mg of a new salycilic aldehyde derivative, namely 9*S*,11*R*-(+)-ascosalitoxin (**2**). The structure and absolute configuration was established as 9*S*,11*R* by DFT B3LYP/DGDZVP calculations which allow comparison of theoretical and experimental optical rotation of the natural product. In addition, chemical transformations of **2** yielded compounds **3** a **4**, being the latter suitable for NOESY and ^1H-^1H NMR coupling constants analyses, which reinforced the stereochemical assignment.



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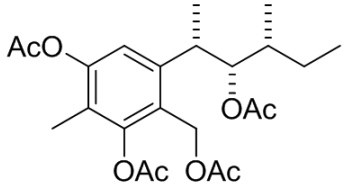

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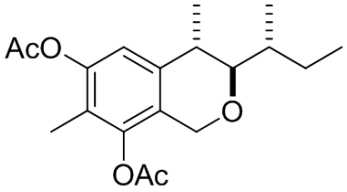

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PI57 Isolation and structural determination of cyclic lipopeptide from cultured freshwater cyanobacterium *Oscillatoria* sp. (UIC 10045)

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Cyanobacteria, commonly known as blue-green algae, have provided a variety of novel bioactive natural products. As part of our ongoing natural product drug discovery project, we evaluated organic extracts of cultured cyanobacteria for cytotoxicity against the HT-29 human colon carcinoma cell line. The organic extract of cyanobacterium *Oscillatoria* sp. (UIC 10045) displayed significant activity in this assay. LC-MS-NMR dereplication indicated the activity to be associated with a potentially new peptide. Bioassay guided fractionation led to the isolation of this peptide. The structure was determined by a combination of 1D/2D NMR spectroscopic techniques and HRESIMS as a dodeca lipopeptide with three non-standard amino acids and one β-amino acid residue. The

structure elucidation and biological activities of the compound will be presented.

PI58 Fungal natural products targeting chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults from the western world. No curative treatments of CLL are presently known so the treatment strategy today is primarily to prolong patient survival,[1] why we have initiated new activities towards discovery of novel compounds with potential tumor specificity. Our starting point is a diverse fungal collection of thousands of *Penicillium* and *Aspergillus* species. These fungi have proven to be a very rich source of various bioactive compounds and yet our dereplication investigations have demonstrated that there are still numerous unknown compounds to be identified within these species. Until now we have found that 11 out of 289 fungal extracts are active against CLL cells. Using our established chemotaxonomic discovery approach we have dereplicated and fractionated these extracts to track the activity into single fractions/compounds.[2,3] This includes analysis of the spectroscopic data generated from LC-DAD-MS to reveal whether the active principles are either structurally known compounds or are likely to be novel compounds. This paper will illustrate our integrated discovery approaches and recent findings of anti-leukemia compounds. 1. Seiffert,M. *et al. Blood* **116**, 4223 – 30 (2010). 2. Nielsen,K.F. *et al. J.f Nat. Prod.* **74**, 2338 – 48 (2011). 3. Månsson,M. *et al. J. Nat. Prod.* **73**, 1126 – 32 (2010).

PI59 Cyanobactins and anticancer bioactivity of cyanobacterial extracts

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Cyanobacteria have been found to be a prolific source of novel bioactive natural products. Among those, recent studies have put the focus on a group of small ribosomal cyclic peptides, the cyanobactins. Their reported bioactivities (including anticancer) have made this group of compounds potential as pharmaceutical leads. Apart from cyanobactins, other anticancer compounds have been isolated from cyanobacteria. Our laboratory maintains a cyanobacterial culture collection (LEGE CC) of > 350 isolates from diverse environments, representing an untapped resource of biological and chemical diversity. We have started a screening program using LEGE CC isolates in order to (1) detect the presence of genes involved in cyanobactin biosynthesis and study the phylogenetic relationship among strains; and (2) obtain crude extracts and test their ability to inhibit the anticancer targets: 20S proteasome and histone deacetylase (HDAC). Heretofore, cyanobactin related genes were found in strains belonging to three cyanobacterial orders (Oscillatoriales, Chroococcales and Nostocales). The phylogenetic analysis performed seems to reveal an uncovered diversity of these peptides since several cyanobactin gene sequences from LEGE CC strains are phylogenetically distant to the ones currently known cyanobactins. Moreover, cyanobacterial crude extracts exhibited 20S proteasome and HDAC inhibition activities, underscoring the potential for further discoveries of new natural products.

PI60 Antiparasitic screening of metagenomic libraries from two Brazilian hotspots: Cerrado and Atlantic Rainforest

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[*] Both authors contributed equally to this work. The soil metagenome from the Brazilian hotspots "cerrado" and Atlantic rainforest were explored to search for bioactive natural products using an anti-Leishmania assay. Two libraries were constructed and tested: one from cerrado soil (500.000 clones), and other from Atlantic rainforest soil (250.000 clones), both in Escherichia coli strain EC300. Screenings against Leishmania major promastigotes (strain MHOM/IL/81/Friedlin) were carried out: libraries were cultivated in plates, followed by L. major overlay. No leishmanicidal activity was observed for the Atlantic rainforest library. For the "cerrado" library 84 L. major growth inhibition halos were observed and six of them were confirmed upon retesting. These six clones were cultivated for chemical profiling by HPLC-DAD and HPLC-MS and are being submitted to DNA sequencing. Therefore, this work shows that the metagenomic approach is a useful tool for searching antiparasitic molecules. The libraries construction, biological results and chemical profiling of positive clones will be presented.

PI61 New cyclyc peptides from marine actinomycetes isolated from Jeju Island in the Republic of Korea

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Marine microorganisms have been further highlighted recently as unique sources of novel small molecules with useful pharmaceutical potential. In our search for novel bioactive molecules, we collected deep-sea mud as well as seashore sediments around Jeju Island, Republic of Korea. Through extensive isolation and chemical screening of marine actinomycete strains from these sea samples, we discovered two strains, SNJ013 and SNJ042, producing structurally-novel peptides. Sungsanamide from SNJ013, collected from a deep-sea sediment sample at a depth of 140 m, was identified as a new lasso peptide with a knotted arrangement. The sequence of the amino acids is quite different from the previously-reported 11 lasso-peptides. New peptides from SNJ042, isolated from seashore sediment, possess 36-membered cyclic rings bearing unusual amino acids such as 6-methoxy-tryptophan and β-hydroxyphenylalanine. These cyclic peptides from SNJ042 displayed significant inhibitory activities against various pathogenic bacteria including *Micrococcus luteus* (MIC = 0.53 μM).

PI62 Four novel metabolites from a water bloom of cyanobacteria

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A sample of the cyanobacterium *Microcystis* sp. (IL-399) was collected from a water reservoir near Kibuttz Hafetz Haim, Israel, on the fall of 2008. The cyanobacterial cell mass was freeze-dried and extracted with a 7:3 MeOH/H_2O solution. The crude extract was separated using various chromatographic methods including flash-chromatography on an ODS column, gel-filtration on Sephadex LH-20 column and finally reversed-phase HPLC, to afford four new protease inhibitors: Micropeptin HH978, micropeptin HH960, micropeptin HH992 and aeruginosin HH553. The structures of the pure compounds were elucidated using 1D and 2D NMR techniques, as well as, high-resolution mass spectrometry. The stereochemistry of the new natural product was determined using Marfey's method for amino acid and chiral HPLC. The inhibitory activity of the natural products was determined for the serine proteases – trypsin and chymotrypsin. The structure elucidation and biological activity of the new natural products will be presented.

PI63 Structure and biological activity of a cyclic lipodepsipeptide phaeofungin discovered with the *Candida albicans* fitness test

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Phaeofungin, a cyclic lipodepsipeptide, was isolated from a fermentation a *Phaeosphaeria* sp. (Phaeosphaeriaceae) from Spain. Antibiosis of the extract was recognized in an assay for *C. albicans* cell-wall perturbing agents. The extracts were chemogenomically assayed in the *C. albicans* fitness test (CaFT) that profiles the activities of antifungal agents. The CaFT profile overlapped with, but was differentiated from that of an antifungal lipodepsipeptide, phomafungin. Differences were confirmed when structure of phaeofungin was elucidated by spectroscopic methods including HRFTMS and 2D NMR. The amino acid identity and configuration were confirmed by acid hydrolysis followed by Marfey's derivatization. The antifungal activities of both lipodepsipeptides demonstrated phaeofungin synergizes with aureobasidin A and caspofungin leading to ATP leakage from cells of *C. albicans*.

PI64 Immunotoxicological safety of preparations obtained from cells of *Penicillium* species

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The cell biomass of *Penicillium chrysogenum* Thom (DSM 5753), *P. glabrum* (Wehmer) Westling (DSM 5752) and *P. roqueforti* Thom (DSM 5504) gained after fermentation is purified from culture medium components and then mechanically opened through a cell mill. The water-soluble filtrate then undergoes sterile filtration and is freeze-dried. The resulting lyophilisate is characterized by electrophoresis (SDS-PAGE), carbohydrate composition of polysaccharides and protein content. The resulted starting material is named "*e volumine cellulae*" (evc) and potentised to homeopathic dilutions or triturations used for the adjuvant treatment of microbial disorders of humans and animals. Possible immunotoxic effects after repeated oral and rectal or intradermal/dermal application were tested in various guideline studies with GLP compliance in genetic defined mice and guinea pigs. These studies include general immunotoxicity, mitogenic effects of naive T-cells, proliferation of antigen-stimulated T-cells, delayed type hypersensitivity reactivity, antigen-specific antibody production, acute systemic anaphylaxis induction, and skin sensitisation studies. It can be concluded that Penicillium chrysogenum evc (Notakehl^R, PLEO^R NOT), Penicillium glabrum evc (Quentakehl^R, PLEO^R QUENT), and Penicillium roqueforti evc (Fortakehl^R, PLEO^R FORT) can be regarded as safe in potency 5X (drops, sips, tablets), 4X (capsules) and 3X (suppositories, ointments). The immunotoxicological safety data are valid only for the investigated fungi strains as well as for the specific, GMP controlled manufacturing process.

PI65 Chemical epigenetics induce additional secondary metabolites in a filamentous fungus

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With the recent advent of complete fungi genome sequences, it has become clear that the number of gene clusters encoding for fungal secondary metabolites greatly outnumbers the known metabolites for these organisms. Small molecule epigenetic modifiers can be used to inhibit various proteins that subdue DNA transcription, upregulating the production of diverse secondary metabolites. This study tested the effect of three epigenetic modifiers, including the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA), the DNA methyltransferase inhibitor 5-azacytidine, and the proteasome inhibitor bortezomib, on the secondary metabolome of a filamentous fungus shown to produce polyketide-derived resorcylic acid lactones. A suite of growth conditions was explored, including a rice medium, the defined medium Czapek Dox, and potato dextrose broth (PDB); PDB demonstrated optimal metabolite production. The fungus (MSX 63935, Pleosporales), collected from leaf litter, was grown in PDB in the presence of several concentrations of each modifier. The organic extracts of these growths were analyzed by ultrahigh performance liquid chromatography (UPLC) and compared to a negative control growth. Each of the modifying agents induced the fungus to produce additional secondary metabolites.

PI66 Isolation of novel anti-TB cyclohexapeptides from actinomycetes

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Thirty-five thousand actinomycete extracts were screened for anti-TB activity, followed by C_{18} cartridge fractionation of 37 prioritized extracts. Based on MICs against replicating and non-replicating M. tuberculosis (Mtb), and IC_{50}s against Vero cells to generate selectivity indices, seven fractions were selected for further separation. ECUM14046, a Streptomyces hygroscopicus strain, when cultured in GSS media and extracted with ethyl acetate, yielded a fraction with potent anti-TB activity. This fraction had a well-defined thin layer chromatography (TLC) profile and was therefore further fractionated using preparative HPLC. The molecular formulas of two purified components, designated as hytramycin-V and hytramycin-I, were determined by high-resolution mass spectrometry (ESI-IT-TOF) as $C_{30}H_{51}N_9O_6$ and $C_{31}H_{53}N_9O_6$, resp. Structure elucidation by 1D/2D NMR revealed both to be cyclohexapeptides with three unusual piperazic acid moieties. The MICs against replicating and especially non-replicating Mtb fall into the range of existing anti-TB drugs, such as streptomycin and capreomycin, and were maintained against Mtb strains that represent the major global clades, as well as H_{37}Rv-isogenic strains that are resistant to individual clinical anti-TB drugs.

PI67 Screening of Aspergillus nidulans metabolites from habitat mimicking media using LC-DAD-TOFMS system

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Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark (DTU)

Fungi are a valuable source of metabolites and other bioactive compounds. These compounds are essential for human society, and it is estimated that around 49% of the drugs used to treat cancer are natural products or derived therefrom. Six different wild types of *Aspergillus nidulans* have been cultured on a range of different habitat mimicking media. Extracts from the fungi were analyzed using a LC-DAD-TOFMS system, and by screening the extracts using an in-house database of known fungal metabolites. Using this database we were able to easily select unknown compounds, and dereplicate these using the spectrometric and spectroscopic data obtained from the analysis. The Analysis revealed several metabolites not previously reported in *A. nidulans* as well as a novel metabolite.

PI68 Discovery of novel secondary metabolites in *Aspergillus aculeatus*

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Polyketides (PKs) and non-ribosomal peptides (NRPs) constitute large classes of diverse secondary metabolites (SMs) and are important sources for pharmaceuticals due to their structural diversity and wide variety of biological activities. Our investigation of the chemical profile of the industrially important black Aspergillus *Aspergillus aculeatus* by

UHPLC-DAD-HRMS has identified several SMs already known from this organism. However, several compounds could not be unambiguously dereplicated wherefore some have been selected, purified and structure elucidated by 1D and 2D NMR spectroscopy, whereby several novel secondary metabolites have been discovered. *A. aculeatus* has recently been genome-sequenced; however no genetic approaches have so far been described to facilitate genetic engineering. We here present a system for non-integrated (AMA1-based) gene expression in *A. aculeatus* based on the USER™ cloning technique. The AMA-1 based gene expression has successfully been applied to express genes in *A. aculeatus* and by this approach the function of a PKS gene has been established. Furthermore the technique was used to activate a silent cluster by expression of a transcription factor, leading to the production of two previously unknown compounds, which we propose to be of meroterpenoid origin.

PI69 DNA barcoding for fungal taxonomy: A primer for the natural products community

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Since members of the fungal kingdom play an important role in natural products research, accurate identification of these species is a critical step in revealing information about the organism under study. Based on a recent study by a multinational and multilaboratory consortium, the nuclear ribosomal internal transcribed spacer (ITS) has been selected as the DNA barcode for fungi, although recommendations for other genes where ITS might fail may be offered in the future. In this study, we sequenced the complete ITS region for species-level identification of selected fungal strains from the Mycosynthetix library that produced a range of bioactive compounds (i.e. cyclodepsipeptides, sesquiterpenoids, verticillins, tetramic acids, and resorcyclic acid lactones). These strains were previously identified using a partial region (~300 – 350 bp) of the D2 divergent domain of the large-subunit nrRNA. We compared the utility of both ribosomal markers by performing a GenBank-Blastn search and Maximum Likelihood phylogenetic analysis to determine the species identities and to predict their molecular phylogenetic affinities, respectively. In addition, we examined the morphological characteristics of the fungal strains to confirm their identity. Our results suggests that ITS has a higher probability of successful identification at the species level, while D2 was slightly poorer.

PI70 Pityriazepin, a new indole alkaloid isolated from *malassezia furfur* yeasts

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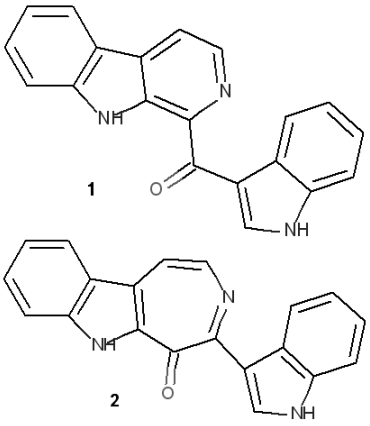
Malassezia furfur strains isolated from diseased skin preferentially biosynthesize indole alkaloids that are active Aryl-hydrocarbon Receptor (AhR) inducers. Studying the induction of AhR by *Malassezia* yeasts, we envisaged the isolation of new metabolites produced by *Malassezia* when grown on L-tryptophan agar. Chemical investigation of the EtOAc extract of a *M. furfur* strain isolated from lessional skin revealed, by HPLC/UV, the presence of a yet unknown yellow compound. Isolation with Column Chromatography and preparative TLC and then structure elucidation with NMR 600 MHz, combined with MS analysis, led us to propose for this metabolite a structure similar to that of pityriacitrin (**1**). Their difference is the transformation of pyridine to an azepine ring, where the carbonyl group is incorporated in the ring. In conclusion, this conversion afforded pityriazepin (**2**), a new natural molecule isolated from *M. furfur* yeasts. Furthermore, the fusion of indole with the azepine ring appears to offer a new natural skeleton.



PI71 Fundacion medina, a public-private model for microbial natural products drug discovery

Genilloud O
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Microbial natural products (NPs) represent one of the most successful sources of drugs to treat human diseases. Despite the current need of novel drugs, NPs discovery programs have been gradually abandoned by the big pharma, and smaller biotechnology companies and research organizations are clearly taking the lead in the discovery of novel NPs. Fundación MEDINA is a non-profit research organization established as a spin-out from the transfer of the NPs drug discovery research programs of Merck & Co in Spain, in partnership with the University of Granada and the Government of Andalucia (Spain). MEDINA leverages the experience of more than 50 years in NPs drug discovery and has inherited one of the world's largest and most productive NPs screening libraries. MEDINA is currently developing new approaches to further exploit the potential of its huge microbial resources as source of novel compounds, to mine for orphan biosynthetic pathways, or to isolate novel species thought to be uncultivable, as part of the drug discovery programs. These activities are developed in combination with high throughput chemical dereplication and semi-automated methods for the isolation and structural elucidation of novel bioactive NPs. These innovative approaches will be presented together with the new challenges faced in the current drug discovery framework to address the isolation of novel bioactive NPs and the generation of novel screening libraries enriched in chemical diversity.

PI72 Bioactive natural products from traditional Indonesian medicinal plant-associated fungi

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The rapid emergence of antibiotic-resistant pathogens has driven the discovery of new drug leads from natural sources, particularly rarely encountered microorganisms. Traditional medicinal plants have long been investigated as sources of bioactive molecules, many of which are polyketides or peptides. It is established that natural products from these structure classes are biosynthesised by microorganisms, therefore we hypothesise that many of these bioactive molecules originally isolated from plants are in fact microbial products. Thus we have initiated a genetic-based screening program of culturable endophytes to identify strains capable of producing bioactive polyketides and peptides. The rich biodiversity and traditional history of Indonesia make it an attractive target for the discovery of novel therapeutic compounds from medicinal plants and their endophytic fungi. Plant-associated fungal isolates from selected Indonesian traditional medicinal plants have been genetically screened for the presence of PKS and NRPS genes as indicators of bioactivity. Preliminary antimicrobial screening against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Mycobacterium avium* has been carried out on the organic extracts of the PKS/NRPS-containing isolates as a means of prioritising strains for downstream analyses. Bioassay-guided fractionation has been carried out on selected potent

isolates to obtain pure compounds. Chemical characterisation of these compounds will be presented.

PI73 Nucleoside antibiotic components from *Streptomyces scopuliridis* RB72

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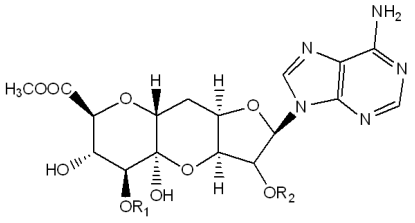
A *Streptomyces* sp. strain producing antimicrobial compounds were isolated from woodland in Daejeon, Korea and identified as *Streptomyces scopuliridis* RB72 according to 16S rRNA analysis. This strain was cultured mass production in bennet's medium with Diaion HP-20. A new nucleoside antibiotic constituents herbicidin K (**1**) and three known nucleoside antibiotic constituents herbicidin A (**2**), herbicidin B (**3**), and herbicidin F (**4**) were isolated from ethyl acetate extracts of *Streptomyces scopuliridis* RB72. The structures of **1** - **4** were elucidated by extensive MS and NMR spectroscopic methods including ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY. Antimicrobial activities of isolated compounds will be discussed.



Herbicidin K (**1**) R_1 : $CH_3CH=C(CH_3)CO$, R_2 : H
Herbicidin A (**2**) R_1 : $CH_3CH=C(CH_2OH)CO$, R_2 : Me
Herbicidin B (**3**) R_1 : H, R_2 : Me
Herbicidin F (**4**) R_1 : $CH_3CH=C(CH_3)CO$, R_2 : Me

PI74 Tyrocidine a from a haliclona sponge derived *Vibrio* sp

Noro JC[1], Kalaitzis JA[1], Williams DE[2], Dalisay DS[2], Andersen RJ[2], Neilan BA[1]
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Taxonomically diverse, sponge-associated microbial communities represent a rich source of potentially novel and bioactive natural products. In our search for bioactive compounds from sponge-associated microbes we isolated and identified a *Vibrio* sp. from a sample of *Haliclona* collected from Milne Bay, Papua New Guinea. The *Vibrio* strain was selected for further investigation on the basis of testing positive for the presence of non-ribosomal peptide synthetase (NRPS) coding genes in our PCR-based screen. Chemical investigation of this *Vibrio* sp. resulted in the isolation and identification of the NRPS product tyrocidine A, and its decapeptide structure was confirmed by 1D and 2D NMR. Tyrocidine A displayed moderate activity against methicillin resistant *S. aureus*, *E. coli* and *P. aeruginosa*. The discovery of tyrocidine A from a marine *Vibrio* sp is intriguing from a microbiological viewpoint as it has long been known to be a product of the Gram-positive, soil-dwelling and spore forming *Bacillus* spp. Aspects of this, and the notion that the marine environment is a largely untapped source of bioactive natural products will be presented.

PI75 Antioxidative and anti-bacterial activities of the essential oils from *Perilla frutescens* var. *Japonica* against some foodborne pathogenic bacteria

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To develop a new effective and safe, natural antibiotics against antibiotic-resistant food-borne pathogenic bacteria, the essential oil was extracted by steam distillation from the leaves of *P. frutescence* var. *japonica*. Its composition was analyzed by GC-MS and the activities against antibiotic-susceptible and -resistant strains of some food-born bacteria which could cause severe clinical symptom in human. In addition we have also determined the capacity of this oil to modulate the resistance of *Bacillus* and *Shigella* to antibiotics. As the results, the essential oil fraction of *P. frutescence* var. *japonica L.* and its main component, perilla ketone showed significant inhibitory activity against most of the tested strains of antibiotic-susceptible and -resistant bacteria resulting MICs ranged from 0.25~> 2.00 mg/ml. The Perilla oils combined with norfloxacin or trimethoperim/sulfamethoxazole showed synergistic or additive effects against the tested *Bacillus* and *Shigella* species with FICIs ranging from 0.15~1.00 in checkerboardtiter test.

PI76 Evaluation of the anticancer potential of marine bacteria isolated from a New Caledonia's extreme environment

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COREUS Research Unit. IRD Noumea, 101 Promenade Roger Laroque, Anse Vata B.P. A5 Noumea 98848 New Caledonia

Marine microorganisms such as bacteria or fungi are well-known for being a source of bioactive natural products with the advantage of sustainable production of these secondary metabolites. In order to evaluate their bioactivity against human cancer cell lines, we have proceeded to the isolation and characterization of 5 marine bacteria associated with a sponge, a coral, a bryozoan and a tunicate from a New Caledonia's extreme environment. Samples of macroorganisms were collected in a hydrothermal site (fluid temperature: 27 °C, pH: 11 and salinity: 2 g/L) at a bathymetry of about 38 meters. Bacterial cultures in Marine Broth were incubated at 30 °C; growth curves for each bacteria as well as gram and motility status (swarming, swimming and twitching) were determined. Mid polar and high polar automated extractions of lyophilized bacterial media were performed with dichloromethane and methanol respectively. Extracts were then screened for their biological activity using HeLa cells. Identification of these bacteria is in progress. Our work may lead to the finding of new bacterial species and/or new therapeutics agents.

PI77 Applying LC-MS de-replication strategies for the discovery of new natural products

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De-replication is a key process in the discovery of new bioactive molecules from natural sources. Fundación MEDINA extensively applies de-replication techniques on bioactive samples to focus its fermentation and isolation resources only on potentially new molecules. With the aim of discovering novel molecules with unknown activities, LC-MS de-replication was applied to a subset of non bioactive samples generated from fungi fermented in 8-medium nutritional arrays. Selected samples were analyzed by LC-LRMS, and the chromatographic components were compared against an in-house database to identify those containing potentially novel molecules in a semi-automated process that allows us to process daily a hundred samples per instrument. LC-HRMS was used to determine the molecular formula of relatively intense and unidentified peaks. When the molecular formula is not found in natural product databases, the molecule is isolated, characterized and added to our compound collection. The method's success will be illustrated by the identification and isolation of three new cyclic tetra-peptides from *Onychochola sclerotica* (Ascomycota, Arachnomycetales) that subsequently demonstrated activity as cardiac calcium channel blockers.

PI78 New phomactin analogues from an unidentified fungicolous fungal isolate obtained from the surface of a polypore

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Our ongoing search for new bioactive natural products from fungicolous fungi led to investigation of the ethyl acetate extract of cultures of an undetermined dematiaceous fungus (MYC-1907) obtained from the surface of a polypore found on a dead hardwood branch collected in Lava Tree State Park, Hawaii. The extract showed antifungal activity against *Aspergillus flavus* and antiinsectan activity against fall armyworm. Chemical investigation of this extract led to the isolation of six new phomactin analogues, in addition to four known compounds (phomactins F, I, and J and *epi*-phomactin I). Previously reported phomactins have shown potent activity as platelet activating factor (PAF) antagonists. The groß structures of the new analogues were established using various 1D and 2D NMR techniques. Relative configurations were established by interpretation of NOESY data, and absolute configurations were assigned using X-ray diffraction analysis and/or electronic circular dichroism (ECD) in conjunction with computational methods. Absolute configurations of three additional new phomactin analogues isolated in our lab from an extract of different isolate (*Mycelia sterilia* MYC-1969) were also proposed by analysis of their ECD spectra. One of the latter phomactins showed significant antifungal activity against *A. flavus* and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.*

PI79 The search for new antibiotic substances from filamentous fungi

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In an environment contaminated with high concentrations of antibiotic pharmaceuticals, bacteria develop multi-resistance more easily. Is it possible that these resistant bacteria would also accelerate the evolutionary response of the surrounding micro-flora that has to combat them for survival? And in that case, can we exploit that response to discover new antibiotics that have developed in that micro-environment? We have explored the antibacterial activity and chemistry of micro fungi from a river situated close to a wastewater treatment plant in the Indian city of Patancheru, where extreme concentrations of several pharmaceuticals, such as the broad spectrum antibiotic ciprofloxacin (28 – 31 mg/mL) and other fluoroquinolones (up to 780 µg/L) occur; [1]From the Indian river sediment, 61 filamentous fungi were isolated, cultivated and extracted. Several strains of the genus *Aspergillus* showed antimicrobial activity against susceptible and multi-resistant bacteria, such as meticillin-resistant *Staphylococcus aureus*, ESBL-producing *E. coli* and vancomycin-resistant *Enterococcus*. The highest activity was observed for *Aspergillus fumigatus*, whose extract contained the secondary metabolite gliotoxin; [2]The production of gliotoxin was triggered by the addition of lipopolysaccharides, indicating a direct correlation between bacterial structural elements and the production of antibiotic compounds; [1]D.G. Larsson *et al*., J Hazard Mater, 2007; [2]K.S. Svahn *et al.*, Infect Ecol Epid, 2012 *in press.*

PI80 Sterols of the white nose bat fungus

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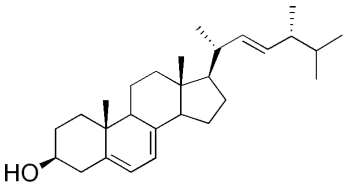
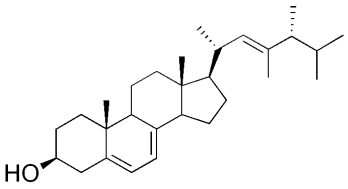
In recent years, the white nose bat fungus, *Geomyces destructans*, has decimated the bat populations of North America. Because fungal sterols and their biosynthesis are the targets of most antifungal compounds used in medicine and agriculture, a thorough sterol analysis was carried out using RP-HPLC separation and 600 MHz NMR analysis. The complete sterol analysis will be presented. While the major sterol was ergosterol, interestingly, an unknown species of *Geomyces* from a bat cave on Long Island contained a sterol with a side chain commonly found in marine dinoflagellates.



ergosterol



4-desmethyl-5,7-dehydrodinosterol

PI81 Asperjinone, a norneolignan, and terrein, a suppressor of ABCG2-expressing breast cancer cells, from *Aspergillus terreus*

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Breast cancer cells express ABCG2 transporters, which mediate multidrug resistance. Discovering a novel compound that can suppress ABCG2 expression and restore drug sensitivity could be the key to improving breast cancer therapeutics. In the current work, one new nor-neolignan, asperjinone (**1**), as well as 12 other known compounds, was isolated from Aspergillus terreus. The structure of the new isolate was determined by spectroscopic methods. Among these isolates, terrein (**2**) displayed strong cytotoxicity against breast cancer MCF-7 cells. Treatment with terrein (**2**) significantly suppressed growth of ABCG2-expressing breast cancer cells. This suppressive effect was achieved by inducing apoptosis via activating the caspase-7 pathway and inhibiting Akt signaling pathway, which led to a decrease in ABCG2-expressing cells and reduction in the side-population phenotype.

PI82 Kipukasin and oxepinamide derivatives from an undescribed *Aspergillus* sp

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In the course of our ongoing studies of fungicolous fungi, an isolate of *Aspergillus* sp. (MYC-2152 = NRRL 62124) was obtained from a basidioma of an *Inonotus* sp. found on a dead soap berry tree in a forest in Kipuka ki (Volcanoes National Park) in the Ka'u District of Hawaii. Sequencing studies later showed that this isolate was a representative of an undescribed species of *Aspergillus* Section Versicolores (GenBank Accession No. JN093265). The ethyl acetate extract obtained from solid-substrate fermentation cultures of this isolate showed showed antifungal activity against *Fusarium verticillioides* and antiinsectan activity against *Spodoptera frugiperda* (fall armyworm). Chemical investigation of this extract afforded two new kipukasin derivatives (named kipukasins H and I) and a new stereoisomer of oxepinamide E. The structures of these compounds were assigned using various 1D and 2D NMR techniques, HRESIMS, and CD spectroscopy. Nine known compounds were also encountered (sterigmatocystin, stephacidin A, 6,8-di-O-methylnidurufin, av-

rainvillamide, aspergamide A, and notoamides D, E, F, and K). The majority of the activity observed for the extract can be accounted for by high levels of sterigmatocystin, although the new oxepinamide E analogue also showed antiinsectan effects.

PI83 Verdeamides A and B from the cultured cyanobacterium *Oscillatoria* sp. (UIC 10109)

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As part of our screening program (P01CA125066), UIC 10109 strain was isolated from a sample collected from Puerto Rico (N18 °20.490' W65 °49.365') and identified as *Oscillatoria* sp. by 16S rRNA gene analysis. Repeated fractionation led to the isolation of two novel cyclic peptides containing a β-amino acid 3-amino-2,5,7,8-tetrahydroxy-10-methylundecanoic acid (Aound). The isolated compounds, verdeamides A and B, were evaluated for cytotoxicity against a set of human cancer cells as well as for inhibition of the 20S proteasome, an established target for cancer treatment. Both compounds exhibited significant cytotoxicity and 20S proteasome inhibition against SW-620, NCI-H23, and MDA-MB-435 cells with ED_{50} values ranging from 0.7 to 4.4 μM. The structure determination by analyses of 1D and 2D NMR and MS/MS fragmentation and biological activities of these peptides will be presented.

PI84 Investigation of interactions between host, pathogens, and probiotic bacteria

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The consumption and value of the eastern oyster (*Crassostrea virginica)* and pacific oyster (*Crassostrea gigas)* has increased dramatically in the last 15 years. The fisheries industry relies upon aquaculture to meet the commercial demand for oysters. Pathogen outbreaks of *Vibrio* spp. can have devastating effects on oyster production in shellfish hatcheries. Currently, probiotics are being explored as new treatments to combat the threat of infectious diseases. We recently isolated a probiotic bacterium belonging to the genus *Phaeobacter* from the inner side of the shell of a healthy oyster in Rhode Island. This bacterium significantly protects oyster larvae against infection by the shellfish pathogens *V. tubiashii and R. crassostreae in vivo*. A chemical investigation of the bacterium using HPLC-UV-bioassay, semi-preparative HPLC, MS, IR, and NMR yielded a tropolone antibiotic. Four knock-out genetic mutants were constructed to investigate the role of the tropolone in the probiotic protective effect. Chemical analysis of bacterial supernatants confirmed that the mutants lacked the ability to produce the tropolone. *In vivo* assays conducted with the mutant strains demonstrate that production of the tropolone antibiotic is required for the probiotic effects of this strain.

PI85 Bioactive constituents from *Bionectria* sp. (MSX 47401): isolation, structure elucidation and biological activities

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The extract of the filamentous fungus *Bionectria* sp. (MSX 47401) showed both promising cytotoxic activity (> 90% inhibition of H460 cell growth at 20 μg/mL) and antibacterial activity for methicillin-resistant *Staphylococcus aureus* (MRSA). A bioactivity-directed purification study led to the isolation and characterization of a new peptaibol (**1**) and three new tetramic acid derivatives (**2 – 4**), along with five known compounds, clonostachin (**5**), virgineone (**6**), virginione aglycone (**7**), AGI-7 (**8**), and tetrahydro-bisabolen triol (**9**). The structures of the new compounds were elucidated primarily by high field NMR (950 MHz), HRESI-MS/MS, and chemical degradations (Marfey's analysis). To the best of our knowledge, compounds **7** and **9** have not been isolated previously from natural sources. In addition, compound **1** represents the second petpaibol reported containing a terminal ester-linked sugar alcohol instead of an amide-linked amino alcohol. Finally, compound **8** displayed moderate cytotoxic activity, whereas compounds **2**, **3**, **6** and **7** showed promising antibacterial (including several MRSA strains) and antifungal properties.

PI86 Two benzoquinones and one terphenyl compound from an unidentified fungus (MSX 47445)

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Three bioactive compounds were isolated from an organic extract of an unidentified fungus (MSX 47445) using bioactivity-directed fractionation methodology as part of a search for anticancer leads from filamentous fungi. Of these, two were benzoquinones [betulinan A (**1**) and betulinan C (**3**)] and the third was a terphenyl compound BTH-II0204 – 207:A (**2**). The structures of the isolated compounds were elucidated using a set of spectroscopic and spectrometric techniques; the structure of the new compound (**3**) was confirmed via X-ray crystallography. Compounds (**1-3**) were evaluated for cytotoxicity against a human cancer cell panel, for antimicrobial activity against an array of bacteria and fungi, and for phosphodiesterase (PDE4B2) inhibitory activities. The putative binding mode of **1-3** with PDE4B2 was examined using a validated docking protocol, and the binding and enzyme inhibitory activities correlated.

PI87 Dihydroisocoumarins produced by *Botryosphaeria parva* an endophytic fungus *from Eugenia jambolana*

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Endophytic fungi are a rich source of new and biologically active natural products. They colonize a relatively unexplored ecological habitat and their secondary metabolism is particularly active, presumably due to metabolic interactions with their hosts. In the course of our continuing investigations of new bioactive metabolites from endophytic fungi, the crude extract of *Botryosphaeria parva* isolated from *Eugenia jambolana* was found to be active in acetylcholinesterase inhibition assay triggering further studies. The fungus was grown in Czapek for 28 days at room temperature and extracted with EtOAc. The crude ethyl acetate extract was fractioned by reversed-phase HPLC, yielding four known isocoumarins, *Rel. (3S, 4S)-4*-hydroxymellein (**1**), 5-hydroxymellein (**2**), 7-hydroxymellein (**3**) and mellein (**4**). The structures of the compounds were established on the basis of comprehensive spectral analysis, mainly using 1D and 2D NMR experiments. Dihydroisocoumarins are important metabolites founded in a wide range of organisms including bacteria, fungi and liquens. This class of substances has several biological activities, such as protease inhibitors, antimicrobial, antiallergic, antimalarics and growth regulators. This is the first report of the *B. parva* producing dihydroisocoumarins and the biological activities are under investigation. *Acknowledgements:* FAPESP, CAPES and CNPq

PI88

Antimalarials from an unidentified plant pathogenic fungus isolated from *Torreya taxifolia*

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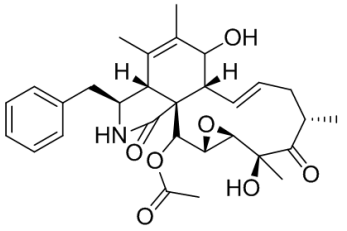
As part of our program to isolate antimalarial compounds from plant pathogenic fungi, an EtOAc extract of fermentation cultures of an unidentified fungus (UM#10 M) isolated from diseased leaves of the conifer *Torreya taxifolia* Arnott. showed potent *in vitro* antiplasmodial and good herbicidal activities. Bioassay-guided fractionation of the culture broth extract yielded two known cytochalasins, 19,20-epoxycytochalasin C (**1**), 19,20-epoxycytochalasin D (**2**), and a new analogue, 18-deoxy-19,20-epoxycytochalasin C (**3**) as active constituents. All three compounds showed potent antimalarial activity and phytotoxicity with no cytotoxicity against a panel of mammalian cell lines.



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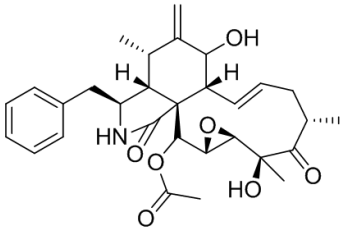

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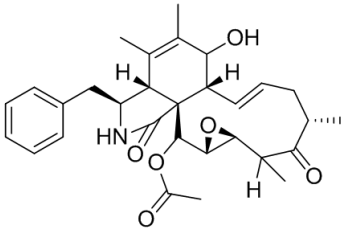

3

PI89

Bioguided antileishmanial activity from *arthrinium* state of *Apiospora montagnei* endophytic fungus extracts

Ramos HP[1], Severiano ME[2], Simão MR[2], Toledo JS[3], Cruz AK[3], Ambrósio SR[2], Said S[1]
[1]FCFRP-Universidade de São Paulo, Av. do Café s/n Ribeirão Preto, SP, Brazil; [2]Universidade de Franca, Av. Dr. Armando Sales de Oliveira, 201. Pq. Universitário, Franca, Brazil; [3]FMRP-Universidade de São Paulo, 3900 Av. Bandeirantes, Ribeirão Preto, SP, Brazil

Leishmaniasis is a public health problem lacking efficient therapies to control this protozoan disease. Endophytic fungi are promising source of bioactive secondary metabolites due in part of their close interaction with the host plants. In the present study, the endophytic fungus *Arthrinium* state of *Apiospora montagnei* isolated from *Smallanthus sonchifolius* (Asteraceae) was cultivated in a liquid medium under standardized culture condition. After that, the biomass obtained was extracted with ethyl acetate to furnish the extract codified as AmE. AmE was evaluated against three *Leishmania* promastigotes strains and showed an IC_{50} value very promising (4.87 μg/ml). In order to identify its active compounds, AmE was chromatographed over silica gel using vacuum liquid chromatography and furnished seven new fractions (AmE1-AmE7). The obtained fractions were also biologically investigated and AmE5 was the most active (0.81 μg/ml). Based on thus, AmE5 was submitted to several chromatography techniques and the two main compounds were obtained. Their chemical structures are being established by NMR data and further investigation against strains of *L. braziliensis* will be performed.

PI90

Biological and chemical assessment of vermitrasporin, an anti-TB active metabolite from the fungus MSX 105528

Hwang CH[1,2], Jaki BU[1,2], Napolitano JG[2], Lankin DC[2], McAlpine J[2], Franzblau SG[1], Cho SH[1], Pearce CJ[3], Pauli GF[1,2]
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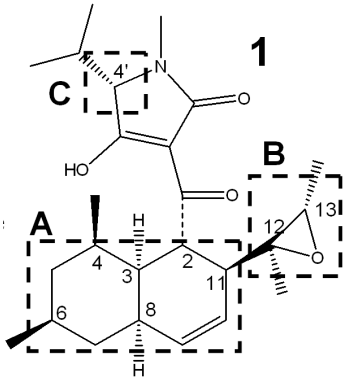
Bioassay-guided fractionation of the methanolic extract of the fungus MSX 105528 led to the isolation of vermitrasporin (**1**), an anti-TB active tetramic acid derivative containing a decalin and an oxirane moiety. Biological evaluation against *Mycobacterium tuberculosis* revealed an MIC of 0.74 μg/ml in the microplate Alamar Blue and 0.56 μg/ml in the TB bioluminescence assay. The MBC was determined to be 0.50 μg/ml. In the low oxygen recovery assay, **1** exhibited activity against non-replicating *M. tuberculosis* (MIC 1.50 μg/ml). Full ^{1}H NMR spin analysis with the PERCHit iterator established all ^{1}H chemical shifts and scalar coupling constants. Therefore, the relative configuration of the decalin moiety of **1** was established as of 2*S**, 3*R**, 4*R**, 6*S**, 8*R**, 11*S** configuration. The relative configuration of the oxirane ring was determined via NOE/ROE analysis, indicating a 12*R**, 13*R** configuration. In summary, all spectroscopic data of **1** are compatible with 8 stereoisomers generated by the three chiral clusters (**A**, **B**, and **C**). Literature data available for the known constitutional isomer, vermisporin, exhibit noticeable spectroscopic differences in both the decalin and oxirane moieties, but data limitations preclude alignment of its structure with that of **1**.



PI91

Evaluation of anti-tuberculosis secondary metabolites from Deep Lake Michigan sediment strain *Micromonospora tulbaghiae*

Wei X, Tanouye U, Carlson S, Hwang CH, Kim M, Cho S, Franzblau SG, Murphy BT
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Our research program focuses on discovering novel therapies to treat TB. Following similar principles that led researchers to turn to the ocean for drug-leads, we have expanded this exploration to freshwater habitats, to which extraordinarily little is known about the endemic microbial and chemical diversity. Upon screening fractions of secondary metabolites from freshwater actinomycetes against *M. tuberculosis* (strain H_{37}Rv), we discovered that a strain (B006) collected from deep Lake Michigan sediment produced several submicromolar anti-TB secondary metabolites. We cultivated B006 in 30 L of freshwater media, extracted the extracellular metabolites, and after several chromatographic steps we isolated six metabolites that exhibited low micromolar potency growth inhibition against a panel of drug-resistant mycobacterial strains. In addition,

one compound and a fraction containing a mixture of derivatives exhibited antibiotic activity toward a panel of the Gram-positive pathogens, though with markedly less potency when compared with activity against mycobacterial strains. The molecules are currently being further profiled with respect to selectivity, cidality, protein-mediated MIC shift and activity against a panel of *M. tuberculosis* global clade representives.

PI92 A new cyclic depsipeptide from a fungicolous Hawaiian Isolate of *Phaeoacremonium* sp

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During our continuing studies of fungicolous fungi, an isolate of *Phaeoacremonium* sp. (NRRL 54515) was obtained from black stromata of a pyrenomycete found on a dead hardwood branch in a Hawaiian forest. *Phaeoacremonium* is a relatively unexplored fungal genus from a chemical standpoint. The ethyl acetate extract of solid-substrate fermentation cultures of this isolate showed antifungal activity against *Fusarium verticillioides*. Chemical studies of this extract led to isolation of a new cyclic depsipeptide. The structure of this metabolite was assigned based on analysis of 2D NMR and HRESITOFMSMS data for the compound and its acetylation product. Several N-methyl amino acyl units were present, but the most distinctive structural feature was the incorporation of a five-carbon sugar unit which was identified and located by 2D NMR analysis of the natural product. Its presence and identity were confirmed by GCMS analysis of the corresponding derivatized hydrolysis product in comparison with standards. GCMS analysis of N-trifluoroacetyl-*s*-butyl ester derivatives of the amino acids obtained upon hydrolysis confirmed the amino acid composition and enabled assignment of their absolute configurations. To our knowledge, this metabolite is the first peptide-type natural product to be reported from a member of the genus *Phaeoacremonium*.

PI93 Active compounds against plasmodium falciparum isolated from Lentinus sp

Guizado J[1], Hoffman T[2], Piepenbring M[3], Cubilla-Rios L[1]
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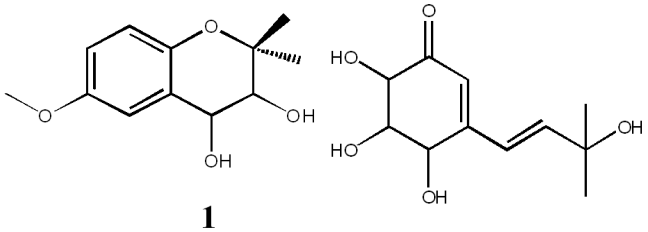
One hundred and thirty two samples of macro and micro fungal strains have been collected as part of an inventory and to investigate their capability to produced active substances against tropical diseases, bacteria and plant pathogenic fungi. After their identification and literature analysis only 13 fungi were selected for further work; the fungal strain *Lentinus sp*. (LC46) was cultivated in PDA and MEA. Both extracts (AcOEt) showed a promising activity against *P. falciparum* (100% GI) and *T. cruzi* (82% GI) at 10 µg/mL. Six compounds have been isolated from the extract obtained from the culture in MEA, the determination of their activity is under evaluation. Two of these compounds have been reported previously **1**, **2** (Nat Prod Commun 2009, 4: 501 – 506.); we present here two tentative structure for the new compounds **3** and **4**.



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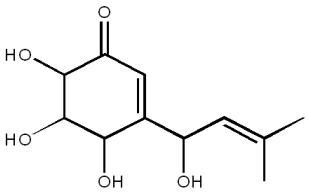

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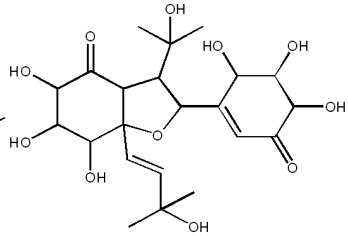

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Acknowledgement: SENACYT grant Col 10 – 060

PI94 Metabolomic manipulation of marine fungi through epigenetic modification via histone deacetylase inhibition

Demers D[1], Beau J[1], Mutka T[2], Kyle D[2], Baker BJ[1]
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Recent studies have shown marine fungi to be interesting targets of natural product investigation due to their ability to be grown under a variety of conditions that alter secondary metabolite production. Epigenetic modification via the histone deacetylase (HDAC) inhibitor sodium butyrate has been shown to increase production of metabolites of interest as well as previously unidentified compounds. In the spring of 2012, a collection of endophytic fungi was isolated from Florida mangroves. Crude extracts of these isolates were submitted for bioassay against infectious diseases including malaria and methicillin resistant *Staphylococcus aureus* (MRSA). This study illustrates examples in which modified versus unmodified extracts yielded both differing bioassay results and metabolomics profiles.

PI95 Enhanced secondary metabolite production by microbial co-cultures

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Microorganisms are a rich source of bioactive natural products beneficial in drug discovery. Competitive interactions are a prominent factor in secondary metabolite production. However, typical isolated cultivation techniques do not harness the complete chemical diversity found in their natural environment. Recently, microbial mixed fermentations have been employed to increase yields of previously described metabolites and induce production of novel secondary metabolites. Isolation of a marine endophytic fungus from a *Xestospongia muta* sponge has been shown to inhibit the growth of *Aspergillus niger*, a common foodstuff and laboratory mold contaminant. A co-culture technique of both fungi was utilized and lead to increased metabolite production from pure cultures. The research herein describes the methods and preliminary results from the co-cultures.

PI96 New lapatin analogues from a fungicolous isolate of *Aspergillus* sp

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[1]Department of Chemistry, University of Iowa, Iowa City, IA 52242; [2]Bacterial Foodborne Pathogens & Mycology Research Unit, Agricultural Research Service, National Center for Agricultural Utilization Research, USDA, Peoria, IL 61604

As part of our continuing search for new bioactive fungal natural products, analysis of an ethyl acetate extract from cultures of a fungicolous fungal isolate obtained in Hawaii (*Aspergillus* sp.; MYC-2075) was undertaken. This extract was selected for investigation because it showed significant activity in dietary assays against fall armyworm. Chemical studies of this extract yielded two new lapatin analogues and several known compounds, including sterigmatocystin and aurantiamine. Lapatins are members of the quinazoline class of fungal metabolites. Other

members of this group, such as spiroquinazoline, alantrypinone, and serantrypinone, are known to display various kinds of effects, including activity as substance P and GABA receptor antagonists. The two new metabolites were identified as lapatin analogues on the basis of similarities between their NMR and HRESIMS data and those reported for lapatins A and B. The structures of these compounds were assigned through the use of 2D NMR techniques.

PI97 Antiproliferative extracts from algae and cyanobacteria found in acidic mine drainage sites

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As part of an ongoing investigation of the bioactivity of natural products found within the highly-biodiverse southern Appalachian region of the United States, samples of algae and cyanobacteria were collected from an acid mine drainage site. Organisms were grown in pH adjusted BG-11 medium, and extracted with DCM/MeOH, and fractionated using Diaion HP-20 with an IPA gradient. Extracts of the samples were evaluated for antiproliferative activity against a mammalian cancer cell line. Thirty percent of all fractions examined to date have moderate antiproliferative activity (IC_{50} < 200 µg/mL) against JEG-3 cells. Of those, ten percent exhibit IC_{50} values ranging from 50 – 100 µg/mL and approximately five percent have significant activity (IC_{50} < 25 µg/mL). Additional fractionation of active fractions using HPLC is ongoing as are activity assays against prokaryotic microorganisms.

PI98 Secondary metabolites from *Colwellia psychrerythraea* 34 H, a cold-obligate Arctic bacterium

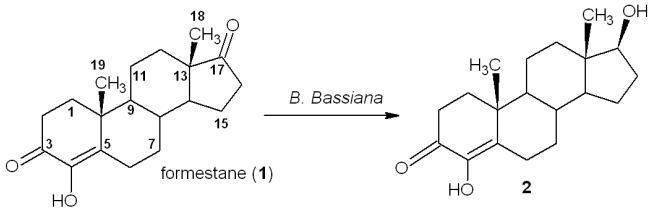
Liu J, Balunas MJ
Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269 USA

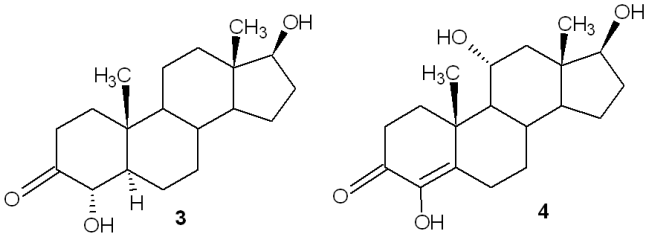
Very little research has been done on natural products from extreme environments, including from the Arctic and sub-Arctic. However, those environments could provide new resources for natural products with potential for encountering novel structures and/or interesting biological activity. To explore the ability of cold-obligate bacteria to produce diverse secondary metabolites, we have been investigating *Colwellia psychrerythraea* 34 H, a psychrophilic bacterium from the Arctic. The full genome sequence of this organism revealed the biosynthetic potential for production of secondary metabolites. Extracts of *C. psychrerythraea* 34 H have shown potent growth inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA). Activity-guided isolation and identification of new biologically active compounds will be discussed.

PI99 Biocoversion of formestane by *Beauveria bassiana* ATCC 7159

Narvaez J, Martin GDA
Department of Chemistry, Biochemistry and Physics, The University of Tampa, 401 West Kennedy Boulevard, Tampa, FL 33606, USA

The bioconversion of 4-hydroxyandrost-4-ene-3,17-dione (formestane) **1** with *Beauveria bassiana* yielded the reduced 4,17β-dihydroxyandrost-4-en-3-one **2**, 4α,17β-dihydroxyandrostan-3-one **3**, and 4,11α,17β-trihydroxyandrost-4-en-3-one **4**. All the metabolites showed significant cytotoxicity activities against the MCF-7 breast cancer cell lines. The bioactivities and structural elucidation of these metabolites by chemical and spectroscopic means are reported herein.





PI100 The discovery of mangosteen (*Garcinia mangostana* L.) leaves extracts exhibiting anti-quorum sensing properties

Chong YM[1], Yin WF[1], Chan KG[1]
[1]Division of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Quorum sensing (QS) refers to the bacterial cell-to-cell communication mechanism that coordinates different behaviours as the population has reached a high cell density. Previous research has shown that higher plants can produce compounds that can attenuate the bacterial pathogenicity by mimicking the chemical structures of the bacterial QS signalling molecules. As an example, our group has previously showed that malabaricone C extracted from the Malaysian *Myristica cinnamomea* attenuate the QS-mediated virulence determinants in the pathogenic bacteria. This study aimed to determine the anti-QS activities of the mangosteen (*Garcinia mangostana* L.) leaves extracts. By using the bioassay guided isolation, the sample was found to inhibit the violacein production in *Chromobacterium violaceum* CV026 as well as inhibit the selected virulence determinants of *Pseudomonas aeruginosa* PA01. Our data validate the use of higher plants as rich source of anti-QS compounds that can attenuate the bacteria virulence which may be valuable source for novel drug discovery. To the best of our knowledge, this is the first documentation reporting the anti-QS properties of the *G. mangostana* L., which bears edible fruits, are the favourite fruits by the locals.

PI101 Antibacterial effects of essential oils from *Anthricus sylvestris* against antibiotic-susceptible and -resistant bacteria

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The increased incidence of various clinical symptoms by antibiotic-resistant food-born bacteria have been more and more important issues, especially associated with the extensive use of antibiotics to cattle, fishes and etc. against antibiotic-resistant food-borne pathogenic bacteria, the essential oil was extracted by steam distillation from the roots of *A. sylvestris* and its activity was evaluated to develop a new effective and safe, natural antibiotics. Its composition was analyzed by GC-MS and the activities against antibiotic-susceptible and -resistant strains of some food-born bacteria. The synergism of this oil combined with antibiotic was determined by checkerboardtiter test. Mysristicin and caryophyllene were found as main components of this oil by GC-MS analysis. The essential oil fraction of *A. sylvestris* against most of the tested strains of antibiotic-susceptible and -resistant bacteria resulting MICs ranged from 0.5~> 4.00 mg/ml. However, both of the main components of this oil showed relatively mild or no activity. The *A. sylvestris* oil fraction combined with antibiotics showed synergistic or additive effects depending on the tested species of bacteria.

PI102 Anticancer plant garden

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[1]Association JPAC, Saint-Pol-de-Léon, France; [2]Laboratoire SPH, Bordeaux University, Talence; France; [3]ManRos Therapeutics, Perharidy, Roscoff, France

Cancer is a major health concern in the world. Consequently there is considerable interest from the population in the progress of research on new therapeutic drugs. It is one of scientists' tasks to make current research understandable to everybody and to share their knowledge with the public. To this aim we have created the "Anticancer Plant Garden", a pedagogic garden located in Saint-Pol-de-Léon, Brittany,

France. Its goals are to illustrate the importance of plants as a source of potent anticancer drugs, to enhance the public awareness on the interest in protecting and studying Nature as a unique, yet fragile, source of novel medications, and to tell the emulating stories of researchers who discovered natural products based therapeutic drugs. The garden will present plants from which molecules are currently used in cancer chemotherapy, plants with antitumor properties still under investigation, plants used as adjuvants, plants which are reported to provide some prevention towards the development of cancers and plants producing carcinogenic compounds. The project is accompanied by a website, a booklet which will present plants, active principles and brief history of their discovery, and a more detailed book on potential anticancer plants. The garden will also accommodate conferences and photography exhibits on plants, cancer issues, prevention and protection of natural resources. The project communication will be carried out by a PhD student preparing a thesis on the discovery of the anticancer drugs Taxotère® and Navelbine®. The garden is supported by the "Association JPAC". Information is validated by several scientific experts.

PI103 Luteolin prevents UV-induced skin damage and MMP-1 activation by interfering with the P38-MAPK pathway and IL-20 release

Wölfle U[1], Heinemann A[2], Esser PR[3], Haarhaus B[1], Martin SF[3], Schempp CM[1]
[1]Competence Center skintegral,; [2]Molecular Dermatology; [3]Allergy Research Group, Department of Dermatology, University Medical Center Freiburg,

Ultraviolet radiation induces DNA damage, oxidative stress and extracellar matrix degradation which can result in skin inflammation, photoaging and photocarcinogenesis. The flavonoid luteolin is a potent antioxidant that is present in higher amounts in the dyers weld, Reseda luteola. We investigated UV-protective and antioxidant properties of a luteolin-rich dry extract from Reseda luteola (RL) in human keratinocytes in vitro, ex vivo and in vivo. Furthermore, we investigated direct and indirect effects of RL on dermal fibroblasts as major targets of photoaging. Spectrophotometric measurements revealed extinction maxima of RL in the UVB and UVA range. UV transmission below 370 nm was < 10%. In human skin, RL effectively reduced the formation of UVB-induced cyclobutane pyrimidine dimers. The antioxidative activity of RL was assessed in the H_2DCFDA-assay performed with UVB-irradiated keratinocytes. RL was much more effective (EC_{50} 3 µg/ml) than N-acetylcysteine (EC_{50} 847 µg/ml). RL also inhibited UVB-induced skin erythema as well as cyclooxygenase-2 upregulation and PGE_2 expression in human skin. Next, we assessed the role of conditioned supernatants from keratinocytes irradiated with solar simulated radiation (SSR) on non-irradiated dermal fibroblasts. In keratinocytes, RL inhibited SSR-induced production of the proinflammatory cytokine IL-20, that is associated with skin aging via interference with the p38 MAPK pathway. Similarly, keratinocyte supernatant-induced IL-6 and MMP-1 expression in fibroblasts was reduced by pre-treatment of keratinocytes with RL. These data suggest that RL may protect human skin from UV-induced damage by a combination of UV-absorbing, DNA protective, antioxidant, anti-inflammatory and extracellular matrix protecting properties.

PI104 ISolation, characterization and biological screening of artemisinin from *A. annua* grown in Nigeria

Jegede IA[1], Okpako L[2], Orisadipe A[1], Okhale S[1], Okogun J[1], Ibrahim H[3], Ilyas N[3], Gamaniel K[1]
[1]National Institute for Pharmaceutical Research and Development (NIPRD) PMB 21, Garki, Abuja, Nigeria; [2]Department of Life Sciences, University of Bradford, UK; [3]Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria

Artemisia annua is currently being cultivated in Nigeria in commercial quantity. Using a modified method[1], Artemisinin was isolated from plants growing in Abuja, Nigeria and characterized along with antimalarial screening. This is towards developing a monograph and providing scientific evidence to Pharmaceutical Industry in the country for the local production of ACTs to combat malaria. The result of these studies will be presented along with the Pharmacognostic investigations of *Artemisia annua* plant material. Antiplasmodial activity (in vitro) of the isolated Artemisinin against chloroquine resistant *Plasmodium falciparum* (strain K1) using parasite lactate dehydrogenase (pLDH) assay[2] yielded a comparable antimalarial activity to the reference drug. Efforts are currently on to determine the artemisinin contents in the different *A. annua* biomasses obtained from different pilot farms in Nigeria. These results along with others being compiled are aimed at encouraging local production of ACTs in the country as Nigeria strives to meet the Millennium Development Goals (MDGs). 1.D.L Klayman et al. (1984). J. of Natural Products. 47,715. 2. Makler, M.T. et al. (1993). American Jour. of Tropical Medicine & Hygiene **48**(6): 739 – 741.

PI105 Theaflavin (TF), chief flavanoid of black tea protect adjuvant induced rheumatoid arthritis in experimental animal models

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Laboratory of Toxinology & Experimental Pharmacodynamics, Department of Physiology, Calcutta University, Kolkata, India

Objectives: Evaluating the anti-arthritic activity of Theaflavin. **Materials and Method:** Rheumatoid Arthritis (RA) was induced by Freund's complete adjuvant. **Subjects:** Male albino Wistar rats (120 ± 10 g), groups: **I**- Sham control, **II**- Arthritis control, **III**- Standard drug, **IV**- TF treated (0.01 mg $100gm^{-1}$ ip for 14 days), **V**- TF treated (0.05 mg $100gm^{-1}$ ip for 14 days). Anti-arthritic activity of TF was examined through physical, urinary, serum, synovial fluid parameters, histological structure, X ray of joints & bone minerals content. Institutional Animal Ethical Committee approved all animal experiments. Results were expressed in terms of mean ± SEM (n = 6) and Level of Significance determined through One Way ANOVA ($P < 0.05$). **Results:** Ankle & paw diameter changed significantly in arthritic group compared to Group **I** and this change was significantly restored in group **IV & V**. Urinary markers hydroxyproline, glucosamine, pyridoline and deoxypridoline levels were significantly restored in Group **IV & V**. Serum enzymes (ACP/ALP), cytokines (osteocalcin, IL 6, TNFα, IL 10, IL 12), synovial fluid cytokines, antioxidant markers, bone ash minerals (Ca^{++}, P and Na) were restored significantly in Group **IV** & **V**. Histological and X-Ray studies showed significant effects. **Conclusion:** Findings showed that TF possess distinct anti-arthritic activity.

PI106 The essential oils of some medicinal plants on the immune system of rainbow trout (*Oncorhynchus mykiss*)

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[1]Shahrekord Branch, Islamic Azad University, Research Center of Medicinal Plants and Ethno-veterinary, Shahrekord, Iran; [2]Management of Aquaculture, Agricultural Organization, Chaharmahal va Bakhtiari Province, Iran; [3]Department of Animal Husbandry, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran; [4]Department of Fish Health, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

A large number of plants and their isolated constituents have been shown to potentiate immunity [1 – 2]. In present study, the essential oils of five species from were used to determinate the effects on immune system in rainbow trout (*Oncorhynchus mykiss*). The experimental in a completely randomized design with 7 treatments and 3 replicates and each replicate fish were divided into 12 pieces. The experimental treatments were: essential oils of *Satureja bachtiarica* Bunge, *Thymus daenensis* Celak, *Satureja khuzestanica* Jamzad, *Dracocephalum multicaule* Benth, *Mentha longifolia* L, normal diet + olive oil, normal diet (control). The results showed significant different ($p \leq 0.05$) between almost characteristics of immune system rainbow trout. The results of showed that the highest levels of immune factors (the percentage of phagocytosis, number and rate of crime phagosite Igm) relating to essential oils of *M. longifolia*, *S. khuzestanica* and *D. multicaule*. In final, the use of essential oils and herbs, especially the *M. longifolia* and *S. bachtiarica* improve the status indicator. **Key word:** *Mentha longifolia*, *Satureja bachtiarica,* immune system, *Oncorhynchus mykiss* [1] Savnur, H.V. 1950. A handbook of Ayurvedic materia medica with principles of pharmacology and therapeutics. Belagaum: Dr. Jaghat & Sons. [2] Zargari, A. 1989 – 1992. Medicinal Plants. Vol. 1 – 6. University Publication, Tehran, Iran.

PI107 Hexane extract of the seeds oy Byrsonima crassifolia accelerates wound healing in streptozotocin-induced diabetic ratas

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[1]Department of Biotechnology and Bioengineering, Cinvestav-IPN, Av. IPN 2508, Col. San Pedro Zacatenco, Mexico D.F, CP 07360; [2]Research Laboratory of Natural Products. School of Chemical Engineering and Extractive Industries-IPN. Unidad Profesional Adolfo Lopez Mateos, Zacatenco, CP 07758, Mexico D.F

Seeds of *Byrsonima crassifoli*a has been shown to possess wound healing activity. Therefore, it may be worthwhile to study the effect of *B. crassifoli*a in diabetic wound healing. Wound healing potential of hexane extract (NS) in the form of simple ointment for treatment of dermal wounds was studied in streptozotocin-induced diabetic rats on excision wound, incision wound and dead space wound. Various parameters as epithelization period, scar area, tensile strength, hydroxyproline, total protein, DNA, hexosamine, uronic acid, antioxidant enzymes (SOD and CAT) were used to evaluate the effect of *B. crassifolia* on wound healing. NS in form topical accelerates the wound healing process by decreasing the surface area of the wound with a significant increase in the rate of wound contraction and tensile strength. Increase granulation tissue dry weight, hydroxyproline, total protein, DNA and SOD and CAT when compared to diabetic control. Our results demonstrated for the first time that *Byrsonima crassifolia* was effective in promoting diabetic wound healing in rats through the processes of tissue regeneration.

PI108 Development of RP-HPLC method for the estimation of β-amyrin and syringic acid from *Marigold* flower

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Marigold (*Tagetes erecta* L.; Family: Asteraceae) has been reported for several pharmacological activities. This experiment was designed to develop a reverse phase HPLC method according to ICH and FDA guidelines to assure the quality standard of marigold flower for medicinal uses and to find out the content of its biomarkers. β-amyrin (yield 0.3% w/w) and syringic acid (yield 4% w/w) were isolated from methanol extract of flower; dissolved in HPLC mobile phase, subsequently diluted to 200 – 1000 µg/mL and used as external standards. Methanol and water acidified with 1% acetic acid (20:80 v/v) was optimized as mobile phase. The Waters Spherisorb 5 µm ODS 2, 250 x 4.6 mm column was used along with isocratic elution for optimum separation and detection at 280 nm. Retention time of β-amyrin and syringic acid were found to be 12.95 and 23.01 min. The response was linear with good correlation between concentration and mean peak area with the coefficient of determinants (r^2) > 0.99. β-amyrin and syringic acid contents in methanol extract were found to be 0.06 and 2.30% w/w and mean % recoveries were calculated to be 99.88 ± 0.07 and 99.60 ± 0.20 respectively. The % RSD of intra and inter-day precision were < 2.0%. The LOD & LOQ were 30.23 µg/mL and 94.75 µg/mL for β-amyrin and 44.13 µg/mL and 176.59 µg/mL for syringic acid. However, it can be concluded that HPLC standardization of marigold with respect to β-amyrin and syringic acid is properly validated as simple, accurate, specific, precise and reproducible method.

PI109 Cytotoxic studies of the essential oils of *Petiveria alliacea* in colon cancer cells

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Tropical plants found in Puerto Rico are popular for their different therapeutic effects. People use to drink teas made from some of these plants as a treatment of different maladies. There is no evidence that these plants have therapeutic effects. This research is focused on trying to find scientific evidence that support the anti-carcinogenic effects of *Petiveria alliacea*. The extracts of the plant are obtained by extraction with dichloromethane. The extracts are then incubated with HT-29 cells (colon cancer cells). Cytotoxic assays will be evaluated quantitatively, measuring cells viability with UV-Vis in the microplate reader with MTT assay. Isolation, characterization and evaluation of the different compounds in essential oils extracts of *Petiveria Alliacea* will be made.

PI110 Desmutagenic and antimutagenic potential of Khaya grandifoliola (C.DC.) Meliaceae

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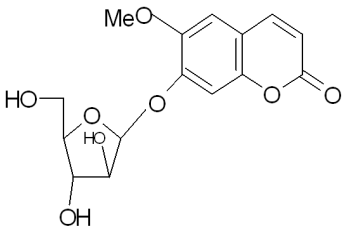
Five phenolics were isolated for the first time from the ethanol extract of *Khaya grandifoliola* leaves. These compounds were identified [1] using spectroscopic analysis (UV, ^{1}H-NMR, ^{13}C-NMR and ESI) as Quercetin 3-*O*-rhamnoglucoside (rutin), Quercetin 3-*O*-rhamnoside, Quercetin 3-*O*-glucoside, Quercetin aglycon and the new 6- methoxycoumarin, 7- arabinofuranoside. Desmutagenic and antimutagenic [2] activities of specimen extracts of immaculate *khaya grandifoliola* leaves and flowers were ascertained by measuring the inhibition of *Salmonella typhimurium* TA 100 His$^+$ revertants induced by ethyl methane sulphonate EMS and ribose lysine RL. A frustration of the induced reversion was observed. The alcoholic extract of both leaves and flowers of *khaya grandifoliola* exhibited desmutagenic and antimutagenic activity against EMS and RL induced reversion.



6- methoxy, coumarin-7-arabinofuranoside

PI111 Ring A, D-SECO limonoids and flavonoid from the Kenyan *Vepris uguenensis* Engl. and their antioxidant activity

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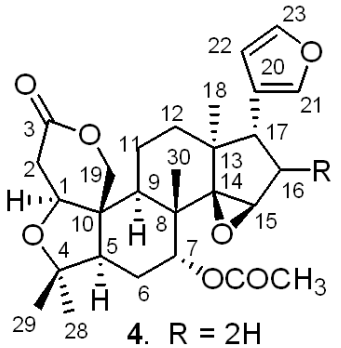
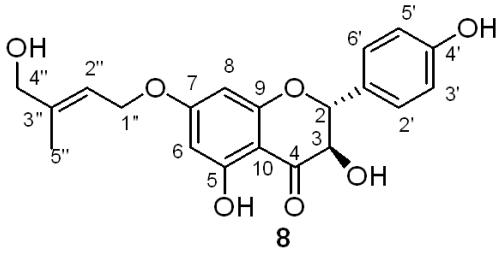
Two new A, D-*seco*-limonoids, accorded the trivial names, uguenensene (**4**) and uguenensone (**5**) and a new C-7 prenylated flavonoid, uguenenprenol (**8**) were isolated from *Vepris uguenensis* (*Rutaceae*). In addition, eleven known compounds, niloticin (**1**), chisocheton A (**2**), kihadalactone A (**3**), limonyl acetate (**6**), methyl uguenenoate (**7**), 7-O-methylaromadenrin (**9**), flindersiamine (**10**), 8α,11-elemodiol (**11**), tricoccin S_{13} acetate, skimmianine, and lupeol were isolated. Antioxidant activity of the isolated compounds showed that **8** and **9** were good antioxidant agents. Significantly high antioxidant activity was also exhibited by 8α,11-elemodiol (**11**). The two new limonoids fit nicely into a biosynthetic scheme with other limonoids isolated from the plant.





PI112 Anti-inflammatory activity and toxicity of the water extract of *Terminalia chebula* rezt in rats

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Terminalia chebula Retz. is a plant in the family Combretaceae known as in Thailand "Sa Maw Thai". The aim of this study was to investigate anti-inflammatory activity and toxicity of the water extract of *T. chebula* fruit prepared according to Thai Herbal Pharmacopoeia. Oral administration of *T. chebula* extract at the doses of 150, 300 and 600 mg/kg caused dose-dependent inhibition of carrageenan-induced acute inflammation (Table 1). Chronic inflammation, *T. chebula* at 600 mg/kg did not reduce both transudative and proliferative phases, body weight gain and thymus weight in the cotton pellet-induced granuloma formation. Inhibitory effect on the synthesis and/or release of inflammatory mediators, especially prostaglandins, may be the main mechanisms of action of *T. chebula* water extract. In addition, *T. chebula* water extract produced neither acute (LD_{50} > 5,000 mg/kg) nor chronic oral toxicity. The extrapolation of these results to humans suggests that *T. chebula* water extract is considered safe for usage at the doses of 300, 600 and 1,200 mg/kg/day. *Acknowledgements:* Royal Golden Jubilee Ph.D. Program and the National Research Council of Thailand.

PI113 Bioguided identification of anticancer and antifungal substances from the South Brazilian orchid *Miltonia flavescens*

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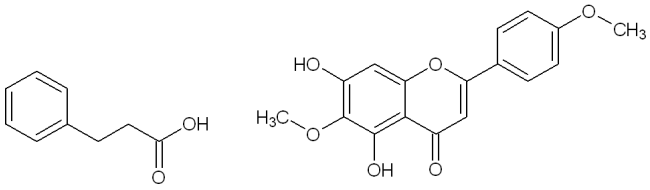
The Orchidaceae family is appreciated worldwide due to the beauty of their flowers. In this context, Brazil is a continental country pleased with the occurrence of hundreds of species. However, little is known about the potential of orchids for therapeutically important studies. Therefore, this study aimed the isolation of bioactive molecules from South Brazilian orchid *Miltonia flavescens* Lindl.. Bioguided studies with the fungus *Cladosporium herbarum* allowed the identification of hidrocinnamic acid as the antifungal active substance. In addition, chloroform partition (CP) showed an interesting activity against cancer cell lines. CP fractionation afforded the isolation of the flavonoid hortensin, wich was active against seven human cancer cell lines, been selective for MCF-7 breast carcinoma with GI50 = 6.8 μg/mL. Acknowledgement: Fundação Araucária (Brazil).



PI114 Two new compounds from Aster yomena

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Aster yomena Makino (Asteraceae) is a perennial herb that is distributed throughout Korea and Japan. The whole plant is used in Korean traditional medicine for the treatment of bronchial asthma, inflammation, and cold. In addition, phytochemical and biological studies of this plant have not been reported so far Herein, we report the isolation and structural elucidation of a new megastigmane palmitate, 5(13)-megastigmen-9-one-3β-palmitate (**1**), and a new oleanane-type triterpenoid, 3β, 23, 28-trihydroxy-12-oleanene-11-one (**2**), together with three known oleanane-type triterpenoids, β-amyrin (**3**), erythrodiol (**4**), and 3β, 23, 28-triol olean-12-ene (**5**) were isolated. Of these compounds, oleanane-type triterpenoids **3** – **5** were isolated from this plant for the first time. Their structures were identified on the basis of 1D and 2D NMR, including ^1H-^1H COSY, HSQC, HMBC, and NOESY spectroscopic analyses.

PI115 Extraction and characterization of the fixed oil of seeds of *Buchholzia corieacea* Engl (Capparaceae)

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The *Buchholzia coriacea* seed oil was extracted using light petroleum ether (60 – 80 ^{0}C) by soxhlet apparatus. The physiocochemical properties and fatty acid composition of the *B. corieacea* seed oil were determined. Preliminary phytochemcial analysis of the seed meal was also carried out. The seed consist of 6.48% yield (dry %) oil. The physiocochemical properties shows acid value (1.18 mg KOH/g oil), iodine value (249.57 mg iodine/g oil), saponificaiton value (168.30 mg KOH/g oil), ester value (167.10 mg KOH/g oil), peroxide value (0.00 mg/g oil), Hydroxyl value (0.70 mg KOH/g oil), Acetyl value (0.70 mg KOH/g oil), unsaponificaiton value (4%), thiocyanide (73.50 mg KOH/g oil) etc. Fatty acid composition analysis of the oil showed presence of palmitic acid (32.60%), stearic acid (10.56%), oleic acid (39.65%), Linoleic acid (38.20%), linolenic acid (2.10%), myristic acid (2.95%). Preliminary phytochemcial analysis of the powdered seed revealed the presence of flavonoids, carbohydrates, oils, alkaloid, glycoside, saponins, resins, acids etc. The result of the investigation is an indication of good quality that can be modified so as to useful in food industry as additives in food as well as in cosmetics and pharmaceutical industries. The present study was aimed at extraction and characterization of the seed oil of *Buccholzia coriacea* for the purpose of searching for oils from non conventional sources, because of increasing needs for oil both for human consumption and industrial applications. **Keywords:** *Buchholzia coriacea*, physicochemical properties, fatty acid composition.

PI116 Quantitative phytochemical, proximate/nutritive composition analysis of *Beta Vulgaris* Linnaeus (Chenopodiceae)

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The quantitative phytochemical analysis, proximate composition and level of some nutrients of *Beta vulgaris* (Beet root) were studied using standard analytical method. Result of the quantitative phytochemical analysis indicates the presence of alkaloids (128.889), steroids (16.4), glycosides (0.652), flavonoids (6.417), terpeniods (115.5), saponins (3.789), and acidity level (5.227) all in mg/100 g. Proximate composition analysis of *Beta vulgaris* (Beet root) indicates that it contains 1.35%, 0.3%, 11.64/mg/100 g, 1.9%, 2.56%, 42 kcal, 87.4% and 1.4% of protein, fats and oils, β-carotene, dietary fibre, total fibre, energy, moisture, and ash value respectively. The elemental analysis also indicates the presence of the following minerals: iron (0.76 mg/100 g), magnesium (18.6 mg/100 g), copper (0.08 mg/100 g), sodium (73.6 mg/100 g), potassium (312.0 mg/100 g), manganese (0.86 mg/100 g), calcium (13.8 mg/100 g) and zinc (0.29 mg/100 g). Vitamins found were vitamin A (2.6 µg/100 g), vitamin C (4.36 mg/100 g), vitamin E (0.18 mg/100 g), vitamin K (3.2 µg/100 g), vitamin B3 (0.35 mg/100 g), vitamin B6 (0.03 mg/100 g), vitamin B2 (90.053 mg/100 g), vitamin B1(0.034 mg/100 g), panthotenic acid (0.151 mg/100 g), and cholesterol (0.04 mg/100 g). This result reveals that the root contain appreciable amounts of nutrients that justifies its use in treatment of different ailments.

PI117 New labdane diterpenes from *Leonotis leonurus* and their biological activities

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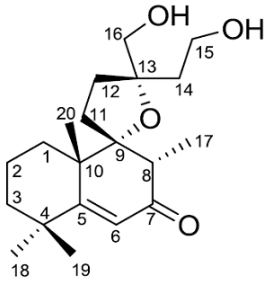
Leonotis leonurus (Lamiaceae), is native to South Africa and has been reported to cause mild marijuana-like sedative effects when smoked or consumed as a tea. Three new labdanes, leonurenones A-C **(1-3)** were isolated and characterized from a commercial source of *Leonotis leonurus*. Genetic methods allowed for identification of the plant material. The new compounds contain an uncommon α,β-unsaturated enone moiety in ring B. Compounds **1** and **2** were evaluated in a competitive inhibition assay at the GABA A neuroreceptor site. The aqueous extract has GABAA activity. However, compounds **1** and **2** isolated herein did not show any activity at this receptor, indicating that these compounds are not by themselves responsible for the activity.



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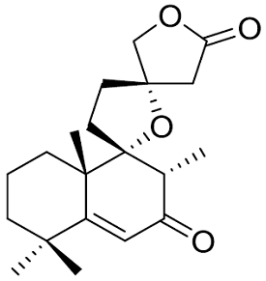

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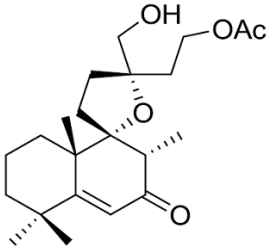

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Keywords: labdane; leonurenone; *Leonotis leonurus;* isolation

PI118 In vitro anti-hyperglycemia properties of the aqueous stem bark extract from Strychnos henningsii (GILG)

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Strychnos henningsii (SH) is a plant commonly used in southern Africa traditional medicine for the management of diabetes mellitus. Previous *in vivo* studies showed that a stem bark extracts improves glycemic control in a diabetic animal model. Meanwhile, the mechanism of action has not been elucidated. The present study therefore investigated various *in vitro* models known to target glucose homeostasis and its direct complications. The plant extract was found to stimulate both basal and insulin glucose uptake in differentiated 3T3-L1 cells but not in Chang liver cells. The effect on 3T3-L1 cells appears independent of PPARγ as the extract did not stimulate adipogenesis. Although, SH extract was inhibitory toward intestinal alpha-glucosidase, the physiological relevance is doubtful based on the recommended dosages. The extract strongly inhibited protein glycation which, at least in part, may be explained by the antioxidant and phenolic content of this plant. Cytotoxicity in Chang liver cells yielded an IC_{50} value of 130 µg/ml raising concern that continual exposure to this herbal remedies may initiate hepatotoxicity. The finding from this study suggests that SH extract may promote glucose homeostasis through enhanced peripheral tissue glucose utilization. .

PI119 Effects of piperovatine and piperlonguminine on mitochondrial function and integrity of the cell membrane in *Trypanosoma cruzi*

Desoti VC[1], Miranda N[1], Ueda-Nakamura T[1,2], Dias-Filho BP[1,2], Garcia Cortez DA[1], Palazzo de Mello JC[1], de Oliveira Silva S[1], Nakamura CV[1,2]
[1]Programa de Pós-graduação em Ciências Farmacêuticas; [2]Laboratório de Inovação Tecnológica no Desenvolvimento de Fármacos e Cosméticos, Universidade Estadual de Maringá, Maringá, Paraná, Brazil

The search for therapeutic agents effective and less toxic for the treatment of Chagas' disease is increasing. Due to the wide diversity of molecular structures found in natural products, they have become targets for new drug discovery. Although the literature reports many studies with extracts and pure compounds obtained from plants with a good potential for treating infection, little is known about their mechanism of action. Thus, the main of this study was to evaluate the alterations caused by piperovatine and piperlonguminine, compounds isolated from *Piper ovatum* Vahl, on mitochondrion function and cell membrane plasma integrity of *T. cruzi* in a way to try to elucidate possible mechanism of action. For this, epimastigotes were treated for 24 h, using IC_{50} and IC_{90} (41.5 and 178.4 µM for piperovatine; 54 and 163.6 µM for piperlonguminine) and incubated with Rh123. Epimastigotes were also treated with high concentrations of piperovatine (256 µM) and piperlonguminine (220.5 µM) and incubated with propidium iodide. Both assays were then evaluated by flow cytometry. The treatment with piperovatine and piperlonguminine with IC_{90} caused decrease in fluorescence intensity total of Rh123 of 53.3% for piperlonguminine and 30.3% for piperovatine, indicating mitochondria depolarization. Additionally, an increase of 62.4% only for piperlonguminine was observed in treated cells, indicate alteration in cell membrane integrity. It is possible to suppose that the tripanocidal action of piperovatine and piperlonguminine maybe involve its effect on the mitochondrial function leading to cell death of the parasite.

PI120 The anti-inflammatory effect of various Thai rice

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Whole grain, rice bran and polished grain of 16 Thai rice cultivars (*Oryza sativa* Linn.) were extracted by ethanol and by 70°C distilled water. Extracts were assayed for cyclooxygenase-1 (COX-1) inhibition *in vitro*, free radical (2,2-diphenyl-1-picrylhydrazyl; DPPH°) scavenging activity

and the content of phenolic compounds. The COX-1 inhibition effect was not found in all extracts. The ethanol extract from rice bran of Kam Chiang-mai (KCBE), a purple-black rice, had the best DPPH° scavenging activity (EC_{50}= 24.72 mcg./mL.), the highest content of cyanidin-3-glucoside (303.65 mcg./g.) and total phenolic compounds (32.72 mg./g.) whereas the water extract from rice bran of Khao Dawk Mali 105 (KDBW), a cream colored rice, had low DPPH° scavenging activity (EC_{50} > 100 mcg./mL.), no detectable cyanidin-3-glucoside and had low level of total phenolic compounds. Both KCBE and KDBW had significant inhibition effect ($p < 0.05$) on edema induced by carrageenan in Sprague-Dawley rats' paws as compared to control. Fifty percent of edema was reduced within 1 hours after carrageenan injection when 500 mg./kg. rat weight of KCBE were gavaged and 59% reduction within 5 hours when 250 mg./kg. of KCBE were gavaged. While 45 and 65% of edema were reduced within 1 and 5 hours, respectively, when 500 mg./kg. of KDBW was gavaged. In conclusion, the results were more or less contradictory to others [1, 2] and further study on the anti-inflammation is worth conducted. *Acknowledgements: Research Unit, Faculty of Medicine, Thammasat University, the National Research Council of Thailand, Higher Education Research Promotion and National Research Project of Thailand.*
References: 1 Choi SP., et al (2010) J Agric Food Chem 58:10007 – 15. 2 Min S-W., et al (2010) International Immunopharmacology 10:959 – 66.

PI121 *Lonicera japonica* thunb. – anti-inflammatory activity of various extracts from herb, stems and leaves

Ortmann S[1], Pferschy-Wenzig EM[1], Zhao YM[2], Miao J[2], Bauer R[1]
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Lonicera japonica Thunb. (Caprifoliaceae) is widely used in traditional Chinese medicine for its antibacterial and anti-inflammatory properties. In order to examine the differences between stems and leaves of *Lonicera japonica* concerning constituents and anti-inflammatory activity, extracts were made with methanol, ethanol and methylene chloride (CH_2Cl_2) using Accelerated Solvent Extraction. The extracts were tested in different *in-vitro* assays: prostaglandin E_2 (PGE_2) inhibition assay with cyclooxygenase-1 and -2 enzymes, leukotriene B_4 (LTB_4) inhibition assay with human granulocytes[3], and a NO production inhibition assay with LPS/IFNγ stimulated mouse macrophages[4]. In addition, TLC and HPLC analyses were made. The chromatograms showed differences depending on the plant part and the solvent. Also the anti-inflammatory activity of the extracts was quite variable. CH_2Cl_2 extracts of herb and leaves were the most active in the NO- inhibition assay, CH_2Cl_2 extracts were the most and ethanol extracts slightly less active in PGE2 and LTB4 inhibition assays. Therefore the CH_2Cl_2 extracts are considered for further investigation.

PI122 Anticancer activity of *Enterolobium cyclocarpum* on cervical and breast cancer cells *in vitro*

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[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria; [2]Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, South Africa; [3]Key Laboratory of Marine Drugs, School of Medicine and Pharmacy, Ocean University of China, People's Republic of China

Enterolobium cyclocarpum (Jacq.) Griseb., a tree growing in Africa, is used in traditional treatment of cancer. The anticancer effects of the methanolic extract of the leaves, were investigated in cervical (HeLa) and breast (MCF7) cancer cell lines using the MTT assay, cell cycle analysis and Annexin V-FITC/PI staining. Significant growth inhibition was observed with IC_{50} values of 2.07 ± 1.30 μg/mL and 11.84 ± 1.18 μg/mL for HeLa and MCF7 respectively. Cell cycle analysis indicated that HeLa cells were arrested in the G_2/M phase while in the MCF7, the cells were arrested in the G_1/G_0 phase. The Annexin V-FITC/PI staining revealed phosphatidylserine translocation and thus apoptosis induction upon treatment with the extract. The results validate the traditional use of the plant.

PI123 Phytochemical and biological investigations on *Mentha longifolia* subsp. *noena*

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[1]Department of General & Analytical Chemistry, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey; [2]TUBITAK, National Metrology Institute, 41470 Gebze, Turkey; [3]Department of Biology, Faculty of Art & Science, Batman University, 72100 Batman, Turkey; [4]Department of Biology, Faculty of Art & Science, Dicle University, 21280 Diyarbakir, Turkey

The aerial parts of *Mentha longifolia* (L.) Hudson subsp. *noeana* (Boiss. ex Briq.) Briq. (Lamiaceae) were collected from South-east Turkey. Uvaol (**1**), stigmast-5-ene-3β-yl formate (**2**), stigmast-5-en-3-one (**3**), β-sitosterol (**4**) were isolated from the petroleum ether extract, ursolic acid (**5**), bis(2-ethylhexyl) benzene-1,2-dicarboxylate (**6**), hexacosyl (E)-ferulate (**7**) from the acetone extract, 5-hydroxy-6,7,3',4'-tetramethoxy flavone (**8**), *cis*-8,11,14-eicosatrienoic acid methyl ester (**9**) from the methanol extract. The compounds (**1-3**, **6-7**, **9**) were isolated for the first time from *Mentha* species. This study was also the first phytochemical and biological reports on this plant. The methanol extract indicated 80% inhibition of lipid peroxidation, the acetone extract possessed moderate DPPH free radical scavenging activity (60% inhibition) at 100 μg/mL[1]. Pulegone (32.3%) was the main constituent of the essential oil which exhibited strong butyrylcholinesterase inhibitory activity (77.36 ± 0.29%) and moderate antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Candida albicans*[1,2]. **References** **1**.Kolak, U., Boğa, M., Uruşak, E.A., Ulubelen, A. (2011). Turk J Chem 35,637 – 645. **2**.Goren, A.C., Bilsel, M., Demir, H., Kocabas, E.E. (2003). Z. Naturforsch. C 58, 687 – 690.

PI124 Superior immunological effects of a resveratrol/glucan/vitamin c combination

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University of Louisville, Department of Pathology, Louisville, KY, 40292

Natural products, useful in preventing and/or treating various diseases, have been sought after throughout the history of mankind. Polysaccharides in general, and glucans in particular, have a long history as immunomodulators. Recently, numerous substances have been shown to have synergistic effects with glucans. We decided to compare the biological activities of insoluble yeast-derived β1,3-D-glucan with a combination of glucan/resveratrol/vitamin C. Our data show that whereas resveratrol or vitamin C alone had only limited effects on immune reactions, the combination significantly increased the phagocytosis of peripheral blood leukocytes, specific antibody response, nitrite anion production and apoptosis. In addition, significant inhibition of cancer growth was found using two different experimental models. We can summarize that whereas resveratrol or vitamin C alone had only limited effects on immune reactions, the combination significantly increased all tested immunological reactions. Our data represent further proof that combined preparations of glucan, resveratrol and vitamin C strongly stimulate the immune reactions. We hypothesise that strong anti-cancer properties showed by the combination of these three compounds are manifested via stimulation of immune reactions and apoptosis. Data presented in this study represent further proof that combined preparations of glucan and and other natural immunomodulators strongly stimulate both branches of immune reactions. A study attempting to reveal the exact mechanisms of these effects is currently under progress.

PI125 The cytotoxic effect of endemic *Centaurea fenzlii* Reichardt on colon cancer cell lines

Yirtici U[1], Yılmaz F[1], Serim G[1], Kirimer N[2], Ulukaya E[3], Icgen B[1], Ergene A[1]
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Being the largest genus of the *Compositae* family with over 169 species and 199 taxons, *Centaurea* L. has been used in folklore for the treatment of wide range of diseases in Turkey. The antitumor activities of the *Centaurea* species in different types of cancer have been identified through several bioactivity studies. However, the *in vitro* anticancer activity of endemic *Centaurea fenzlii* Reichardt. has never been evaluated

and published yet. In this study, the cytotoxic effect of this plant was tested *in vitro* against Caco-2 (human epithelial colorectal adenocarcinoma) cells by using MTT, and ATP assays. For the positive control, the cells were treated with the chemotherapeutic drug 5-fluorouracil which has been widely used in the treatment of a range of cancers. The aerial parts of the plants were collected during the flowering period and the extracts were obtained with n-hexane, dichloromethane and methanol, respectively. The extracts were applied to Caco-2 cell lines at increasing doses. The IC_{50} values of samples were determined and their direct cytotoxic effects were measured. The extracts displayed noteworthy cell growth and proliferation inhibitory activity. Moreover, the cytotoxic effect of crude dichloromethane extract on Caco-2 cell lines was the highest compared to other extracts. This cytotoxicity screen has provided important preliminary data promoting the selection of the plant species and their different extracts with potential antitumor properties for a therapeutic option in colon cancer.

PI126 Chromatographic profiling and identification of two new iridoid-indole alkaloids by UPLC-MS and HPLC-SPE-NMR analysis of an antimalarial extract from *Nauclea pobeguinii*

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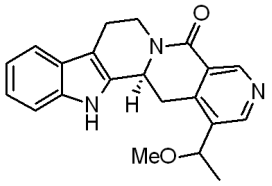
The total 80% EtOH extract of stem bark of *Nauclea pobeguinii* (Rubiaceae), which is active against uncomplicated *falciparum* malaria as shown in previous clinical studies, was analysed by means of UPLC-MS and HPLC-SPE-NMR. Apart from the main constituent, strictosamide, a series of minor constituents was identified, including two new iridoid-indole alkaloids, i.e. naucleidinic acid (**4**) and 19-*O*-methyl-3,14-dihydroangustoline (**7**), together with 8 known iridoid-indole alkaloids, i.e. naucleidinal, magniflorine, naucleofficine D, two diastereoisomers of 3,14-dihydroangustoline, strictosidine, desoxycordifoline, 3α,5α-tetrahydrodeoxycordifoline lactam, and a phenol glycoside 3,4,5-trimethoxyphenol-β-D-apiofuranosyl-(1 – 6)-β-D-glucopyranoside (kelampayoside A).



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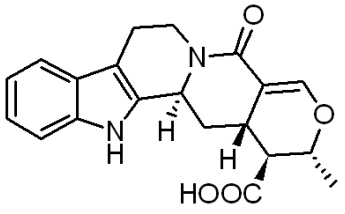

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PI127 Antioxidant constituents from the stems and fruits of Momordica *Charantia*

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A new cucurbitane-type triterpene glycoside taiwacin A (**1**), a new pentanorcucurbitane, taiwacin B (**2**) and a known cucurbitane-type triterpene glycoside (**3**), and a known steroid glycoside (**4**), were isolated from the stems and fruits of *Momordica charantia*, respectively. These four compounds, **1**, **2**, **3** and **4** revealed ABTS radical cation scavenging activity with an IC_{50} values of 119.1 ± 4.3, 204.5 ± 1.2, 159.7 ± 11.0 and 98.1 ± 2.4 μM, respectively. Compounds **1** and **3** displayed an inhibitory effect on xanthine oxidase activity with IC_{50} values of 24.1 ± 3.4 and 158.3 ± 35.6 μM, respectively. Compounds **2-4** significantly displayed $O_2^{\cdot-}$ scavenging activity with an IC_{50} values of 12.5 ± 2.2, 16.5 ± 0.1 and 27.3 ± 1.4 μM, respectively and the relative oxygen radical absorbance capacity values of **2** and **3**, using ORAC-pyrogallol red (PGR) assay, were determined to be 0.88 ± 0.02 and 0.55 ± 0.09, respectively. These findings showed that **1-4** may be used as antioxidants.

PI128 Potential new sources of natural dyes from the traditional pharmacopoeia of New-Caledonia

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Nowadays interest is growing in finding nature friendly sustainable technologies which can be used as alternatives to fossil-based raw materials and energy. In this context, our laboratory recently began research programs to explore and learn local population plants usages to make a selection of "new" source of colorant. 74 plants were harverest and tested for their potentiality for dyeing different textile fibres. After evaluation of their color strength and color fastness to light and washing, 21 plants were selected for a chemical study. High performance liquid chromatography (HPLC), mass spectroscopy analysis and 2D NMR experiments are used to evaluate the separation potential of natural products and to determine the chemical structure of isolated compounds. Furthermore, 31 crude extracts from different parts of interest species were tested for antimicrobial, antitumoral and antioxidant activities. 7 extracts were found to have inherent activities against *E. coli*, *S. aureus*, *S. epidermis* and *C. albicans* strains, one against KB cells and 10 out of 20 showed antioxidant activities. As a matter of fact, increasing our knowledge on plants and their traditional usages could lead to a development and a protection of natural and cultural inheritance.

PI129 Nutritional suitability of linseed and oil hemp from fatty acids profile perspective

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Linseed (*Linum usitatissimum* L.) and oil hemp (*Cannabis sativa* L.) can provide a complete and balanced source of fatty acids. The nutritional quality of linseed is given by the oil quality which conteins ω-3 and ω-9 fatty acids and the pleasant taste while oil hemp although has an optimal ratio of 3:1 between the ω-6/ω-3 fatty acids, because of the national legislation has limited use in Romania. The biological material used consisted of two linseed varieties (Floriana and Alexin), one monoecious (Diana) and one dioecious (Silvana) hemp variety. All varieties are authorized in our country, according to the Official Catalogue of varieties of crop plants in Romania. The Soxhlet method with a Velp block of mineralization was used to determine the oil content of the studied varietes and for the investigation of fatty acid profile was used the gas chromatography GC-MS whit Shimadzu GC MS QP 2010.The study aims to investigate the fatty acid composition in linseed and oil hemp in order to obtain an overall picture about their nutritional suitability, from the point of view of fatty acids.The fatty acids separated and identified from the linseed and hemp oil were: alpha-linolenic acid (ω-3), linoleic acid (ω-6), oleic acid (ω-9), palmitic acid and stearic acid. Linseed and oil hemp are highly suitable dicotyledonous crops for the human health because of the fatty acid profile.

PI130 Anti-inflammatory effects of *Byrsonima crassifolia*

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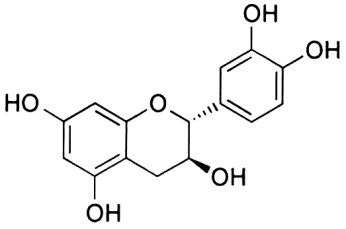
The anti-inflammatory effects of many plants and plant-derived compounds have been studied and documented. In a screening of Venezuelan plant extracts for anti-inflammatory activity, the crude bark extract and fractions of *Byrsonima crassifolia* (Malpighiaceae) were tested in various assays, including their effect on the production of the inflammatory mediators, tumour necrosis factor (TNF-α), interleukin-6 (IL-6) and nitric oxide (NO) by LPS-activated RAW 264.7 macrophages, serum levels of the same mediators in Balb/c mice challenged with LPS (lipopolysaccharide) and cellular levels of NF-κB, an important nuclear factor involved in the inflammatory response. Of the 23 fractions obtained from solvent extraction and reverse phase HPLC, fraction 17 showed the greatest overall effect on NO production *in vitro* (53% at 30 μg/ml) and *in vivo,* but did not affect TNF-α, IL-6 or NF-κB. Mass and NMR spectroscopy analysis showed fraction 17 to be (+)-catechin, a flavonol with antioxidant properties, but which has also been reported to show anti-inflammatory activity.



PI131 Anti-inflammatory effects of different preparations of cat's claw

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South America has two predominant species of Cat's Claw (Uña de Gato), *Uncaria tomentosa* and *U. guianensis*, which are both used in traditional medicine to treat inflammation. In this study, two commercial preparations of *U. tomentosa* and a hydroethanolic preparation of *U. guianensis* were evaluated and compared in various assays, including their effect on the production of the inflammatory mediators, tumour necrosis factor (TNF-α), Interleukin-6 (IL-6) and nitric oxide (NO) by LPS-activated RAW 264.3 macrophages, serum levels of the same mediators in mice 1 h after challenge with LPS (lipopolysaccharide) and cellular levels of NF-KB, an important nuclear factor involved in the inflammatory response. All the extracts, as well as several of the fractions obtained by reverse phase HPLC, showed an inhibitory effect on NO production, particularly a *U. guianensis* subfraction (UgAIV) *in vitro* (85% at 30 μg/ml) and one commercial *U. tomentosa* fraction B *in vivo* (73% at 5 mg/Kg). The UgAIV subfraction also showed the greatest inhibitory effect on the TNF-α and IL-6 responses both *in vitro* and *in vivo,* as well as inhibiting NF-κB activation. Further subfractionation and biological evaluation of UgAIV are currently being carried out to determine the compound(s) responsible for these activities.

PI132 Toxicity of the ethanol extract from *Antidesma acidum* Retz

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Toxicity tests of 95% ethanol extract of the root from *Antidesma acidum* were studied in male and female rats. The oral acute toxicity test at 5,000 mg/kg revealed that the ethanol extract did not produce toxic effects on signs, general behavious, mortality and groß appearance of internal organs of rats. Furthermore, the oral subacute toxicity test at the dose of 1,000 mg/kg/day displayed no significant changes in body and internal organs weights, normal hematological and clinical blood chemistry values. Histological examination also showed normal architecture of all internal organs. In conclusion, *A. acidum* extract did not produce any toxicity in oral acute and subacute toxicity studies.

PI133 Preparation of pentacyclic triterpenoid-rich *Centella asiatica* extract and its pharmacological activities

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Centella asiatica is used as a traditional medicine for the treatment of skin diseases and wounds. It has been reported that pentacyclic triterpenes, mainly, asiatic acid, madecassic acid, asiaticoside, and madecassoside are the active constituents that possess various pharmacological activities, including wound healing, anti-ulcer, anti-inflammatory and anticancer. A new method for preparation of pentacyclic triterpenes-rich *C. asiatica* extract (PRE) was established and its antibacterial and anti-inflammatory activities were evaluated. A simple method for preparation of the PRE, which contained not less than 65% w/w total pentacyclic triterpenes involved a macroporous resin (Diaion® HP-20) column eluted with ethanol, and a decolourisation step with activated charcoal. Antibacterial activities of PRE against *Streptococcus* spp. were stronger than asiaticoside, and madecassoside, and almost equal to those of madecassic acid, but lower than asiatic acid. PRE showed strong anti-inflammatory activity *via* inhibition of nitric oxide production by murine macrophage-like RAW264.7 cells assay, with IC_{50} value of 64.6 μg/ml. In addition, the anti-inflammatory activity of PRE was stronger than madecassic acid, asiaticoside, and madecassoside, but lower than asiatic acid.

PI134 Comparison of biological activities and chromatographic fingerprints of root extracts from two *Acanthopanax* species

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Root aqueous and 75% ethanolic extracts of *Acanthopanax trifoliatus* (*At*) which is called in Thai as phak-paem and *Acanthopanax senticosus* (*As*) or Siberian ginseng were tested for *in vitro* antioxidant activities and inhibitory effect to acetylcholinesterase. It was found that 75% ethanolic refluxing extract from *At* roots exhibited the strongest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with EC_{50} value of 33.78 ± 0.70 μg/ml while at the concentration of 100 μg/ml, aqueous decoction extract from *As* roots had the highest inhibitory effect (90.65 ± 0.12%) to peroxidation of linoleic acid determined by thiobarbituric acid reactive substances (TBARS) method. However, using ferric reducing antioxidant power (FRAP) assay, all extracts showed similar potency in reducing antioxidant power with FRAP value around 3 g

$FeSO_4$ equivalent/100 g extract. At the concentration of 2000 µg/ml, 75% ethanolic extract from *At* roots also exhibited the strongest inhibitory effect to acetylcholinesterase using Ellman's method with percentage of inhibition of 97.46 ± 4.41%. High performance liquid chromatography (HPLC) and thin layer chromatography (TLC) of root extracts of *At* and *As* showed specific fingerprints suggesting the similar major chemical constituents of phenolic compounds including chlorogenic acid and dicaffeoylquinic acid derivatives.

PI135 Antioxidant and antihyperlipidemic activity of Tephrosia purpurea callus culture

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This study was undertaken to investigation the effect of callus culture of *Tephrosia purpurea* (CCTeph) on blood glucose, tissue lipid profile, and lipidperoxidation in alloxan induced diabetes. The callus culture was successfully developed and maintained on MS medium supplemented with different concentrations and combinations of plant growth regulators. Aqueous extract of leaf and its callus culture were administered orally (300 mg/kg body weight) for 21 days. The levels of lipid peroxides [TBARS, and Hydroperoxide] and tissue lipids [cholesterol, triglyceride, phospholipides and free fatty acids] blood glucose level was estimated in alloxan induced diabetic rats. The effects were compared with glibenclamide.Treatment with CCTeph and glibenclamide showed significant reduction in blood glucose level, in tissue lipids and lipid peroxide formation. The effect produced by CCTeph was comparable with that of glibenclamide. The decreased lipid peroxides and tissue lipids clearly indicate the antihyperlipidemic and antioxidant activity of CCTeph apart from its antidiabetic effect. The present study provided evidence first that CCTeph has Antihyperlipidemic and antioxidant activity, suggesting the potential of the tissue culture technique to substitute for wild *Tephrosia purpurea* in the pharmaceutical industry.

PI136 Inhibitory effect of ethanolic extract of *Annona squamosa* L. leaves on the expression of EGFR

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The precise causes of psoriasis, a relatively common, chronic, inflammatory and hyperproliferative skin disease, are not known, thus making it very difficult for therapy. We previously found an *in vitro* anti-psoriatic activity in ethanolic extract of *Annona squamosa* L. leaves. EGFR is overexpressed in psoriatic skin, thus considering as one of essential determinants of psoriasis. The objective of this study was to investigate the molecular effect of *Annona squamosa* L. leaf extract on the EGFR expression using HaCaT keratinocyte cell line as a model. Based on RT-PCR, concentrations at 3.15 and 1.575 µg/mL (IC_{50}= 6.3 µg/mL) significantly reduced the EGFR mRNA expression *(P< 0.05)* although all three concentrations showed a similar tendency of inhibitory effect. Western blot analysis found that all concentrations of the extract tested greatly inhibited the expression of EGFR *(P< 0.05)*. Confocal immunofluorescence microscopy subsequently confirmed this finding. Taken together, this might suggest that the ethanolic extract of *Annona squamosa* leaves could exert its biological effect by suppressing the expression of EGFR biomarker.

PI137 Adjuvant therapeutic use of supercritical-ethanol extracts of *Curcuma* species with cancer drugs in rhabdomycosarcoma cell lines

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Rhabdomyosarcoma is a cancerous tumor that grows in the soft tissues of the body, particularly in the muscles that attach to bone and help the body to move. The two most common forms of this tumor are embryonal and alveolar, the latter being refractory and difficult to treat mainly because of cellular drug resistance. We investigated the synergistic effect of supercritical -ethanol extracts of *Curcuma* species along with conventional chemotherapeutic drugs in alveolar and embryonal rhabdomyosarcoma cell lines. *Curcuma amada* (mango ginger-CA) extract showed the highest levels of cytotoxicity as compared to *C. longa* (turmeric) and *C. xanthorrhiza* (Japanese turmeric) extracts. CA showed synergistic cytotoxic effects with vinblastine (VBL) and cyclophosphamide (CP), as indicated by the combination index values of < 1 for VBL+CA, CP+CA and VBL+CP+CA combinations in both embryonic and alveolar rhabdomyosarcomas. When CA was combined with cancer drugs CP and VBL, caspase 3 activity increased significantly higher than that of individual agents which correlated with the percentage of apoptotic cells induced by the drugs and combinations. CA in combination with VBL and CP induced higher percentage of apoptosis than single agents in both cell lines. CA also induced significant quantitative changes in gene expression patterns (*Bcl-2, Bax, Bak and p53*) associated with intrinsic pathway of apoptosis. These results suggest that CA can be evaluated further as an adjuvant with cancer drugs for the treatment of rhabdomysarcoma patients.

PI138 New Clerodane diterpenes from Zuelania guidonia

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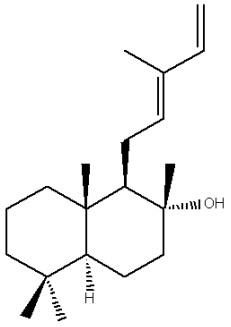
We studied the content of cytotoxic compounds from *Zuelania guidonia* (Flacourtiaceae) leaves collected in Santa Rosa, Guanacaste (Costa Rica). We isolated two labdanes **(1,2)** and twenty clerodanes **(3 – 22),** sixteen of which have not been previously reported. Interestingly, clerodane glycosides are described for the first time in this family. The isolation was carried out using HPLC techniques and the structural elucidation was performed with 1D and 2D NMR and mass spectrometric techniques. The absolute stereochemistry of the elucidated clerodanes was proposed to be 5S, 8R, 9R and 10S based on previous X-ray studies for compounds from this family. The relative stereochemistry of the remaining chiral carbons was determined using NOE and 13C-NMR spectroscopy.



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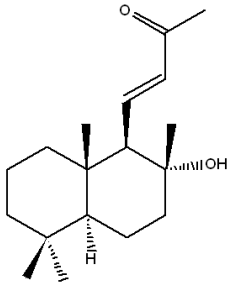

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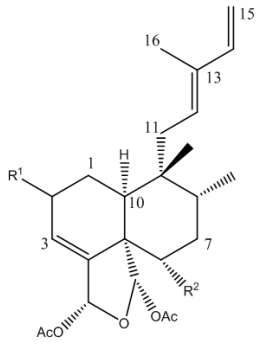

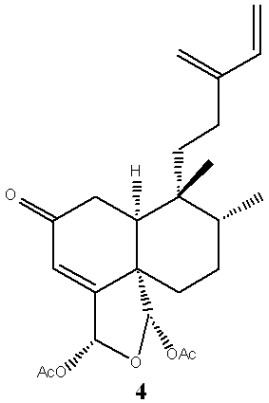

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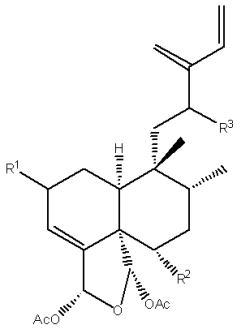
3 R^1 = - - - $OCO(CH_2)_8CH_3$, R^2 = OH

8 R^1 = - - - OH, R^2 = O-Bz

15 R^1 = — OH, R^2 = O-Cin



5 R^1 = - - - $OCO(CH_2)_8CH_3$, R^2 = OH, R^3 = - - - OH

6 R^1 = — O-Cin, R^2 = OH, R^3 = H

7 R^1 = - - - $OCO(CH_2)_6CH_3$, R^2 = OH, R^3 = - - - OH

9 R^1 = - - - OH, R^2 = O-Cin, R^3 = H

10 R^1 = — OH, R^2 = O-Cin, R^3 = H

13 R^1 = - - - OH, R^2 = $OCOCH_2CHOH(CH_2)_8CH_3$, R^3 = H

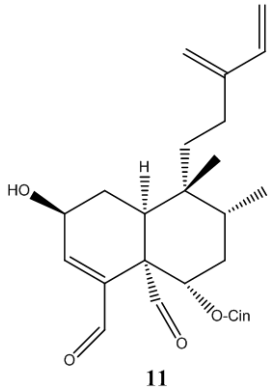
14 R^1 = - - - OH, R^2 = O-Bz, R^3 = H

16 R^1 = - - - OH, R^2 = O-Bz, R^3 = - - - OH

17 R^1 = — OH, R^2 = $OCOCH_2CHOH(CH_2)_8CH_3$, R^3 = - - - OH

18 R^1 = - - - OH, R^2 = $OCOCH_2CHOH(CH_2)_8CH_3$, R^3 = - - - OH

22 R^1 = - - - $OCO(CH_2)_8CH_3$, R^2 = O-Xyl, R^3 = — OH



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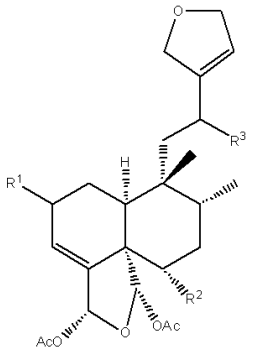

12 R^1 = - - - OH, R^2 = OH, R^3 = H

19 R^1 = - - - OH, R^2 = O-Cin, R^3 = - - - OH

20 R^1 = - - - $OCO(CH_2)_8CH_3$, R^2 = O-Xyl, R^3 = — OH

21 R^1 = - - - $OCO(CH_2)_8CH_3$, R^2 = O-Xyl, R^3 = - - - OH

PI139 Novel Kaempferol triglycosides from *Olax manni* leaves: Structures and biological activities

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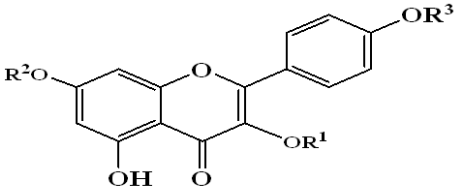
Four (4) new kaempferol triglycosides, namely, Kaempferol 3-*O*- α-D-apiofuranosyl (1 -- 2) α-L- arabinofuranosyl- 7-*O*- α-L- rhamnopyranoside (**1**), Kaempferol 3-*O*- β-D- glucopyranosyl (1 -- 2) α- L- arabinofuranosyl- 7-*O*- α-L- rhamnopyranoside (**2**), Kaempferol 3-*O*- α-L- arabinofuranosyl (1 -- 2)- β-D- galactopyranosyl- 7-*O*- α-L- rhamnopyranoside (**3**), Kaempferol 3-*O*- α-L- rhamnopyranosyl (1....2)- α-L- arabinofuranosyl- 7-*O*- α-L- rhamnopyranoside (**4**) in addition with fifteen (15) known flavonoid glycosides (**5 – 19**) were isolated from the leaves of *Olax manni*. All the compounds were isolated for the first time from the genus, *Olax*. The structures of these compounds were elucidated by HPLC, LC-ESIMS, ^{1}HNMR, ^{13}CNMR, DEPT, HMQC and HMBC. The compounds showed moderate antifungal activity against *Aspagillus fumigatus* and mild cytotoxicity against mouse lymphoma cell line (L5178Y).

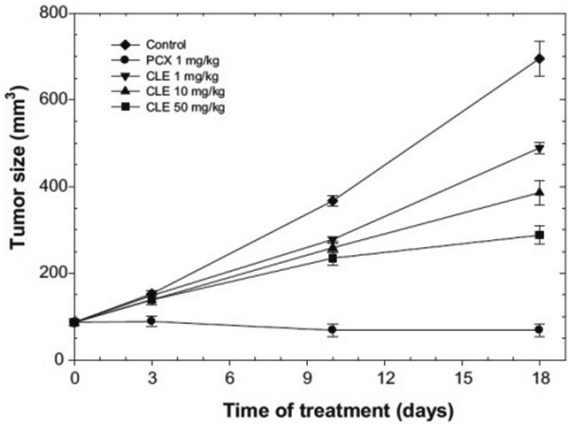


	R^1	R^2	R^3
1	Apio (1 -2) Arab -	Rham-	H
2	Glu (1-2) Arab-	Rham-	H
3	Arab (1-4) Gal-	Rham-	H
4	Rham (1-2) Arab-	Rham-	H

PI140 Antitumor effect of *Croton lechleri* Mull. Arg. (Euphorbiaceae)

Alonso-Castro AJ[1,2], Ortiz-Sánchez E[2], Domínguez F[3], López-Toledo G[2], Chávez M[4], de Jesús Ortiz-Tello A[2], García-Carrancá A[2,5]
[1]Facultad de Química, UNAM, DF México, [2]INCAN, DF México, [3]CIBIOR, Puebla, México, [4]CMN Siglo XXI, IMS S, México DF, [5]Instituto de Investigaciones Biomédicas, UNAM, DF

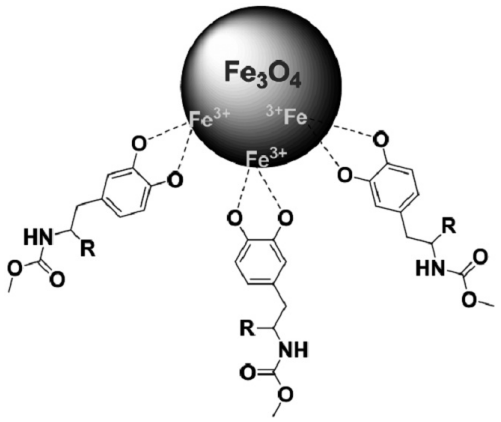
We evaluated the acute toxicity in mice, the cytotoxic and antitumoral effects of methanolic extracts of *Croton lechleri* leaves (CLE). CLE showed low IC_{50} values on HeLa (17 μg/ml) cells but lack toxic effects against human normal cells. Induction of cell death in HeLa cells by CLE was confirmed by an increase of apoptosis (Annexin/PI) by 30% compared to untreated cells. The LD_{50} was 356 mg/kg by intraperitoneal route (i.p.) and 500 mg/kg by oral route. CLE administrated at 1, 10 and 50 mg/kg i.p. inhibited the tumor growth by 38%, 48% and 59%, respectively, in mice bearing HeLa tumor.



PI141 Selective adsorption of phytochemicals with carboxyl or *o*-phenolic hydroxyls by Fe_3O_4 nanoparticles

Chen B[1], Zhang S[1], Shen Y[1,2], van Beek TA[2]
[1]Key Laboratory of Chemical Biology & Traditional Chinese Medicine Research, Ministry of Education, Hunan Normal University, Changsha 410081, China; [2]Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands

Catechol (1,2-diphenols) derivatives are often used as dispersant anchors for Fe_3O_4 nanoparticles because of the high affinity of catechols for the nanoparticles (see Figure, *J. Phys. Chem. 2011, 115, 682 – 691*). This "grafting to" approach implies that Fe_3O_4 nanoparticles could be applied to selectively adsorb some phytochemicals with carboxyl or ortho-phenolic hydroxyls. This possibility was explored. Using ~30 nm Fe_3O_4 nanoparticles, ginkgolic acid, ascorbic acid, cichoric acid, citrinin (with carboxyl) and some flavones containing a catechol moiety in *Scutellaria baicalensis* Georgi roots could be successfully adsorbed from methanol, acetonitrile or water solutions. After separating the nanoparticles from the herbal extract solution with a magnet, the adsorbed phytochemicals could be simply desorbed at a low pH, e.g. with formic acid solution. The adsorptive capacity of nanoparticles was ~1.0% (w/w). The whole purification process is solvent-efficient and also energy-efficient when using a permanent magnet. The method can be used as a first step in the large scale production of fine chemicals.



PI142 MAO-A inhibitors from *Hypericum thasium* Griseb

Demirkiran O
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n-BuOH fraction from 80% ethanol extract of *Hypericum thasium* Griseb. have yielded two new compounds 3′,4,5′-trihydroxy-6-methoxy-2-*O*-α-arabinosylbenzophenone (**1**), and 3′,4,5′,6-tetrahydroxy-2-*O*-α-L-arabinosylbenzophenone (**2**), along with a known flavonoid glycoside quercetin-3-*O*-α-arabinose (**3**). The structures of the new compounds were elucidated by 1D and 2D NMR analysis as well as HR EIMS. The isolated compounds (**1 – 3**), as well as quercetin, and kaempferol previously isolated from EtOAc fraction were screened against MAO-A inhibitory activity. When tested against the MAO-A quercetin and kaempferol displayed IC_{50} values of 19.6, and 17.5 μM, respectively. The IC_{50} values for MAO-A inhibition by compounds (**1 – 3**) were 310.3, 111.2, and 534.1 μM, respectively. Standart inhibitor (clorgyline) exhibited MAO-A inhibition with an IC_{50} value of 0.5 μM.

PI143 Enhancing effect of *Thevetia peruviana* flower extract on TNF-α induced apoptosis in human cervical cancer cells

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Tumor necrosis factor (TNF-α) is a pleiotropic cytokine triggers several intracellular signaling pathways including mitogen-activated protein kinases, nuclear factor-κB (NF-κB) and extracellular signal-regulated kinase, which involve in survival and apoptosis of cancers. This study was conducted to investigate the effect of ethanolic extract from the flowers of *Thevetia peruviana* on TNF-α induced apoptosis in human cervical cancer (HeLa) cells by determinations of cell viability and apoptosis using WST-1 cell proliferating assay and immunoblot analysis, respectively. Pretreatment of HeLa cells with various concentrations (3 – 300 μg/mL) of flower ethanolic extracts of *T. peruviana* enhanced TNF-α induced antiproliferation in HeLa cells. Extracts at concentration of 30, 100 and 300 μg/mL together with TNF-α significantly decreased viability of HeLa cells compared with that treated with only TNF-α. These extracts strongly promoted TNF-α induced cleavage of caspase-3 and PARP-1. Compared with HeLa cells treated with *T. peruviana* extract alone, only extract at concentration of 30 μg/mL together with TNF-α strongly decreased cancer cell viability. The results suggested that ethanolic extract from the flowers of *Thevetia peruviana* showed enhancing effect on TNF-α induced apoptosis in HeLa cells by activation of caspase cascade.

PI144 Chemoprevention of herb tea, Tabebuia avellanedae on in vitro and in vivo carcinogenesis system

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[1]Kanazawa University Graduate School of Medical Science, Kanazawa, JAPAN; [2]Kinki University, Nara, JAPAN

As part of an ongoing project to investigate the anti-tumor and anti-tumor promoting properties of *Tabebuia avellanedae*, extract and its active compound, kind of Naphthoquinone (NQ801) was carried out. *Tabebuiaavellanedae* (TA), which is native in South America, is well known in traditional folk medicine used for the treatment of various disease. The inner bark of this plant produced in Brazil is distributed in Asia as a herb tea and healthy purpose. The application of a new screening procedure which utilizes the synergistic effects in enabled rapid and easy detection of naturally occurring substances (anti-tumor promoters, with inhibition of Epstein-Barr virus (EBV) activation, using Raji cells. These compounds were evaluated for their in vitro inhibitory effect on EBV-Early Antigen activation induced by TPA and exhibited potent inhibitory effects (70~50% inhibition at 100 μg concentration) on this assay. We have now extended these investigations to a tumorigenesis model in which we initiated the tumors with DMBA initiation and promoted with 1.7 nmol of TPA in two-stage mouse skin test. The control animals exhibited a 100% papilloma incidence at 20 weeks after promotion. However, treatment with the tested compounds (85 nmol) along with tumor promoter, reduced the percentage of tumor bearing mice by between 13.3 – 33.3 at 20 weeks.These results provide a basis for further development of these botanical supplements for human cancer chemoprevention.

PI145.1 Efficient evaluation of healthy tea, Gromwell seed against tumor promoting stage

Tokuda H[1], Arai T[1], Suzuki R[1], Strong JM[1], Schneider A[1], Suzuzki N[1]
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In our continuous search for anti-tumor promoting, chemopreventive active potency from natural source material, a kind of healthy tea, Gromwell seed (*Coix lachryma-jobi*) ext. have been screened using the *in vitro* synergestic assay indicated by inhibitory effects on the induction of Epstein-Barr virus early antigen (EBV-EA) by TPA. In assay,Gromwell seed aqueous extract and Hot aqueous extract exhibited the potential inhibitory effects on EBV-EA activation without strong cytotoxicity on Raji cells. In our experimental system, the inhibitory effects of both Gromwell extracts were greater than that of beta-carotene, which is known anti-tumor promoting agent and/or chemopreventive agent. These compounds were evaluated for their in vitro inhibitory effect on EBV-EA activation induced by TPA. The percentage of the inhibition of TPA-induced EBV-EA activation for these material were 60% and 30% at concentration 100 μg. Based on the results obtained in vitro, we studied the inhibitory effect of compounds, in an in vivo two-stage carcinogenesis test of mouse skin papillomas using DMBA as an initiator and TPA as a potential promoter. The control animals showed a 100% incidence of papillomas at 20 weeks after DMBA-TPA tumor promotion, while treatment with compounds reduced the percentage of number of tumor to 60% after 20 weeks. Results from in vitro and in vivo studies showing chemopreventive activity against TPA promoting stage and These data suggest that compounds might be a functional material for chemopreventive tea as well as fruits and vegetables.

PI145.2 Inhibitory effect of *Copaifera langsdorffii* on 1,2-dimethylhydrazine induced genotoxicity in rat colon

Morais Alves J[1], Marques Senedese J[1], Tinti de Castro P[1], Eleutério Pereira D[1], Ambrósio SR[1], Kenupp Bastos J[2], Crispim Tavares D[1]
[1]University of Franca, Brazil; [2]Faculty of Pharmaceutical Sciences, State University of São Paulo, Brazil

Copaiba oils are produced by exudation from the trunks of trees belonging to the genus *Copaifera*. *Copaifera langsdorffii* known as "copaiba", "capaiva" or "pau-de-oleo" belongs to the Leguminosae family. The effects attributed to copaiba oils in folk medicine include anti-inflammatory, anti-tetanus, anti-tumour, anti-blenorrhagea and urinary antiseptic activities. In the present study, we evaluated the effects of *C. langsdorffii* oil on the formation of 1,2-dimethyl-hydrazine (DMH)-induced aberrant crypt foci (ACF) in the colon of the male Wistar rat. The animals received subcutaneous (sc) injections of DMH (40 mg/kg body weight, b.w.) twice a week for two weeks to induce ACF. *C. langsdorffii* oil was administered to the rats five times a week for four weeks by gavage at doses of 12.5, 25 and 50 mg/kg b.w/day each, during and after DMH treatment. All animals were sacrificed in week 5 for the evaluation of ACF. The results showed a significant reduction in the frequency of ACF in the group treated with the *C. langsdorffii* oil plus DMH when compared to those treated with DMH alone, suggesting that *C. langsdorffii* oil suppress the formation of ACF and have a protective effect against colon carcinogenesis. Financial Support: Foundation for Research Support of São Paulo State (FAPESP, grants number 2009/17237 – 8 and 2011/13630 – 7).

PI146 Analysis of the pentane fraction obtained from *Daucus carota* oil extract and its activity against colon cancer cell lines

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Phytotherapy has been widely used throughout the years for the treatment of a variety of diseases such as colon and colorectal cancers. Recent studies in our laboratories have shown that *Daucus carota* (Linnaeus) ssp. carota possesses potential anti-tumor activity. Oil extract (1:1 methanol/acetone) of *Daucus carota* grown in Lebanon was subjected to a chromatographic separation using a pentane based mobile phase to yield four fractions. GCMS analysis of the first fraction, F1, reveals 11 components present in excess of 1%, such as α-longipinene (2.97%), humulene (13.2%), (10S,11S)-himachala-3(12)-4-diene (2.56%), caryophyllene (7.41%), β-selinene (32.6%), β-himachalene (3.96%) and caryophyllene oxide (2.33%). The anticancer effect of the F1 fraction was tested against colon cancer cell lines, HT-29 and Caco-2, and the results demonstrated that the F1 fraction had an anti-proliferative effect against both cell lines. The F1 fraction also exhibited dose dependent cytotoxicity against all tested cell lines with IC_{50} values of 25 μg/mL for HT-29 cells and 15 μg/mL for Caco-2 cells. A comparitive study of *Daucus carota* from various geographic locations including Lebanon and Europe revelead significant differences regarding the major components present in the methanol/acetone oil extract. In conclusion, the composition of the *Daucus carota* oil extract is dependant on geographical location and that the F1 fraction of the oil extract obtained from *Daucus carota* grown in Lebanon posseses anticancer activity.

PI147 Endogenous GA3 levels of artichoke plants: At different parts and various developmental stage

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The hormone gibberllin (GA) plays an important role in many aspects of plant growth and development like floral development, stem elongation. This study was conducted to reveal the level of endogenous GA3 in different parts of artichoke (*Cynara scolymus* L.) cv. Sakız, an important Turkish local cultivar, was used as plant material. The levels of endogenous GA3 on Ga3 treated and non treated plants were determined by High Performance Liquid Chromotography (HPLC) method. The results of the study revealed that the highest level of GA3 was determined on the plants treated with in 25 ppm GA3 and at the miniature head parts of the plant. On the other hand it was found that the lowest level of GA3 was determined on the plants treated with in 50 ppm GA3 and at the mature head.

PI148 Anti-inflammatory compounds of *Smilax corbularia* Kunth

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Smilax corbularia Kunth (Smilacaceae) is Thai traditional medicine plant locally known as 'Hua-Khao-Yen Neua' were examined for their inhibi-

tory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines. The ethanolic extract of *Smilax corbularia* Kunth exhibited the most inhibitory activity, with an IC_{50} value of 83.90 μg/ml. From this extract, three compounds [engeletin (1) astilbin (2) and quercetin (3)] were isolated and further investigated for their inhibitory properties of NO production. It was found that **3** possessed the highest activity (IC_{50}= 9.17 μg/ml), whereas **1** and **2** exhibited mild activity. The ethanolic extract, water extract of *Smilax corbularia* Kunth and its compounds **1 – 3** were also evaluated for the inhibitory effect on LPS-stimulated PGE_2 release from RAW 264.7 cells. The results found that **1, 2** and **3** possessed potent activity against PGE_2 release with IC_{50} values of 19.67, 19.72 and 19.85 μg/ml respectively. The present study support the use of *Smilax corbularia* Kunth by Thai traditional medicine for treatment the inflammatory diseases

PI149

Analysis of chromone glycosides in saposhnikovia divaricata for the establishment of a monograph of the german pharmacopoeia

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[1]University of Regensburg, Institute of Pharmacy, Pharmaceutical Biology, Universitätsstr. 31, 93040 Regensburg, Germany; [2]University of Aberdeen, Department of Chemistry, Meston Building, Meston Walk, Aberdeen, AB24 3UE; [3]Federal Institute for Drugs and Medical Devices, Kurt-Georg-Kiesinger-Allee 3, 53175 Bonn, Germany

Medicinal plants from Traditional Chinese Medicine (TCM) are getting popular in Europe. Therefore it was decided to develop TCM monographs for the German Pharmacopoeia (DAB). Saposhnikoviae divaricatae radix is used in TCM to treat common cold and headache. Prim-O-glucosylcimifugin and 4'-O-β-D-glucosyl-5-O-methylvisamminol are considered to be among the active compounds of *Saposhnikovia divaricata* (Turcz.) Schischk. Thus, these chromones were selected as suitable reference compounds for TLC/HPTLC fingerprint identification and quantitative analysis in the assay. A new solvent system allows the HPTLC differentiation of the medicinal herbal drug from its most common adulterants. For quantification, a HPLC method published by Xin was modified and validated, resulting in good separation of the chromones. In comparison, also a densitometric HPTLC method was developed for these compounds. Quantitative HPLC and HPTLC were compared to evaluate their suitability for a new Saposhnikovia monograph.

PI150

Characterization of the anti-inflammatory effect from the essential oil of *Citrus latifolia*

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Federal University of Rio de Janeiro; [1]Institute of Biomedical Science; [2]Natural Product Research Center. Av. Carlos Chagas Filho 373, CCS Building. Rio de Janeiro, Brazil

The aim of this work was to evaluate the anti-inflammatory effect of the essential oil *Citrus latifolia* leaves (CL) in the carrageenan (Carr)-induced inflammation into the subcutaneous air pouch (SAP). Male mice (20 – 25 g) received oral administration of CL (10, 30 or 100 mg/kg) 1 h before Carr injection. After 24 h the mice were euthanized and the exsudate from SAP were collected to several measurements (listed below). Authorization for animals assays was ICBDFBC015. Statistical analyses was performed by ANOVA and Bonferroni's test (*p < 0.05). Results are:

Group	mg/kg	Leukocyte (x 10^6 cell/mL)	NO (μM)	TNF-γ (pg/mL)	INF-γ (pg/mL)
PBS	-	1.1 ± 1.1	35.6 ± 5.8	35.7 ± 6.6	381.1 ± 29.5
Carr	-	58.6 ± 6.8	207.1 ± 31.8	674.1 ± 66.1	914.8 ± 66.6
CL	10	40.7 ± 5.4*	174.9 ± 21.1	630.4 ± 55.9	488.8 ± 45.4*
	30	22.6 ± 9.3*	77.6 ± 23.4*	394.4 ± 81.6*	27.5 ± 13*
	100	11 ± 6.8*	65.2 ± 28.4*	386.2 ± 45.8*	18.4 ± 13.8*

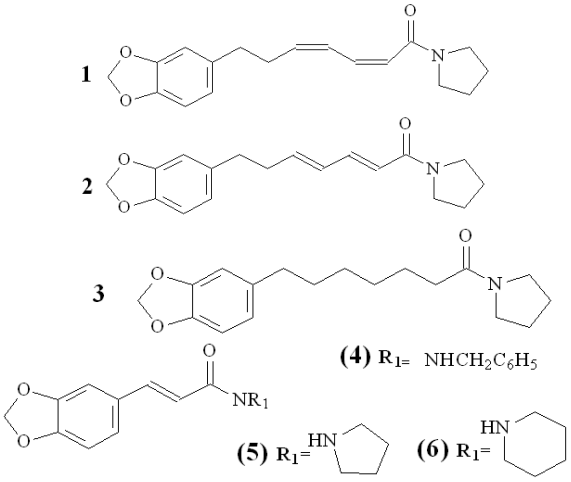
Conclusions: The essential oil from *C. latifolia* develops an anti-inflammatory effect, reducing several parameters of an inflammatory process.
Financial support: CNPq, FAPERJ, Instituto Vital Brazil.

PI151

Antileishmanial activity of amides from *Piper amalago* L. derivative, and synthetic analogs

Cortez DAG[1], Carrara VS[1], Serra LZ[1], Demarchi IG[2], Lonardoni MVC[2], Cardozo-Filho L[3], Cunha-Júnior EF[4], Torres-Santos EC[4], Corrêa AG[5], Monteiro JL[5], Cortez LER[6]
[1]Departamento de Farmácia; [2]Departamento de Análises Clínicas; [3]Departamento de Engernharia Química, Universidade Estadual de Maringá, Maringá, PR; [4]Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro; [5]Departamento de Química, UFSCar, São Carlos; [6]Cesumar, Avenida Guedner, 1610 Maringá – PR, Brazil

Amides (**1 – 2**) were isolated from the chloroform extract of *Piper amalago* L. leaves. A derivative (**3**) and synthetic analogs (**4 – 6**) were prepared and all the compounds were tested against the promastigote and intracellular amastigote forms of *Leishmania amazonensis*. The cytotoxicity toward the J774A1 macrophages and the ability to induce nitric oxide production were also investigated. Compound **2** was the most active of all the compounds against the promastigote and intracellular amastigote forms with IC_{50} values of 15 μM and 14.5 μM, respectively. None of the compounds modulated the production of nitric oxide, suggesting a direct mechanism of action on *Leishmania*.

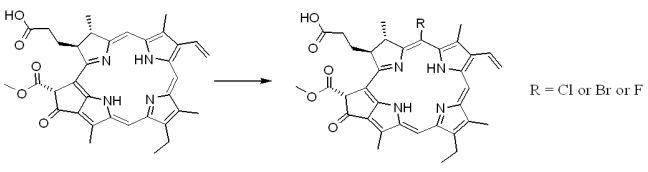


PI152

Potential anticancer activity of young Carpinus betulus leaves

Cieckiewicz E[1], Angenot L[1], Gras T[2], Kiss R[2], Frédérich M[1]
[1]University of Liège, CIRM, Laboratory of Pharmacognosy, Liège, Belgium; [2]Free University of Brussels, Institut of Pharmacy, Laboratory of Toxicology, Campus de la Plaine, Bruxelles, Belgium

As part of our ongoing research for anticancer compounds from the Walloon Region forest[1], EtOAc extract from *Carpinus betulus* leaves was phytochemically studied, leading to the bioguided isolation of pheophorbide a. Evaluation of the growth inhibitory activities of pheophorbide a using MTT colorimetric assay and phase-contrast microscopy in various human cancer cell lines confirmed its photoactivable properties[2]. To improve these properties, the 20-meso position of pheophorbide a was halogenated (Cl or Br or F) and the resulting 20-halogenated pheophorbide a were also evaluated for their growth inhibitory activities.



[1]Frédérich M, Marcowycz A, Cieckiewicz E, Mégalizzi V, Angenot L, Kiss R. Planta Med 2009, 75, 1634 – 1637. [2] Cieckiewicz E, Angenot L, Gras T, Kiss R, Frédérich M, Phytomedicine 2012, 19, 272 – 283.

PI153

In vitro morphogenetic responses and comparative analysis of phthalides in the highly valued medicinal plant *Ligusticum porteri*

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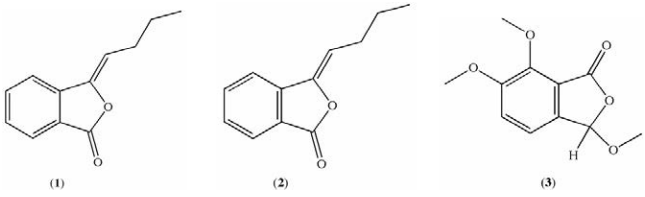
[1]Labotarorio de Etnobotánica, Jardín Botánico del Instituto de Biología, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Coyoacán, 04510, México D.F;
[2]Laboratorio de Cultivo de Tejidos Vegetales, Jardín Botánico del Instituto de Biología, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Coyoacán, 04510, México D.F;
[3]Laboratorio 124, Departamento de Farmacia, Facultad de Química, Anexo E, Universidad Nacional Autónoma de México, Circuito Escolar, Copilco, 04510, México D.F

The morphogenetic response of *Ligusticum porteri* was investigated as a part of a conservation strategy and was compared to that of *Petroselinum crispum*. After *in vitro* germination of seeds, plantlets were excised into different explants and cultured in an induction medium supplemented with 2,4-dichlorophenoxyacetic acid (0 – 18.09 μM) or α-naphthaleneacetic (0 – 21.48 μM) acid in combination with 6-benzylaminopurine (0 – 13.31 μM) to induce callus formation. GC-MS analysis of calli, mature aerial parts and roots extracts of both species led to the identification of 3-butylidenephthalide (**1**), 3-*n*-butylphthalide (**2**) and 3,6,7,-trimethoxy-isobenzofuran-13(H)-one (**3**). This is the first report on phthalides production from *in vitro* cultures of *L. porteri*.



PI154

Punicatannins A and B: α-glucosidase inhibitory ellagitannins from pomegranate (*Punica granatum*) flowers

Yuan T[1], Ferreira D[2], Seeram NP[1]
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[2]Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, United States The fruit of Punica granatum L., commonly known as pomegranate, has been extensively studied but there is paucity of data on compounds in its flowers. Interestingly, pomegranate flowers have been utilized for treating diabetes in traditional medicine systems including Unani and Ayurveda. Moreover, a pomegranate flower extract was reported to lower blood glucose levels in the Zucker rat diabetes model. However, to date, the bioactive compounds responsible for these effects remain unidentified. Herein, α-glucosidase inhibitory bioassay guided isolation of pomegranate flowers yielded nine hydrolysable tannins including two new ellagitannins named punicatannins A and B. The punicatannins contained an unprecedented oxidized hexahydroxydiphenoyl moiety not previously observed in ellagitannins. Their structures and absolute configurations were determined by extensive spectroscopic analyses (including NMR, HRMS, and ECD) and chemical methods. Punicatannin A was 30 times more potent than the clinical α-glucosidase inhibitory drug, acarbose. These results suggest that ellagitannins are the bioactive constituents in pomegranate flowers responsible for its blood glucose lowering effects.

PI155

Glycolipids from *Ipomoea murucoides* as modulators of antibiotic activity in multidrug-resistant strains

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Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, 04510 DF, Mexico

The $CHCl_3$-soluble extract from flowers of *Ipomoea murucoides*, through preparative scale recycling HPLC, yielded three new pentasaccharides of 11-hydroxyhexadecanoic acid, named murucoidins XVII-XIX, which were characterized by NMR and mass spectrometry. These compounds were tested for *in vitro* antibacterial and resistance modulating activity against *Staphylococcus aureus, Escherichia coli* Rosetta gami, and two nosocomial pathogens, *Salmonella typhi* and *Shigella flexneri.* They exerted a potentiation effect of the clinically useful antibiotics tetracycline, kanamycin, and chloramphenicol against the tested bacteria strains by increasing antibiotic susceptibility up to 16-fold at concentrations of 25 μg/mL (Figure 1). Financial Support: Dirección General de Asuntos del Personal Académico, UNAM (IN217310) and CONACyT (101380-Q).

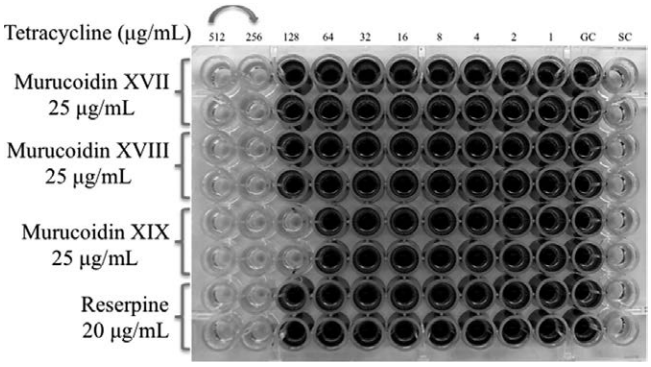

Fig. 1: Modulation assay of tetracycline against *S. flexneri.*

PI156

Profiling of Alkaloids and Eremophilanes in Miracle Tea (*Packera candidissima* and *P. bellidifolia*) Products

Fragoso-Serrano M, Figueroa-González G, Pereda-Miranda R
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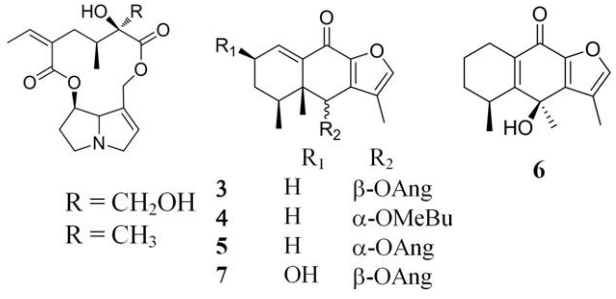
Commercial preparations of the Mexican herbal drug known as "miracle tea" (*Packera candidissima* and *P. bellidifolia*) have been profiled qualitatively by HPLC and GC-MS. Pyrrolizidine alkaloids were identified in the alkaloid extracts. The content of free PAs and their N-oxides was determined for a total of 22 samples and the results showed that the amount of these hepatotoxic compounds (0.0005 – 0.94% free PAs; 0.0004 – 0.55% N-oxides), through the presence of retrorsine (**1**) and senesionine (**2**) as the main constituents, may reach toxic levels. Eremophilanes (**3-7**) were the major components found in the hexane-soluble fraction. Financial Support: CONACyT (101380-Q).



1 $R = CH_2OH$
2 $R = CH_3$

	R_1	R_2
3	H	β-OAng
4	H	α-OMeBu
5	H	α-OAng
7	OH	β-OAng

6

PI157

Preparation of phenylbutanoid-rich *Zingiber cassumunar* extracts and simultaneous HPLC analysis of phenylbutanoids

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Zingiber cassumunar are used increasingly as ingredients in marketed phytomedicines. Therefore, methods for the preparation of active constituent-rich extract, which yield products with batch-to-batch consistency, are required. Four anti-inflammatory phenylbutanoids, (*E*)-4-(3,4-dimethoxy-phenyl)but-3-en-l-ol, (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-l-yl acetate, (*E*)-1-(3,4-dimethoxy-phenyl)butadiene and (*E*)-3-(3,4-di-

methoxyphenyl)-4-[(*E*)-3,4-dimethoxystyryl]cyclohex-1-ene, isolated from *Z. cassumunar*, were used as standard markers for quantitative determination and preparation of phenylbutanoid-rich *Z. cassumunar* extracts (PZEs). A reversed-phase HPLC method was established for the simultaneous determination of the phenylbutanoids in *Z. cassumunar* extracts. The parameters of linearity, repeatability, reproducibility, accuracy, specificity, and sensitivity of the method were evaluated. Systematic extraction studies to maximize phenylbutanoid content revealed that hexane was the most appropriate solvent for extraction. A one-step purification of the hexane crude extract of *Z. cassumunar*, using silica gel vacuum chromatography, provided the PZEs. The content of phenylbutanoids in the PZEs was up to 48.3% w/w dry weight.

PI158 Purgins II-III, glycolipid ester-type dimers from *Ipomoea purga*

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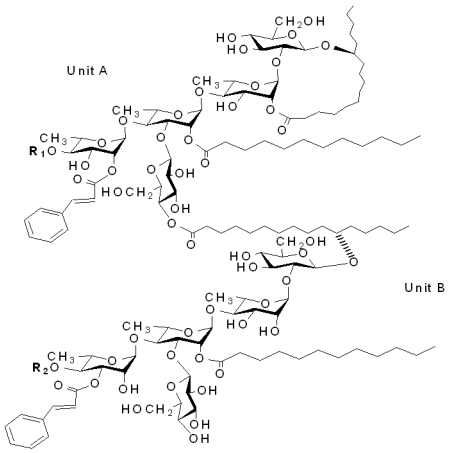
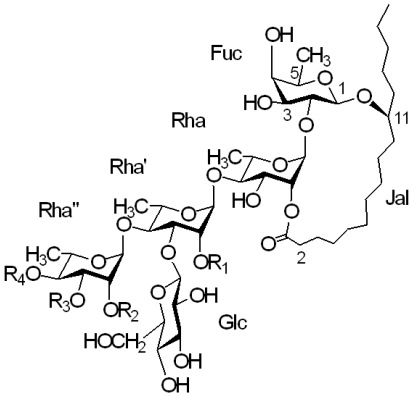
Purgins II and III (**1** and **2**), glycolipid ester-type dimers, along with purginosides III-IV (**3** and **4**) were isolated from the chloroform-soluble extracts of the aerial parts of *Ipomoea purga.* They were purified by preparative-scale recycling HPLC. Compounds **1** and **2** are the first dimers isolated from the morning glory family (Convolvulaceae) containing operculinic acid B as their oligosaccharide cores (monomeric units A and B).



	R_1	R_2
1	mba	mba
2	hexa	hexa



	R_1	R_2	R_3	R_4
3	deca	H	hexa	Cna
4	deca	H	H	hexa

PI159 New chlorinated daphnane diterpenoids orthoester from *Trigonostemon cherrieri* as potent antiviral agents

Bourjot M[1], Allard PM[1], Martin MT[1], Leyssen P[2], Guéritte F[1], Litaudon M[1]
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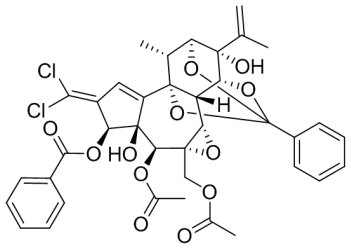
The phytochemical investigation of *T. cherrieri* Veillon (Euphorbiaceae), an endemic plant from New Caledonia, led to the isolation of a series of thirteen new chlorinated and highly oxygenated daphnane diterpenoids orthoester (DDOs) in minute quantities. DDOs have been detected by using a combination of LC/UV, LC/MS and microprobe NMR spectroscopic methods. The first member of the series, trigocherrin A, exhibited a potent and selective antiviral effect on the replication of the Chikungunya virus in cell culture.[1] We will report in this communication their isolation, characterization and antiviral activities against HIV, Chikungunya, Semliki forest and Sindbis viruses. The structure-activity relationships will be discussed. Reference: [1]Allard *et al.* (2012), Org. Lett. 14 (1): 342 – 345



Trigocherrin A

PI160 Quantitative analysis of nine compounds from the stem barks of *Fraxinus rhynchophylla* by HPLC-DAD

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College of Pharmacy & CICT, Chungbuk National University, Cheongju 361 – 763, Korea

A high-performance liquid chromatography-diode array detector (HPLC-DAD) method was established for quantitative evaluation of nine constituents of *Fraxinus rhynchophylla* such as four coumarins, esculin (**1**), fraxin (**2**), esculetin (**3**), esculin (**4**), three lignans, syringaresinol 4,4'-O-β-D-diglucoside (**5**), pinoresinol 4-O-β-D-glucoside (**6**), pinoresinol (**9**), one secoiridoid, oleuropein (**7**), and one coumarinolignan, cleomiscosin B (**8**). The HPLC-DAD condition was optimized for the simultaneous analysis of nine constituents using a reversed-phase C18 column with a gradient water-acetonitrile solvent system. The wavelength was set at 220 nm. The developed method was also validated for linearity, intra/inter precision and recovery. The content of these constituents in *F. rhynchophylla* samples from different extraction conditions with various extraction time, different extraction environment and extraction solvents was quantitated using developed method. Our study suggested that addition of water to extraction solvent, longer extraction time and extraction at 70 °C significantly increased yield of extraction.

PI161 A skin lightening, sun protector and antioxidant agent: origa'light®

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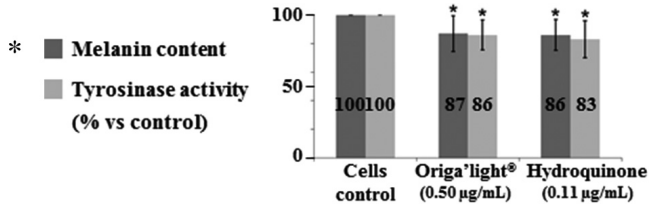
Today's, there is an amazing increase in the demand for skin lightening products. We have developed a new natural extract inhibiting melanin production: an oregano leaf extract, Origa'light®. This extract has a significant and dose-dependent lightening activity. It shows a strong inhi-

bition of melanogenesis by a decrease of melanin content (-13%) and tyrosinase activity (-14%) in B16 murine melanocytes*. Moreover, with a SPF of 7.14 and a proved photostability, it has interesting photoprotective properties. It has also strong antioxidant activities against DPPH and superoxide anion radicals (IC_{50}= 16.0 µg/mL and 7.4 µg/mL). Thus, Origa'-light® is an excellent cosmetics multi-active ingredient: skin lightening, sun protector and antioxidant.



PI162 Leontopodic acid in edelweiss related species

Slacanin I[1], Rey C[2], Rey S[2], Gafner F[3]
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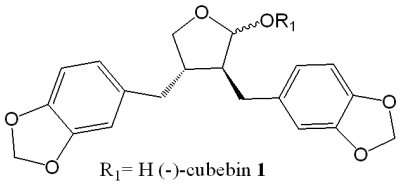
In earlier times Edelweiss *Leontopodium alpinum* Cass. had been known under several names such as *Gnaphalium leontopodium*, *Gnaphalium alpinum*, *Filago alpina*, *Filago leonto-podium* and *Antennaria leontopodium*. In 1822 Alexandre de Cassini assigned Edelweiss to the genus *Leontopodium* and named it *Leontopodium alpinum*. In 2005 Schwaiger et al identified and elucidated the structure of unique caffeoyl-D-glucaric acid derivatives leontopodic acid and leontopodic acid B in Edelweiss (*L. alpinum*) and other species from the genus *Leontopodium*. In 2010, Blöch et al investigated and published the phylogenetic relationship of species of the genus *Leontopodium* with those of *Gnaphalium, Filago, Antennaria* and others. In the Swiss alps and the Val d' Aoste (Italy), we collected plant species related to Edelweiss in order to analyze them for the presence of leontopodic acids and to assess the chemosystematic significance of those compounds. Leontopodic acids were present in most species analyzed. Our data show a borderline between the genera containing leontopodic acids and those that do not. Leontopodic acids therefore appear to be interesting chemosystematic markers for the genera *Leontopodium*, *Gnaphalium, Filago* and *Antennaria*. The combination of phytochemical analysis and molecular phylogeny relationships is an interesting tool for the discovery of bioactive natural products in commercially interesting plants.

PI163 Antibacterial activity of (-)-cubebin isolated from *Piper cubeba* and its synthetic derivatives against microorganisms that cause endodontic infections

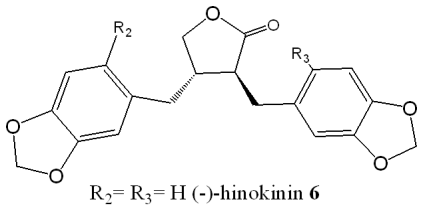
Rezende KCS[1], Lucarini R[1], Símaro GV[1], Souza CD[1], Esperandim VR[1], Martins CHG[1], Vinholis AHC[1], Cunha WR[1], Bastos JK[2], e Silva MLA[1]
[1]Núcleo de Ciências Exatas e Tecnológicas da Universidade de Franca, Av. Dr. Armando Salles Oliveira, 201, CEP14404 – 600 Franca, SP, Brazil; [2]Departamento de Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto – USP, Av. Do Café S/N, CEP 14030 – 000, Ribeirão Preto, SP, Brazil

The aim of this study was the obtainment of (-)-cubebin **1**, its semisynthetic derivatives and the subsequent evaluation of their antibacterial activity. It was used for the synthesis of: (-)-*O*-methylcubebin **2**, (-)-*O*-benzylcubebin **3**, (-)-*O*-acetylcubebin **4**, (-)-O-(*N,N*-dimethylamino-ethyl)-cubebin **5**, (-)-hinokinin **6** and (-)-6.6'-dinitrohinokinin **7**. The evaluation of the antibacterial activity have been done by broth microdilution technique for determination of the minimum inhibitory concentration (**MIC**) and the minimum bactericidal concentration (**MBC**) against *Porphyromonas gingivalis*, *Prevotella nigrescens*, *Actinomyces naeslundii*, *Bacteroides fragilis* and *Fusobacterium nucleatum*. From this study, it was possible to make an analysis regarding the relationship between structure and antimicrobial activity of derivatives against microorganisms that cause endodontic infections. Among the results presented in both trials, the most promising were **MIC**= 50 mg/mL against *P. gingivalis* by **2** and **3**, and **MIC**= 100 µg/mL against *B. fragilis* by **6**. The cytotoxicity assays demonstrated that **1** does not display toxicity, as well as its derivatives, which contributes to its direct use in biological assays.

Sponsors: CNPq, FAPESP and CAPES



R_1= H (-)-cubebin **1**
R_1= -CH_3 (-)-*O*-metilcubebin **2**
R_1= -CH_2-Ph(-)-*O*-benzylcubebin **3**
R_1= -CH_2-CO-CH_3 (-)-*O*-acetylcubebin **4**
R_1= -C_2H_4-N-$(CH_3)_2$ (-)-O-(N,N-dimethylamino-ethyl)-cubebin **5**



R_2= R_3= H (-)-hinokinin **6**
R_2= R_3= -NO_2 (-)-6,6'-dinitrohinokinin **7**

PI164 Identification of anti-inflammatory fraction & isolation of active compound formononetin from root of *Astragalus membranaceus*

Lai PKK[1,2], Chan JYW[1,2], Cheng L[1,2], Lau CP[1,2], Han SQB[1], Leung PC[1,2], Fung KP[1,2,3], Lau CBS[1,2]
[1]Institute of Chinese Medicine; [2]State Key Laboratory of Phytochemistry and Plant Resources in West China (CUHK); [3]School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

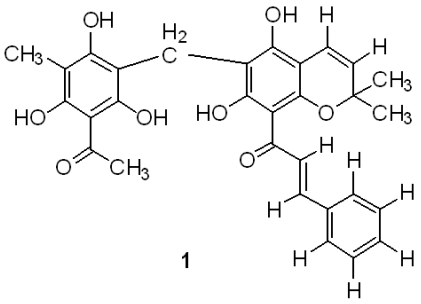
The present study aimed to identify active fraction(s) and compound(s) from AR (root of *Astragalus membranaceus*) aqueous extract that are responsible for the anti-inflammatory effect using *in vitro* bioassay-guided fractionation. The effect was monitored by inhibition of nitric oxide (NO) released from lipopolysaccharide (LPS)-stimulated mouse macrophage RAW 264.7 cells after treated with AR extract, sub-fraction or isolated component(s). Two active fractions (P2 – 3-2 – 2-2 and P2 – 3-2 – 2-3) were found to significantly inhibit NO production. Formononetin was isolated from P2 – 3-2 – 2-3 and was shown to inhibit NO production. Besides, the anti-inflammatory effect of combined fraction (P2 – 3-2 – 2-2 and P2 – 3-2 – 2-3 in 1:1 ratio) was evaluated by measuring (i) release of prostaglandin E_2 (PGE_2), interleukins (IL-1β, IL-6) and tumor necrosis factor (TNF-α) using ELISA; (ii) expression of inducible nitric oxide synthase (iNOS) by RT-PCR; (iii) expression of iNOS, cyclooxygenase-2 (COX-2) and mitogen-activated protein kinase (MAPK) pathway by Western blot; (iv) activation of nuclear factor kappa B (NFκB) translocation by electrophoretic mobility shift assay. The combined fraction could significantly inhibit LPS-induced expression of iNOS, COX-2, MAPK and NFκB, as well as production of PGE_2, IL-1β, IL-6 and TNF-α. In conclusion, two major anti-inflammatory AR fractions were identified and formononetin was one of the active components.

PI165 Rottlerin: An antibacterial agent and inhibitor of plasmid-mediated antibiotic resistance transfer

Mbaebie B[1], Shinde V[2], Shinde K[2], Guzman JD[1,3], Bhakta S[3], Stapleton P[1], Gibbons S[1]
[1]Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, WC1N 1AX London; [2]Department of Pharmacognosy,

Poona College of Pharmacy, Pune 38, India. [3]Department of Biological Sciences Institute of Structural and Molecular Biology Birkbeck, University of London WC1E 7HX London Analysis of the phytochemical and antimicrobial properties of *Mallotus phillipensis* revealed rottlerin (**1**) as the primary active agent in a chloroform extract from the plant. Rottlerin had notable activities against *Staphylococcus aureus* (0.625 – 4 µg/ml) and good to moderate activities against *Escherichia coli* (32 µg/ml) and *Mycobacteriun bovis* (100 µg/ml) respectively. Remarkably, rottlerin at sub-inhibitory concentrations inhibited by > 1000-fold the transfer of broad-host range plasmid pKM101 between *E. coli* isolates. The anti-

plasmid activity appeared to be specific, the transfer plasmids of different types, such as TP114, were not inhibited by such the same extent although the complete range of activity has yet to be determined. Rottlerin appears to have dual properties: direct anti-bacterial activity and the capacity to inhibit antimicrobial resistance spread. This is vital in the fight against multi-resistant bacteria and exploiting the mechanism of plasmid transfer could potentially lead to the development of more universal antimicrobial agents.



1

PI166 stimulation of cellular proliferation of human fibroblasts and human keratinocytes cell by *Eclipta prostrata* and *Litsea glutinosa* extracts

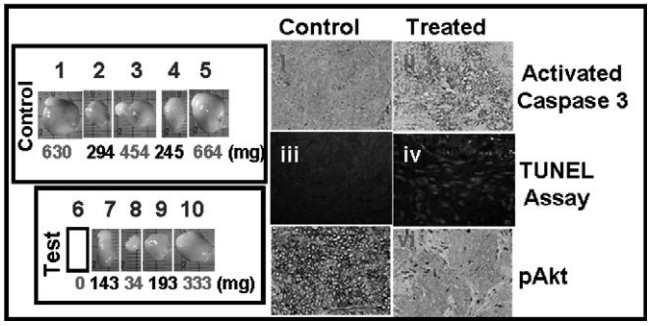
Nualkaew N[1], Wang R[2], Hensel A[2]
[1]Faculty of Pharmaceutical Sciences, Khon-Kaen University, Khon-Kaen 40002, Thailand; [2]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Münster D-48149, Germany

Stimulation of cellular proliferation is central mechanism within the complex physiology of wound healing. This study aimed to investigate the influence of extracts (EtOH 95%) from the aerial parts from Eclipta prostrata Linn. (Asteraceae) and of an aqueous extract from Litsea glutinosa C.B.Robinson (Lauraceae) leaves on the proliferation of primary human dermal fibroblasts and immortalized human keratinocyte cell line (HaCaT). Both plants are extensively used within Thai traditional medicine for wound healing. Results indicated that extracts from E. prostrata (50 µg/mL) stimulated fibroblast cell proliferation in a dose-dependent manner to 196% ($p < 0.01$). Also keratinocyte proliferation was stimulated at 1 µg/mL to 139% ($p < 0.01$). L. glutinosa leaves are rich of polysaccharide. Fractionation of an aqueous extract yielded the high molecular raw polysaccharides (RPS) and the low molecular weight fractions (LMW). RPS was fractionated on DEAE-Sephacel ion exchange stationary phase. The neutral fraction exhibited 153% proliferation ($p < 0.01$) in HaCaT cell at concentration of 100 µg/mL but showed no significantly effects against dermal fibroblasts. The LMW fraction stimulated fibroblast proliferation to 256% ($p < 0.01$) at concentration of 10 µg/mL, and increased HaCaT proliferation to 163% ($p < 0.01$) at concentration of 50 µg/mL. The L. glutinosa LMW fraction with highly stimulation effect is interesting for further studies. The increasing of cells proliferation of both plants extracts was along with their traditional uses for wound healing.

PI167 Systematic analyses validates *in vitro* and *in vivo* anticancer properties of the Ayurvedic plant *Achyranthes aspera*

Sarkar M[1], Subbarayan PR[2], Philip S[3], Kumar P[5], Ahmed M[3], Ardalan B[1], Lokeshwar BL[3,4]
[1]Department of Biology; [2]Department of Medicine; [3]Department of Radiation Oncology; [4]Department of Urology, University of Miami Miller School of Medicine, Miami; [5]Rameshwar Research and Development Corporation, Meerut, India

Leaves of the plant *Achyranthes aspera* (Family: *Amaranthacea*) is used to treat pancreatic cancer (PaCa) by ayurvedic practitioners. Due to traditional use, its anti-tumor properties and clinical usage remains anecdotal. Here we demonstrate *A. aspera* preferentially inhibited the proliferation of cultured human pancreatic cancer cells, and growth of human pancreatic cancer in athymic mice. Preliminary mechanistic analyses indicate induction of caspase-3, dephosphorylation of Akt-1, inhibition of angiogenic and metastatic specific genes. Detailed results will be discussed.



PI168 Bioactivity-guided isolation of antioxidant and quinone reductase-inducing constituents from Maqui berry

Li J[1], Chai H[1], Deng Y[1], Keller WJ[2], Kinghorn AD[1]
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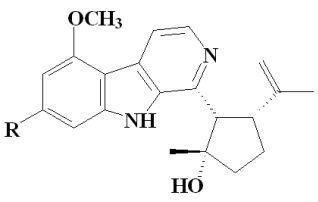
Aristotelia chilensis (Mol.) Stuntz (Elaeocarpaceae), Maqui berry, is a native South American evergreen shrub that produces small edible purple or black berries. In a search for naturally occurring cancer chemopreventive agents, the ethyl acetate-soluble extract of *A. chilensis* showed significant hydroxyl-radical (·OH) scavenging activity, and thus was fractionated via bioactivity-guided isolation. Fifteen known compounds (**1**-**15**) were isolated, with phloroglucinaldehyde 2-*O*-*ß*-D-glucopyranoside (**12**) being isolated as a new natural product, and eleven compounds (**1**, **3**, and **7**-**15**) obtained from *A. chilensis* for the first time. The structures of these compounds were identified on the basis of spectroscopic methods. All pure isolates were evaluated for their hydroxyl-radical scavenging and quinone reductase (QR) induction activities. Twelve compounds (**1**-**12**) showed potent antioxidant activity in the hydroxyl-radical scavenging assay, with cyanidin 3-*O*-*ß*-D-glucopyranoside (**2**, ED_{50}= 0.13 µM) being of the greatest potency. Four compounds (**3**, **7**, **9**, and **11**) exhibited relatively high QR-inducing activity, with protocatechuic acid (**9**, CD = 4.1 µM) found to be the most potent. Thus, *A. chilensis* seems worthy of further investigation for the discovery of cancer chemopreventive agents.

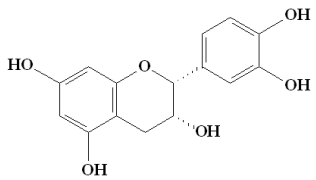
PI169 New β-carboline alkaloids from *Galianthe ramosa* (Rubiaceae)

de Freitas CS[1], Silva D[1], Kato L[1], de Oliveira CMA[1], Schuquel IT[2], da Silva CC[2], de O Santin S[2], Delprete PG[3], Silva Neto BR[4], Soares CMA[4], Pereira M[4]
[1]Instituto de Química – [4]Instituto de Ciências Biológicas-Universidade Federal de Goiás, Campus Samambaia, 74001 – 970- Goiânia, GO; [2]Depto. Química, Universidade Estadual de Maringa, Av. Colombo, 5790, 87020 – 900 Maringá, PR, Brazil; [3]Herbier de Guyane, (IRD),UMR AMAP, B.P. 165, 97323 Cayenne Cedex, French Guiana, France

Galianthe Griseb. (Rubiaceae, tribe Spermacoceae) is a genus comprising 50 endemic species in South America, its main center of diversity is located in central and southern Brazil. In our continuing chemical and biological investigations concerning Rubiaceae species from Cerrado, we report the isolation of new compounds **1** and **2**, together with the known epi-catechin (**3**), ursolic and oleanolic acid. The alkaloid **2** inhibited the enzyme malato synthase from pathogenic fungus *Paracoccidioides brasiliensis* wich is considered an important target since it is not found in human.



R=OCH_3=Alkaloid (**1**)
R=H=Alkaloid (**2**)



Epi-catechin (**3**)

PI170 Phytochemical screening, isolation of betulinic acid and trigonelline from stems of an indigenous plant, from the Guyana flora

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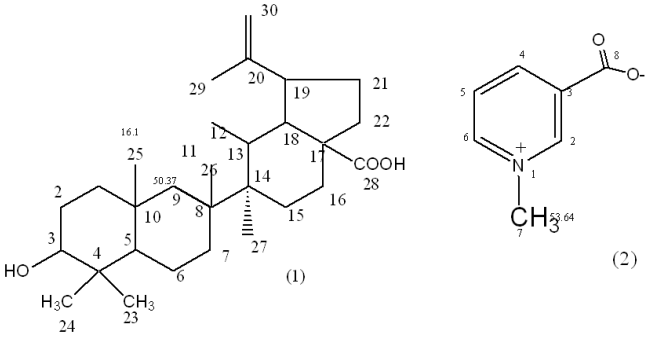
[1]Department of Chemistry, University of Guyana, South America; [2]Department of Chemistry, University of the West Indies, St. Augustine campus, Trinidad and Tobago

The stems of an indigenous plant of the Guyana (South America) flora were screened for natural products using solvents of varying polarity: C_6H_{12}, CH_2Cl_2, EtOAc, CH_3CH_2OH. Phytochemical screening revealed the presence of Emodols, Tannins, Flavones and other reducing compounds in the non-hydrolysed C_2H_5OH extract. C_6H_{12} extracts revealed the presence of sterols, triterpenes, coumarins and reducing compounds. From the CH_2Cl_2 extract, a white solid crystallized, which after further purification and spectroscopic elucidation, yielded the lupine type pentacyclic triterpene, betulinic acid **(1)** (betulinic acid (3-beta-hydroxylup-20-(29)-en-28-oic acid). The EtOAc extract showed the presence of emodols, tannins, flavones, reducing compounds and alkaloid salt. Flash column chromatography yielded Trigonelline **(2)** as one of the major fraction. For the hydrolysed C_2H_5OH extract, positive tests were noted for anthrasenosides and coumarins. The non-hydrolysed C_2H_5OH extract revealed the presence of emodols, tannins, reducing compounds and flavones.



PI171 The anti-metastatic activity of a chinese herb *Andrographis paniculata* in esophageal cancer – a preclinical study

Yue GGL[1,2], Lee JKM[1,2], Chan JYW[1,2], Pui Fung K[1,2,3], Chiu PWY[4], Lau CBS[1,2]
[1]Institute of Chinese Medicine; [2]State Key Laboratory of Phytochemistry and Plant Resources in West China (CUHK); [3]School of Biomedical Sciences; [4]Department of Surgery, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

Esophageal cancer is usually diagnosed at advanced stage with high postoperative recurrence and systemic metastasis which leads to poor prognosis. In the present study, the *in vitro* anti-metastatic effects of the herb *Andrographis paniculata* (AP), a herbal medicine widely used in both Chinese and Indian traditional medicines, were evaluated in two esophageal cancer cell lines, EC-109 and KYSE-520. The cytotoxicities of AP aqueous and ethanolic extracts were determined by MTT assay. The effects of AP extracts at non-toxic concentrations were assessed using scratch wound and cell invasion assays. Our results showed that AP ethanolic extract (50 μg/ml) and aqueous extract (800 – 1600 μg/ml) significantly decreased the closed wound area in both cell lines, suggesting inhibitory effects on motility of cells. Unlike the ethanolic extract which has no inhibitory effect, AP aqueous extract (1600 μg/ml) significantly inhibited the invasion of EC-109 and KYSE-520 cells through the Transwell. Besides, using real-time PCR, mRNA expression of metastasis-related gene TM4SF3 was significantly decreased in AP aqueous extract-treated EC-109 cells. To conclude, this is the first report of the inhibitory effects of AP aqueous extract on both migration and invasion of esophageal cancer cells EC-109 and KYSE-520, and also suppression of the metastasis-related gene TM4SF3 expression.

PI172 Phytochemical and anti-inflammatory studies of aqueous root extract of *Cryptolepis sanguinolenta* (Periplocaceae)

Odoh UE[1], Inya-agha SI[1], Ezugwu CO[1], Osadebe PO[2], Adimegwu JU[1]
[1]Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, Nigeria; [2]Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka, Nigeria

The anti-inflammatory activities of methanol root extract of *Cryptolepis sanguinolenta* was determined against paw edema induced by egg albumin, formalin, carrageenan and dextran in rats. Phytochemical analysis and acute toxicity test (LD_{50}) of the ethanol extract was also carried out. Results show that the methanol root extract of *Cryptolepis sanguinolenta* has significant ($P < 0.05$) dose-dependent anti-inflammatory activity in the entire model studied. The extract at the doses of 100, 200 and 400 mg/kg showed an inhibition (25.20, 38.21 and 50.41%), (28.27, 33.79 and 46.21%), (43.01, 54.30 and 67.00%), and (39.00, 47.00, and 54.00%) against acute paw edema-induced by egg albumin, formalin, carrageenan and dextran respectively at 3 h. The LD_{50} of the extract was 1265.40 mg/kg. Phytochemical analysis of the extract show that it contain carbohydrates, alkaloids, glycosides, saponins, resins, tannins, proteins, steroids and terpenoids. The present study justifies the use of *Cryptolepis sanguinolenta* as an anti-inflammatory agent in traditional medicine.

PI173 Pharmaceutical properties of 14,15-epoxygeranylgeraniol from *Pterodon emarginatus* seeds

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[1]Institute of Physics, Goiás Federal University, Goiânia, Brazil; [2]Biological Institute of São Paulo, São Paulo, Brazil; [3]Section of Plant Physiology and Biochemistry, Institute of Botany, São Paulo, Brazil

Plants of the genus *Pterodon* (Fabaceae), commonly known as 'sucupira', have been used frequently in popular medicine in Brazil for its anti-rheumatic, analgesic, and anti-inflammatory properties. But, the antitumor effects on glioblastoma cells obtained from brain tumors are still unknown. Furthermore, important pharmaceutical properties have never been investigated. The aim of this work was to investigate the action of the extracts and the 14,15-epoxygeranylgeraniol, a diterpene abundant in hexane extract of *P. emarginatus*, on glioblastoma tumor cells and in the antioxidant and α-glucosidase inhibition activities. Therefore, the hexane (HE), dichloromethane (DE) and ethanol (EE) extracts were obtained from seeds powder in each solvent. The 14,15-epoxygeranylgeraniol was obtained from HE fractionation in silica gel columns and the TLC, CG-MS and RMN techniques were employed to characterize this diterpene. The cell viability assay showed that the proliferation of U87MG human glioblastoma cells was inhibited by both extracts and the 14,15-epoxygeranylgeraniol fraction. Both the HE and its diterpene fraction presented antioxidant activity and α-glucosidase enzyme inhibition when compared to the quercetin and acarbose solutions control, respectively. These results are promising and can lead to the identification of new bioactive molecules, chemotherapeutic agents or target enzymes blockers. Support by CAPES/PNPD.

PI174 Pyrrole alkaloids from the fruits of *Lycium chinense* and their hepatoprotective activity

Youn UJ[1], Kil YS[1], Kang U[1], Lee YJ[1], Shin HJ[1], Nam JW[1], Han AR[1], Sung SH[2], Kim J[2], Lee SM[3], Lee D[4], Lee JH[5], Seo EK[1]
[1]College of Pharmacy, Ewha Womans University, Seoul 120 – 750, Korea; [2]College of Pharmacy, Seoul National University, Seoul 151 – 742, Korea; [3]School of Pharmacy, Sungkyunkwan University, Suwon 440 – 746, Korea; [4]School of Life Sciences and Biotechnology, Korea University, Seoul 136 – 713, Korea; [5]Department of Korean Medicine, Dongguk University, Geongju 780 – 714, Korea

Four new pyrrole alkaloids, methyl 2-(2-formyl-5-methoxymethylpyrrol-1-yl)propanoate (**1**), methyl 2-(2-formyl-5-methoxymethylpyrrol-1-yl)-3-(*p*-hydroxyphenyl)propanoate (**2**), dimethyl 2-(2-formyl-5-methoxymethylpyrrol-1-yl)succinate (**3**), and dimethyl 2-(2-formyl-5-methoxymethylpyrrol-1-yl)pentanedioate (**4**), were isolated from EtOAc extracts of the fruits of *Lycium chinense* Miller (Solanaceae). The structures

of 1-4 were elucidated by analyses of various spectral data including the ^{1}H, ^{13}C, HSQC, and HMBC NMR spectra. Compounds 1-4 exhibited hepatoprotective activity in vitro with 86.6 ± 7.9, 86.7 ± 3.0, 93.1 ± 8.3, and 97.7 ± 3.9 cell viabilities at 1 μM, respectively, in cultured HepG2 liver cell injury induced by CCl_4.

PI175 Inhibition of pancreatic cancer cells by furocoumarins from *Poncirus trifoliata*

Jayaprakasha GK, Murthy KNC, Patil BS
Vegetable and Fruit Improvement Center, Department of Horticultural Sciences,

Texas A&M University, College Station, TX 77845 – 2119. *Poncirus trifoliata* (syn. *Citrus trifoliata*), is a member of Rutaceae family. Fruits of *Poncirus trifoliata* (trifoliata orange) are well known for traditional medicines in Asia especially for treating allergic diseases, inflammation, ulcers, anti HIV-1 and hepatoxicity. In the present study, lyophilized trifoliata oranges were extracted with chloroform and dried extract was fractionated on flash chromatography to obtain four compounds. The purity of the compounds were analyzed by HPLC and identified by 1D, 2D NMR spectral data as bergamottin, imperatorin, isoimperatorin and epoxyimperatorin. These compounds were tested for inhibitory activity of pancreatic cancer (Panc-28) in culture models. Among the tested compounds, epoxyimperatorin was found to exhibit highest proliferation inhibition (35%) of cells at 12.5 μM after incubation of 24 h and more than 80% cells were inhibited after 72 h treatment with 50 μM. The magnitude of proliferation inhibition was followed by imperatorin and bergamottin. Studies on expression of protein by immunoblotting demonstrate that furocoumarins of *Poncirus trifoliate* are capable of inducing apotosis through activation of caspase-3, Bax/bcl_2, death inducing protein (p53) and caspase-8. The magnitude and nature of cytotoxicity of activity suggest that both epoxyimperatorin and imperatorin may have greater potency and it is worth exploring their mechanism using *in vivo* system. The present research is based on work supported by the "Designing Foods for Health" through USDA NIFA # 2010 – 34402 – 20875.

PI176 Bioactive diterpenoids from *Callicarpa longissima*

Liu YW[1], Cheng YB[1], Liaw CC[1], Chen CH[1], Guh JH[1], Hwang TL[2], Shen YC[1]
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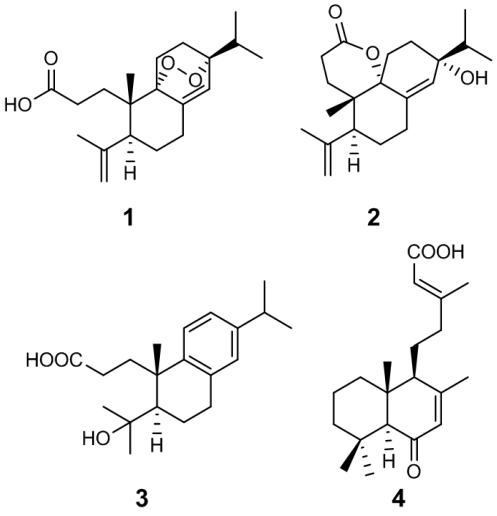
Investigation of the leaves and twigs of C. longissima resulted in the isolation of four new compounds (1 – 4), named callilongisins A-D, and five known compounds, including (E)-6β-hydroxylabda-8(17), 13-dien-15-oic acid and artemetin. Compounds 1 – 3 are 3,4-seco-abietane-type diterpenoids and compound 4 is an analog of a labdenoic-type diterpene. The structure of compound 1 was confirmed by X-ray crystallographic analysis. Cytotoxicity of against a human prostate cancer cell line (PC3) and anti-inflammatory activities of the isolated compounds were evaluated.



PI177 Phenolic compounds and possible effects of *Phillyrea latifolia* L. on weight loss in the rats fed on high energy diet

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[1]Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, 34116, Beyazit/Istanbul, Turkey; [2]Yeni Yüzyıl University, Faculty of Pharmacy, Department of Pharmacognosy, 34010, Zeytinburnu/Istanbul, Turkey; [3]Istanbul University, Faculty of Science, Department of Biology 34134, Vezneciler/Istanbul, Turkey

Phillyrea latifolia L. is used for weight loss in folk medicine in the Mediterranean area. In this study, the determination of active compounds in *P.latifolia* leaves and experimental evaluation of the leaves aqueous extract's possible effects on weight loss, food intake and biochemical-histological changes in the rats fed on high energy diet were aimed. The dried-powdered leaves from Istanbul Ayazaga were extracted with ethanol. Luteolin 7-O-glucoside and chlorogenic acid were isolated from EtOAc extract. The groups (n = 6) were rats fed on high energy diet (HED; for 15 weeks), rats treated with *P.latifolia* (PLE; 220 mg/kg p.o. for 5 weeks) and a control group. When compared to HED group, PLE administration did not cause any weight loss ($p > 0.05$), and there were no effects on food intake ($p > 0.05$) and plasma leptin hormone levels ($p > 0.05$) but significant changes were observed in plasma glucose ($p < 0.05$) and total cholesterol ($p < 0.01$). PLE showed an improved effect on structural integrity and decreased leukocyte infiltration in liver and intestine tissues. In conclusion, although *P.latifolia* does not have any effect on weight loss, it can have beneficial effects on obesity related cellular problems and may become a good source of antidiabetic medication.

PI178 Cytotoxic antioxidant & antimicrobial activity of labdane type diterpene & tow flavones from *Salvia sharifi* Rech. & Esfan

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Salvia is a fascinating plant genus and one of the wide spread members of the Labiatae (Lamiaceae) family, which comprises about 900 herbs and shrubs, growing in the temperate and warmer zones of the world. Some of these species feature prominently in the pharmacopoeias of many countries throughout the word. The range of traditional applications of the herbs in domestic medicine seems to be endless. Two flavones namely Ladanein and 6-hydroxy-5,7,4'-trimethoxy-flavone and one known diterpene namely ent-13-epi-manoyloxide were isolated from the ethyl acetate and methanol extracts of the lowered aerial parts of *Salvia sharifi*. The compounds were purified using several chromatographic methods. Their structures were established using one and two-dimensional NMR spectroscopy and mass spectrometry. Isolated compounds have been screened for cytotoxic, antioxidant and antimicrobial activity. The diterpene showed higher cytotoxic activity than the flavones while the later compounds were better antioxidant than the isolated diterpene.

PI179 *In vitro* growth inhibitory effects of 13, 28-epoxyoleanane triterpene saponins in cancer cells

Haddad M[1,2], Lelamer AC[1], Moreno Y Banuls L[3], Carraz M[1,2], Vasquez P[4], Vaisberg A[4], Castillo D[4], Sauvain M[1,2], Rojas R[4], Kiss R[3]
[1]Université de Toulouse, UPS, UMR152 Pharma-Dev, Toulouse, France; [2]IRD UMR 152 Pharma-Dev; Mission IRD, casilla 18 – 1209, Lima, Peru; [3]Laboratoire de Toxicologie, Faculté de Pharmacie, Université Libre de Bruxelles (ULB), Brussels, Belgium; [4]Laboratorios de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Peru,

Two new 13,28-epoxyoleanane triterpene saponins, magnosides A (1) and B (2), were isolated from the 95% ethanolic extract of *Cybianthus magnus* (Mez) Pipoly roots. Their structures were deduced by a combination of spectral analyses and chemical evidences as compared to data reported in the literature. The bioactivity of compounds 1 and 2 was

evaluated *in vitro* against different cellular models including *Mycobacterium tuberculosis*, *Leishmania amazonensis* axenic amastigotes, mouse peritoneal macrophages and eight cancer cell lines. While neither of the tested compounds displayed any activity against *M. tuberculosis*, both exhibited anti-leishmanial activity against axenic amastigotes as well as *in vitro* growth inhibitory activity against all tested cancer cell lines with IC_{50} growth inhibitory concentrations ranging between 4 and 33 μM. The compounds displayed similar growth inhibitory activity in cancer cell lines sensitive to pro-apoptotic stimuli versus those displaying various levels of resistance to such stimuli. Quantitative videomicroscopy analyses revealed that compounds **1** and **2** are cytotoxic.

PI180

Effects of dichloromethane fraction from fruits of *Piper tuberculatum* Jacq on gastric ulcer and reflux esophagitis in rats

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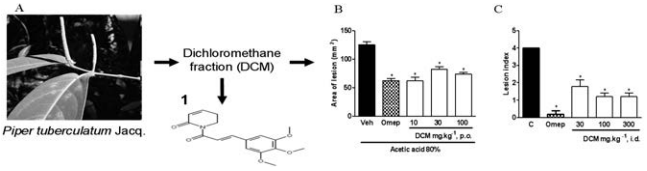
[1]Department of Pharmacology, Federal University of Parana, Curitiba, PR, Brazil; [2]Department of Medicine, Federal University of Rondonia, Porto Velho, RO, Brazil; [3]Department of Physiological Sciences, Federal University of Santa Catarina, Florianopolis, SC, Brazil

This study evaluated the healing of chronic gastric ulcers and oesophageal protection effects of dichloromethane fraction (DCM) from fruits of *Piper tuberculatum* Jacq **(A)**. DCM (10, 30 and 100 $mg.kg^{-1}$, p.o) accelerated the healing of 80% acetic acid-induced chronic gastric ulcer **(B)** together with enhancement of mucus content, proliferating cell nuclear antigen (PCNA) immunostaining and prevention of reduced glutathione levels in gastric mucosa. Moreover, the DCM (30, 100 and 300 $mg.kg^{-1}$, i.d.) was able to reduce esophageal injuries **(C)** and myeloperoxidase activity in the acute reflux esophagitis model. These results suggest that *Piper tuberculatum* Jacq may provide an alternative therapeutic source for gastric ulcer and oesophagitis treatment. However, further studies must be carried out to better clarify these actions, and if the isolated compound, piplartine (**1**), could be involved in these effects.



PI181

β-glucogallin: A novel aldose reductase inhibitor from *E. officinalis* used in traditional ayurveda to treat diabetes

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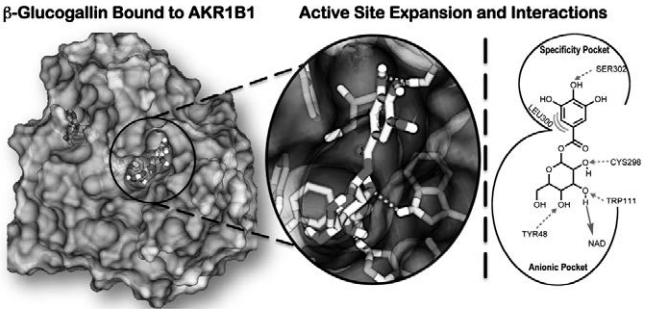
The plant *Emblica officinalis* has been used for thousands of years as an Indian Ayurvedic preparation to treat diabetes. In the eye, human aldose reductase (AKR1B1) metabolism of glucose to sorbitol is linked to cataract formation. We present the isolation and characterization of β-glucogallin (BGG) from *E. officinalis*, as a potent and selective inhibitor (IC_{50}= 17 μM) of AKR1B1. Molecular modeling demonstrates that BGG favorably binds the active site of AKR1B1. BGG effectively inhibits sorbitol accumulation by 73% under hyperglycemic conditions in AKR1B1 expressing lenses excised from transgenic mice. This study supports the continued development of natural products such as β-glucogallin as therapeutic leads in the development of novel therapies to treat diabetic complications such as cataract.



PI182

Antiproliferative activity and ESI-MS/MS chemical profile of the crude extract and fractions from of *Campomanesia adamantium*

Pascoal ACRF[1], Ruiz ALTG[2], de Carvalho JE[2], Stefanello ME[3], Sawaya ACHF[1], Salvador MJ[1]
[1]Biociências e Tecnologia de Produtos Bioativos, Universidade Estadual de Campinas; [2]Divisão de Farmacologia e Toxicologia, CPQBA, Universidade Estadual de Campinas, Campinas, SP, Brazil; [3]Departamento Química, Universidade Federal do Paraná (UFPR), Curitiba,PR, Brazil

Leaves of *Campomanesia adamantium* were collected in Curitiba-PR, Brazil, dried, pulverized and subjected to maceration process with ethanol. The crude extract was fractionated by liquid-liquid extraction with hexane, dichloromethane and butanol. Samples were analyzed by direct insertion mass spectrometry with electrospray ionization (ESI-MS). The ion corresponding to compounds of interest were selected and submitted to collision induced dissociation. The compounds were identified by comparison of their ESI-MS/MS fragmentation spectra with that of standards. Antiproliferative activity was evaluated for a panel of human tumor cells, including U251, MCF7, NCI-H460, OVCAR-03, HT-29, 786 – 0, NCI-ADR/RES and non- cancer cell HaCat by sulforhodamine B assay. Samples were showed antiproliferative activities against some cell lines with GI 50 (concentration able to inhibit 50% growth) between 1.19 μg/mL and 112.48 μg/mL. In ESI-MS/MS analyses it was possible to identify gallic acid, 2', 4'-dihydroxy-6'-methoxychalcone, 2', 4'-dihydroxy-5'-methyl-6'-methoxychalcone, quercetin, quercitrin and isoquercitrin. Those compounds may be responsible for the antiproliferative activities from crude extraction and fraction.

PI183

Metabolite profiling of jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices

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Many dark-colored fruit juices, rich in anthocyanins, are thought to be important for human health. Joboticaba (*Myrciaria cauliflora*) fruits, native to Brazil, have phenolics including anthocyanins, and are processed into juice and other products. The phenolic constituents in the fruits of jaboticaba were studied by high-performance liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry. Twenty-two compounds were identified or tentatively determined by detailed analysis of their mass spectral fragmentation patterns; eleven compounds including seven gallotannins, two ellagic acid derivatives, syringin and its glucoside were detected for the first time in the fruit. The compositional differences among the fruit extracts and their commercial products were also compared by principal component analysis; two anthocyanins, delphinidin 3-*O*-glucoside and cyanidin-3-*O*-glucoside, as well as two depsides, jaboticabin and 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid present in the fruit extracts were not detected unexpectedly in commercial jaboticaba juice or jam. Therefore stability of anthocyanins in jaboticaba fresh fruits and products has been compared directly with that of other dark-colored fruit products made from blueberry and grape, and the same trend of decreasing amounts of anthocyanins was observed in all tested products.

PI184 Desmutagenic and antimutagenic potential of *Khaya grandifoliola* (C.DC.), meliaceae

Hashem F[1], Aboutabl ES[2], El-Souda S[3], Moharam M[4], Mammoun A[1]
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Five phenolics were isolated for the first time from the ethanol extract of *Khaya grandifoliola* leaves. These compounds were identified using spectroscopic analysis (UV, ^{1}H-NMR, ^{13}C-NMR and ESI) as Quercetin 3-*O*-rhamnoglucoside (rutin), Quercetin 3-*O*-rhamnoside, Quercetin 3-*O*-glucoside, Quercetin aglycon and the new 6- methoxycoumarin, 7- arabinofuranoside. Desmutagenic and antimutagenic activities of specimen extracts of immaculate *khaya grandifoliola* leaves and flowers were ascertained by measuring the inhibition of *Salmonella typhimurium* TA 100 His^{+} revertants induced by ethyl methane sulphonate EMS and ribose lysine RL. A frustration of the induced reversion was observed. The alcoholic extract of both leaves and flowers of *khaya grandifoliola* exhibited desmutagenic and antimutagenic activity against EMS and RL induced reversion.

PI185 Aqueous extract of *Momordica dioica* stimulates insulin release from isolated rat islets

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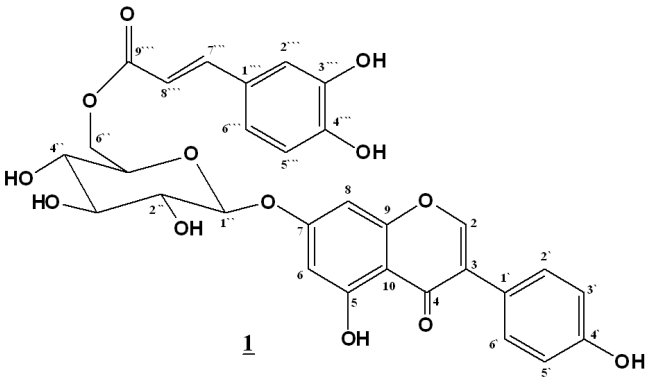
Momordica dioica (Cucurbitaceae) has been used for management of diabetes by ethnic groups in Bundelkhand region of central India. Increase in serum insulin in diabetic Wistar rats by aqueous extract of fresh unripe fruits *M. dioica* has been reported earlier by our group (1). To confirm insulin secretagogues potential of *M. dioica*, aqueous extract (AE) and methanol soluble fraction (MSF) and methanol insoluble fractions (MIF) of AE were subjected to *In Vitro* insulin release assay from isolated pancreatic islets of normoglycemic Wistar rats. ATP dependant potassium channel opener drug nicorandil was employed to investigate possible mechanism of insulin release. Isolated pancreatic islets of rat in HBBS containing 1% BSA were incubated with glucose (3.3/16.7mM) and 1 mg AE, MSF, MIF or 1 mg of nicorandil. Release of insulin in external media was measured by ELISA. Glucose at 16.7 mM level stimulated insulin release as compared to 3.3mM, proving the sensitivity of the assay. AE and MSF of *M. dioica* significantly ($p < 0.01$) increased insulin release were as MIF was not able to enhance insulin release. The results indicated that the active principle was present in methanol soluble fraction of aqueous extract. Trypan blue staining and LDH release assay from isolated islets indicated that the extracts were non cytotoxic at tested concentration and the insulin release was not merely by alteration of membrane permeability by *M. dioica* extracts. Nicorandil did not inhibit *M. dioica* mediated stimulation of insulin release, indicating that mechanism of insulin release by *M. dioica* was independent of k-ATP and Ca voltage gated channels of pancreatic β-cells.

PI186 A new isoflavone glucoside from Blepharis ciliaris

El-Shanawany MA[1], Sayed HM[1], Ibrahim SRM[1], Fayed MAA[1], Radwan MM[2], Ross SA[2,3]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt; [2]National Center for Natural Products Research,; [3]Department of Pharmacognosy, University of Mississippi, School of Pharmacy, University 38677, USA

Phytochemical study of the aerial parts of *Blepharis ciliaris*. led to the isolation of one new isoflavone glucoside [genstein-7-O-(6''-O-E-caffeoyl-β-D-glucopyranoside)] (**1**), along with seven known compounds: methyl veratrate, methyl vanillate, protocatechuic acid, naringenin-7-O-(3''-acetyl-6''-E-p-coumaroyl-β-D-glucopyranoside), naringenin-7-O-(6''-E-p-coumaroyl-β-D-glucopyranoside), api-genin-7-O-(6''-E-p-coumaroyl-β-D- glucopyranoside), and acteoside. Their structures were established on the basis of detailed analyses of chemical, and spectral data. The antioxidant activity was evaluated.



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PI187 High throughput huaman pancreactic lipase screening of natural product library for lipase inhibition activity

Jiao P[1], Zhao J[1], Yeop Lee J[1], Tseng-Crank J[1], Corneliusen B[1], Yimam M[1], Hodges M[1], Hong M[1], Maurseth C[1], Oh M[2], Kim H[2], Hyun E[2], Jia Q[1,2]
[1]Unigen, Inc. USA, 2660 Willamette Drive NE, Lacey, WA 98516, USA; [2]Unigen, Inc. Korea, 200 – 1 Songjung-Ri, Byeongcheon-Myeon, Cheonam-Si, Chungnam 330 – 863, South Korea

Pancreatic lipase, the primary enzyme that is involved in converting dietary triglycerides to monoglycerides and free fatty acids in the digestive system, is considered a validated pharmaceutical target for the treatment of obesity. In order to discover natural lipase inhibitors, a high-throughput (HTP) human pancreatic lipase inhibition assay was developed by utilizing recombinant human pancreatic lipase (hPL). The assay was adapted to a 96-well plate assay format with a Z' value of 0.905. The HTP lipase assay was used to screen 13,979 plant and marine extracts generated from biomasses collected mainly from Asia, North America, and Africa. 1,343 extracts exhibited strong lipase inhibition with *in vitro* activity over 80%. The primary hits were further filtered by IC_{50} values, tannin index testing, and HTP screening. The confirmed hits were further studied by following the lipase inhibition activity to isolate and identify active compounds. Active compound enriched plant extracts were prepared and evaluated in high-fat diet (HFD) induced obese animal models for weight loss effects. Two plants *Punica granatum* and *Marchantia polymorpha* will be discussed in detail in this presentation.

PI188 Isolation, structural elucidation and cytotoxicity of new xanthones from bioactive extracts of *Calophyllum soulattri*

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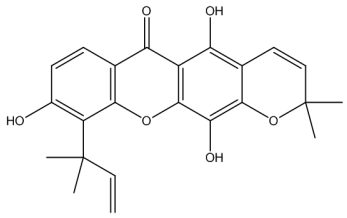
Calophyllum is widely distributed in tropical areas in the world, primarily in the Indo-Pacific region. Some species of *Calophyllum* have been used in folk medicine. Previous studies indicated the existence of a variety of secondary metabolites such as xanthones, coumarins, triterpenoids and flavonoids which possess various bioactivities. Extensive chromatography techniques applied on the bioactive hexane and dichloromethane extracts of stem bark of *Calophyllum soulattri* afforded two new prenylated xanthones, soulattrin **(1)** and phylattrin **(2)** along with four other xanthones, macluraxanthone **(3)**, caloxanthone C **(4)**, brasixanthone B **(5)** and trapezifolixanthone **(6)**. The structural elucidations of these compounds were achieved on the basis of spectroscopic analysis of 1D and 2D NMR spectral data (^{1}H, ^{13}C, DEPT, COSY, HMQC & HMBC) while molecular masses were determined via MS techniques. Compound **1 – 6** were evaluated for their cytotoxicity towards nine human cancer cell lines, SNU-1 (stomach), HeLa (cervix), Hep G2 (liver), NCI-H23 (lung), K562 (leukimia), Raji (lymphoma), LS 174T (colon), IMR-32 (neuroblastoma) and SK-MEL-28 (skin) cells *in vitro* using the MTT method. All the xanthones isolated exhibited significant cytotoxic activity with low IC_{50} values ranging from 0.27 to 9.89 μg/mL.



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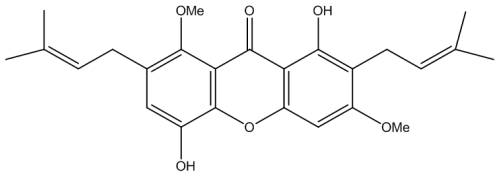

(2)

PI189 Seasonal changes in silicon accumulation of wild populations of horsetail (*Equisetum arvense L.*)

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Horsetail (*Equisetum arvense* L.) is a perennial herb with segmented stem. Life cycle consist spring and summer stems. Summer stalks are 0.10 to 0.40 m high, green, ribbed, branched and barren. There is an object of the collection known as *Herba equiseti*. Each plant species has its specific limiting borders. This border limits its compatibility or tolerance to exposure in various environmental factors habitat. Monitoring of selected species and populations was realized in the period of 2009 – 2011 years on three different natural locations in Laborecká vrchovina (Slovakia). Samples of horsetail (*Equisetum arvense* L.) were collected by destructive methods in all three locations (L1, L2 and L3). For individual collections was in the dry biomass determined silicon content by AAS. The highest content of silicon was obtained in the sixth collection, which ranged from $26.44 \pm 1.32\ g.kg^{-1}$ to $48.19 \pm 2.40\ g.kg^{-1}$. The lowest silicon content was measured at the second collections in different locations and years, from $15.25 \pm 0.76\ g.kg^{-1}$ to $25.16 \pm 1.25\ g.kg^{-1}$. The main sources for statistical variability in the accumulation of silicon were the collection, which has the largest impact. **Key words:** Environmental factor, *Equisetum arvense*, Statistical variability, Silicon. **Acknowledgments** - The work was supported by the Agency of Ministry of Education, science, research and sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/2010 – 11).

PI190 Qualitative-quantitative characteristics of anthocyanins in fruits

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Various fruit colours are derivated from only four pigment groups: the chlorophylls, carotenoids, betalains and anthocyanins. Of these pigments, anthocyanins are the most prominent. The aim of actual research is study of natural substances (anthocyanins) isolation using extraction and lyophilisation with adjustment of various parameters, and their determination. Plant species were selected based on content of monitored substances and raw material availability, either from nature. To find the best extraction method, the fruits of the plant material were exposed to the various multiple extraction solvents with different types and acid content, including variable length extraction. Analysis of anthocyanins concentrations in different pH was done by spectrophotometry and isolation of the natural substances in silica gel column chromatography with the use of re-application of ethanol. Qualitative-quantitative parameters of therapeutically active secondary metabolites in the extracts and lyophilisates were determined the by LC/MS and LC/MS/IT TOF. **Key words:** Anthocyanins, Plants, Extraction, Lyophilisation **Acknowledgments** - The work was supported by the Agency of Ministry of Education, science, research and sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/2010 – 11).

PI191 Anti-*Mycobacterium tuberculosis* activity of extracts with a new benzoic acid derivative from *Piper diospyrifolium* by supercritical carbon dioxide

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Piper diospyrifolium leaves extracts obtained by supercritical fluid extraction (SFE-CO_2), a new benzoic acid derivative and their methylated derivative were evaluated against *Mycobacterium tuberculosis*. The SFE-CO_2 was performed at temperatures ranging from 20 °C to 40 °C and pressure from 140 to 250 bar. The SFE-CO_2 extracts was purified by chromatography methods and a new compound 4-methoxy-3-[(1E)-3-methylbuta-1,3-dien-1-yl]-5-(3-methylbut-2-en-1-yl) benzoic acid was determined by spectroscopic methods and the methylated derivative was prepared. Anti-*M. tuberculosis* activity was carried out using resazurin microtiter assay plate (REMA)[1]. The cytotoxicity assay was carried out in macrophages J774G8 by sulforrhodamine B colorimetric assay[2]. Benzoic acid derivative, their methylated derivative and the SFE-CO_2 extracts exhibited activity in *M. tuberculosis* $H_{37}Rv$ with MIC values of 125 µg/mL. The cytotoxicity results showed selectivity index range from 0.6 to 1.0. Additional studies using benzoic acid derivative should be conducted for a better evaluation of anti-mycobacterial activity of these compounds.

PI192 Biological activities of 13,28-epoxyoleanane triterpene saponins from two Peruvian myrsinaceae

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Two 13,28-epoxy-oleanane triterpene saponins (**1**) and (**2**), were isolated from the roots of *Myrsine coriaceae* and *M. andina*. Their structures were deduced by combined spectral analysis and chemical evidences based on data reported in the literature. Compounds **1** and **2** were evaluated against different cellular models such as *Mycobacterium tuberculosis*, *Leishmania amazonensis*, mouse peritoneal macrophages, five human cancer cell lines and two murine cell lines. Compound **1** was found to exhibit antileishmanial activity (IC_{50}= 16 µg/mL) whereas compound **2** was inactive. Furthermore, compound **1** exhibited stronger inhibition activity on human cancer cells (IC_{50}= 15 µg/mL) and on murine cell lines (IC_{50}= 10 µg/mL) than compound **2** (IC_{50} > 82 µg/mL and 42 µg/mL, respectively). As the only difference between **1** and **2** is due to a substitution of an aldehyde group by a hydroxymethyl moiety, these results showed the crucial role of the aldehyde function at C-30 for the cytotoxicity. In contrast, none of the tested compounds revealed activity against *M. tuberculosis*.

PI193 Potential cancer chemotherapeutic agents from *Indigofera spicata*

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Indigofera spicata Forssk. (Leguminosae) has become widespread in tropical and sub-tropical countries as a ground cover plant. In the present investigation, the bioactivity-guided fractionation of a cytotoxic chloroform extract of the flowers, fruits, leaves, and twigs of *I. spicata*, collected in Vietnam, using the HT-29 human colon cancer, 697 human acute lymphoblastic leukemia, and the Raji human Burkitt's lymphoma cell lines, led to the isolation of seven compounds. Of the isolates, (+)-10-deacetyl-purpurin and (2*S*)-2,3-dihydrotephroglabrin are new compounds. The compounds, *cis*-(6aβ,12aβ)-hydroxyrotenone and rotenone, exhibit potent cytotoxicity for 697 and HT-29 cells with IC_{50} values of less than 1 μM. Tephrosin was found to be cytotoxic and cytostatic for 697 cells. Compounds were also tested in a quinone reductase induction assay with tephrosin showing a CD value of 0.4 μM. (Support in part by grant P01 CA125066 from the National Cancer Institute, NIH, Bethesda, MD, is acknowledged).

PI194 *In vivo* antimalarial activity of twigs extracts from *Keetia Leucantha*

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In our search for more effective drugs against Plasmodium falciparum and as a continuation of our investigation of plants used traditionally in Benin, a special attention was devoted to Keetia leucantha which is a West African tree. This plant is used in traditional medicine in Benin to treat malaria and is cited in a list of 88 traditional remedies compiled by the "Direction de la Protection Sanitaire" of the Beninese Ministry of Health. The dichloromethane extract of twigs of Keetia leucantha was previously shown to have in vitro antiplasmodial activity (IC_{50}= 11.3 μg/ml on the chloroquine-sensitive strain 3D7 and 15.8 μg/ml on the chloroquine-resistant strain W2). We analysed the in vivo activities of twigs extracts of Keetia leucantha based on the 4-day suppressive test of Peters. The dichloromethane (given intraperitoneally) and aqueous (given per os) extracts exhibited significant (56.8 % and 53.0 %, respectively, $p < 0.0001$) parasite inhibition in mice at 200 mg/kg/day. The in vivo antimalarial activities of the aqueous and dichloromethane extracts of Keetia leucantha highlighted the possible value of this plant in traditional medicine and could be partially explained by the presence of triterpene acids (ursolic and oleanolic acids) with antiplasmodial activity.

PI195 Cytotoxic withanolide constituents of Physalis longifolia

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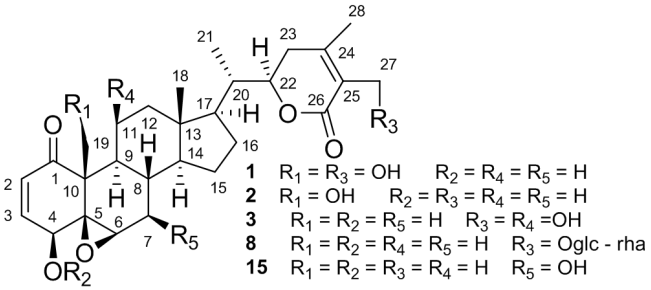
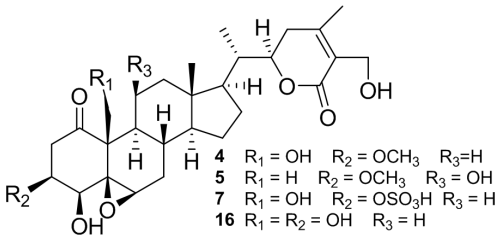
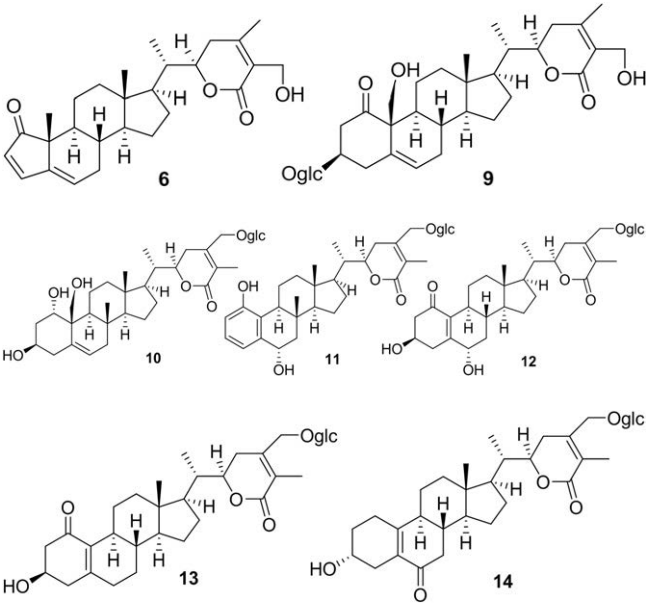
Twenty three withanolides including sixteen new **1-16** (named as withalongolides A-P) were isolated from the aerial parts of *Physalis longifolia* Nutt. (Solanaceae). Their structures were elucidated through spectroscopic techniques including X-ray crystallography (for **1-3**, **6**, and **15**). Nine withanolides and six derivatives showed potent cytotoxicity against a panel of cells (JMAR, MDA-1986, B16F10, DR081 – 1, SKMEL-28, Hs578T, and MRC-5) with IC_{50} values in the range of 0.067 ~ 17.4μM.







PI196 Effect of siam weed extracts on anti-inflamatory and antioxidant activities

Pandith H[1], Thongpraditchote S[2], Wongkrajang Y[2], Zhang X[3], Gritsanapan W[1], Baek SJ[3]
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Siam weed (*Chromolaena odorata* (L.) King and Robinson), a medicinal herb used for stop-bleeding, wound healing and anti-inflammation, contains various bioactive components. Among them, scutellarein tetramethyl ether (STE) may act as a major bioactive component for blood coagulation and anti-inflammatory activity. However, molecular mechanism by which STE affects anti-inflammatory activity has not been elucidated in details. In this study, we examined expression of several inflammatory proteins and measured the anti-oxidant activity with macrophage cells. Cyclooxygenase-2 and inducible nitro oxide synthase are pro-inflammatory proteins. The level of protein and mRNA expression of these enzymes induced by lipopolysaccharide was evaluated in the presence or absence of Siam weed extracts (SWE) or STE. The treatment of SWE and STE dramatically suppressed these enzymes in dose dependent manner. We also found that SWE increased anti-oxidant ac-

tivity, as assessed by GSH activity. Our results indicate that SWE and/or its bioactive component STE exhibit anti-inflammatory activity and further provide a potential uses in the treatment of inflammatory-related diseases.

PI197 Isolation of cytotoxic polyphenolic derivatives from the root bark of *Mesua ferrea*

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Phytochemists nowadays have growing interests on medicinal plants which have pharmacological activities. Therefore, investigation on biological active secondary metabolites from the root bark of *Mesua ferrea* (Clusiaceae) was scrutinized. A series of polyphenolic derivatives were obtained from our ongoing research where three of which were new: mesuaferrin A **1**, mesuaferrin B **2** and mesuaferrin C **3**. The known compounds were identified as caloxanthone C **4**, 1,5-dihydroxyxanthone **5** and tovopyrifolin C **6**. Structures of these compounds were elucidated by extensive spectroscopic methods which include 1D and 2D-NMR, GC-MS and IR techniques. Preliminary insights on *in vitro* cytotoxicity and structure-activity relationships of all the isolated metabolites against a panel of human cancer cell lines including Raji (human B lymphocyte), SNU-1 (human gastric carcinoma), K562 (human erythroleukemia cells), LS-174T (human colorectal adenocarcinoma), HeLa (human cervical cells), SK-MEL-28 (human malignant melanoma cells), NCI-H23 (human lung adenocarcinoma), IMR-32 (human neuroblastoma) and Hep-G2 (human hepatocellular liver carcinoma) were performed using MTT assay. Compounds **1** - **5** exhibited significant (IC_{50} values ranging from 0.1 to 9.4 μg/mL) cell proliferation inhibition against all the tested cancer cells. The phytochemical and pharmacognosy investigation showed adverse effects of polyphenolic compounds suggesting that *Mesua ferrea* could be a phytotheraupic source of lead compounds in drug discovery.

PI198 New diterpenes from *Clerodendrum splendens* G. Don (Verbenaceae)

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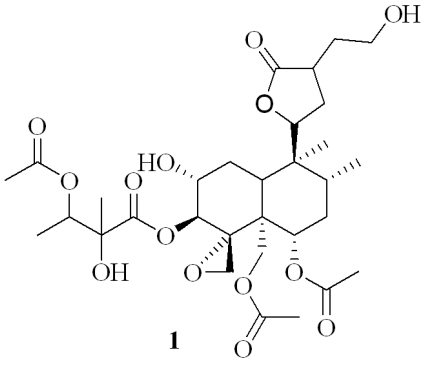
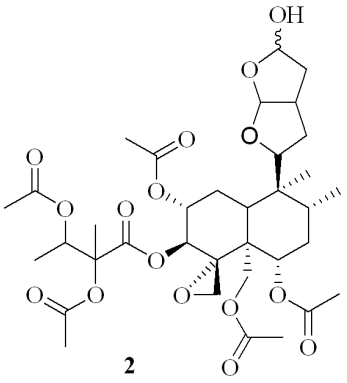
Clerodendrum splendens G. Don (Verbenaceae) is used in traditional medicine to treat many infections, inflammatory diseases, skin disorders, ulcers and venereal diseases. The *Clerodendrum* genus is one of the major sources of *neo*-clerodane diterpenoids, a large group of naturally occurring compounds isolated mainly from Compositae, Labiateae, and Verbenaceae. As part of an ongoing research program on plants acclimatized at the El Zoharia Research Garden of Cairo it was performed a phytochemical screening of *C. splendens* leaves. The study led to the isolation and structural characterization by spectroscopic and spectrometric analyses of some new diterpenes, including compounds **1** and **2**.



1



2

PI199 Epigallocatechin gallate, myricetin and ellagic acid glycosides with anti-inflammatory activity from *Memecylon edule* leaves

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Memecylon edule Roxb. (Melastomataceae) is a shrubby tree commonly found in the Northeast forest of Thailand. The leaf of this plant is traditionally used as an anti-burning remedy for its anti-inflammatory effects. The crude extract of this plant showed significant anti-inflammatory effect in our previous report. In this present, the chemical constituents of *M. edule* were isolated and tested for anti-inflammatory activity. Epigallocatechin gallate, myricetin and ellagic acid glycosides were isolated from *Memecylon edule* leaves by column chromatography and interpreted by spectroscopic method. They are the main component of the 50% methanol fraction. Their anti-inflammatory effects were evaluated by inhibition of pro-inflammatory cytokine (TNF-α) release using enzyme-linked immunosorbent assay. The results demonstrated that epigallocatechin gallate (100 uM), myricetin (100 uM) and ellagic acid glycosides (40 uM) exhibited anti-inflammatory activity which showed %inhibition of TNF-α of 19.46, 39.14 and 47.78, respectively. These results suggested that they are the compounds responsible for the inflammatory effects of this plant which previously observed for the crude extract.

PI200 The synergistic effect of *Morus alba* and *Nelumbo nucifera* mixture to the antityrosinase and anti-elastase activities

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Combinations of crude drugs have been widely used in Thai traditional medicine for the purposes of synergistic, additive, or antagonistic effects. This concept was applied to investigate the antityrosinase and antielastase activities of the combination of plant extracts, *Morus alba* (Moraceae) root bark which is known for antityrosinase, and *Nelumbo nucifera* (Nelumbonaceae) rhizome which possesses anti-elastase activity. It was found that the mixture of 95% ethanolic extract of *M. alba* (ME) (80 μg/ml) and 20 μg/ml aqueous extract of *N. nucifera* (NE) showed the inhibitory effect to tyrosinase (90.4%) which significantly differed ($P < 0.01$) from 100 μg/ml ME (60.0%), 100 μg/ml NE (1.5%), and 40 μg/ml kojic acid (73.5%). For anti-elastase activity, the mixture of 20 μg/ml ME and 80 μg/ml NE exhibited 57.3% inhibition which was different ($P < 0.05$) from those of 100 μg/ml ME (13.4%), or 100 μg/ml NE (44.8%), but not significantly differed from 114 μg/ml epigallocatechin gallate (61.6%). The results clearly indicated the synergistic effect of the mixture of 2 plants extract to the antityrosinase and anti-elastase activities and could be served as a new herbal formulation for cosmetic purposes. The mechanisms of these synergistic effects needed to be further clarified.

PI201 Biologic activities and chemical composition of the *Hymenaea courbaril var. stilbocarpa* (Jatobá) extracts

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Jatobá is an economically important species of the Neotropical region. Our aim was to study the chemical composition and evaluate the anticholinesterase (AChE), antifungal (*Cladosporium cladosporioides*), and free radical scavenging (DPPH) activities. The extracts of leaves (L, 176 g, Botanical Institute, SP, Brazil) and seeds tegument (T, 10 g) were prepared with 70% ethanol (H) and then with 70% acetone (C). The dried extracts LH (5.7%), LC (1.2%), TH (30%) after partition with H_2O:MeOH/n-hexane (h) and ethyl acetate (c) furnished: Lh (1.3 g); Lc (0.2 g); Ch (2.7 g); Cc (0.6 g); Th (0.04 g) and Tc (0.06 g). The assays by TLC showed activity for: AChE (Lh, Lc, Cc, Tc and Th) and antifungal (Lh, Lc, Cc, Ch, Th and Tc). Determination of the EC_{50} (microplate, DPPH, 512 nm and quercetin IC_{50}= 1,70 µg mL^{-1}) showed data for: EC_{50} (Lc)= 2.0 µg mL^{-1}; TC (dried extract, 22%)= 3.5 µg mL^{-1}; TH = 0.9 µg mL^{-1} and Tc = 0.1 µg mL^{-1}. Analysis by GC/MS after derivatization (BSTFA), identified (leaves) the isocitric acid (tms) at retention time in minutes (Tr)= 28.5 min, isopimaric (38.4 min), oleic (35.3 min), protocatechuic (28.1 min) acids and quercetin (49.6 min). In tegument: gallic (30.7 min) and protocatechuic acids. The presence of these compounds may explain the biologic activities of the jatobá extracts.

PI202 Anti-dermatophytic activity and the underlying action mechanism of macrocarpal C isolated from Eucalypti Globuli Folium

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Eucalypti Globuli Folium (EGF), the fresh leaves of *Eucalyptus globules* Labill., has long been used in China to treat influenza, headache, cough, eczema and dermatomycosis. The objectives of present study were to identify the active anti-dermatophytic component from EGF using bioassay-guided fractionation, and to reveal the action mechanism of the isolated compound. Our results showed that ethanolic extract of EGF was more potent than its aqueous counterpart in inhibiting the growth of dermatophytes, with MIC values of 3.9 µg/ml and 15.6 µg/ml on *T. mentagrophytes* and *T. rubrum*, respectively. Using bioassay-guided fractionation, macrocarpal C (a formylated phloroglucinol compound) was identified as the active component, with MIC values of 4.3 µM and 137.7 µM on *T. mentagrophytes* and *T. rubrum*, respectively. Further mechanistic study showed that fungal conidia treated with macrocarpal C (0.625-fold to 5-fold of MIC) for 24 or 48 hours had significant increase in SYTOX Green uptake in both dose- and time-dependent manners. This is the first report to demonstrate the inhibition of macrocarpal C on the growth of dermatophytes by destroying the cell integrity and increasing membrane permeability. Therapeutic effect of macrocarpal C on guinea pig model of tinea pedis will be evaluated in the future.

PI203 Antihepatitis B virus constituents from *Solanum erianthum*

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Eleven constituents including α-linolenic acid (**1**), 13*S*-hydroxy-9(*Z*),11(*E*)-octadecadienoic acid (**2**), 9*S*-hydroxy-10(*E*),12(*Z*),15(*Z*)-octadectrienoic acid (**3**), 9(*Z*),11(*E*)-octadecadienoic acid (**4**), octadecanoic acid (**5**), loliolide (**6**), dihydroactinidiolide (**7**), solasonine (**8**), solamargine (**9**), camelliaside C (**10**), 5-methoxy-(3'',4''-dihydro-3'',4''-diacetoxy)-2'',2''-dimethylpyrano-(7,8:5'',6'')-flavone (**11**) were isolated from title plant. **9** showed the most potent activity against HBsAg (IC_{50}: 1.57 µM). **11** was the only active constituent (IC_{50}: 36.11 µM) against HBeAg. **9** revealed strong inhibition in DNA replication (IC_{50}: 2.17 µM). **1** showed prominent selected index (SI) in anti-HBsAg (7.75) and inhibition of DNA replication (7.18). This is the first report that unsaturated fatty acid **1**, steroidal alkaloid glycoside **9** and flavone **11**, showed excellent activity against HBV.

PI204 New anti-inflammatory aromatic and triterpene components from *Antrodia camphorata*

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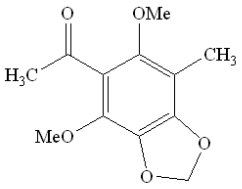
Antrodia camphorata (Polyporaceae) is a parasitic fungus on the inner wall of the heartwood of *Cinnamomun kanehirai*.The fruiting bodies of *A. camphorata* are called "jang-jy" or "niu-chang-chih" in Taiwan. Traditionally, the fungus has been used for the treatment of food and drug intoxication, diarrhea, abdominal pain, hypertension and liver cancer.The components of this fungi have showed activity of anti-inflammation, immune-modulation, anti-*Helicobactor pylori*, neuroprotective from Aβ damage. In this time, we present the result of chemical studies from a mixture of fruting body and mycelia of solid cultures of *A. camphorata*, and 4 new benzenoids, **Ar 1**, **Ar 2**, **Dimer 3**, **Dimer 4**, two new tritertenoids, **T 5**, and **T 6** together with two known benenoids, **Ar 7**, and **Ar 8**, were isolated and elucidated. Compounds, **Dimer 3**, **T 5**, **T 6**, **Ar 7**, and **Ar 8** were evaluated for anti-inflammatory activities, and exhibited the potential inhibition against LPS-induced NO production with IC_{50} values 3.1±0.3, 5.2±0.2, 2.6±0.2, 13.3±1.3, and 3.3±0.4 µg/ml, respectively.



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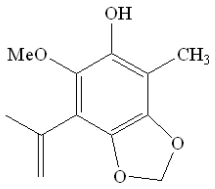

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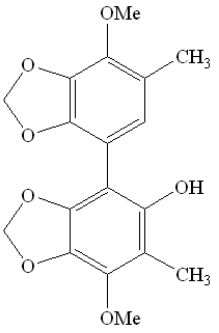
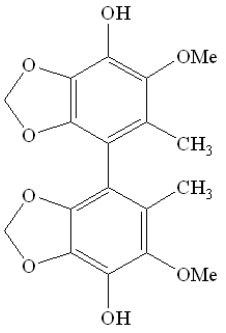
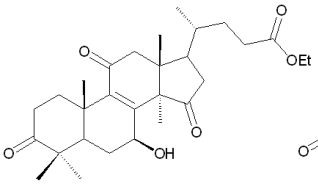


Dimer 3



Dimer 4



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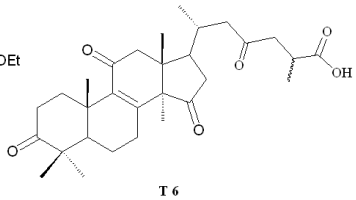

T 6

PI205 Native plants in Serbia – Opportunity for new functional foods and herbal drug

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Scientific research confirmed a wide range of biological and pharmacological activities for a variety of natural products. However many of them exhibit some of the unwanted side effects and drug interactions. It is therefore necessary to conduct complex investigations, which will serve as a scientific ground for application of certain plant products in

producing new drugs or dietary supplements. Here we report on the phytochemical and biochemical study of the several wild growing plant species belonging to the Plantaginaceae, Polygonaceae, Cupressaceae, Alliacae families, with respect to their antioxidant and antiinflamatory activities. Most of plants were investigated for the first time. Especially attention was devoted towards the plants that can be used in diet, serving as the functional food, likewise are species of genus *Rumex, Polygonum, Plantago, Juniperus*. LC-MS/MS and GC-MS technique was applied to evaluate chemical profile. Antioxidant potential was determined using various assays related to free radical ($DPPH^{\cdot}$), reactive oxygen ($HO^{\cdot}$, $O_2^{\cdot-}$) and reactive nitrogen species ($NO^{\cdot}$) scavenging ability, as well as lipid peroxidation (LP) and reducing power (FRAP assay). Anti-inflammatory activity was determined by means of inhibition of cyclooxygenase-1 (COX-1) and 12-lipoxygenase (12-LOX), enzymes involved in metabolism of arachidonic acid. The results obtained show that many of explored native plants has significant antioxidant and antiinflamatory activity, which is often associated with the high content of phenolic compounds. In conclusion, this study strongly supports exploration of nature as a source of novel herbal medicine and food with desired health benefits.

PI206

Eucalyptus gunii Hook. F. new source of valuble bioactive compounds

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Due to antiseptic and anti-inflammatory properties, leaves of plants from genus *Eucalyptus* have found their place in traditional and modern herbal medicine. In this study, a composition, antioxidant, antimicrobial and antimutagenic activity of *E. gunnii* both essential oil and phenolic compounds were determined for the first time. The oil was obtained by hydrodistillation of leaves in 1.76% yield, and chemically profiled by GC-MS technique. The phenolic componds were isolated by maceration in 70% MeOH (1.63 mg/100 g extract). The oil was found to contain 77.4% of oxygenated monoterpenes, and 2.5% of sesquiterpenes, with the most abundant terpenoids being 1,8-cineol (67.8%) and α-pinene (14.1%). In MeOH extract phenolic acids, flavonoids and ellagitannins were identified. Antioxidant activity was evaluated by diffrenet test concerning: DPPH, $O_2^{\cdot-}$, NO, LP, reducing power, XOD. Both essential oil and phenolic compounds demonstrated from moderate to very strong antioxidant activity, depending on the test applied. Methanolic extract exhibited higher antioxidant, but lower antibacterial activity. The oil exhibited high antibacterial activity on *S. epidermidis* ATCC 12228 and *B. subtilis* ATCC 10774 strains. Both essential oil and phenolic compounds showed significant antimutagenic activity. A strong inhibition of *t*-BuOOH-induced mutagenesis is observed in non-toxic concentration range, which can be attributed to radical-scavenging action of antioxidants.

PI207

Sesquiterpenoids from Common Ragweed (*Ambrosia artemisiifolia* L.), an invasive biological polluter

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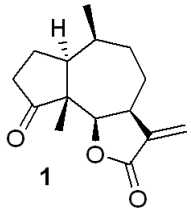
Common ragweed (*Ambrosia artemisiifolia* L.) is an invasive species native to North America, nowadays widespread in most temperate regions of the world. Allergy to *Ambrosia* is increasingly important from a clinical point of view but, surprisingly, little is known on the secondary metabolites of this plant and on their potential involvement in the symptoms associated to the allergic reaction to its pollen. We have isolated eight novel sesquiterpenoids from the aerial parts of *A. artemisiifolia,* whose structures will be reported in this communication, and evidenced the presence of large amounts of exomethylene-γ-lactones, e.g. damsin (**1**) and isabelin (**2**). Since compounds of this type, as well as their acidic precursors, might also be present in pollen, their reactivity with thiols was investigated and correlated to the activation of TRPA1, highly expressed in the aerial pathways and involved in airways sensory irritation, a common complication of allergy to *Ambrosia*.



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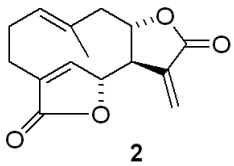

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PI208

Phytochemical profile and biological activities of *Allium carinatum* Ssp. *Carinatum* L. extract

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Members of genus *Allium* have been used for thousands of years for their medicinal properties and characteristic flavor. Chemical composition and biological activities of most *Allium* species, including *A. carinatum* ssp. *carinatum* L., are still unexplored. Therefore, the aim of this study was to evaluate phytochemical profile and biological activities of methanolic extract of *A. carinatum* ssp. *carinatum*. Chemical composition evaluation included the content of total phenolics, flavonoids and anthocyanins, quantitative LC-MS/MS and GC/MS analysis. Antioxidant activity was evaluated by LP, DPPH and NO assays, antimicrobial activity by disk diffusion and MIC assays, while anti-inflammatory activity was observed measuring the inhibitory potential on eicosanoids production. High contents of total phenolics, flavonoids and monomeric anthocyanins were found. The dominant phenolic compounds in the herb extract are ferulic acid, quercetine-3-*O*-Glc, kaempferol-3-*O*-Glc and rutin. Dimethyl-disulphide was detected as the only volatile compound. The extract suppressed production of 12-HETE, 12-HHT and TXB_2, but not of PGE_2, meaning that extract inhibits TXS and 12-LOX activity. Extract have not shown any antimicrobial activity towards nine bacterial strains, while antioxidant activity was weak compared with synthetic antioxidants.

PI209

Evaluation of anti-inflammatory activity of the hexane extract of seeds of *Byrsonima crassifolia* in experimental animal models

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The effect of extracts on inflammation were studied in formaldehyde, 12-O-tetradecanoylphorbol (TPA), carrageenan and histamine induced edema. In addition, cotton pellet granuloma test was used to investigate the effects of NS on inflammation. The hexane extract (NS) significantly inhibited paw edema induced by carrageenan, formaldehyde and histamine as well as ear edema induced by TPA in rat after oral or topical administration at doses of 50, 100 and 200 mg/kg. The antiedema potency of hexane extract was compared with indomethacin, dexamethasone and diclofenac sodium as standard drugs indicated that extract had good anti-inflammatory activity. Taken together, these results suggest that NS exerts anti-inflammatory activity with the partial contribution of inhibitory action of some inflammatory responses. In conclusion, hexane extract from seeds of *Byrsonima crassifolia* effectively controls acute and chronic inflammation in experimental models, which can be mediated by reducing the release of mediators and weakening the inflammation effects of these mediators.

PI210 Anti-inflammatory potential of *Lavandula viridis* esential oil

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As part of our ongoing work on the valorization of Portuguese lavenders we explored both the anti-inflammatory potential of Lavandula viridis essential oil (EO) and its mechanism of action. The EO was isolated by hydrodistillation and analysed by GC and GC/MS using fused silica capillary columns with two different stationary phases. The oil was characterized by high contents of oxygen-containing monoterpenes (69.5%), being the main constituents 1,8-cineole (34.5%), camphor (13.4%), α-pinene (9.0%) and linalool (7.9%). To evaluate the anti-inflammatory potential of the oil an in vitro model of lipopolysaccharide (LPS)-stimulated macrophages was used. Several inflammatory parameters were evaluated: nitrite oxide (NO) production by Griess reaction; inducible NO synthase (iNOS) and cycloxygenase-2 (COX-2) proteins expression, as well as intracellular signaling pathways activation, by western blot with specific antibodies, and mRNA expression of iNOS and pro-inflammatory cytokines (IL-1 and IL-6) by RT-PCR. L. viridis EO was able to inhibit iNOS and COX-2 proteins expression, NO production, (NF)-kB pathway and the extracellular signal-regulated kinase (ERK) ½ activation, without affecting cell viability. mRNA expression of iNOS, IL-1 and IL-6 was also modulated. These positive results highlight the potential of this oil and justify its use as an anti-inflammatory therapeutic strategy. In vivo assays have been considered to confirm the effectiveness of our in vitro results.

PI211 Studies on bioactive constituents from the leaves of *Zanthoxylum avicennae*

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[1]School of Pharmacy, Taipei Medical University, Taipei 110, Taiwan; [2]Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan; [3]Graduate Institute of Pharmaceutical Technology & Department of Pharmacy, Tajen University, Pingtung 907, Taiwan

Zanthoxylum avicennae (Lam.) DC (Rutaceae) is an evergreen shrub distributed in Vietnam, Philippines, southern China, and Taiwan. *Z. avicennae*, locally called 'Ying Bu Bo', is used as a folk medicine for treatment of rheumatism, abdominal pain, jaundice, chronic hepatitis, and common cold in China. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for *in vitro* inhibitory activity on neutrophil pro-inflammatory responses, and *Z. avicennae* has been found to be an active species. Investigation on EtOAc-soluble fraction of the leaves of *Z. avicennae* has led to the isolation of four new coumarins, 5'-methoxy- collinin (**1**), 5'-methoxyauraptene (**2**), 7-((2'*E*,5'*E*)-7'-methoxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin (**3**), 6-methoxy-7-((2'*E*,5'*E*)-7'-methoxy-3',7'-dimethylocta-2',5'- dienyloxy)-coumarin (**4**), along with 16 known compounds (**5–20**). Compounds **8**, **13–15**, **18**, and **19** exhibited inhibition ($IC_{50} \leq 7.67$ μg/ml) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Compounds **8**, **14**, and **18** inhibited fMLP/CB-induced elastase release with IC_{50} values ≤ 6.30 μg/ml.

PI212 Antimalarial natural products from traditional chinese medicinal herbs

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Malaria is a disease caused by parasites of the genus Plasmodium. The latest statistics show that malaria kills in excess of 1 million people and causes over 600,000 cases of acute illness per year. Historically, natural products have played a major role in the treatment of malaria. For centuries the Chinese medicinal plant, Artemisia annua has been used as an antimalarial herbal remedy. Subsequent chemical investigation of A. annua has led to the discovery of the antimalarial drug, artemisinin. Over 300 medicinal herbs are commonly used as antimalarial remedies in China. In this study, 100 herbals extracts were investigated for their antimalarial activity; 72 extracts showed > 50% inhibition, while 54 samples showed > 90% inhibition against a chloroquine-sensitive Plasmodium falciparum line (3D7). Chemical investigation of the active extract from Picrorhiza kurroa has yielded 2 new and 9 known natural products. This presentation will discuss the activity profiles of the 100 medicinal herb extracts and the isolation and structure elucidation of several antimalarial natural products.

PI213 Galloyle derivatives and biological activities of *Acrocarpus fraxinifolius* weight and arn leaves

Abou Zeid AH[1], Mohammed RS[1], Soliman FM[2], Sleem AA[3], El-Dakrory YM[1]
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Acrocarpus fraxinifolius is commonly known as mundane and shingle tree. It is a stately deciduous tree, attaining heights of 30 – 60 m; stem cylindrical, free of branches for up to 75% of its total height. It can achieve a diameter of over 200 cm. The total ethanol extract of the dried powdered leaves of the plant as well as the successive extracts, petroleum ether, chloroform, ethyl acetate, and aqueous ethanol extracts were prepared. Three galloyle derivatives named 2,3 digalloyle-α,-β glucoside, quercetin-(2''-galloyl-)3-*O*-β-glucopyranoside, myrecetin-(2''-galloyl-)3-*O*-β-glucopyranoside as well as *p*-hydroxy-benzoic acid, gallic acid, ellagic acid, quercetin-3-*O*- β – galactopyranoside, quercetin-3-*O*-α -rhamnopyranoside, kaempferol and quercetin were isolated from aqueous ethanol extracts. Different extracts were screened for their antidiabetic and invivo antioxidant activities. LD_{50} of the total ethanol extract was found to be 5.3 g/kg b.wt. Total ethanol and aqueous ethanol extracts at 100 mg/kg exhibited 80.2% and 75.5% potency respectively as compared with metformin (100% potency) in decreasing the glucose level after four weeks. The total ethanol and aqueous ethanol extracts showed invivo antioxidant activity 42.96% and 55.63% potency, respectively as compared with vitamin E (100% potency).

PI214 New cardenolides from *Kalanchoe tubiflora* (Harvey) Hamet

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Two new cardenolides kalantubolide A (**1**), and kalantubolide B (**2**), three known bufadienolides (**3-5**), and thirteen known compounds (**6-18**) were isolated and characterized from the EtOH extract of *Kalanchoe tubiflora* (Harvey) Hamet. The structures of these cardenolides were assigned based on spectroscopic analyses that included 1D and 2D NMR techniques, such as HMQC, HMBC, and NOESY. The biological evaluation indicated that **1-5** showed cytotoxicity against four human tumor cell lines. In addition, the bufadienolides (**4 – 6**) blocked the cell cycle in the G2/M-phase and induced apoptosis in HL-60 cells.

PI215 APPA provides disease modification in preclinical osteoarthritis

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[1]AKL International, United Kingdom; [2]Bolderbiopath, Colorado, USA

APPA, a proprietary combination of apocynin and paeonol, was evaluated for inhibition of cartilage destruction in a well-accepted rat model of osteoarthritis. **Methods:** Male Lewis rats were anesthetized and aseptic procedures utilized to induce a medial meniscal 'tear', under an IACUC-approved protocol. APPA was orally administered at 80 mg/kg BID (n = 15/group) and animals were euthanized at 3 weeks post surgery. Joints were harvested, fixed in formalin, decalcified, halved in the frontal plane, paraffin embedded, sectioned at three 200um intervals and stained with Toluidine Blue. Joints were scored according to the OARSI criteria, by a Veterinary pathologist blinded to treatment groups. The pathology in the total Joint score was significantly reduced by 21% ($p = 0.01$, Mann Whitney U test) when compared to the vehicle. Tibial and femoral cartilage degeneration scores were also significantly re-

duced (p = 0.01 and p = 0.03, respectively, Mann Whitney U test). Rats showed no adverse effects at the 80 mg/kg dose and gained weight through the study. **Conclusions:** APPA was well tolerated, and had no adverse effects when dosed at 80 mg/kg BID. Significant decreases in measures of cartilage degradation were observed for a number of well-described histologic parameters. These differences were statistically significant with modest group sizes and relatively short follow-up time points. These results, along with decreased lameness in dogs with clinical OA, indicate that APPA should be further investigated for both pain relief and disease modification.

PI216 Phenolic profile of sercial and Tinta Negra Portuguese varieties: Novel phenolic compounds in *Vitis vinifera* L. grape

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Grapes from *Vitis vinifera* L. belong to the world's largest fruit crops, and are consumed by population and applied, mainly, on wine production. This study represents the first phytochemical research of phenolic components of Sercial and Tinta Negra *V. vinifera* L. from Madeira Island (Portugal). The phenolic profiles of Sercial and Tinta Negra *V. vinifera* L. grape skins (white and red varieties, respectively) were established using high performance liquid chromatography-diode array detection-electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-MS^n), at different ripening stages (*véraison* and maturity). A total of 40 phenolic compounds were identified, from these, as far as we know, 10 compounds were reported for the first time in *V. vinifera* L. grapes, namely protocatechuic acid-glucoside, *p*-hydroxybenzoyl glucoside, caftaric acid vanilloyl pentoside, *p*-coumaric acid-erythroside, naringenin hexose derivate, eriodictyol-glucoside, taxifolin-pentoside, quercetin-glucuronide-glucoside, malylated kaempferol-glucoside, and resveratrol dimer. This data represents valuable information that may be useful to oenological management and to valorise these varieties as sources of bioactive compounds.

PI217 Antisickling properties of some indigenous and exotic plant species in Nigeria

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Sickle cell anaemia is an inherited chronic disease in which the red blood cells become sickle- shaped instead of disc-shaped. It is a genetic disease caused by abnormal haemoglobin called sickle haemoglobin (HbS), which polymerizes under deoxygenated condition and deforms the red blood cells. Medicinal plants have been used in Nigeria and most parts of developing countries in the treatment of the painful crises associated with SCD especially among the lower socio-economic class who cannot afford the high cost of western medicine as well as traditionalists who simply believe in their efficacy. Such plants being used locally in South West Nigeria for the management of SCD include: *Telfairia occidentalis*, *Parquetina nigrescens* and *Jatropha tanjorensis*. These plants were collected and extracted with Chloroform, petroleum spirit, ethyl acetate and ethanol using soxhlet apparatus. Cold extraction was also made by maceration in ethanol. Extracts were evaporated to dryness and reconstituted in distilled water to get 4 mg/ml concentration. The extracts were tested for both inhibitory and reversal activities. Cold extracts of *T. occidentalis, J. tanjorensis and P. nigrescens* gave 95%, 71% and 65% reversal respectively while *J. tanjorensis* gave the highest inhibitory property. The ethyl acetate, chloroform and petroleum spirit extracts destroyed the RBCs.

PI218 In vitro cytotoxic and antioxidant activity of some *Hypericum* species belonging to drosanthe section

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The *Hypericum* genus of Hypericaceae is represented in Turkey by 100 taxa, 45 of which are endemic. Total phenolic compounds and total flavonoid contents in methanolic extracts of the flowering aerial parts of three of the endemic species of Turkey, namely, *H. spectabile* (HSM), *H. pseudolaeve* (HPSM) and *H. thymbrifolium* (HTM), were determined and compared to *H. perforatum* (HPM). The antioxidant activities of these extracts were determined by using biochemical assays, namely, the liposome peroxidation activity, superoxide radical scavenging activity, DPPH radical scavenging activity and reducing power. The activities correlated well with total phenolic and flavonoid contents. The cytotoxic activity of these extracts were also determined by using MTT cell cytotoxicity and WST-1 cell viability screening assay on HeLa cells. Concerning the antioxidant activities of the extracts, it may be concluded that HSM, HTM and HPM, containing the highest amounts of total phenolics showed the strongest antioxidant properties whereas HPSM, containing the least phenolics, were weakest in activity. The cytotoxic activity results showed that, all extracts except HPSM exhibited low cytotoxic properties against HeLa cell line. It was concluded that the extracts might be a potential sources of antioxidant phytochemicals with associated health benefits.

PI219 Anti-mycobacterial natural products from *Juniperus communis*

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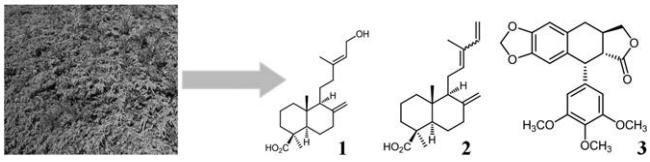
Juniperus communis is used extensively by the indigenous peoples of North America to treat numerous ailments, including tuberculosis. Bioassay guided fractionation of a *J. communis* extract resulted in the isolation of isocupressic acid (**1**), *cis*- and *trans*-communic acids (**2**), and deoxypodophyllotoxin (**3**). Evaluation of the anti-mycobacterial activity of these compounds against *Mycobacterium tuberculosis* H37Ra indicated that they had MICs of 78 μM, 31 μM and 1004 μM and IC_{50}s of 45.7 μM, 15.0 μM and 287 μM respectively. Three endophytes isolated from *J. communis* needles exhibited anti-mycobacterial activity and further work is currently underway to identify the natural products responsible for the activity.



PI220 New myrsinane-related diterpenes from *Euphorbia falcata*

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Euphorbiaceae species are well known for the chemical diversity of their isoprenoid constituents. Among isoprenoids, diterpenoids are of particular interest because of their restricted occurrence and broad structural diversity, including the high variety of carbon skeletons. Myrsinane, premyrsinane and cyclomyrsinane diterpenes containing 5/7/6-, 5/7/

6/3- and 5/6/7/4-fused ring systems, respectively, occur in the plants in highly oxygenated form, mainly as polyesters. Premyrsinanes and cyclomyrsinanes are relatively rare, to date only from nine *Euphorbia* species were reported such diterpenoids. The present paper reports the isolation of five diterpenes from the chloroform-soluble fraction of the MeOH extract prepared from the whole plant of *E. falcata* by combination of CC, VLC, CPC, PLC and HPLC. The compounds were identified by extensive spectroscopic analysis as penta- and hexaesters of myrsinane, premyrsinane and cyclomyrsinane polyols, esterified with acetic, benzoic, *n*-propanoic and isobutanoic acids. All isolated compounds are new natural products; one of them contains a rare hemiacetal moiety. Acknowledgements: This work was supported by the New Hungary Development Plan projects TÁMOP-4.2.1/B-09/1/KONV-2010 – 0005 and TÁMOP-4.2.2/B-10/1 – 2010 – 0012. A. Vasas acknowledges the award of a János Bolyai scholarship of the Hungarian Academy of Sciences.

PI221 (-)-Cubebin and (-)-Hinokinin: Evaluation of immunomodulatory effects in Chagas' disease

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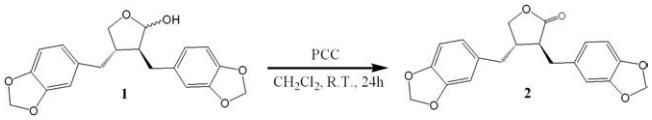
Chagas' disease is transmitted by the protozoan *Trypanosoma cruzi* and in Brazil Benznidazole (Rochagan®) is the only drug with trypanocidal activity available in the market. (-)-Cubebin **1** was isolated from *Piper cubeba* L. and (-)-hinokinin **2** was obtained from **1** by partial synthesis, followed by purification via high-performance liquid chromatography (HPLC) furnishing **2** with 98% yield. For detection of changes in cellular response we quantified the production of nitric oxide for animals treated orally with the compounds, where the results showed that the substances **1** and **2** and increased production of nitric oxide (males control 39,102 μM and treated 62,974 μM; females control 57,358 μM and treated 83,769 μM). These results showed **1** and **2** as immunomodulating agents of the immune response by increasing nitric oxide production by infected animals and subjected to treatment with these substances. Sponsors: **CNPq, FAPESP and CAPES.**



PI222 *In vitro* assay of ethanol extract from *Syzygium cumini* (L.) Skeels leaves against bacteria that cause dermatitis in pets

Gonçalves GG[1], Duda PH[1], Silva RMS[1], Oliveira LDR[2], Lazzari AM[1], Trevisan T[3], Mulinari FF[1], Melo FR[1]
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Dermatitis is an inflammation of deep slayers of skin, which could cause cell lesion generated for different agents. The increase of resistance against bacteria that causes dermatitis in pets have been worried veterinaries in Brazil. Thus, becomes necessary the discovery of new agents effective, as the rich mixture of compounds found in plant extracts. Here we intent to evaluate an ethanol extract of *Syzygium cumini* leaves at 0,2 mg/ml EtOH, which was obtained from fresh leaves. The agar diffusion method was used and the bacteria tested were isolated from dermatitis of pets treated at UPIS Veterinary Hospital. Gram negative and Gram positive strains were used *in vitro* assays. After 24 h, growing inhibition halo of 19 mm was observed when we used 6 mg/μl of extract against *Staphylococcus intermedius* and a 16 mm halo was formed, using the same sample concentration, against *Pseudomonas* spp. This last strain, showed resistance against 16 different antimicrobial drugs. At the same concentration, this extract do not caused cell death *in vitro* assay using sheep blood. MIC of 2 μg/μl of extract was determinate to *S. intermedius* and *Pseudomonas* spp. After toxicity tests, this extract could be used *in vivo* assays, aiming treatment of dermatitis in pets.

PI223 Anti-proliferative efeects of β-cyclodextrin inclusion complexes of the coumarinolignans isolated from *Acer mono*

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The cyclodextrins (CDs) are cyclic oligomers of glucose that are water-soluble because of the large number of hydroxyl groups on CDs. They are biocompatible, non-immunogenic, and have low toxicities in animals and humans, and thus have been widely used to improve solubility of a variety of hydrophobic drugs as well as to enhance their bioavailability by forming water-soluble inclusion complexes via hydrophobic interactions between drugs and hydrophobic cavity of CDs. Two hydrophobic coumarinolignans, cleomiscosins C and D were isolated from the heartwood of Acer mono, however, biological evaluation of these compounds was not carried out due to their low solubility in aqueous system. Therefore, their inclusion complex molecules with β-cyclodextrin were prepared for improvement of their very poor water solubility. Anti-proliferative effects of these complex molecules were successfully estimated on human colon cancer cells, HCT-116 in vitro. The inclusive complexes of cleomiscosins C and D with β-cyclodextrin exhibited excellent water solubility, which made it feasible to measure their cytotoxicity in cell-based assay system, and cleomiscosin D showed significant anti-proliferative effects with IC_{50} value of 6.6 μg/mL.

PI224 Antioxidant and antibacterial activity of essential oil of iranian endemic medicinal herbs

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Numerous chemical compounds in plants with bioactive properties containing secondary metabolites are used in several industries such as pharmaceutical, chemical, cosmetic and especially in food industry. Recently, the existence of antioxidant and antimicrobial compounds in plants causes to take in researcher's interest. This study was designed to examine the *in vitro* antimicrobial and antioxidant activities of the essential oils of six herbs; *Heracleum lasiopetalum* Boiss., *Satureja bachtiarica* Bunge., *Thymus daenensis* Celak., *Dracocephalum multicaule* Benth., *Kelussia odoratissima* Mozaff., *Thymus carmanicus* Jalals and *Satureja khuzestanica* Jamzad.. The Essential oils were also tested for their antioxidant activities using DPPH assay. The antibacterial activity of the essential oil was tested by agar disc diffusion assay against *Pseudomonas aeruginosa*. The results showed that essential oils of *T. daenensis*, *T. carmanicus*, *S. khuzestanica* and *S. bachtiarica* have effects highest antibacterial and the essential oils of *T. carmanicus* and *S. bachtiarica* were the most potent antioxidant.

PI225 Secondary metabolites from two endemic *Stachys cretica* subspecies and their antioxidant properties

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The genus *Stachys* is one of the largest genera of the Lamiaceae family including about 300 species growing wild in the tropical regions of both hemispheres and represented by more than 80 species in the Flora of

Turkey. We aimed to elucidate the structure of major phenylethanoid glycosides and flavonoids present in two endemic subspecies namely *Stachys cretica* ssp. *lesbiaca* and *S. cretica* ssp. *trapezuntica* and evaluate their antioxidant activities through several biochemical assays including inhibition of lipid peroxidation in soybean phosphatidylcholine liposomes induced with Fe3+/ascorbate, scavenging effect on DPPH· and superoxide radicals, reducing power and ABTS radical cation decolorization. Succesive coloumn chromatography of the ethyl acetate subfraction of two subspecies led to isolation of acteoside, leucosceptoside A, martynoside as major phenylethanoid glycosides and isoscutellarein-7 – 0-[6'''-acetyl-allosyl(1→2)]glucopyranoside, 3'-hydroxy-4'-0-methylisoscu- tellarein-7 – 0-[6'''-acetylallosyl(1→2)]glucopyranoside and 4'-0-methylisoscutellarein-7 – 0-[6'''-acetyl-allosyl(1→2)]glucopyranoside as flavonoids. The antioxidant activities of the compounds were compared to that of quercetin as a typical example of a naturally occurring flavonol. All isolated metabolites showed moderate antioxidant activity. Among the tested compounds acteoside was found to be the most active.

PI226

Sesterterpenoids and diterpenoids from the aerial parts of *Scutellaria coleifolia* Levl

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Scutellaria plants belong to the Lamiaceae family and include about 350 species, which are known to contain bioactive diterpenoids and flavonoids.[1] As part of our study for searching new drug seeds, we have investigated chemical constituents of *Scutellaria coleifolia*. The EtOAc-soluble fraction of the aerial parts of this plant (130 g) was separated by repeated column chromatography to give fourteen new compounds, along with three known compounds. Compounds **1** and **2** were sesterterpenoids with a γ-lactone moiety, structurally similar to manoalide derivatives. Although manoalide derivatives were isolated from marine sponges[2], compounds **1** and **2** appear to be the first example of this type of compounds isolated from a higher plant. The structure elucidation and biological activities of compounds **1**-**7** will be presented. **References** 1. X. Shang *et al*., *J. Ethnopharmacology* **2010**, 128, 279 – 313. 2. S.-J. Piao *et al*., *J. Nat. Prod*. **2011**, 74, 1248 – 1254.

PI227

Two new biphenyl-type neolignan derivatives from the flower buds of *Magnolia biondii*

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The dried flower buds of *Magnolia biondii* Pamp (Magnoliaceae), commonly known as Xin-yi in China. The dried flower buds of *M. biondii* are one of the most widely used medicinal plants officially listed in the Chinese Pharmacopoeia. *M. biondii* are used for the treatment of nasal empyema and headache in China. Pharmacologic studies have revealed that *M. biondii* has platelet- activating factor (PAF) receptor antagonistic activity on isolated platelet membranes. Investigation on EtOAc-soluble fraction of flower buds of *M. biondii* has led to the isolation of two new biphenyl-type neolignan derivatives, 4-allyl-2-(2-(hydroxymethyl)benzofuran-5-yl)phenol (**1**) and 5,5'-diallyl-2'-ethoxybiphenyl-2-ol (**2**), along with 7 known compounds. The structure of new compound **1** and **2** were determined through spectroscopic and MS analyses. This symposium describes the structural elucidation of **1** and **2**.

PI228

Activity of tyrosol glucosyltransferase in *Rhodiola kirilowii* transgenic root cultures

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Rhodiola kirilowii (*Crassulaceae*) is plant of Asia growing in mountain regions. *R. kirilowii* is used in traditional medicine for the enhancement of the ability of anti-anoxia. Moreover it shows anticoagulative properties and decreases the level of blood sugar. *R. kirilowii* also protect people against cardiopulmonary function problems when moving to high altitude [1]. Biologically active compounds of *R. kirilowii* are salidroside and rosavins. The aim of this work was to study the activity of tyrosol glucosyltransferase (TGase) in *R. kirilowii* transgenic root culture. These roots was successfully established by transformation with *Agrobacterium rhizogenes* LBA 9402 [2]. The activity of TGase was measured every 72 hours throughout the 30 day cycle of culture using tyrosol as a precursor and without precursor addition (control) [3]. Simultaneously, the content of tyrosol and salidroside were determined. All assays were performed by RP HPLC DAD method. Neither TGase activity nor salidroside were detected in roots cultured without precursor. Addition of tyrosol to transgenic root culture in the first day of culture resulted in significant increase of both TGase activity and salidroside concentration. Since the highest enzyme activity (0.17 U/μg) as well as salidroside concentration (1 mg/g DW) was observed on 18th day of culture, that day was chosen as optimal day for performance of biotransformation reaction. The addition of tyrosol on 18th day of culture in further studies resulted in increase of salidroside concentration up to 2.3 mg/g DW in root culture. In conclusion, it seems that the activity of TGase in transgenic root culture of *R. kirilowii* was induced by precursor addition.

PI229

Structure determination of allo-RA-V and neo-RA-V, RA-series bicyclic peptides from *Rubia cordifolia*

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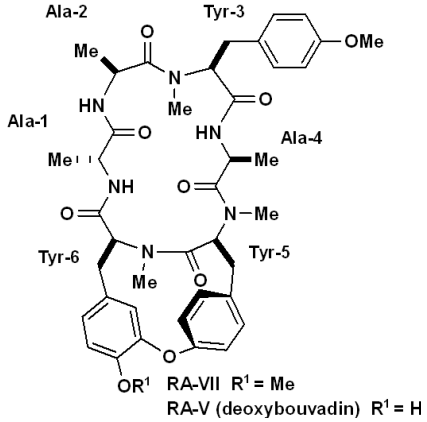
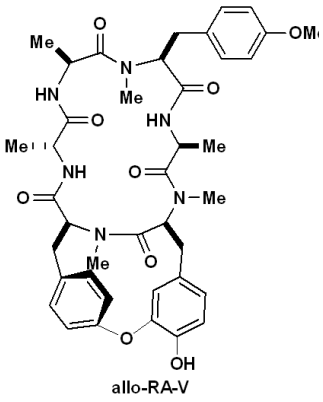
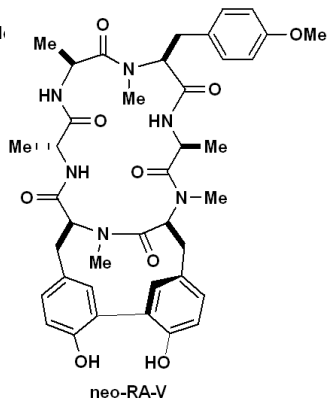
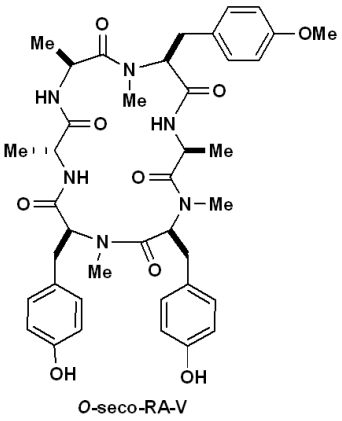
Two bicyclic hexapeptides, allo-RA-V and neo-RA-V, and one cyclic hexapeptide, *O*-seco-RA-V were isolated from the roots of *Rubia cordifolia*. Their groß structures were elucidated on the basis of spectroscopic and X-ray crystallographic analysis. The absolute stereochemistry of allo-RA-V and neo-RA-V were confirmed by their total syntheses, and the absolute stereochemistry of *O*-seco-RA-V by chemical correlation with deoxybouvardin (RA-V). Comparison of 3D structures highly active RA-VII with less-active peptides, allo-RA-V and neo-RA-V suggests that the orientation of the Tyr-5 and/or Tyr-6 phenyl rings plays a significant role in the biological activity.









PI230 Exploitation of *Juglans regia* pericarps: Chemical analysis & in vitro anti cancer and immunomodulating activities

Tsasi G[1], *Samara P*[2], *Tsitsilonis O*[2], *Skaltsa H*[1]
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In search of new anticancer agents of plant origin, the secondary metabolites of *Juglans regia* L. pericarps and their cytotoxic activities were investigated. The chemical analysis revealed the presence of 23 compounds, identified by spectroscopic methods belonging to phenolic acids, naphthoquinones, diarylheptanoids, ellagic acid, dihydrophaseic acid [1], as well as the flavonoids sudachitin, cirsilineol, apigenin, eriodictyol, 5, 6, 4′-trihydroxy 7, 3′-dimethoxy- flavone and five triterpenoids, belonging to α-/β-amyrin derivatives. Preliminary screening showed that sclerone and 3-MeO-juglone exhibit cytotoxic activity against a panel of cancer cell lines (MTT assay). Both compounds increased NK cell cytotoxicity as well as they enhanced T cell proliferation as assessed by ^{3}H-thymidine incorporation assays. The chemical and biological investigation of *J. regia* L. pericarps is still under progress. **References:** 1. Kavroulaki, E. et al. (2008) Planta Med. 74: 1040 **Acknowledgements:** This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

PI231 Secondary metabolites of *Origanum dubium* Boiss. from Cyprus

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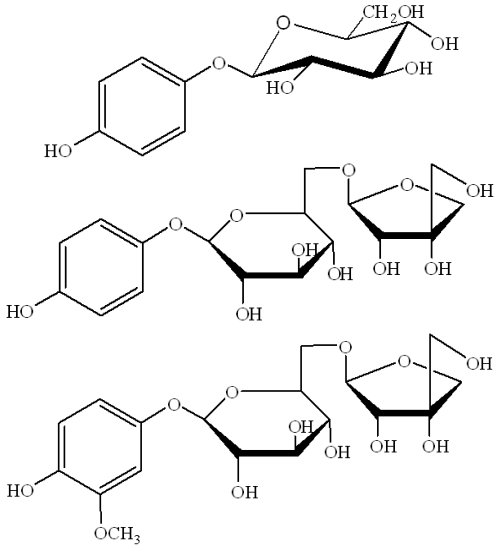
Origanum dubium Boiss. (Lamiaceae) is a flavouring and medicinal herb widely used in Cyprus. Lipophilic and polar extracts of the aerial parts were investigated for their secondary metabolites. Overall, 20 constituents were isolated. The structure of the isolated compounds was elucidated by spectroscopic methods and identified as: carvacrol, carvacrol acetate, thymoquinol-2-*O*-β-glucopyranoside, thymoquinol-5-*O*-β-glucopyranoside, spathulenol, oleanolic acid, ursolic acid, arbutoside, seguinoside-B, osmantolide, *p*-coumaric acid, apigenin, luteolin, xanthomicrol, apigenin-7-*O*-β-D-glucopyranoside, 6-hydroxykaempferol 3-methylether-6-*O*-β-D-glucopyranoside, naringenin, eriodictyol, 12-*O*-hydroxyjasmonic acid, 12-*O*-hydroxyjasmonic acid- 12-*O*-β-glucopyranoside. None of them was previously reported as constituents of *O. dubium* Boiss., while the phenolic derivatives-hydroquinone glycosides are reported for the first time as constituents of the Lamiaceae family.



Hydroquinone glycosides from O. dubium Boiss.

PI232 Essential oil composition of Tanacetum parthenium from Iran

Nazari F[1], *Khiry H*[2]
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Tanacetum parthenium (L.) Schultz-Bip. (feverfew) belongs to the family Asteraceae and is distributed all over the world. Twenty six different species has been reported from the genus of *Tanacetum* grow in various region of Iran, twelve of which are endemic. This plant is a folk medicine used in Europe and has a long history in the Iranian traditional medicine for the treatment of migraine, arthritis, psoriasis, cancer and menstrual cramps, and also has been used as sedatives, anti-microbial anti-inflammatory agents, and other common problems related to stress. Air-dried aerial parts of the plant materials grown Hamedan in the west of Iran were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h to yield. The oil obtained was dried over anhydrous sodium sulfate. Essential oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples. Nineteen compounds were characterized in the essential oil of *T. parthenium*, representing 98.42% of the oil, of which chrysanthenyl acetate (50.69%), Camphor (28.08%), *1,8-cineole* (6.43%) were found to be the major components.

PI233 Antioxidant activity some endemic Iranian medicinal plants (Apiaceae)

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Herbs and their products have been used in the food industry for their flavouring and biological activities since ancient times [1]. Oxidation, deterioration, and microbial reactions occurring in food products may cause economic loss [2]. In this study, seven plants were collected from their natural location in Chaharmahal va Bakhtiari and determined. By maceration method with methanol solvent, extracts were obtained. Antioxidant activity of methanolic extracts was evaluated by three assays: DPPH, FRAP & TEAC. Among the plant extracts were evaluated in FRAP assay, *Echiophora platyloba* DC showed relatively strong ferric ion reducing activity with 3.38 mg/ml. In TEAC assay, IC_{50} value of *E. platyloba* was determine to be 1.70 mg/ml. In comparing different extracts, *E. platyloba* was found to be better antioxidants than the other plants, correlating with its high total phenolics and flavonoids content. **Key words:** *Echiophora platyloba* DC, DPPH, FRAP and TEAC assays. [1] Conforti, F., Menichini, F., Formisano, C., Rigano, D., Senatore, F., Apostolides Arnold, N., Piozzi, F. 2009. Comparative chemical composition, free radical-scavenging and cytotoxic properties of essential oils of six Stachys species from different regions of the Mediterranean Area. *Food Chemistry*, 116, 898 – 905. [2] Yanishlieva, N. V., Marinova, E. Y., Pokorny, J. 2006. Natural antioxidants from herbs and spices. *European Journal of Lipid Science and Technology*, 108, 776-793.

PI234 *Tribulus terrestris* ethanolic extract ameliorates cypermethrin induced reproductive toxicity in male wistar rats

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Role of ethanolic extract of *Tribulus terrestris* (EETT,100 mg/kgbw) against α-cypermethrin (3.38 mg/kgbw) induced reproductive toxicity in male Wistar rats was investigated in 28 days study. At the end of the experiment, rats were sacrificed, testis and epididymis were removed and sperm characteristics, sex hormones and various biochemical parameters were studied. Decrease in weight of testis and epididymis, testicular sperm head count, sperm motility, live sperm count, serum testosterone (T), follicle stimulating hormone (FSH), leutinizing hormone (LH), catalase (CAT), superoxide dismutase (SOD), glutathione S transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), total protein content and increase in sperm abnormalities and lipid peroxidation (LPO) level was observed in rats exposed to cypermethrin. EETT co-administration ameliorated α-cypermethrin induced damage. EETT treatment increased testis and epididymis weight, sperm head counts, sperm motility, live sperm counts, T, FSH, LH, GSH, CAT, SOD, GST, GR, GPx and total protein content as compared to control group. The study suggested that *Tribulus terrestris* plant possess reproductive system enhancement and antioxidant activity.

PI235 Antioxidant activity some endemic Iranian medicinal plants (Lamiaceae)

Setayesh M[1], Ghasemi Pirbalouti A[2,3], Siahpoosh A[1], Abdizadeh R[4]
[1]School of Pharmacy, Ahwaz Jondishapur University of Medical Sciences, Ahwaz, Iran, [2]Shahrekord Branch, Islamic Azad University, Researches Centre of Medicinal Plants & Ethno-veterinary, P O Box: 166, Shahrekord, Iran, [3]Laboratories for Natural Products, Medicinal and Aromatic Plants, Department of Plant and Soil Sciences, University of Massachusetts, Amherst, MA 01003, USA, [4]Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Oxidation, deterioration, and microbial reactions occurring in food products may cause economic loss. Deterioration of lipids is catalysed by different internal and external factors, such as free radicals, metal ions, light, and heat. In this study, some of the Iranian endemic plants were collected from their natural location in Chaharmahal va Bakhtiari. By maceration method with methanol solvent, extracts were obtained. Antioxidant activity of methanolic extracts was evaluated by three antioxidant assays: DPPH, FRAP & TEAC. In DPPH assay, comparing all the plant extracts for their IC_{50} values, *Dracocephalum multicaule* Montbr & Auch and *Thymus daenensis* Celak was the most effective scavenging of free radical, with value of 35.30 and 39 mg/ml. Among the plant extracts were evaluated in FRAP assay, *T. daenensis* showed relatively strong ferric ion reducing activities with 2.37 mg/ml. in TEAC assay, IC_{50} value of *T. daenensis* was determine to be 1.21 mg/ml. Extract of *T. daenensis* exhibited the highest phenolics, and extract of *D. multicaule* exhibited the highest flavonoids.

PI236 Isolation, characterization and bioactivity of sesquiterpene lactones from *Eupatorium lasiophthalmum* Griseb

Maldonado E[1,3], Svensson D[1], Oredsson S[2], Sterner O[1]
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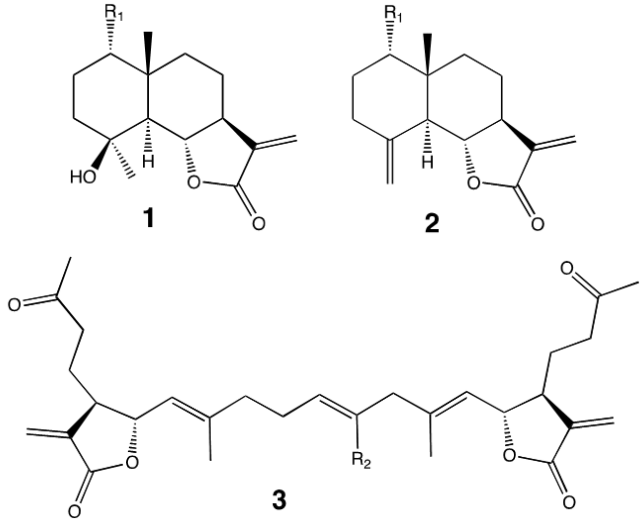
Sesquiterpene lactones (SLs) are the most characteristic bioactive constituents of *Eupatorium* species. They have shown to exhibit a broad variety of different biological activities, specially cytotoxic and antitumor effects[1, 2]. As part of our search of structurally interesting and biologically active compounds from Bolivian medicinal plants, a phytochemical investigation of *Eupatorium lasiophthalmum* G. has resulted in the isolation of two new eudesmanolides **(1 – 2)** and one triterpene **(3)** together with fourteen known SLs. The structures of the new compounds and their cytotoxicity towards five breast cancer cell lines (MCF-7, SK-BR-3, HCC 1937, L 56Br-C 1 and JIMT-1) and one normal-like breast-derived cell line (MCF-10A) will be discussed.



PI237 Isolation, structure elucidation, and biological evaluation of 16,23-epoxycucurbitacin constituents from *Eleaocarpus chinensis*

Pan L[1], Yong Y[2], Deng Y[1], Lantvit DD[3], Ninh TN[4], Chai H[1], Carcache de Blanco EJ[2], Soejarto DD[3,5], Swanson SM[3], Kinghorn AD[1]
[1]Division of Medicinal Chemistry and Pharmacognosy; [2]Division of Pharmacy Practice and Administration, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA; [3]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA; [4]Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; [5]Department of Botany, Field Museum of Natural History, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA

Eight new 16,23-epoxycucurbitacin derivatives, designated as elaeocarpucins A-H (**1-8**), and five known cucurbitacins (**9-13**) were isolated from the separate methanol extracts of the fruits and stem bark of *Elaeocarpus chinensis* (Gardn. & Champ.) Hook. ex Benth. (Elaeocarpaceae) collected in Vietnam. Isolation work was facilitated using an LC/MS dereplication procedure, and bioassay-guided fractionation was monitored using HT-29 human cancer cells. Compounds **1-13** were evaluated in vitro against the HT-29 cell line and using a mitochondrial transmembrane potential assay. Elaeocarpucin C (**3**), produced by partial synthesis from compound **13**, was found to be inactive when evaluated in an in vivo hollow fiber assay using three different cancer cell types (dose range 0.5 – 10 mg/kg/day, ip).

PI238 New salvinorin A – Derived ligands to opioid receptors

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The neoclerodane diterpenoid salvinorin A is a major metabolite isolated from the leaves of psychoactive plant *Salvia divinorum*. It is a highly selective κ-opioid receptor (KOR) agonist and is the most potent naturally occurring hallucinogen. It gained significant scientific interest, being the only non-nitrogenous KOR agonist with no apparent structural similarity to other ligands. Previously, extensive efforts were made to understand how salvinorin A binds and activates the receptor. Our goal is to design a series of salvinorin A-ligands with high affinity to KOR in order to further explore the ligand-receptor interactions at the molecular level. In continuation of our research towards irreversible ligands, we synthesized a series of new C-2 modified salvinorin A derivatives and evaluated them for binding affinity to opioid receptors. A majority of the analogs have shown high affinity to KOR, and some of them have exhibited dual affinity to κ- and μ-opioid receptors.

PI239 Compounds with antileishmanial activity isolated from the stems of *Pentalinon andrieuxii*

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Bioassay-guided fractionation was conducted on a chloroform-soluble extract of the stems of *Pentalinon andrieuxii*, a Mayan traditional antileishmanial plant collected in a rainforest region of Mexico, using a *Leishmania* (L.) *mexicana* strain of parasites. This led to the isolation of five C_{21} sterol derivatives, neridienone A (**1**), 6,7-dihydroneridienone A (**2**), cybisterol (**3**), 12β-hydroxypregn-4-ene-3,20-dione (**4**), and pentalinonoside (**5**), two coumarins, fraxidin (**6**) and fraxetol (**7**), as well as betulinic acid (**8**) and (+)-pinoresinol (**9**). All the isolates were evaluated in vitro for their ability to inhibit the growth of promastigotes of *L. mexicana*. Compounds **1-4**, and **6** were found to exhibit notable leishmanicidal activity in this assay. (Supported by grant RC4 AI092624, from NIAID, NIH).

PI240 Sensitivity enhancement in LC-ESI-MS method using mobile phase additives to quantify Simalikalactone E

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A quassinoid, Simalikalactone E (SkE), extracted from Quassia amara L. (Simaroubaceae) leaves in our laboratory, displayed strong *in vitro* and *in vivo* antimalarial activities and also a very promising effect against chronic myeloid leukemia. In order to optimize the SkE purification process form the plant (present at very low concentration) and further pharmacokinetic studies in biological fluids, a robust and sensitive quantification method was needed. We present here the development of a LC-MS method using single ion monitoring to response the requirement of quantification of SkE. The effects of mobile phase additives using sodium, lithium and ammonium adducts formation were investigated. The ESI in the positive ion mode with aqueous mobile phase containing 0.25 nM of sodium acetate was proved to be the most efficient to improve the sensitivity of the detection. The calibration curve was linear over the concentration range of 0.896 and 8.96 ppm with $R^2 > 0.998$. The limits of detection and quantification were12.3 ppb and 38.1 ppb respectively. The intra-day precision and accuracy were less than 3.39% and 7%, respectively, while the inter-day precision and accuracy were less than 3.55% and 7.75%, respectively. We will finally present the application of this method for the quantification of SkE in all steps of the purification process.

PI241 Cordifolide A, a sulfur-containing clerodane diterpene glycoside from *Tinospora cordifolia*

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Cordifolide A (**1**), a novel unprecedented sulfur-containing clerodane diterpene glycoside, together with other two new diterpene glycosides, cordifolides B (**2**) and C (**3**), and four known analogues, was isolated from a methanol-soluble extract of the stems of *Tinospora cordifolia*. The structures of the new compounds were determined on the basis of spectroscopic data interpretation, with that of cordifolide A (**1**) confirmed by a single-crystal X-ray crystallographic analysis. All isolates were evaluated for their in vitro immunomodulatory activity using mouse bone marrow-derived dentritic cells (BMDCs). (Supported by grant RC4 AI092624, from NIAID, NIH)

PI242 Growth inhibition of human colon carcinoma cells by diterpenes and tetralones of *Zygogynum calothyrsum*

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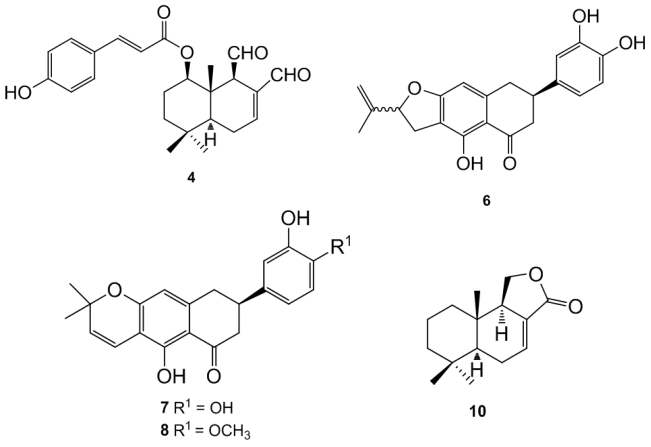
Bioassay guided phytochemical investigation of *Zygogynum calothyrsum* using human colon carcinoma cells COLO205 and KM12 led to the isolation of three new drimane-type diterpenes, 11-hydroxy-11,12-epoxydrimane (**1**), 5,11-dihydroxy-11,12-epoxydrimane (**2**) and 11,12-di-

methoxy-11,12-epoxydrimane (**3**), the known 1β-*p*-coumaroyloxypolygodial (**4**) together with two new tetralones, 3'-dehydroxyisozygolone (**5**) and calothyrlone A (**9**), three known tetralones, isozygolone A (**6**), zygolone A (**7**) and 4'-*O*-methylzygolone A (**8**) and a known cinnamolide (**10**). Compounds **1**, **7** and **8** demonstrated higher cytotoxicity against COLO205 (GI_{50}= 17.8, 16.7 and 11.4 μM, respectively) and KM12 (GI_{50}= 13.5, 14.2 and 17.2 μM, respectively) than other compounds.



PI243

A tricyclic sesquiterpene from *Eriophyllum lanatum* stabilizes the tumor suppressor protein Pdcd4 by Inhibiting the E3-Ligase β-TrCP1

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Pdcd4 is a novel tumor suppressor that inhibits translation rather than transcription, and its cellular abundance is controlled by proteasomal degradation. A luciferase-based high-throughput screen for agents that can stabilize cellular Pdcd4 levels under tumor-promoting conditions was run with extracts from the NCI Natural Products Repository. This identified an extract of the woolly sunflower (*Eriophyllum lanatum*) as active, and subsequent isolation efforts provided a tricyclic sesquiterpene lactone as the active principle. This compound did not inhibit phosphorylation of Pdcd4 that occurs via the PI3K-Akt-mTOR-$p70^{S6K}$ cascade, and it did not inhibit the proteasome itself. It was subsequently shown that the active sesquiterpene specifically inhibited E3 ubiquitin ligase β-TrCP1 activity, and thus stabilized Pdcd4 and other β-TrCP1 targets such as IκBα. This compound also suppressed the tumor-associated activity of several Pdcd4- and IκB-regulated transcription factors, (AP-1 and NF-κB), and it attenuated the migration of tumor cells. These studies identified the sesquiterpene lactone as a novel inhibitor of β-TrCP1, and they suggest that blocking a specific E3-ubiquitin ligase may allow more targeted modulation of protein degradation compared to general proteasome inhibition.

PI244

Effect of 20-hydroxyecdysone on glucose and lipid metabolism in vitro

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Ecdysteroids such as 20-hydroxyecdysone (20HE) are commonly found in the highly nutritious Andean pseudocereal quinoa (*Chenopodium quinoa* Willd.). 20HE and an ecdysteroid-enriched extract from quinoa have been shown to improve insulin sensitivity and decrease adiposity in diet-induced obese mice. One proposed mechanism of action for this in vivo effect is 20HE's negative impact on hepatic glucose output. However, associated anti-diabetic and anti-obesity mechanims, including enhanced insulin sensitivity in peripheral tissues and changes to adipogenesis or lipid metabolism in adipose tissue, have not been reported. Here, we tested the effect of this molecule on these parameters by measuring (a) glucose uptake and insulin sensitivity in L6 rat myotubes, (b) lipid accumulation in 3T3-L1 mouse adipocytes, and (c) lipolysis in 3T3-L1 adipocytes. We also confirmed the impact of 20HE on glucose production in HII4E rat hepatocytes and showed that several fractions of a crude ethanolic extract from quinoa containing ecdysteroids similarly decrease glucose output in this model. Our data suggest that the complex mixture of secondary metabolites found in quinoa may play a synergistic role in the improvement of physiologic parameters associated with metabolic syndrome, and quinoa may be an important functional food for the treatment and prevention of diabetes and obesity.

PI245

6-hidroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (1), a new α-glucosidase inhibitor from *Brickellia cavanillesii*

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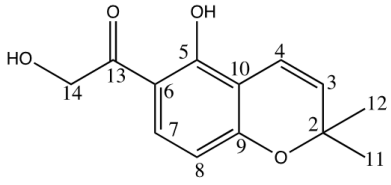
An aqueous extract from the aerial parts of *Brickellia cavanillesii* showed potent inhibitory activity (IC_{50}= 0.169 mg/mL *vs* 1.12 mg/mL for acarbose) against yeast α-glucosidase. Bioassay guided fractionation of the active extract led to the isolation of several compounds including a new chromene, namely, 6-hidroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (**1**). This compound was identified by spectral means. It inhibited the activity of yeast-α-glucosidase with IC_{50} of 0.28 mM. Kinetic analysis revealed that compound 1 behaved as non-competititive inhibitor with a *Ki* of = 0.13 mM.



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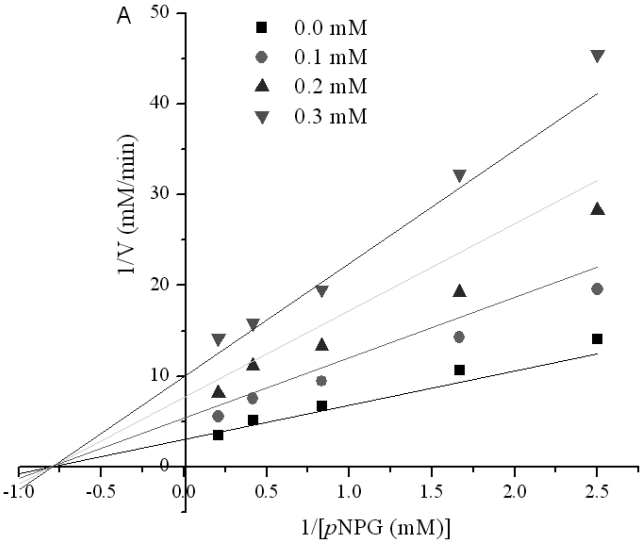

PI246

Leishmanicidal effect of derivatives of dibenzylbutirolactonic lignans from Piper cubeba

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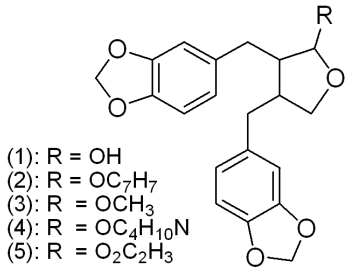
Leishmaniasis is an infection characterized for being a parasitic disease with great incidence in the world. Recent publications have highlighted numerous biological activities attributed to the dibenzylbutirolactonic lignan (-)-cubebin (1). Ongoing studies have focused on its structural optimization, in order to obtain derivatives with greater pharmacological potential, low costs and low toxicity. The aim of this study was the obtainment of (1), its semisynthetic derivatives and the evaluation of their leishmanicidal activity. From the extract of seeds of *P. cubeba* was obtained (1), and it was used for the synthesis of: (-)-*O*-benzylcubebin

(2), (-)-*O*-methylcubebin (3), (-)-*O*-(N,N-dimethylamino-ethyl)-cubebin (4) and (-)-*O*-acethylcubebin (5). The *in vitro* biological activity of the lignans against *Leishmania amazonensis* promastigote forms was carried out with MTT method. It could be verified that (2), (3) and (4) showed leishmanicidal potential (61,6%, 56,9%, 66,3%, respectively), as these effects were comparable to those observed for positive control, Amphotericin, at 12,5 μM (76,4%). The cytotoxicity assay showed that the *in vitro* activity of the compounds may not be related to cytotoxic effects, since they don't present significant cytotoxicity (IC_{50}: 328,2; 383,3; 355,7; 323,1;370,4 μM, respectively). Sponsors: FAPESP, CNPq and CAPES.



PI247 Headspace analysis of the volatile flower scent of *Zeyheria montana* Mart. (Bignoniaceae)

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Floral scent plays a key role for the chemical communication between fragrant flowering plants and animal pollinators. *Zeyheria montana* Mart. (syn. *Z. digitalis*) is a medicinal plant native from the Brazilian savannah "Cerrado". The flowers are tubular in shape, yellow, nectariferous and pollinated mainly by hummingbirds. Despite the biological interest displayed by this plant, phytochemical studies so far have been done with its roots and leaves. Thus, this work was aimed to perform the chemical characterization of the floral scent in *Z. montana* by means of GC/MS analysis. Recently-opened flower samples were enclosed within a 20 mL borosilicate glass vial and analyzed on a Thermo Scientific Focus system, equipped with an ISQ 230ST mass spectrometer and a Triplus automatic sampler. Separations were achieved by using an OV5-MS column, under optimized conditions. Tentative identification of the volatiles was based on the comparison of their mass spectral data and retention index against those from NIST data base or literature. This approach revealed, for the first time, that the floral scent of *Z. montana* comprises a complex mix of at least 32 compounds. They were recognized as normal chain aldehydes, polyunsaturated ketones, non-terpenic hydrocarbons, phenylpropanoids, and sesquiterpenes. Further biological investigations are underway to elucidate the chemical-biological dynamic of its reproductive biology.

PI248 New glucosylated caffeoylquinic acid derivatives in the invasive soda apple, *Solanum viarum* L

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The eggplant and its relatives within the "spiny solanums" (*Solanum* subgenus *Leptostemonum*) have been shown to contain diverse and abundant caffeoylquinic acid derivatives, which have antioxidant and other biological activity with potential benefit to human health. We explored the diversity of caffeoylquinic acid derivatives in fruit extracts of *Solanum viarum,* an invasive species commonly known as the soda apple, which is within subgenus *Leptostemonum* and native to Brazil. Two new glucosylated caffeoylquinic acids, viarumacids A and B, were detected and isolated and their structures elucidated by means of spectroscopic methods including 2D NMR techniques and mass spectrometry. The compounds were tested for their antioxidant activities by ABTS and DPPH assays, and were found to have activities similar to 5-CQA.

PI249 Structure and cytotoxicity of steroidal glycosides from *Allium schoenoprasum*

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A phytochemical analysis of the whole plant of *Allium schoenoprasum*, has led to the isolation of seven new spirostane-type glycosides (**1-7**) while **1-6** were obtained as three pairs of inseparable (*R*)/(*S*)-isomers derivatives **1/2**, **3/4**, **5/6**, and four known steroidal saponins. Their structures were elucidated mainly by 2D NMR spectroscopic analysis and mass spectrometry as (20*S*,25*R*)-spirost-5-en-3β,12β,21-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**1**) and its (25*S*)-isomer (**2**), (20*S*,25*R*)-spirost-5-en-3β,11α,21-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**3**) and its (25*S*)-isomer (**4**), laxogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**5**) and its (25*S*)-isomer (**6**), and (25*R*)-5α-spirostan-3β,11α-diol 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside (**7**). Four of the isolated compounds were tested for cytotoxic activity against the HCT 116 and HT-29 human colon cancer cell lines.

PI250 Isolation and characterisation of antiproliferative compounds from *Neoharmsia baronii*

Dengada AH[1], Brodie PJ[1], Callmander M[2], Rakotobe E[3], Rasamison VE[3], Kingston DGI[1]
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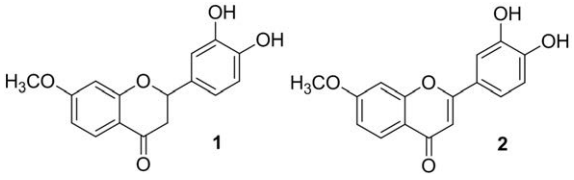
The plant *Neoharmsia baronii* is endemic to Madagascar, and has been listed as a critically endangered species under the IUCN Red List of Threatened Species. Work on this species is thus important so as to obtain as much information as possible before it becomes extinct. As part of an International Cooperative Biodiversity Group (ICBG) program, an ethanol extract of a small sample of this plant was found to have antiproliferative activity against the A2780 ovarian cancer cell line. Bioassay-guided fractionation of this extract led to the isolation of two flavonoids with significant antiproliferative activity. The structures of the flavonoids isolated, fustin **1** and fisetin **2**, were determined using NMR and mass spectrometric techniques. Further isolation and characterization of additional compounds with promising anticancer activity is ongoing.



PI251 Two new triterpenoid saponins from *Pittosporum senacia*

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The Pittosporaceae family is represented by 9 genera, which contains about 250 species, distributed in tropical and subtropical regions. The use for local traditional medicine from species of the Pittosporaceae family has been reported in multiple cases. Some different endemic subspecies of *Pittosporum senacia* are known to have an indigenous medicinal role in tropical regions for treatment of rheumatism and throat infections. A previous work on the volatile constituents of the leaves of *P. senacia* var. *coursii* was reported, whereas triterpene saponins have never been characterized in this species. In this communication we report

about the isolation and characterization of two new triterpene saponins, senaciapittosides A (**1**) and B (**2**) from *Pittosporum senacia*. The structures were elucidated by 600 MHz NMR analysis including 1D and 2D NMR (^{1}H-, ^{13}C NMR, COSY, TOCSY, ROESY, HSQC and HMBC) spectroscopy and mass spectrometry as 3-*O*-[β-D-glucopyranosyl-(1→2)]-[α-L-arabinopyranosyl-(1→3)]-[α-L-arabinofuranosyl-(1→4)]-β-D-glucuronopyranosyl-olean-12-en-28-*O*-β-D-glucopyranosyl ester (**1**), and 3-*O*-[β-D-glucopyranosyl-(1→2)]-[α-L-arabinopyranosyl-(1→3)]-[α-L-arabinofuranosyl-(1→4)]-β-D-glucuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-acetoxyolean-12-en-3β,15α,16α,21β,22α,28-hexol (**2**).

PI252 Antiproliferative compounds from the fabaceae family from the Madagascar dry forest

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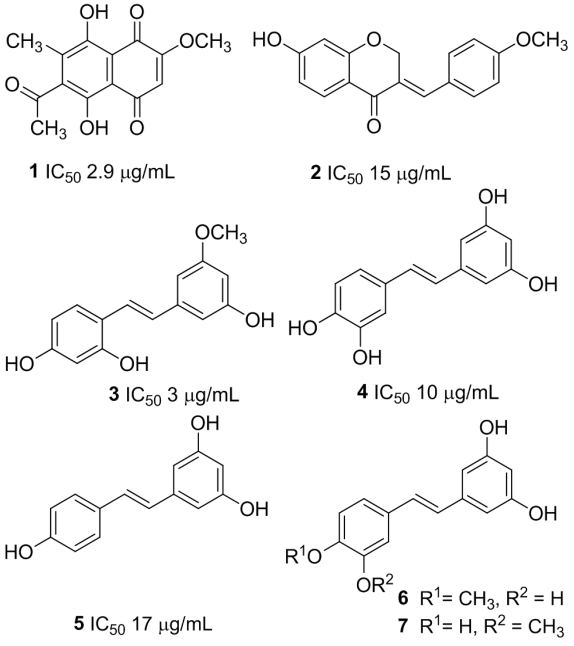
As a part of the International Cooperative Biodiversity Group (ICBG) program, ethanol extracts of the leaves and roots of a plant initially identified as *Bussea sakalava* (Fabaceae) were selected for evaluation as potential anticancer agents based on activity against the A2780 ovarian cancer cell line. Recent taxonomic studies have indicated that the plant is not *B. sakalava*. Bioassay-directed fractionation of the leaf extract afforded the new naphthoquinone (**1**), and a combination separation methods on the root extract afforded the known isoflavonoid bonducellin (**2**), one new stilbenoid (**3**) and the four known stilbenoids piceatannol (**4**), resveratrol (**5**), rhapontigenin (**6**), and isorhapontigenin (**7**). The structure elucidations of all compounds were based on NMR spectra and mass spectroscopic data, and the structure of **1** was confirmed by single crystal X-ray analysis. Compounds **1-5** showed antiproliferative activity against the A2780 human ovarian cancer cell line as shown.



1 IC_{50} 2.9 μg/mL

2 IC_{50} 15 μg/mL

3 IC_{50} 3 μg/mL

4 IC_{50} 10 μg/mL

5 IC_{50} 17 μg/mL

6 R^1= CH_3, R^2 = H

7 R^1= H, R^2 = CH_3

PI253 Mechanistic study of *O*-methylcedrelopsin and dehydrogeijerin

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[1]Department of Pharmacology; [2]Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229

Plants that can survive in the harsh environment of South Texas have the potential to produce molecules with activity against diverse biological targets. The Mountain torchwood, *Amyris madrensis*, was evaluated because the crude lipophilic extract had potent activity against prostate cancer cells. Multiple cytotoxic compounds were isolated using bioassay guided fractionation including 14 previously identified coumarins. Two coumarins, *O*-methylcedrelopsin and dehydrogeijerin, were evaluated for antiproliferative activities against cancer cell lines. *O*-methylcedrelopsin has an IC_{50} of 7.8 and 27.8 μM in HeLa and PC-3 cells respectively. Dehydrogeijerin also showed activity in the low micromolar range with an IC_{50} of 16.0 μM in HeLa cells and 43.0 μM in PC-3 cells. Both *O*-methylcedrelopsin and dehydrogeijerin caused a dose dependent accumulation of cells in G_2/M as determined by flow cytometry. They have no effects on interphase microtubule structures or on the assembly of purified tubulin, suggesting a mechanism different from microtubule binding compounds. The compounds do however initiate the formation of abnormal mitotic spindles which closely resemble those formed following treatment of cells with mitotic kinase inhibitors. The hypothesis that *O*-methylcedrelopsin and dehydrogeijerin target mitotic kinases, specifically polo like kinase 1 (PLK1) or cyclin dependent kinase 1 (CDK1) is being tested. Two structurally similar coumarins, 7-*O*-prenylscopoletin and cedrelopsin, were also identified but they have 10-fold lower antiproliferative potency. These structures will be informative in defining the moieties optimal for inhibiting mitotic progression. *Supported by NIDCR (COSTAR) DE14318 and DoD W81XWH-08 – 1-0395.*

PI254 Antioxidant activities of *Ziziphus mauritiana* seed extracts from different extraction methods

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Ziziphus is a genus in Rhamnaceae family which the fruits and seeds have been widely used since ancient time for the treatments of insomnia, dream-disturbed sleep, excessive sweating and thirst. *Z. mauritiana* is a tropical fruit tree widely distributed in many Asian countries including Myanmar and Thailand. However, the available scientific report on biological activities of this plant is very limited. The seeds of *Z. mauritiana* were extracted using soxhlet extraction methods with different solvents including petroleum ether, hexane, ethanol methanol and decoction with distilled water, respectively. The extracts were tested for *in vitro* antioxidant activities using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay and ferric reducing antioxidant power (FRAP) method. From the results, ethanolic extract from soxhlet extraction exhibit high DPPH scavenging activity with EC_{50} of 33.55 ± 4.43 μg/ml and the highest ferric reducing antioxidant power. Phytochemical analysis of *Z. mauritiana* seed extracts was also conducted.

PI255 Antiparasitic and anticancer carvotacetone derivatives from *Sphaeranthus bullatus*

Machumi F[1], Yenesew A[2], Midiwo JO[2], Heydenreich M[3], Kleinpeter E[3], Khan S[1], Tekwani BL[1], Walker LA[1], Muhammad I[1]
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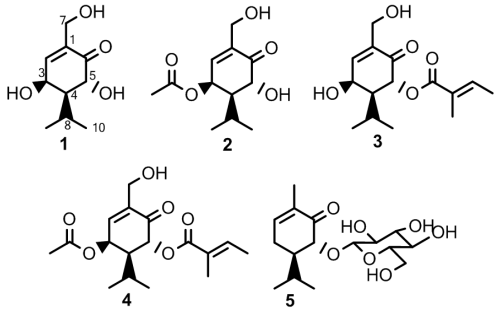
The 1:1 $MeOH/CH_2Cl_2$ extract of aerial parts of *Sphaeranthus bullatus*, an annual herb native to tropical East Africa, showed activity against chloroquine sensitive D6 (IC_{50} 9.7 μg/ml) and chloroquine resistant W2 (IC_{50} 15.0 μg/ml) strains of *P. falciparum*. Seventeen secondary metabolites were isolated and evaluated for their *in-vitro* antiplasmodial, antileishmanial and anticancer activities revealing activity on four carvotacetone derivatives **1-4**; with antiplasmodial activity of IC_{50} 3.4, 0.6, 0.8, 1.4 μg/ml respectively against D6 strains of *P. falciparum*; antiplasmodial activity of IC_{50} 2.8, 0.7, 0.9, 2.0 μg/ml respectively against W2 strains of *P. falciparum*; antileishmanial activity of IC_{50} 17.0, 0.7, 3.0 and 0.7 respectively against the parasite *L. donovanii*, and anticancer activity of IC_{50} < 5.3 μg/ml against SK-MEL, KB, BT-549 and SK-OV-3 cells for **2-4**. In addition, cytotoxicity of the active compounds was evaluated against monkey kidney fibroblasts (VERO) and pig kidney epithelial ($LLC\text{-}PK_{11}$) cells.



PI256

In vitro antifungal activity of *Otacanthus azureus* (Linden) Ronse essential oil alone and in combination with azoles

Houël E[1], Rodrigues A[2], Jahn-Oyac A[1], Bessière JM[3], Eparvier V[2], Deharo E[4], Stien D[2]
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We determined the chemical composition and investigated the antifungal activity of *Otacanthus azureus* (Linden) Ronse essential oil alone or combined with azoles antifungals against a range of human yeasts and dermatophytes. The oil was shown to be composed in majority of sesquiterpenes. Using broth microdilution techniques, it was found to exert interesting *in vitro* antifungal activities, more particularly against human dermatophytes, with minimum inhibitory concentrations as low as 4 μg/ml against a clinical isolate of *Trichophyton rubrum*. The analysis of the combined effect of this oil with azoles highlighted a pronounced synergism between the oil and ketoconazole or itraconazole, against *Candida albicans*, *C. parapsilosis* and *Trichophyton mentagrophytes*, with fractional inhibitory concentration indices in the 0.1 – 0.5 range. Interestingly, the oil showed no cytotoxicity on VERO cells (ED 50> 100 μg/ml). According to these results, *O. azureus* essential oil may be considered a promising natural product in the treatment of human mycoses, more particularly those originating from dermatophytic fungi. Also, it is likely to reduce the minimum effective dose of ketoconazole and itraconazole against *Candida* species, thus minimizing the side effects of these drugs, and the risk to develop resistances.

PI257

Compounds inhibiting hyperglycemia and cancer cell proliferation from *Morus alba* L

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In traditional use, the root bark *Morus alba* L, Cortex Mori (Sangbaipi) is for the treatment of lung-heat, cough, hematemesis, dropsy, beriberi, difficulty in micturition and so on; the twig after processed, Ramulus Mori (Sangzhi), for dispelling wind-damp, easing joint movement, promoting diuresis and reducing swelling and edema, dysuria and painful limbs. Recently, *M. alba* was reported to have anti-HIV, anti-tumor and anti-hyperglycemic effects. Our results showed that some benzofuran and flavonoid compounds are responsible for these dual inhibitory effects on cancer cells' proliferation and hyperglycemia. Moracin M, steppogenin-4'-*O*-β -*D*-glucosiade, and mulberroside A presented anti- hyperglycemic effects on alloxan-diabetic mice *in vivo*. Steppogenin-7,4'-di-*O*-β-*D*-glucosiade inhibted the proliferation of human ovarian cancer HO-8910 cells *in vitro*. Moracin C and Moracin M inhibited the priliferation of adenocarcinomic human alveolar basal epithelial cells A549 and human breast cancer cells MCF-7.

PI258

Stimulating effects of cheonggukjang extract on macrophage-mediated immune responses

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In Asia, there are various fermented soybean products like Natto, Doenjang, Tempeh and so on. Cheonggukjang is soybean paste fermented by *Bacillus subtilis* and a traditional healthy food in Korea. Here, we investigated the regulatory effects of Cheonggukjang extract on macrophage-mediated immune responses. When Cheonggukjang extract treatment was used in combination with interferon-γ (IFN-γ), there was a marked cooperative induction of nitric oxide (NO) and tumor necrosis factor-α (TNF-α) production in macrophages. Cheonggukjang extract increased the expression of inducible NO synthase mRNA, protein and TNF-α mRNA in macrophages. These alterations of Cheonggukjang extract-treated cells were associated with the activation of nuclear factor-κB (NF-κB). Cheonggukjang extract increased the phosphorylation and transcriptional activity of p65 in macrophages. These results suggest that Cheonggukjang extract increased NO and TNF-α production through phosphorylation of p65 following IκBα degradation and NF-κB activation. Treating macrophages with pyrrolidine dithiocarbamate and BAY11 – 7082, an inhibitor of NF-κB, decreased the synergistic effects of Cheonggukjang extract. In conclusion, our results demonstrate that Cheonggukjang extract can effectively promote the activation of macrophages, suggesting that Cheonggukjang extract may possess the potential to regulate immune responses.

PI259

Studies on the constituents of *Cudrania tricuspidata*

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Cudrania tricuspidata (Moraceae), which is a deciduous tree, widely distributed in Korea and China, has been traditionally used for the treatment of lumbago, hemoptysis, and contusion. As part of our search for bioactive plant metabolites, we have investigated constituents of this plant. The MeOH extract of the roots of *C.tricuspidata* was partitioned with EtOAc and H_2O. The EtOAc-soluble fraction was separated repeatedly by various column chromatografies to yiled a new prenylated xanthone (**1**) and two new prenylated flavonoids (**2** and **7**), together with nineteen known compounds. The structures of these compounds were elucidated by extensive spectroscopic analyses including 1D-, 2D-NMR and MS. The antibacterial activities against MRSA as well as the antifungal activities against *Aspergillus niger*, *Candida albicans*, *Penicillium* sp., *Rhizopus* sp. and *Trichophyton* sp. of isolated compounds were evaluated. The structure elucidation and the antimicrobial activities of these compounds will be presented.

PI260

Studies on the chemical constituents of *Evodia rutaecarpa* and *Evodia rutaecarpa* passed with *Liquorice* and biological activities

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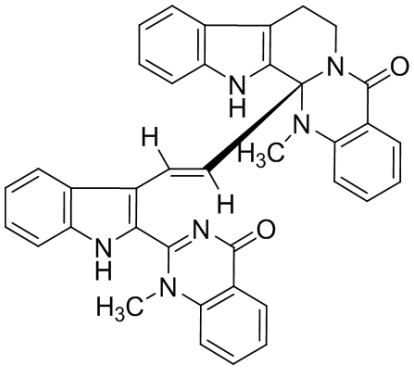
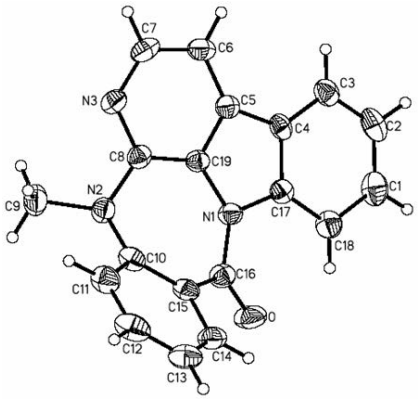
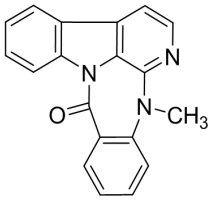
17 indole quinoline alkaloids, 8 limonins, 7 flavonoids, 7 triterpenoids and 11 other compounds were obtained from *E. rutaecarpa* and *E. rutaecarpa* passed with *Liquorice*. 6 of them are new compounds. About 19 compounds were tested by replanting tumor to mice, angioma of zebrafish model for antiangiogenic drug screening and human tumor cells in vitro. Thus compounds evodiagenine (**2**), evodialimonin (**3**), rhetsinine (**4**), dehydroevodiamine (**5**), wuchuyuamide I (**7**) and 1-methyl-2-undecyl-4(1 H)-quinolone (**8**) showed moderate activity against human tumor cells, dievodiamine (**1**) and evodiamine (**6**) showed good activity. The inhibitory rate of **4** on sarcoma S_{180} was 57.78% at the dose of 5 –

10 mg/kg·d in vivo. Moreover, the inhibition of the active selectivity of cycloxygenase **1** and **4** showed a stronger effect.







PI261 Effect of salicylic acid on eleutheroside accumulation in the adventitious root culture of *Eleutherococcus koreanum*

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Department of Forest Genetic Resources, Korea Forest Research Institute, Republic of Korea

Eleutherococcus koreanum is a useful medicinal plant that has been used to alleviate many ailments including diabetes, hypertension and neuralgia. This plant has known to contain various beneficial compounds including eleutherosides (B, E, and E_1), coumarins, and polyacetylenes. This study was carried out to investigate the dose-dependent effect of **salicylic acid** (SaA) on both the adventitious root growth and the accumulation of various eleutherosides in the bioreactor culture of *Eleutherococcus koreanum*. The highest biomass production (3.5 g DW/L) was observed in the absence of SaA. Although the root growth was significantly decreased by increasing the SaA concentration, SaA stimulated the production of both eleutheroside B and E. The highest level of eleutheroside B (14.2 μg/g DW) and E (141.8 μg/g DW) were obtained at 120 and 80 μM of SaA. Total eleutheroside was increased up to 374.7 μg per liter when SaA was not applied. In addition, when the adventitious roots were cultured with 80 μM of SaA, eleutheroside B was decreased by increasing the SaA concentration. The highest levels of eleutheroside E and E_1 were observed at the 9th day, respectively.

PI262 Two *ent*-labdane diterpenoids from *Andrographis paniculata*

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Plant species are the principle ingredients of traditional medicine and their use dates back to the beginning of human civilization. In recent years an extensive screening of ethno-medicinal plants is preformed in order to develop new and effective drugs for human illnesses. The plant *Andrographis paniculata* Nees, collected from Indian Himalaya, is a known Indian medicinal plant used in multiple clinical applications. Infusion of fresh leaves is used to relieve abdominal pain, irregular bowel syndromes and loss of appetite. Leaves and roots are used as treatment of infectious diseases due to their antibacterial, antifungal and antiviral properties and creams are prepared from leaves for the treatment of skin eruptions, scabies, ulcers and wounds. The crude extract of *A. paniculata* was found to inhibit the bacterial growth of Methicillin sensitive *Stphylococcs aureus* (MSSA) and Methicillin resistant *Stphylococcs aureu* (MRSA) with respect to the commercial antibiotic Oxacillin. While searching for the antibacterial principals we isolated two new *ent*-labdane ditrepenoids. Here we report the isolation and structure elucidation of the two new *ent*-labdane ditrepenoids: 21-methoxy-3,19-dihydroxy-8(17),11,13-*ent*-labdatrine-16-oic acid and symmetric bis 16,19-(15-O-β-glucose-3-hydroxy-19-oxy-8(17),13-*ent*-labdadiene-16-oate) dilactone along with 12 known *ent*-labdane derivatives from the active crude extract. The structure, including absolute stereochemistry, of the pure compounds was determined by 1D and 2D NMR techniques, high resolution mass spectrometry measurements, and application of Mosher method. The structure elucidation of the two new compounds will be presented.

PI263 Identification of β-secretase inhibitors in various plant resources

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The number of patients of dementia is increasing and has been considered as the serious social problem. In the treatment of dementia, especially in Alzheimer's disease (AD), the acetylcholinesterase inhibitor has been utilized as a first medicine. The inhibitors increases the availability of acetylcholine in central synapse, however the inhibitors cannot reverse the progression nor cure completely. In these circumstances, we focused on the β-secretase inhibitors, which deeply related to the β-amyloid peptide which form senile plaques which said to destroy nerve cells in brain. Thus the longstanding consumption of β-secretase inhibitors in natural plant resources might block the pathogenesis and/or progression of AD. The high sensitive and accurate detection of β-secretase inhibitor has been developed and utilized to screen spices and crude drugs used in the traditional oriental medicines. Among them, then extracts of several spices showed inhibitory activities and their active principle were determined as terpenes and an amine. The strategy for the investigations and the activities of β-secretase inhibitors will be presented in detail.

PI264 Study of morphological and anatomycal structure of arctium leiospermum leaves and roots

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The two species of Arctium grow in Kazakhstan: Arctium leiospermum Juz. et Serg. (AL) and Arctium tomentosum Mill. (AT). Only the roots of AT are used in medicine. The objective is to study the leaves and roots of the AL as a medicinal plant material. A comparative study of the AL and AT leaves and roots showed the presence of distinctive characteristics:

- AL leaves: almost naked, the bottom is slightly hairy;
- AT leaves: green above, tomentose below;
- AL baskets: green, glabrous, rarely purple, sitting on a short, 1 – 3 cm long, peduncles, involucre leaves all end with a hook;
- AT baskets: hairy, sitting at a long peduncles, inner leaves, involucre with a small cusp planted, but no hook-curved.

Anatomical structure of AL leaves is also different from the structure of the AT leaves.

- AT leaves: epidermal cells are not elongated, less sinuous walls, much mechanical tissue.
- AL leaves: epidermal cells are elongated, with very sinuous walls, a little mechanical tissue.

These is no significant difference in morphological and anatomical structure of AL and AT roots. Content of biologically active substances in AL roots is 5 – 10% higher than in AT roots.

PI265

New cromones from *Cnidium monnieri* fruits inhibit adipocyte differentiation in 3T3-L1 cells

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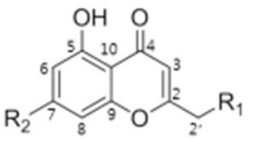
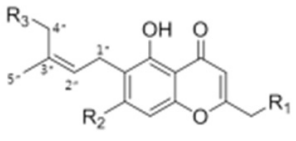
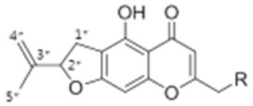
Seven new chromone glycosides, monnieriside A **(3)**, B **(10)**, C **(12)** D **(13)**, E **(15)**, F **(16)**, G **(17)** and one new phenolic compound, methylpicraquassioside B **(23)** were isolated from the *n*-BuOH soluble fraction of *Cnidium monnieri* fruits (Umbelliferae), together with fourteen known compounds, undulatoside A **(1)**, cnidimol C **(2)**, saikochromoside A **(4)**, cnidimoside A **(5)**, cnidimoside B **(6)**, 2-methyl-5-hydroxy-6-(2-butenyl-3-hydroxymethyl)-7-(β-D-glucopyranosyloxy)-4 H-1-benzopyran-4-one **(7)**, cnidimol D **(8)**, hydroxycnidimoside A **(9)**, umtatin **(11)**, 6′-hydroxylangelicain **(14)**, cnidioside A **(18)**, cnidioside B **(19)**, picraquassioside A **(20)**, methylpicraquassioside A **(21)** and picraquassioside B **(22)**. The structures of isolated compounds were determined on the basis of spectroscopic analysis including 1D, 2D NMR and HRESI-MS. Among the compounds isolated, compounds **5, 6, 9** and **10** significantly inhibited adipocyte differentiation as measured by fat accumulation in 3T3-L1 cells using Oil Red O staining.



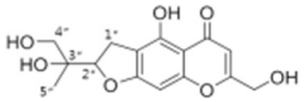
	R_1	R_2
1	H	OGlc
2	OH	OH
3	OGlc	OH
4	OGlc	OCH_3



	R_1	R_2	R_3
5	H	OH	OGlc
6	H	OCH_3	OGlc
7	H	OGlc	OH
8	OH	OH	OH
9	OH	OH	OGlc
10	OH	OGlc	OH



11 R=OH
12 R=OGlc



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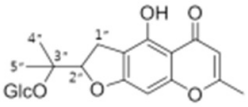

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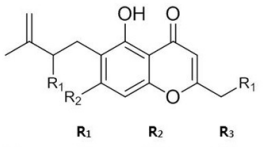
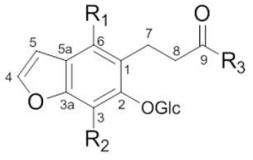


	R_1	R_2	R_3
15	H	OH	OGlc
16	OH	OH	OGlc
17	OH	OGlc	OH



	R_1	R_2	R_3
18	H	H	OH
19	H	OCH_3	OH
20	OCH_3	H	OH
21	OCH_3	H	OCH_3
22	OCH_3	OCH_3	OH
23	OCH_3	OCH_3	OCH_3

PI266

Mauritanicain – a new serine protease from the latex of Euphorbia mauritanica L

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A new protease called Mauritanicain was isolated from the latex of *Euphorbia mauritanica* L. (Euphorbiaceae), with a high proteolytic activity against casein. The activity was only inhibited by specific serine protease inhibitors, classifying it to the serine protease family. It is stable at temperatures from 20 – 90 °C with an optimum in activity at 55 – 60 °C and pH 6.5 – 7.5. The protease with a molecular weight of about 95 kDa shows a preference to cleave its substrates (exemplarily shown for β-lactoglobulin) behind the amino acids lysine (K), leucine (L) and alanine (A).

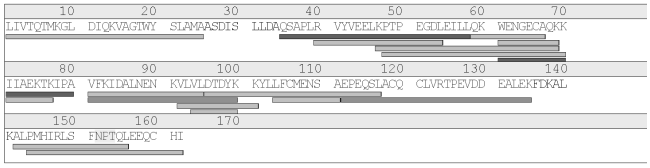


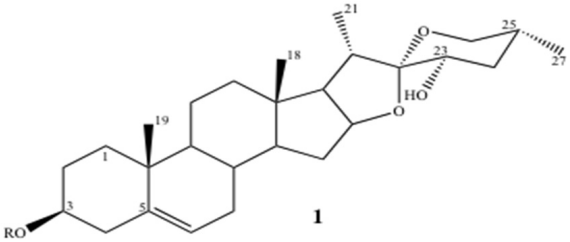
Fig. 1: Sequence of β-lactoglobuline (horse heart) and the theoretic cropped pieces after a digestion after L, K, A; sequence coverage: 90.7 %

PI267

Phytochemical and biological studies of two synonymy species of *Solanum*: *S. incanum* and *S. heteracanthum*

Jaovita Manase M[1], Mitaine-Offer AC[1], Pertuit D[1], Miyamoto T[2], Tanaka C[2], Delemasure S[3], Dutartre P[3], Mirjolet JF[4], Duchamp O[4], Lacaille-Dubois MA[1]
[1]EA 4267 (FDE/UFC), UFR de Pharmacie, Université de Bourgogne, Dijon, France; [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan; [3]Cohiro, UFR de Médecine, Dijon, France; [4]Oncodesign, Dijon, France

A new spirostanol saponin (**1**), with four known saponins, dioscin (**2**), protodioscin (**3**), methylprotodioscin (**4**), indioside D (**5**) and one known glycoalkaloid solamargine (**6**) were isolated from the two synonymous species, *Solanum incanum* and *S. heteracanthum*. **1** was established as (23S,25R)-spirost-5-en-3β,23-diol 3-O-{β-D-xylopyranosyl-(1→2)-O-α-L-rhamnopyranosyl-(1→4)-[O-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside}, by using a combination of 1D and 2D NMR techniques including ^1H, ^{13}C, COSY, TOCSY, NOESY, HSQC and HMBC experiments and by mass spectrometry. **1**, **3**, **4** and **5** were evaluated for cytotoxicity and antioxidant activities against six cancer cell lines (HCT 116, HT-29, SW480, DU145, EMT6 and THP-1).



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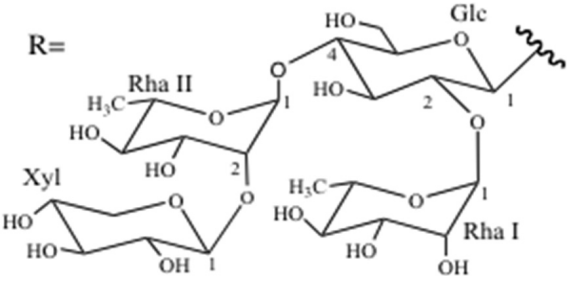

PI268 Chemical compostion and biological activities of *Oenothera biennis* L. and *Oenothera paradoxa* Hudziok aerial parts' extracts

Granica S, Czerwińska ME, Piwowarski JP, Kiss AK
Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Banacha 1, 02 – 097 Warsaw, Poland

Oenothera biennis L. and *Oenothera paradoxa* Hudziok, commonly known as evening primrose, are two perennial herbs cultivated in Europe and North America as a source of seeds used for the production of oil rich in unsaturated fatty acids. There are no studies concerning the polyphenol composition of *O.*paradoxa herb, and those concerning aerial parts of *O. biennis* are limited. The aqueous-methanol extracts were prepared and analyzed by HPLC-MS^3 and then anti-oxidative properties of extracts towards different reactive oxygen species were checked using both cellular and non-cellular models. Anti-hialuronidase activity was also evaluated. The HPLC analyses allowed for identification of 40 compounds in both extracts belonging to different classes of polyphenols, including tannins, phenolic acids and flavonoids. Both extracts (examined at a concentration between 2 and 50 μg/mL) performed scavenging properties towards reactive oxygen species, especially against H_2O_2 (SC_{50} = 2.7 and 2.8 μg/mL). Anti-hialuronidase assays have shown that extracts of evening primrose exhibit inhibitory activity on the pro-inflamatory enzyme (IC_{50} ~ 20 μg/mL). The study conducted showed that both plant materials may be considered as a source of extracts rich in polyphenols, exhibiting reactive oxygen species scavenging activity and anti-inflammatory activity.

PI269 Extracts from *Epilobium* Sp. herbs inhibit proliferation, PSA secretion and induce apoptosis in prostate cancer cells (LNCaP)

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Extracts from *Epilobium* sp. herbs (*Onagraceae*) have traditionally been used in the treatment of prostate diseases. The aim of the study was to investigate the effect of standardized aqueous extracts from *Epilobium angustifolium* L., *Epilobium parviflorum* Schreb. and *Epilobium hirsutum* L. herbs on hormone-dependent prostate cancer cells (LNCaP). The extracts were characterized by the HPLC-DAD-MS/MS method. LNCaP cells were incubated with increasing concentration of the extracts (20 – 70 μg/ml). Cell proliferation was measured using bisbenzimide (Hoechst 33258). Apoptosis was detected using Annexin V-FITC. PSA secretion and caspase-3 activity were quantified by ELISA assays. Extracts from *Epilobium* sp. herbs contained significant amounts of oenothein B as well as flavonoids (derivatives of quercetin, kaempferol, myricetin) and phenolic acids. Exposure of LNCaP cells to the extracts resulted in a significant decrease of cell proliferation (IC_{50}~35 μg/mL) and increase of cells apoptosis (from the level 26,9 ± 3,3% to 80%). The tested extracts reduced PSA secretion from the level of 19.9 ± 0.9 to 4.3 ± 1.3 ng/mL. The extracts increased the activity of caspase-3 from the level of 0.3 ± 0.07 to 1.26 ± 0.32 ng/mg of protein. Extracts from *Epilobium* sp. herbs inhibit LNCaP cells proliferation, PSA secretion and induce apoptosis via caspase-dependent pathway. Our results partly confirm the use of *Epilobium* sp. herbs in the treatment of prostate diseases.

PI270 Alkaloids from Choisya ternata and their human antiplatelet activity

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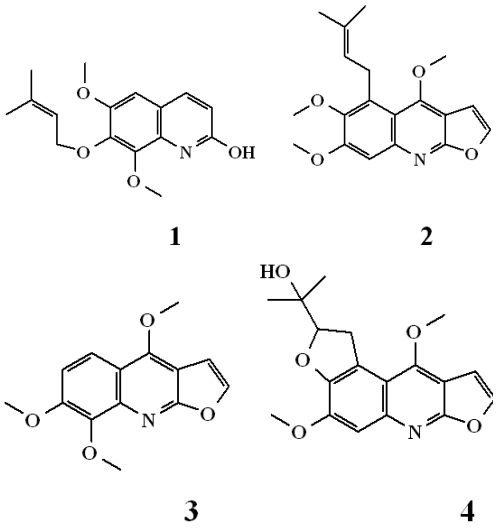
One novel alkaloid was isolated from the hexane extract of *Choisya ternata* Kunth. (Rutaceae) leaves, choisyaternatine (1), together with the known alkaloids tecleamaniensine A (2) and skimmianine (3). From the dichloromethane extract, skimmianine (3) and choisyine (4) were also isolated. Tecleamaniensine A is reported for the first time in this species. The structures of isolated compounds were elucidated by extensive spectroscopic analyses and comparison with previously reported spectroscopic data. The structure of choisyaternatine was further confirmed based on X-ray data analysis. Anti-platelet activity of hexane extract, choisyaternatine and skimmianine was also evaluated.



PI271 A comparison of the effects of oleuropein and its derivative from olive oil, oleacein, on functions of human neutrophils

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Polyphenols extracted from extra virgin olive oil are believed to play a vital role in the prevention of cardiovascular diseases. However the precise mechanisms by which they exert this effect have not been completely defined. Taking into account that neutrophils are suggested to be involved in the mechanism of vascular and heart diseases [1], we have analyzed the effect of oleuropein and its derivative from olive oil oleacein (3,4-DHPEA-EDA) in a concentration range 10 – 100 μM on neutral endopeptidase (NEP) activity, elastase release, MMP-9 and IL-8 production in stimulated neutrophils and L-selectin expression in unstimulated neutrophils. Oleacein was the inhibitor of NEP activity (IC_{50}= 43.2 ± 4.0 μM) and L-selectin expression (IC_{50}= 7.3 ± 1.8 μM) in contrast to oleuropein, which was not active. The inhibition of MMP-9 and IL-8 production and elastase release was comparable for both compounds (15 – 35%). Oleacein, as the constituent of extra virgin olive oil, inhibits NEP activity and L-selectin expression more strongly than oleuropein and modulates other functions of neutrophils, which may explain the role of olive oil in the prevention of cardiovascular diseases, in particular those associated with inflammation. References: 1. Ernst, E, et al. (1987) JAMA 257: 2318 – 2324.

PI272 Antibacterial and antiprotozoal effect of *Artemisia annua* extracts

Ivarsen E[1], Fretté XC[1], Engberg RM[2], Thøfner ICN[3], Christensen JP[3], Grevsen K[4], Schou TW[5], Liebhart D[6], Hess M[6], Christensen LP[1]
[1]Instute of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark; [2]Department of Animal Science, Aarhus University; [3]Department of Veterinary Disease Biology, University of Copenhagen; [4]Department of Food Science, Aarhus University; [5]DHI, Copenhagen; [6]Clinic for Avian, Reptile and Fish Medicine, University of Veterinary medicine, Vienna, Austria

Two of the most common infections in poultry, are blackhead, caused by the parasite Histomonas melagridis (HM), and necrotic enteristis (NE) caused by the bacteria Clostridium perfringens (CP). At present there is no treatment of blackhead disease, and the preventive treatment towards NE may soon be banned in the EU. Extracts of aerial parts of Artemisia annua (AA) showed antimicrobial activity in overnight cul-

tures of CP strains isolated from diseased broilers. The hexane extract (HEX) gave the strongest inhibition (MIC = 185ppm) while the dichloromethane extract (DCM) gave a weaker inhibition (MIC = 270ppm). The dietary incorporation of HEX reduced the population of CP and the severity of the associated small intestinal lesions ($P > 0.05$) in broilers when applying a NE disease model. The antibacterial compounds from HEX and DCM, chrysosplenol and ponticaepoxide, were isolated. This is the first report of activity against CP for these compounds. HEX, DCM and artemisinin were also tested against HM. The two latter showed highest antiprotozoal effect in vitro (MLC = 1.0 mg/ml and IC_{50} = 1.3 mg/ml respectively), and were tested in vivo in infected poultry. However, no effect against HM at the given concentrations was observed.

PI273 Oleacein from olive oil inhibits myeloperoxidase and metalloproteinase-9 production by atherosclerotic plaque

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Recently we have shown that oleacein (secoiridoid) inhibits myeloperoxidase (MPO) release by human neutrophils, which may explain the protective effect of extra virgin olive oil against cardiovascular diseases [*Food Chemistry* 2012, 131: 940 – 947]. MPO is also localized in human atherosclerotic lesions, where is responsible for inflammatory reactions and destabilization of atherosclerotic plaque. The aim of the study was to establish the direct effect of oleacein on MPO and metalloproteinase-9 production by human carotid plaque. Carotid plaques (n = 10) were collected immediately after the endarterectomy of patients with transient ischemic attacks. Matching pieces of each plaque were incubated in PBS or in PBS with oleacein in the concentration of 10 µM by 24 h in the presence of LPS (1 µg/ml). The effect of oleacein on MPO and metalloproteinase-9 production was measured by ELISA assays. Oleacein inhibited MPO and metalloproteinase-9 production by 25% and 42% respectively. Oleacein, in a concentration that could be reached in blood after intake of 50 g of olive oil per day, could protect atherosclerotic plaque against rapture by inhibiting MPO and metalloproteinase-9 production. This suggests the role of oleacein rich olive oil in the prevention of myocardial infarction.

PI274 Phenolic profile and biological activity of *Plantago bellardii* all

Beara I[1], Lesjak M[1], Četojević-Simin D[2], Simin N[1], Balog K[1], Francišković M[1], Mimica-Dukić N[1]
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Ancient use of plantains (genus *Plantago* L., Plantaginaceae) as herbal remedies is a consequence of their broad range of curative properties [1]. *Plantago bellardii* All. is distributed in south Europe, but there are no detailed data about biological activity of this species. In order to valorize medicinal use of *P. bellardii,* some tests on antioxidative, anti-inflammatory and cytotoxic activities of methanolic extract of this plantain, collected from area of Ulcinj (Montenegro) have been undertaken. The phenolic composition was determined by LC-MS/MS, where the content of vanillic (2.0 mg/g of dw) and chlorogenic (38.2 mg/g of dw) acid, with quercetin-3-*O*-glc (2.5 mg/g of dw) and rutin (51.6 mg/g of dw) was the highest. The radical scavenger capacity (RSC) was evaluated towards several radicals [1], indicating similar activity to synthetic antioxidant BHT (butylated hydroxytoluene). Anti-inflammatory potential was examined by means of cyclooxygenase-1 (COX-1) and 12-lipooxygenase (12-LOX) inhibition [1], quantifying 12-HHT (12-hydroxy-5,8,10-heptadecatrienoic acid) and 12-HETE (12-hydroxy-5,8,10,14-eicosatetraenoate) products by RP-HPLC-MS/MS (IC_{50} = 6.6 and 2.34 mg/mL, respectively). The extract showed moderate cytotoxic activity against HeLa (cervix epitheloid carcinoma), MCF7 (breast adenocarcinoma), HT-29 (colon adenocarinoma) and MRC-5 (human fetal lung) cell lines, while results were obtained using SRB (Sulforhodamine B) assay [1]. Consequently, this species could be considered as a promising herbal remedy.
[1] Beara I. et al. (2012) *LWT-Food Sci. Technol.* 47: 64 – 70.

PI275 Four diarylheptanoids from *Hedychium coronarium* rhizomes

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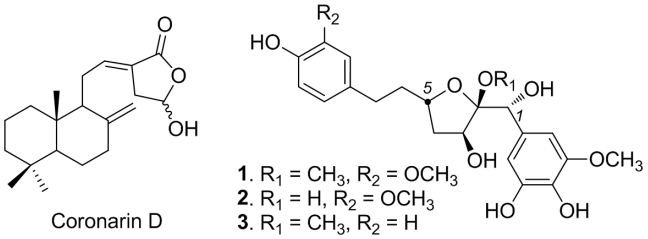
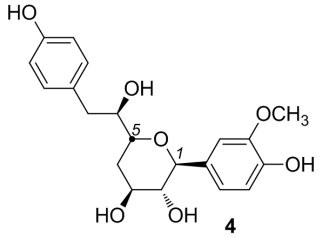
Several cytotoxic compounds have been identified from the rhizome of *Hedychium coronarium* (Zingiberaceae), a plant widely distributed in subtropic and tropic areas. To supply some of these active ingredients for chemical modification, its constituents were reinvestigated, leading to the isolation of four new but very minor diarylheptanoids (**1**-**4**) together with the major coronarin D. Their structures were elucidated based on spectroscopic analyses.





PI276 Screening of plant extracts for potential effects on the metabolic syndrome

El-Houri RB[1], Christensen KB[1], Kotowska DE[2], Olsen LCB[3], Bhattacharya S[4], Fretté XC[1], Grevsen K[4], Færgeman N[3], Oksbjerg N[4], Kristiansen K[2], Christensen LP[1]
[1]Institute of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Denmark; [2]Department of Biology, University of Copenhagen, Denmark; [3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark; [4]Department of Food Science, Aarhus University, Denmark

The metabolic syndrome (MS) is a cluster of risk factors for cardiovascular diseases and the major underlying factors are obesity and insulin resistance (IR). The massive worldwide rise in incidences of obesity and IR has initiated a search for health-promoting plant-derived compounds with potential effects on these. Nine different plant species were tested in a series of bioassays including PPARγ transactivation, adipocyte differentiation, glucose uptake in fat and muscle cells, as well as fat accumulation in the nematode *C. elegans*. Eight of these were shown to contain compounds with potential bioactivities in one or several of the bioassays. Tested at 100 µg/mL the extract of golden root (*Rhodiola rosea*) showed promising activity in all bioassays. Extracts of golden root contain flavonoids and phenylethanol derivatives (e.g., salidroside) that may be responsible for the observed activity. The dichloromethane extract of carrots (*Daucus carota*) also exhibited promising bio-activities and a bioassay-guided investigation identified the polyacetylene falcarinol as one of the active components. Further investigations of the bioactive principles in all the extracts are ongoing to reveal their potential application within treatment and/or prevention of MS-related disorders.

PI277 New A-type Trimeric and tetrameric procyanidins from Peanuts skins

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Peanut skins constitute up to 4% w/w of peanuts and are a unique source of A-type procyanidins. Methanol extract of the skins was purified by Centrifugal Partition Chromatography (FCPC Kromaton) and RP HPLC to afford pure procyanidins. They contain both epicatechin (EC) and catechin (C) subunits, which are joined through 4β-8 or 4α-6 bonds. The extract contains dimers A_1 and A_2 and certain tetramers > monomers > trimers. Two new tetrameric procyanidins containing two A-type bonds and one trimer, in addition to two known trimers, were isolated. The structures were established to be EC-(4β-8', 2β-O-7')-EC-(4α'-6")-EC-(4β"-8"', 2β"-O-7"')-EC (**1**), EC-(4β-8', 2β-O-7')-EC-(4α'-6")-EC-(4β"-8"', 2β"-O-7"')-C (**2**), EC-(4β-6', 2β-O-7')-EC-(4β'-8", 2β'-O-7")-C (**3**), EC-(4β-8')-EC-(4β'-8", 2β'-O-7")-C (**4**), EC-(4β-6')-EC-(4β'-8", 2β'-O-7")-C (**5**) by means of NMR and MS. Compounds (**4**) and (**5**) were previously isolated from *Vaccinium vitis-idaea*. Their structures differ only in the location of a linkage between upper and middle units. At 275K both **4** and **5** exist as two clearly distinguishable by NMR rotamers. The NOESY spectra at 275K with different mixing time were recorded, showing the NOESY correlations within one of the rotamers, when mixing time did not exceed 100 ms and correlations between different rotamers with mixing time of 500 ms.

PI278 Gniditrin is the main diterpene ester in the bark of daphne species

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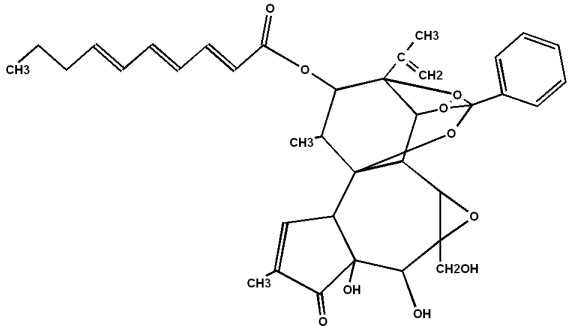
Daphne species are noted as poisonous members of the plant family Thymeleaceae. The plants are evergreen or deciduous shrubs distributed mainly in Europe, Asia, and North Africa. Daphne species are a rich source of diterpenes which are known to cause gastrointestinal diseases when consumed orally or rashes after handling of fresh twigs. The diterpene ester mezerein is often reported as a major toxic substance in various cited literature. In the reported work methanolic extracts of the bark of various *Daphne spp.* were tested for the presence and identification of gniditrin as the main diterpene ester in these aforementioned species. Gniditrin was isolated from the methanolic extract of *D. alpina* using two-step HPLC and the isolated fraction was characterized for structural features using ^{1}H-, ^{13}C-, H-H-cosy-, hmbc- and hmqc- NMR spectroscopy. This is the first report for a complete NMR spectroscopic evaluation of gniditrin.



PI279 Bioactive steroidal glycosides from *Solanum torvum*

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During the course of our anti-inflammatory activity screening of extracts of Formosan plants, the EtOAc- and *n*-BuOH-soluble fractions of *Solanum torvum* Swartz (Solanaceae) showed inhibition on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB at 10 μg/mL. Through bioassay-guided fractionation, fifteen steroidal glycosides (**1**-**15**), including three new ones (**1**-**3**) were isolated. Among fifteen metabolites, four 23-hydroxy-spirostanol glycosides (**7**, **9**-**11**) and one spirostanol saponin **14** were first found in this plant. All compounds and their biological data will be reported herein.

PI280 Antimicrobial and sanitizer activity of *Baccharis dracunculifolia* DC. (Asteraceae) extract

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Baccharis dracunculifolia (Asteraceae) is a woody shrub, native from the southeast of Brazil, widely used in folk medicine for the treatment of inflammatory and gastrointestinal diseases. To obtain the *B. dracunculifolia* extract (BDE) the plant was collected, dried, grounded and macerated using ethanol:water 96:4 (v/v). The extract was lyophilized and used to evaluate the antimicrobial and sanitizer activities. Therefore, the antimicrobial activity was performed by the minimum inhibitory concentration (MIC) assay, against microorganisms *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella choleraesuis*. Penicillin and streptomycin were used as positive controls. The other assay (sanitizer activity) was analyzed using a 40% alcohol solution containing 0.2% BDE. Thus, it was performed against the bacteria listed above using carriers cylinders contaminated with cultures of the same microorganisms. These cylinders were exposed to the test solution for 15 minutes and transferred to tubes containing nutrient medium. Results were positive when was not observed bacterial growth in the subculture tubes. Assays showed that the extract presented antimicrobial activity only against *S. aureus* (MIC ≤ 200 μg/mL). However, the 40% alcohol solution containing 0,2% BDE demonstrated the ability to eliminate all microorganisms tested and controls were carried out using only 40% alcohol solution. It was also observed that the antibacterial activity of BDE was pronounced when associated with 40% alcohol, which should be allowed the entry of BDE active constituents into bacterial cells, improving the antimicrobial activity. Assays using isolated substances from the BDE are being conducted to determine (s) which active (s) constituent (s) is responsible for the antimicrobial activity.

PI281 Three new cyclic diarylheptanoids from *Santalum album*

Lee JW[1], Lee C[1], Jang H[1], Lee IS[2], Jeon WK[2], Lee MK[1], Hwang BY[1]
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Santalum album L. is a tropical evergreen tree which is one of the most widespread members of the Santalaceae family in India, Malaysia, South China, and Australia. It is commonly known as sandalwood. The essential oil of sandalwood has been used as an aromatherapy as an antidepressant, sedative, antifungal, anti-inflammatory, insecticide, and lung antiseptic. Previous phytochemical studies on this plant have been reported that it contained sesquiterpenoids, phenylpropanoids, diarylheptanoids, and lignans. As part of our continuing search for the discovery of new bioactive constituents from medicinal plant, we investigated an ethylacetate-soluble fraction of the MeOH extract of *S. album*. Three new cyclic diarylheptanoids along with six known compounds were isolated and identified. Their structures were determined on the basis of results of spectroscopic analysis, including 1D- and 2D-NMR spectroscopic data.

Here, we report the isolation and structure elucidation of these three new cyclic diarylheptanoids.

PI282 *Glycyrrhiza uralensis* containing toothpaste: Characterization of the extract and reduction of oral malodor in a clinical study

Villinski JR[1], Bergeron C[1], Dobrovolny M[1], Stewart B[2], Williams M[2], Schneider C[3], Tanabe SI[4], Desjardins J[4], Grenier D[4], Gafner S[1]
[1]Tom's of Maine, Kennebunk, ME 04043; [2]Colgate-Palmolive Global Technology Center, Piscataway, NJ 08854; [3]Memorial University of Newfoundland, St. Johns, Newfoundland, Canada A1C 5S7

[4]Université Laval, Quebec City, QC, Canada The CO_2 extract of *Glycyrrhiza uralensis* roots was found to be a promising material to help reduce bad breath based on its *in-vitro* ability to reduce the production of malodorous volatile sulfur compounds (VSC's) at concentrations above 6.25 µg/mL. In addition to the isoflavones and coumarins isolated earlier from the *G. uralensis* extract,[1] we were able to identify four minor constituents as licoriphenone, 6,8-diprenylgenistein, gancaonin G and isoglycyrol. Based on the *in-vitro* data, we believe that the isoflavans licoricidin and licorisoflavan A are in part responsible for the bioactivity of the extract. Toothpaste containing the *G. uralensis* extract was submitted to a clinical study to determine the efficacy in humans. Malodor scores were determined by trained and calibrated judges and gas chromatography. Three hours after brushing for one minute with the test toothpaste, they were evaluated by the two methodologies. The subjects exhibited statistically significant ($p < 0.05$) reduction in organoleptic scores (31.7%), breath VSC (ppb) scores (48.4%) and breath VSC (ng/mL) scores (43.4%) as compared to baseline.

PI283 New antiproliferative trihydroxyalkylcyclohexenones from Pleiogynium timoriense

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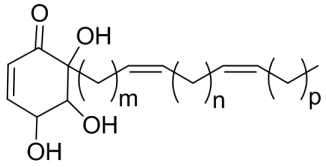
The former Merck collection of natural product extracts is now managed by the Natural Products Discovery Institute, and the authors have initiated a collaborative investigation of this collection as a potential source of new drug leads. In our initial investigation, a dichloromethane partition of the ethanol extract of the bark of *Pleiogynium timoriense* was selected for investigation because of its strong antiproliferative activity against the A2780 human ovarian cancer cell line (IC_{50} 1.3 µg/mL). The three new bioactive trihydroxyalkylcyclohexenones **1 – 3** have been isolated by bioassay-directed fractionation using solid phase extraction and HPLC methods. The structures were determined using spectroscopic and chemical methods. All three compounds exhibited antiproliferative activity against the A2780 human ovarian cancer cell line with IC_{50} values of 0.2, 1.9 and 0.5 µg/mL, respectively. The absolute configurations of the isolated compounds were determined using spectroscopic evidence and chemical modification.



1 m+n+p=13
2 m+n+p=15
3 m+n+p=17

PI284 Total synthesis of (+)-scholarasine A

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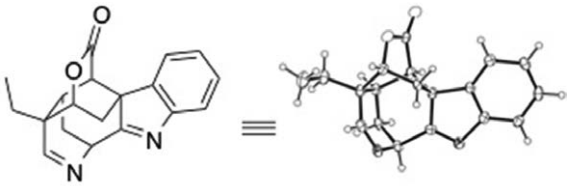
The total synthesis and assignment of the absolute configuration of (+)-scholarisine A, a monoterpenoid indole alkaloid containing an unprecedented cage-like structure, has been achieved via a 20-step sequence. Highlights of the synthesis include a reductive cyclization, involving hydrogenation of a nitrile and opening of an epoxide by the resulting amine; a modified Fischer indole protocol utilizing hydrazine protection; a late stage two-step oxidative-lactonization; and an intramolecular cyclization to furnish the indolenine ring system of (+)-scholarisine A.



(+)-Scholarisine A

PI285 Triterpene glycosides from *Planchonella obovata* leaves

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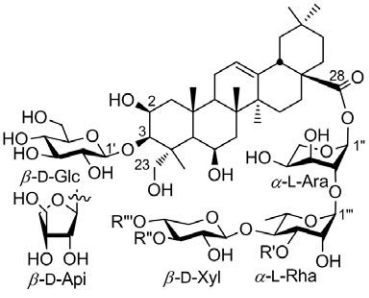
School of Pharmacy, College of Medicine, National Taiwan University, 1, Jen-Ai Rd., Sec. 1, Taipei 10051, Taiwan, Republic of China

Continual chemical investigation of the BuOH-soluble fraction of the leaves of *Planchonella obovata* (R. Br.) Pierre (Sapotaceae) led to the isolation of eight triterpene glycosides as well as nine flavonoids. Their structures were elucidated based on spectroscopic analyses, in particular using 1D TOCSY to identify the various sugar moieties. The configuration of each monosaccharide in the glycon part was determined by GC method. Among these, compounds **3-5** are new natural products. 3'''-*O*-β-D-Apiofuranosylarganin F (**4**) and Mi-saponin A (**6**) showed moderate cytotoxic activity against HL-60 leukemia cell line with the IC_{50} values of 16.88 and 15.50 µM, respectively.



	R'	R''	R'''
1	H	Rha	Glc
2	Api	Rha	Rha
3	Api	H	H
4	Api	Api	H
5	H	H	H
6	H	Rha	H
7	H	Api	H

PI286 Secondary metabolites from the stem bark of *Strychnos aff. dariensis*

Travasarou A, Vougogiannopoulou K, Fokialakis N, Skaltsounis AL
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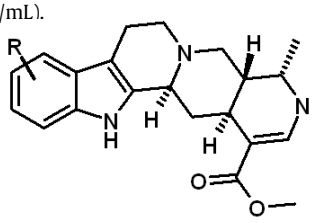
The genus *Strychnos* (Loganiaceae) is well-known as a rich source of various bioactive indole alkaloids. In continuation of our phytochemical studies on plants from Amazonia [1], we examined *Strychnos aff. dariensis*, collected in Peru. This species has been traditionally used in South America [2] as an arrow poison and is applied till nowadays as a drug from Yanesha tribe in Peru [3]. Extraction with EtOAc and MeOH and then treatment in different pH values, resulted to a first fractionation of the plant material. Further phytochemical investigation of this plant led to the isolation and structure elucidation by NMR and HRMS of 14 compounds that belong to the categories of phenolic acids (*p*-hydroxybenzoic acid and vanillic acid), flavonoids (luteolin, 3',4',7-trihydroxyflavone, 3-methoxy quercetin, strychnobiflavone and minaxin), lignans (syringaresinol-β-D-glucoside, balanophonin and ficusal) and alkaloids (venoterpine, condensamine, diaboline and 11-methoxy diaboline). The presence of the indole alkaloid 11-methoxy-diaboline as a major constituent was in accordance with the literature [4] that reports that in species of *Strychnos* with a relatively low percentage of alkaloids it constitutes the main component. References: 1. Vougogiannopoulou et al. (2010) Org. Letters *12*:1908 – 1911, 2. Philippe, G. et al (2004) Toxicon 44:405 – 416, 3. Valadeau, C. et al. (2010) J Ethnopharmacol 127:175 – 192, 4. Ohiri, FC. et al (1983) J Ethnopharmacol 9:167 – 223.

PI287 Indole alkaloids from *Duroia macrophylla* (Rubiaceae)

Nunez CV[1], Roumy V[2], Mesquita DWO[1,3], Mesquita ASS[1,3], Sahpaz S[2], Bailleul F[2], Hennebelle T[2]
[1]Laboratório de Bioprospecção, Coordenação de Pesquisas em Produtos Naturais, Instituto Nacional de Pesquisas da Amazônia, Aleixo, Manaus, Amazonas, 69060 – 001, Brazil; [2]Laboratoire de Pharmacognosie, EA 4481, Université de Lille, 59006 Lille, France; [3]Departamento de Engenharia de Produção Agroindustrial, Campus de Cacoal, Universidade Federal de Rondônia, RO, Brazil

Duroia macrophylla Huber (Rubiaceae) is a tropical tree, known as "puruí", which occurs in the Amazon region. The study of the methnolic extract led to the isolation of the indolic alkaloids 9-methoxyajmalicine and 10-methoxyajmalicine (cabucine) which were identified by NMR and MS data analyses. The methanolic extract was toxic against *Artemia salina* (IC_{50}= 40 µg/mL).



PI288 Flavonoids from *Biophytum petersianum*, a Malian medicinal plant

Nguyen C[1], Pham AT[1], Malterud KE[1], Diallo D[2], Wangensteen H[1]
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We have previously reported that the tropical herb *Biophytum petersianum* Klotzsch. is used in Mali against cerebral malaria, pain, and as a wound healing agent. 6-[6-Deoxy-α-L-mannopyranosyl)-β-L-ribo-hexopyranos-3-ulos-1-yl]-apigenin (cassiaoccidentalin A) was isolated from the plant. This is a very rare flavonoid, having been reported once before in nature. A second flavonoid has been assigned the structure apigenin 6-(2-propenoic acid). This is a new natural product. Protocatechuic acid (3,4-dihydroxybenzoic acid) was also isolated and identified. This is a fairly common compound in nature. The methanolic extract of the plant was a good inhibitor of xanthine oxidase (IC_{50} 26 ± 6 µg/ml), an enzyme that is important for peroxidative processes by catalyzing the formation of superoxide radical anion. Most of the inhibitory activity was found in the ethyl acetate fraction. The crude extract was only moderately active as a scavenger of the DPPH radical or as inhibitor of 15-lipoxygenase. Protocatechuic acid was inactive as XO scavenger and 15-LO inhibitor, but scavenged DPPH radical (IC_{50} 71 ± 13 µM); cassiaoccidentalin A was a poor radical scavenger and showed moderate activity as XO inhibitor (IC_{50} 75 ± 6 µM) and 15-LO inhibitor (IC_{50} 112 ± 9 µM). It appears that other, so far unidentified XO inhibitors are present in the ethyl acetate extract. Apigenin 6-(2-propenoic acid) was not present in sufficient amount for activity assays.

PI289 Synthesis of ellagic acid peracetate and antitumor efficacy with enhancement of immunity

Ren Y[1], Wei M[2], Still PC[1], Chen X[3,4,5], Himmeldirk K[3], Kinghorn AD[1,6], Yu J[6,7]
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A synthetic method was developed for the total synthesis of ellagic acid (EA) and ellagic acid peracetate (EAPA). A subcutaneous B16 melanoma tumor model of C57BL/6 immunocompetent mice was used to evaluate the antitumor efficacy of the two chemicals. After the treatment of EA and EAPA for three weeks, using a dose of 0.5 mg/kg per mouse, tumors were removed, weighed, photographed, and the average tumor size was calculated and compared. The expression of CD 107a and the production of IFN-γ in natural killer cells and the levels of white blood cells and other immune cells were determined, with the weights of bodies, livers, and spleens of normal mice also being evaluated. The results showed that administration of EAPA significantly suppressed B16 melanoma growth in mice without affecting natural killer cell activity and was more effective than EA. EAPA increased white blood cell quantity in several organs or tissues including peripheral blood, bone marrow, and liver, and such effects were greater than those of EA. Furthermore, neither compound showed toxicity to mice. This study suggests that EAPA may be investigated further as a new immunity-stimulatory anticancer drug candidate with potential low toxicity for cancer treatment (Partial support is from grant P01 CA125066 from the National Cancer Institute, NIH, Bethesda, MD, MetaCor Pharmaceuticals Inc., and the Edison Program of the State of Ohio).

PI290 Antileishmanial natural prenylated anthranoids

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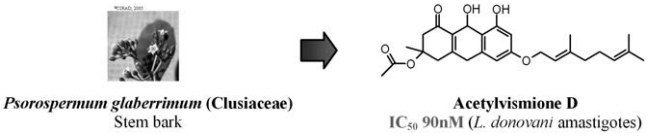
Chemotherapy is the main tool for the control of leishmaniases, a family of diseases including a variety of clinical manifestations classically labeled as visceral, cutaneous, and mucocutaneous leishmaniases. However, the different forms of leishmaniases require expensive treatments, and currently used medicines show toxicity together with numerous side effects. In an effort to discover new lead compounds against leishmaniasis, we screened plant extracts of the Clusiaceae family that are traditionally used for the treatment of parasitic diseases in Cameroon. Prenylated anthranoids have been isolated by bioassay-guided fractionation from *Psorospermum glaberrimum*. Acetylvismione D showed particularly both strong *in vitro* leishmanicidal activity against *L. donovani,* well above that of the reference drug, miltefosine (Miltex®) (IC_{50} = 90 nM and 0.46 µM, respectively) and low *in vitro* cytotoxicity against mammalian cells (L6).



***Psorospermum glaberrimum* (Clusiaceae)**
Stem bark

Acetylvismione D
IC_{50} 90nM (*L. donovani* amastigotes)

PI291 Cornoside A, a new monoterpenoid glucosid with an unusual skeleton from *Cornus controvers*

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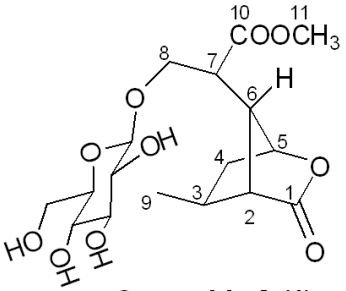
A novel monoterpenoid glucoside with an unprecedented 2-oxabicyclo-[2.2.1]-heptan-3-one rearranged ring system was isolated from *Cornus controversa* and named cornoside A (**1**). The structure and relative stereochemistry was elucidated on the basis of extensive spectroscopic analysis. Cornoside A represents an unprecedented rearrangement iridoid. In addition, the novel compound exhibited potent LXR agonistic activity and moderate PPARγ agonistic activity.



Cornoside A (1)

PI292

Cytotoxicity, NF-κB P65 inhibition, and *in vivo* antitumor efficacy of sesquiterpene lactones from *Piptocoma rufescens*

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Several new and known sesquiterpene lactones (SQLs) were isolated from *Piptocoma rufescens* Cass. (Asteraceae) using column chromatography guided by cytotoxicity to HT-29 cells. The structures of the SQLs were established from translation of their IR, UV, NMR, and mass spectra, and the absolute configurations were determined by analysis of a combination of single-crystal X-ray diffraction, Mosher ester reactions, specific rotation values, NOESY NMR data, and CD spectra. All SQLs were screened in terms of their cytotoxicity against HT-29 cells, and some were tested in a NF-κB p65 inhibition assay. The antitumor potential of three highly cytotoxic SQLs, goyazensolide, 15-deoxygoyazensolide, and ereglomerulide was evaluated in an *in vivo* hollow fiber assay. The results showed that all the SQLs isolated were highly cytotoxic toward HT-29 cells, with 15-deoxygoyazensolide (IC_{50}, 0.26 μM) being the most potently active compound. Several SQLs exhibited NF-κB p65 inhibitory activity. Goyazensolide showed significant *in vivo* antitumor potency, when tested at a dose of 12.5 mg/kg (i.p.) in mice, but neither 15-deoxygoyazensolide nor ereglomerulide was active in this *in vivo* assay system, when evaluated up to a dose of 25.0 mg/kg (ip) in mice. (Support from grants U01 CA52956 and P01 CA125066 from the National Cancer Institute, NIH, Bethesda, MD, is acknowledged).

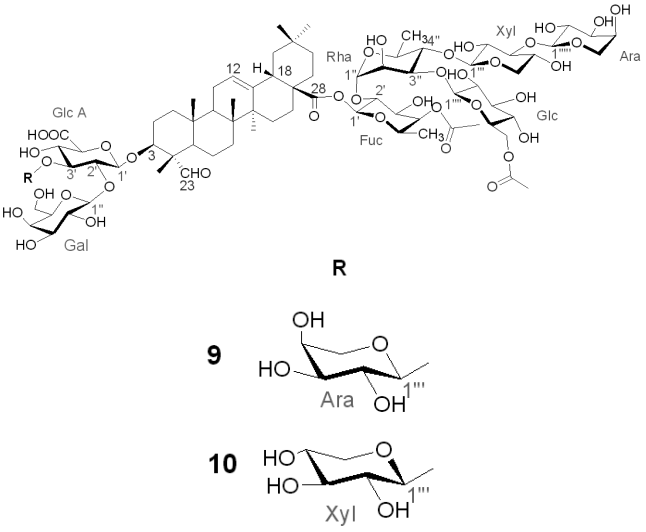
PI293

Acylated triterpenoid saponins from roots of *Gypsophila trichotoma*

Voutquenne-Nazabadioko L[1], Gevrenova R[2], Borie N[1], Harakat D[1], Weng A[3], Thakur M[3], Henry M[4]

[1]Institut of Molecular Chemistry of Reims, UMR CNRS 7312, Reims Champagne-Ardennes University, Reims cedex 2, France; [2]Department of Pharmacognosy, University of Medicine, 2 Dunav street, 1000 Sofia, Bulgaria; [3]Institut für Laboratoriumsmedizin und Pathobiochemie, Charité – University of Medicine, Berlin, Germany; [4]Department of Botanic and Mycology, Henri Poincaré Nancy 1 University, 5 rue Albert Lebrun, Nancy Cedex, France

Eleven new triterpenoid saponins were isolated from the roots of *Gypsophila trichotoma* Wend. (Caryophyllaceae) together with one known compounds. The structures were established on the basis of extensive 1D and 2D NMR analysis, completed by analysis of HR-ESI-MS and ESI-MS^n. The cytotoxicity of the saponin extract from *G. trichotoma* was evaluated on a rat alveolar macrophage cell line NR8383 and a human macrophage cell line U937. The synergistic effect of the aminoacyl saponins, previously isolated from *G. trichotoma*, was tested for its ability to enhance the cytotoxicity of the targeted toxin in HER14 cells.



PI294

Solanum torvum, a source of new steroidal saponins

Pérez Colmenares A[1,2], Rojas LB[2], Mitaine-Offer AC[1], Pouységu L[3], Quideau S[3], Paululat T[4], Usubillaga A[2], Lacaille-Dubois MA[1]

[1]EA 4267 (FDE/UFC), Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université de Bourgogne, 7 Bd Jeanne d'Arc, 21079 Dijon Cedex, France; [2]Research Institute, Faculty of Pharmacy and Bioanalysis, University of Los Andes, 5101, Mérida, Venezuela; [3]Institut des Sciences Moléculaires, CNRS-UMR 5255 & Institut Européen de Chimie et Biologie, Université de Bordeaux, 2 rue Robert Escarpit, 33607 Pessac Cedex, France; [4]Universität Siegen, OC-II Naturwissenschaftlich-Technische Fakultät, Adolf-Reichwein-Str. 2, D-57076 Siegen, Germany

Seven new steroidal glycosides have been isolated from the leaves of *Solanum torvum* Swartz, a species growing in Merida, Venezuela. Their structures were established by 2D-NMR spectroscopic techniques (^1H,^1H-COSY, TOCSY, NOESY, HSQC, and HMBC) and mass spectrometry as (25*S*)-5α-furost-20(22)-ene-3-one-6α,26-diol 6-*O*-β-D-xylopyranosyl 26-*O*-β-D-glucopyranoside (**1**), (25*S*)-22-methoxy-5α-furost-20(22)-ene-3-one-6α,26-diol 6-*O*-β-D-xylopyranosyl 26-*O*-β-D-glucopyranoside (**2**), (25*R*)-22-methoxy-5α-furostane-3β,6α,26-triol 6-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl 26-*O*-β-D-glucopyranoside (**3**), (25*S*)-5α-spirostan-3-one-6α-ol 6-*O*-β-D-xylopyranoside (**4**), (25*S*)-5α-spirostan-3β,6α-diol 6-*O*-β-D-glucopyranoside (**5**), (25*S*)-5α-spirostan-3β,6α-diol 6-*O*-β-D-xylopyranoside (**6**), (25*S*)-5α-spirostan-3β,6α,27-triol 6-*O*-β-D-glucopyranoside (**7**). We will present in this communication the isolation and structure elucidation of these new compounds.

PI295

Constituents from *Bupleurum chinense* and their effects on PPARα and related nuclear receptors

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The peroxisome proliferator-activated receptor α (PPARα) is expressed in liver at high level, and has become an important target for lipid metabolism modulating drugs. Due to the adverse effects of available PPARα modulators it's important to find new agonists with fewer side effects. Medicinal plants used in the traditional Chinese medicine (TCM) are a relevant source for the identification of new pharmaceutical leads. The roots of *Bupleurum chinense* (Chai Hu) have been used in TCM as a hepatoprotective and antipyretic remedy. Its dichloromethane extract showed strong PPARα agonistic activity. By activity-guided isolation, four polyacetylenes, eight triterpenoid saponins, and one linoleoyl lysolecithin were obtained and examined for PPARα activation and selectiv-

ity of action by checking agonism towards PPARγ, PPARβ/δ and LXRβ. Their structures were determined by spectroscopic methods as saikodiyne A (**1**), 2(*E*),8(*E*)- pentadecadiene-4,6-diyne-1,10-diol (**2**), pentadeca-8(*E*)-en-4,6-diyne-1,10-diol (**3**), pentadeca-2(*Z*), 9(*Z*)-dien-4,6-diyne-1,8-diol (**4**), 16-oxo-mullersaponin I (**5**), saikosaponin B2 (**6**), saikogenin D (**7**), 23-deoxy-saikosaponin B2 (**8**), prosaikogenin D (**9**), and 1-linoleoylglycerol-3-phosphorylcholine (**10**) bupleuroside III (**11**), and bupleuroside IV (**12**). Compounds **3**, **4**, and **8** are new chemical entities, and **2**, **5** are identified for the first time from a natural source.

PI296 A new bioassay-guided fractionation from root of *Bryonia dioica*: A promising strategy for drug discovery

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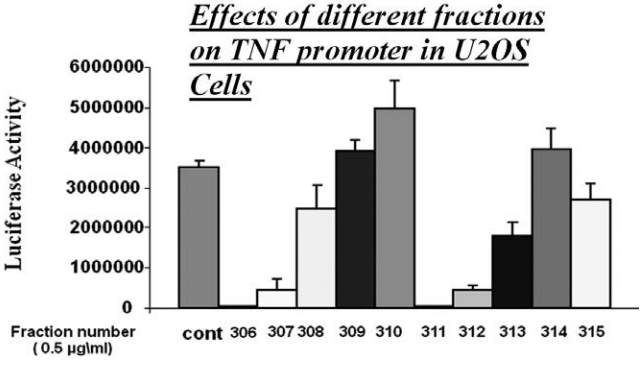
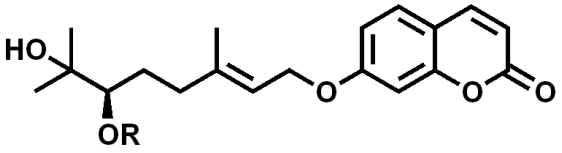
The discovery of bioactive natural compounds is largely related to the development and the sensitivity of biological methods. In our search for new antitumor and anti-inflammatory molecules, we have developed a new strategy to identify active compound from the roots of *bryonia dioica* (cucurbitaceae), which are widely used in traditional medicine to treat chronic diseases such as arthritis and rheumatism. Tumor necrosis factor alpha (TNF-α) is a central cytokine that drives the inflammation; hence inhibition of TNF-α offers an attractive treatment strategy. We used the human TNF promoter as biotool to identify active molecules. Crude extract was separated on an appropriate column chromatography and obtained fractions were tested to select the active fractions. Bryonia crude extract was found to exhibit a strong inhibitory effect on the proliferation of human U2OS osteosarcoma cells. By bioassay-guided fractionation, we isolated active fractions with a strong inhibitory action on the transcriptional activity of TNF promoter. Further fractionation was carried out: in purified active sub-fraction, we isolated and confirmed the structure of cucurbitacin E by NMR and HRMS as an active molecule. These results are very promising since they show that our bioassay-guided fractionation methodology could be applied for the detection of active compounds.



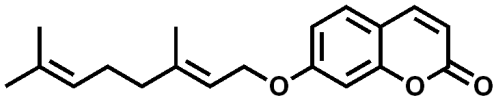
PI297 Semisynthetic studies identify mitochondrial poisons from botanical dietary supplements – *Aegle marmelos* geranyloxycoumarins

Li J[1], Mahdi F[1], Du L[1], Jekabsons MB[2], Zhou YD[1], Nagle DG[1]
[1]Department of Pharmacognosy, School of Pharmacy; [2]Department of Biology, University of Mississippi, University, MS 38677, USA

Bioassay-guided isolation of a Bael tree *Aegle marmelos* lipid extract yielded two unstable acylated geranyloxycoumarin mixtures (**1** – **2**), six geranyloxycoumarins (**3** – **8**), (+)-9'-isovaleroxylariciresinol (**9**), and dehydromarmeline (**10**). In a T47D cell-based reporter assay, **1** and **2** potently inhibited hypoxia-induced HIF-1 activation (IC_{50} values 0.18 and 1.10 µg mL^{-1}, respectively). Insufficient material prevented full delineation of the fatty acyl side chain olefin substitution patterns in **1** and **2**. Therefore, five fatty acyl geranyloxycoumarin ester derivatives (**11** - **15**) were prepared from marmin (**3**) and commercial fatty acyl chlorides. Derivative **14** potently inhibited HIF-1 activation (IC_{50} 0.92 µM). The octanoyl (**11**) and undecanoyl (**12**) ester derivatives also suppressed HIF-1 activation (IC_{50} values 3.1 and 0.87 µM, respectively). These geranyloxycoumarin derivatives disrupt mitochondrial respiration at complex I. One surprising observation was that, while less potent, the purported cancer chemopreventative agent auraptene (**8**) acts as a mitochondrial poison.



1 R = = -$COC_{22}H_{37}$ and -$COC_{22}H_{39}$

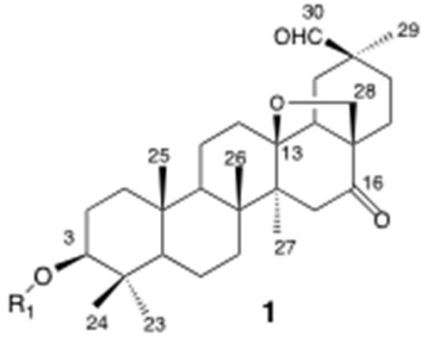


8 auraptene

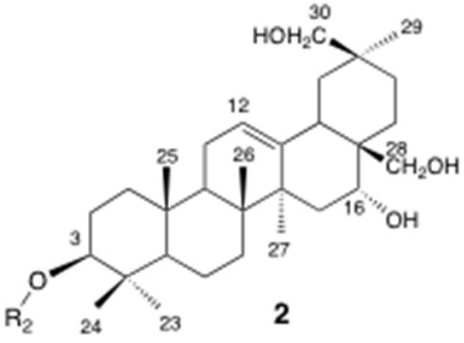
PI298 Two new triterpene saponins from *Cyclamen africanum*

Bencharif-Betina S[1,3], Miyamoto T[2], Tanaka C[2], Kabouche Z[3], Mitaine-Offer AC[1], Lacaille-Dubois MA[1]
[1]EA 4267 (FDE/UFC), Laboratoire de Pharmacognosie, Université de Bourgogne, Dijon, France; [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812 – 8582, Japan; [3]Laboratoire d'Obtention des Substances Thérapeutiques, L.O.S.T., Faculté des Sciences, Université de Constantine, Algérie

Two new oleanane-type triterpene saponins, afrocyclamin A and B (**1**, **2**) were isolated from a methanol extract of the roots of *Cyclamen africanum* Boiss. & Reuter, together with three known triterpenoid saponins, lysikokianoside (**3**), deglucocyclamin I (**4**) and its dicrotalic acid derivative (**5**). The structures were elucidated, on the basis of 1D-, 2D-NMR experiments and mass spectrometry as 3-*O*-{β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β-hydroxy-13β,28-epoxy-olean-16-oxo-30-al (**1**) and 3-*O*-{4-*O*-[3-hydroxy-3-methylglutaryl]-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16α,28,30-tetrahydroxy-olean-12-ene (**2**).



1

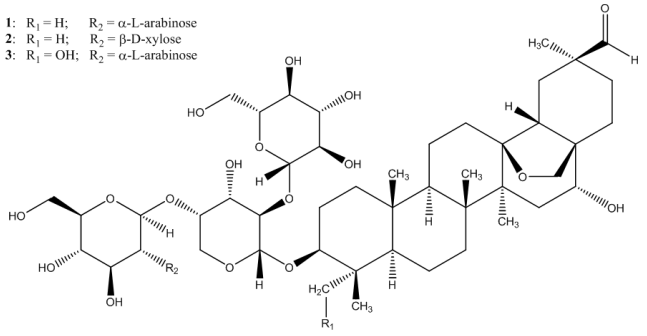


2

PI299 Triterpene-saponins of the roots of *Soldanella alpina* L

Schwaiger S[1], Heiderstaedt S[1], Eschmann J[2], Stuppner H[1], Gafner F[3]
[1]Institute of Pharmacy/Pharmacognosy, Center for Molecular Biosciences Innsbruck, University Innsbruck, Innrain 80/82, 6020, Austria; [2]Gärtnerei Eschmann, Waltwil 51, CH-6032 Emmen, Switzerland; [3]Mibelle Biochemistry, Bolimattstrasse 1, CH-5033 Buchs AG, Switzerland

The genus *Soldanella* L. (Primulaceae) is one of only 27 genera endemic to Europe and occurs in 16 species in the European Alpine system. The presented study represents the first phytochemical investigation of a member of the genus. Investigation of the roots of *S. alpina* L. afforded three triterpene-saponins which were identified as 3β,16α-dihydroxy-13β,28-epoxyolean-30-al 3-*O*-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-arabinopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside)] (**1**); 3β,16α-dihydroxy-13β,28-epoxyolean-30-al 3-*O*-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside)]=deglucocyclamin (**2**) and 3β,16α,23-trihydroxy-13β,28-epoxyolean-30-al 3-*O*-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-[*O*-α-L-arabinopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside)] (**3**). Compound **1** and **3** represent two novel natural products, while compound **2** was identified as deglucocyclamin, which was previous described from different cyclamen species.



PI300 Synthesis and characterization of β-glucogallin as an aldose reductase inhibitor from *Emblica officinalis*

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As the prevalence of diabetes mellitus continues to grow at epidemic proportions, the development of novel therapeutics to prevent the progression of diabetic retinopathy and cataract is paramount to eliminating secondary blindness in diabetic patients. Aldose reductase (AKR1B1) has been implicated in the progression of diabetic complications through the catalytic reduction of glucose to sorbitol. However, clinical trials of aldose reductase inhibitors have failed, most prominently due to toxicity as a result of nonspecific inhibition of other members of the aldo-keto reductase (AKR) superfamily. We recently identified the polyphenolic compound 1-*O*-galloyl-β-D-glucose (β-glucogallin) as the major active component of aqueous extracts from *Emblica officinalis* fruits, which have been shown to effectively treat cataracts in a diabetic rat model. Fruits of *E. officinalis* have been consumed for millennia in traditional Ayurvedic preparations. Herein we present the stereospecific synthesis of β-glucogallin from gallic acid, as well as its characterization as a potent (IC_{50}= 17 μM) as well as a specific inhibitor of AKR1B1. Molecular modeling demonstrates how β-glucogallin is able to bind both active site and specificity pocket residues of AKR1B1 and is utilized to drive rational drug design using the polyphenolic glycoside scaffold. This work supports the continued use of natural products such as β-glucogallin as therapeutic leads to treat diabetic complications.

PI301 *Terminalia arjuna*: Exploring its fungicidal potential

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Natural fungicides are desirable alternatives to anthropogenic fungicides that have negative effects on workers who use them and the environment. We explored the fungicidal potential of *Terminalia arjuna* (TA), a tree whose bark extract has recently been found to have pesticidal properties but whose fungicidal potential has not been well-researched. Enhancing the interest in its potential as a fungicide is the fact that TA has clinically-demonstrated cardiotonic properties. We chose to explore the effects of TA on the growth of a fungus pathogenic to Hudson Valley agriculture, *Geotrichum candidum*. Bioassays were conducted with an ethanolic extract of TA against *G. candidum* inoculates on Potato Dextrose Agar (PDA) media to determine the lowest concentration of TA extract demonstrating fungicidal activity. Minimum inhibitory concentration was observed at 9.89 uM. The crude ethanolic extract was further fractionated into hexane, diethyl ether, ethyl acetate, and aqueous fractions and bioassays were conducted. The results of our study will be presented.

PI302 Discovery of plant-lipid mixtures that synergistically stimulate innate immune system

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Juzen-taiho-to (JTT) is an herbal medicine known to exhibit safe and effective immunostimulatory activity. It is clinically used to improve the immune functions of cancer patients undergoing chemotherapy and radiation therapy in Japan. The chemical constituents responsible for its therapeutic effects are poorly characterized due to the chemical complexity of this formulation and possible synergism. In the course of the study to identify immunostimulants in JTT, we discovered plant-lipid mixtures that exhibit immunostimulatory activity. One such example is a mixture of glucocerebroside-ceramide, two lipid components identified from JTT. Glucocerebroside and ceramide synergistically stimulate THP-1, a human leukemia monocytic cell line. Our finding suggests that lipid constituents in JTT exhibit immunostimulatory activity as a mixture, but not as a single entity. Further mechanistic studies on those plant lipids would enable us to better understand the molecular basis of safe and effective immunostimulation exhibited by JTT.

PI303 In vitro anti-inflammatory and anti-proliferatIve effects of *Prangos platychloena* Boiss.ex Tchih. on colorectal cancer cell line

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We investigated anti-carcinogenic and anti-inflammatory activities of *Prangos platychloena* plant. We monitored such activities in two different extracts from the plant, methanolic and water, at 10 – 1000 μg/ml concentrations, in colorectal cancer cell line (CCL-221) and colon cancer cell line (Caco-2) using Trypan Blue Exclusion Test. We investigated the anti-inflammatory activity of the extracts on interleukin 8 (IL-8) and interleukin 6 (IL-6) secretion after stimulating the cancer cell by tumor necrosis factor-α (TNF-α). We used different concentrations of the plant, ranging from 10 to 1000 μg/mL. We observed that the most effective anticancer activity was at 1000 μg/ml concentration. At 1000 μg/mL concentration, the water extract showed anti-carcinogenic properties with maximum inhibition of 72% in CCL-221 cell, and maximum inhibition of 59% in Caco-2 cell. We observed no cytotoxic effect against normal cell line (Human fibroblast). Finally, we observed that IL-8 decreased from 519.07 pg/ml to 28.3 pg/ml with water extract of *P.platychloena*, to 92.73 pg/ml with its methanol extract in ccl_221, IL-6 decreased from 63 pg/ml to 1pg/ml with the water extract, and to 4 pg/ml with methanol extract. These results showed that *P. platychloena* could be used in pharmaceutical applications because of its remarkable anticancer and anti-inflammatory effects on CCL-221 and Caco-2 cancer cell lines.

PI304

In vitro schistosomicidal activity of *Usnea steineri* extract and its major constituent (+)-usnic acid against *Schistosoma mansoni*

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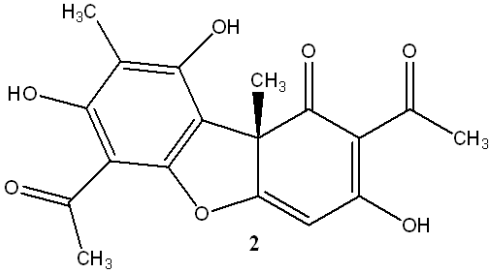
Universidade de Franca, Franca, SP, Brazil

The lichen genus *Usnea* is widespread throughout the world and is known to elaborate a number of interesting metabolites with biological properties. The schistosomicidal activity of the acetone extract of *Usnea steineri* **(1)** and of its major constituent (+)-usnic Acid **(2)** were evaluated *in vitro* against *Schistosoma mansoni*. The extract of *U. steineri* at 100 ug/mL in 24 h and usnic acid at 200 uM in 120 h caused death of all adult worms. In addition, both **1** and **2** caused tegument alteration, as peeling and bubbles, which was similar at observed to praziquantel (PZQ). Also, **1** and **2** reduced egg production and development in all concentrations. It is the first time that the schistosomicidal activity *in vitro* has been reported for extract of *U. steineri* and usnic acid. Therefore, these results suggest that extract of *U. steineri* and usnic acid are promising and could be used for the development of schistosomicidal agents. [Sponsors: Capes, CNPq, FAPESP]



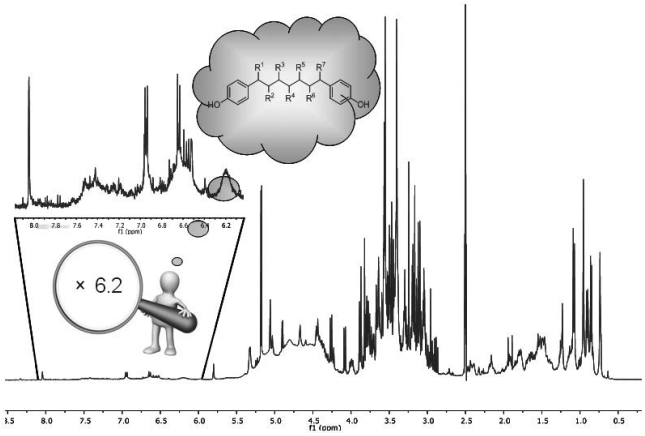
2

PI305

Constituents from the botanical dietary supplement wild yam

Dong SH, Nikolic D, Simmler C, Qiu F, van Breemen RB, Pauli GF, Chen SN
UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA 60612

Dioscorea villosa, its rhizomes/roots known as "wild yam", is a species of a twining tuberous vine that is native to North America. The general fame of *Dioscorea* species is based on their steroidal saponins which can be chemically converted to progesterone contraceptives and cortisone. Almost all previous phytochemical and pharmacological research on wild yam focused on the isolation and bioactivity evaluation of steroids. So far, twelve steroidal saponins and two flavan-3-ol glycosides have been reported as major secondary metabolites from the rhizomes/roots of *D. villosa*. This narrow spectrum of known phytoconstituents limits the current understanding of the significance of wild yam as an important dietary supplement. Aimed at the metabolomic mining of potentially interesting bioactive constituents, this study explored a new fractionation methodology on the basis of 1D-^{1}H and 2D-COSY NMR and diversified chromatography. The resulting isolation of ten diarylheptanoids including both open-chain and new cyclized (pyranoid) species from the MeOH extract marks the first report of this metabolite class from *D. villosa*. LC-MS profiling confirmed that diarylheptanoids represent genuine secondary metabolites of wild yam. To date, the only other report of the occurrence of diarylheptanoids in the genus *Dioscorea* came from *D. spongiosa*. Well-known from the Zingiberaceae and prominent for their pharmacological activities such as anti-cancer, estrogenic, anti-bacterial, anti-oxidative, anti-inflammation, and anti-osteoporotic activity, the metabolomic mining of a variety of diarylheptanoids in wild yam might offer a new lead for the development of this and possibly other *Dioscorea* botanicals.



PI306

Isolation of cytotoxic constituents from *Carex vulpinoidea* seeds

Niesen D[1], González-Sarrías A[1], Ma H[1], Yuan T[1], Henry GE[2], Seeram NP[1]
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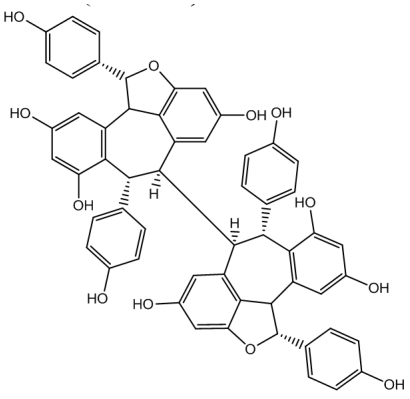
Recent studies from our group have identified bioactive stilbenoids, including resveratrol oligomers, from previously uninvestigated *Carex* species collected in Pennsylvania, United States. Here, *Carex vulpinoidea* seeds were evaluated using cytotoxicity-assay guided isolation against human colon cancer (HCT-116; Caco-2) cells. Hopeaphenol **(1)** (IC_{50}= 2 – 5 μM), a resveratrol tetramer, was identified as a major bioactive compound along with other polyphenols including luteolin **(2)**, 3,5,5',7' tetrahydroxyflavanone **(3)** and methylated naringenin **(4)**.



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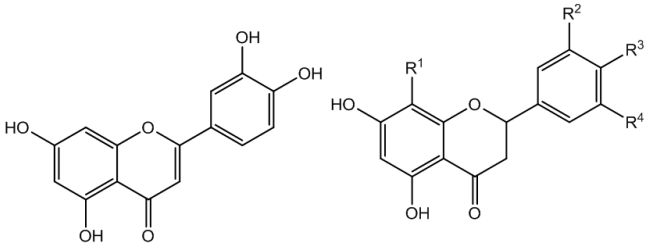

2

3 R^1=H, R^2=OH, R^3=H, R^4=OH
4 R^1=CH3, R^2=H, R^3=OH, R^4=H

PI307 Proanthocyanidins from cranberry fruit (*Vaccinium macrocarpon*) modulate colon tumor cell proliferation by multiple pathways

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[1]UMass Cranberry Health Research Center and Department of Chemistry and Biochemistry; [2]Department of Bioengineering, University of Massachusetts-Dartmouth, North Dartmouth, MA 02747

A-type proanthocyanidins (PACs) isolated from cranberry fruit (*Vaccinium macrocarpon*) are observed to decrease the proliferation of HCT116 and HT-29 colon cancer cells. FACS analysis shows that cell cycle arrest in G2 is significantly increased by exposure to PACs for as little as six hours. To determine the pathways affected by treatment, cells were exposed to cranberry PACs at 6 and 18 hours. Total RNA was extracted from treated and untreated control cells. Transcriptional profiling using an Illumina microarray system revealed altered expression of several members of the mitogen activated protein kinase family (MAPK) in treated cells, leading to decreased transcription of genes in the nucleus. Quantitative (Q)-PCR confirmed changes in expression of these genes, and changes in expression of MAPK pathway proteins (MAPK1/ERK, MAP2K1) were confirmed by Western blotting. PACs also appear to modulate apoptosis -linked genes (Bid, Bax and Bcl-family) and inflammation-linked genes (IL-4, IL-8, TNF-α, COX-2, CEACAM-1) suggesting multiple cellular targets for these compounds.

PI308 Anticancer studies of gallotannins from Maple (*Acer*) Spp

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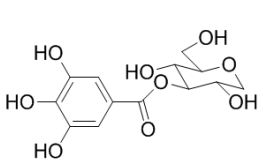
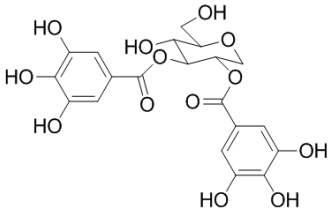
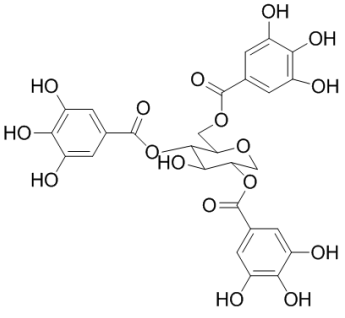
Gallotannins are hydrolyzable tannins found in higher plants. Here we conducted anticancer and structure activity related studies of twelve gallotannins isolated from sugar (*Acer saccharum*) and red (*Acer rubrum*) maple species. The gallotannins, ginnalins A-C and maplexins A-I, differ in the number of galloyl groups connected to a 1,5-anhydro-D-glucitol core. The gallotannins were evaluated for antiproliferative effects against human colon (HCT-116) and breast (MCF-7) cancer cells. While the gallotannins with one galloyl group were not active, those with two galloyls were more active than those with three galloyls (IC_{50}= 28 – 50 vs. 64 – 112 μg/mL). Also, maplexins C-D, which contained two galloyls, induced apoptosis and arrested cell cycle (in S-phase) of the cancer cell lines. Thus, the anticancer effects of maple gallotannins are influenced by the number of galloyl groups and are mediated by apoptosis and cell cycle arrest.



maplexin A



maplexin C



maplexin E

PI309 Isolation of new sesquiterpene lactones from Inula britannica with centrifugal partition chromatography

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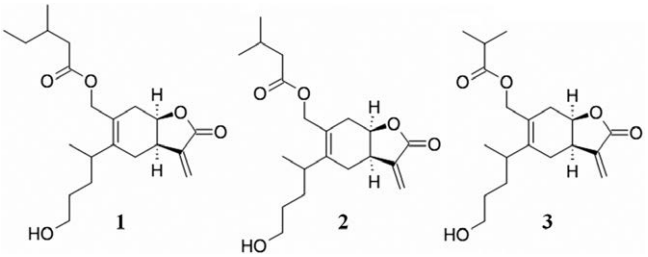
Inula britannica produces a variety of biologically active sesquiterpene lactones mainly pseudoguaianolides and seco-eudesmanolides. In order to further study the chemical constituents of *I. britannica* we used centrifugal partition chromatography followed by semi-preparative HPLC to isolate 10 sesquiterpene lactones. Three new seco-eudesmanolides (**1** – **3**) were isolated in the course of these experiments. The structure elucidation of these compounds was accomplished with high resolution MS, ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, HMBC, and NOESY.



PI310 The antimicrobial activity screening of three *Verbascum* species in Marmara region

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[1]Department of Pharmacognosy, Istanbul University, Beyazit, Istanbul 34116; [2]Department of Pharmaceutical Microbiology, Istanbul University, Beyazit, Istanbul 34116; [3]Department of Pharmacognosy, Yeni Yuzyil University, Zeytinburnu, Istanbul 34010

Three *Verbascum* species in Marmara Region, *V. lagurus*, *V. gnaphalodes* and *V. xanthophoeniceum*, are collected. The aerial parts were extracted with methanol, chloroform, ethyl acetate and water. These extracts were compared chromatographically and for their antimicrobial activity with *V. phlomoides* and *V. densiflorum*, which are preferred to be used in phytotherapy. Two fenolic acids (chlorogenic acid and caffeic acid), three flavonoids (luteolin, luteolin-7-glucoside and diosmetin-7-glucoside) and three phenylethanoid glycosides (verbascoside, angoroside A and forsitoside B) were isolated from ethyl acetate extract of *V. lagurus*, which was shown higher antimicrobial activity among the other *V. lagurus* extracts. *V. lagurus* seems to be similar to medicinal species. All of the extracts of *V. lagurus*, showed activity against *S. aureus* with MIC values of between 156 – 625 mg/L. Ethyl acetate extract, expressed also higher antibacterial activity against *Staphylococcus epidermidis*, while only the methanol extract was exhibited antibacterial activity against *Pseudomonas aeruginosa*. Antifungal screening result has indicated that methanol, ethyl acetate and aqueous extracts possess significant antifungal activity against *Candida albicans*. According to these results, the ethyl acetate extract displayed good antimicrobial activity against *S. aureus* and *C. albicans*. This is the first investigation on *V. lagurus* and *V. gnaphalodes*, and first isolation of diosmetin-7- glucoside from *Verbascum* species.

PI311 HTS-based antioxidant evaluation of native plants of Kansas

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[1]Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045, USA; [2]High Throughput Screening Laboratory, University of Kansas, Lawrence, KS 66045, USA; [3]Kansas Biological Survey, University of Kansas, Lawrence, KS 66047, USA

Over 600 samples prepared from native Kansas plant biodiversity, were evaluated by High Throughput Screening (HTS) methods for enzymatic and non-enzymatic antioxidant potentials. The majority of the 111 species in 35 plant families examined have little or no reported antioxidant

assessment. The enzymatic Antioxidant Response Element (ARE) assay determined the most potent species as *Physalis longifolia*, *P. angulata* (Solanaceae) and *Impatiens capensis* (Balsaminaceae) with EC_{50} values of 0.48 μg/mL, 1.93 μg/mL, and 3.22 μg/mL, respectively. The non-enzymatic Total Antioxidant Capacity (TAC) assay demonstrated that *Mirabilis glabra* (Nyctaginaceae), *Larrea tridentata* (Zygophyllaceae), and *Eriogonum helichrysoides* (Polygonaceae) were the most active antioxidant species with Trolox equivalent AUC values of 22.14, 22.13, and 21.10, respectively. Furthermore, these results, in conjunction with our previous *P.longifolia* study, support an Nrf2/ARE pathway and cell apoptosis relationship. The results of these experiments will be presented.

PI312 Structural elucidation of complex carbohydrates from cranberry

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[1]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677; [2]National Center for Natural Products Research, The University of Mississippi, University, MS 38677

Complex carbohydrates from plant sources, such as the fruits of cranberry (*Vaccinium macrocarpon* Aiton), potentially promote human health in a variety of ways. However, complete studies of carbohydrate function often hinge on a detailed understanding and thorough elucidation of a carbohydrate's chemical structure. Several methods have been used in the structural elucidation of cranberry oligosaccharides, such as methods of derivatization followed by analysis using gas chromatography-mass spectrometry (GC/MS). In this manner, the identity and quantity of saccharide monomers within cranberry oligosaccharides were investigated through the hydrolysis of the oligomer and subsequent synthesis of trimethylsilyl- and alditol acetate-monomeric derivatives. Further analysis using partially methylated alditol acetate-monomeric derivatives revealed the linkage position on each monomer. A more complete structural picture was then assembled through the compilation of data from the analysis of tandem MS/MS fragmentation patterns and 1D and 2D NMR experiments. Complex carbohydrates have enormous potential for structural complexity which requires numerous techniques for their full structure elucidation. This poster outlines the techniques used to break down oligosaccharides from cranberry products as a means of assembling a detailed picture of their chemical structure.

PI313 Cranberry leaves (*Vaccinium macrocarpon*) as a source of antimicrobial metabolites

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[1]UMass Cranberry Health Research Center, Department of Chemistry and Biochemistry, University of Massachusetts-Dartmouth, North Dartmouth, MA 02747; [2]Microbiotix, Inc., Worcester, MA 01605

Secondary metabolites from the leaves of North American cranberry (*Vaccinium macrocarpon*) are under investigation for structure and inhibitory activity against bacterial biofilm formation and cancer cell growth. Compounds were separated by column chromatography using various stationary phases, and characterized by MS and NMR. In addition to several quercetin glycosides, proanthocyanidin subfractions ranging in oligomer size from dimer to octamers, were isolated from the aqueous extracts and characterized by MALDI-TOF MS. Cranberry leaf PACs inhibited biofilm formation by *Staphylococcus aureus* (MBIC = 1.5 – 3.1 μg/mL) as well as the growth of several *Candida* species and HeLa cells. The quercetin glycosides also inhibited *S. aureus* biofilms (MBIC = 25 μg/mL). Constituents of the nonpolar extracts include β-sitosterol and ursolic acid derivatives to be identified. Cranberry leaves may be a useful source of antimicrobial compounds.

PI314 Anticancer activity of resveratrol analogs from *Eugenia rigida* DC

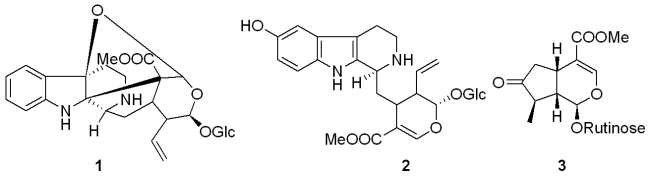
Zaki MA[1,2], Samoylenko V[1], Khan S[1], Abd slam RM[2], Hetta MH[2], Shin U[3], Pelletier J[3], Walker LA[1], Muhammad I[1]
[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, USA; [2]Department of Pharmacognosy, School of Pharmacy, Beni-Suef University, Beni-Suef, Egypt; [3]McIntyre Medical Sciences, McGill University, Montreal H3G 1Y6, Quebec, Canada

Two new natural resveratrol analogs, namely 3,5,3',4'-tetramethoxy-(*trans*)-piceatannol (**1**) and 3,5,3'.4'-tetramethoxy-(*cis*)-piceatannol (**2**), together with the known 3,5,4'-trimethoxy-(*trans*)-resveratrol (**3**) have been isolated from the leaves of *Eugenia rigida*. The structures of **1** and **2** were characterized using NMR and MS techniques. The *trans* (**1**) and *cis*-isomers (**2**) were evaluated for anti-cell proliferative activity against selected human cancer cell line and non-cancerous VERO cells. The *cis*-isomer showed anti-cell proliferative activity with IC_{50} values ranging between 5.3 – 16.6 μM, while the *trans*- isomer (**1**) was inactive up to 33.3 μM. Furthermore, the activity against intracellular reactive oxygen species (ROS) generation was also determined. The *trans*-isomer showed an inhibition of 50% in ROS generation at 33.3 μM in PMA-induced HL-60 cells, while *cis*-isomer did not exhibit any effect. Finally, both the isomers were studied for the inhibition of protein biosynthesis *in vitro* in a translation system derived from the Krebs cells.

PI315 Cymoside, an original hexacyclic monoterpene indole alkaloid and others compounds from *Chimarrhis cymosa* (Rubiaceae)

Lemus C, Kritsanida M, Canet A, Michel S, Deguin B, Grougnet R
Laboratoire de pharmacognosie, Université Paris Descartes, Sorbonne Paris Cité, Faculté des sciences pharmaceutiques et biologiques, 4 avenue de l'Observatoire F-75006 Paris, France

"*Dedicated to the memory of Pr François Tillequin*" Monoterpene indole alkaloids are a group of more than 3000 compounds, source of potent bioactives molecules. Our interest has therefore focused on the genus *Chimarrhis* (Rubiaceae) which has been poorly studied. Indeed, phytochemical studies deal only with the species *Chimarrhis turbinata*, which led to the description of some original monoterpene indole alkaloids [1]. We report here the structure determination of secondary metabolites from *Chimarrhis cymosa*, a tree endemic from Lesser Antilles: Cymoside (**1**), a strictosidine derivative with a hexacyclic-fused core, together with 10-hydroxystrictosidine (**2**), three known monoterpene indole alkaloids, two bis-monoterpene indole alkaloids, one well known iridoid and a novel diglycosilated iridoid (**3**).



1 2 3

PI316 *Hypericum foliosum* leaf – Chromatographic fingerprint of a promising antidepressant medicinal plant

Machado A[1], Ramalhete N[1], Serrano R[1], Wolfender JL[2], Gomes ET[1], Silva O[1]
iMed.UL, Faculty of Pharmacy, University of Lisbon, Av. Professor Gama Pinto, 1649 – 019 Lisbon, Portugal; [2]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30, Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland

Hypericum foliosum leaf is an endemic species of the Azores archipelago that in previous studies was showed a promising antidepressant activity, similar to the activity of St. John's Wort, a well-established herbal medicine belonging to the same botanical family and used as antidepressant in Europe. Hereby we present results of the analytical studies performed

in order to obtain the chromatographic fingerprint of the active H. foliosum aerial part extract and of most active fractions obtained from this (methanol and ethyl ether ones). Obtained results of the analytical studies performed by TLC, LC-UV/DAD and LC-DAD/MS in the methanolic fraction permitted the identification of its phenol acids and flavonoid constituents, as main compounds, namely quinic acid, 3,4-dimethylbenzoic acid, (+)catechin, chlorogenic acid, quercetin-3-O-sulphate, miquelianin, xanthone and biapigenin.

PI317 Effects of 13-acetoxyrolandrolide on colon cancer cells

Muñoz Acuña U[1], Matthew S[1], Wittwer J[1], Pan L[2], Kinghorn AD[2], Carcache de Blanco EJ[2]
[1]Division of Pharmacy Practice and Administration, College of Pharmacy, The Ohio State University, Lloyd M. Parks Hall 500 W. 12th Avenue, Columbus, OH 43210

[2]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Lloyd M. Parks Hall 500 W. 12th Avenue, Columbus, OH 43210. The compound 13-acetoxyrolandrolide was previously isolated from Rolandra fruticosa (L.) Kuntze (Asteraceae). Potent cytotoxic activity was found against HT-29 colon cancer cells compared with paclitaxel, EC_{50}= 0.16 μM and EC_{50}= 0.0006 μM, respectively. Initial screening showed that the NF-κB inhibition was IC_{50}= 7.1 μM. The mechanism of action through which transcription factor NF-κB was further investigated and the expression of up-stream mediators such as IKKα and IKKβ was analyzed by immunoblotting. In this study, 13-acetoxyrolandrolide demonstrated similar effects to staurosporine, inducing loss of the mitochondrial membrane potential (ΨΔm). Cell cycle analysis showed a significant increase of HT-29 cells in the G_1-phase after treatment, and 68% of treated cells were found in this G_1-phase compared to 55% in the untreated cells. In addition, high intracellular levels of ROS were also detected in treated cells. These findings suggest that the mitochondrial activity of cancer cells was affected and NF-κB was inhibited, possibly through an oxidative pathway. Thus, chemical optimization of 13-acetoxyrolandrolide might lead to the discovery of a new potential cancer chemotherapeutic agent for the treatment of colon cancer.

PI318 Crude extract and 9-methoxyisomoschatoline from *Guatteria hispida* as photosensitizers in antimicrobial photodynamic inactivation (PDI)

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[1]Curso de Farmácia, DBV, Instituto de Biologia, UNICAMP, Campinas (SP), Brazil; [2]LABORGANICS, DQI, UFS, São Cristóvão (SE), Brazil; [3]DQ, UFAM, Manaus (AM) Brazil; [4]DFQ, Instituto de Química, UNICAMP, Campinas (SP), Brazil

Guatteria hispida (R. E. Fr.) Erkens & Maas (Annonaceae) is a small tree that occurs in the Brazilian Amazon forest. This study was undertaken to evaluate the effect of methanol crude extract and alkaloid 9-methoxyisomoschatoline isolated from the bark of *G. hispida* as photosensitizers in antimicrobial photodynamic inactivation (PDI) of bacteria and yeast. Methanol extract and isolated alkaloid (9-methoxyisomoschatoline) were tested, in sub-inhibitory concentration, against *Staphylococcus aureus* ATCC 14458, *Escherichia coli* ATCC 10799, *Proteus vulgaris*, *Candida albicans* ATCC 1023, *C. albicans* ATCC 10231, *Candida dubliniensis* ATCC 778157 and *C. dubliniensis* ATCC 777. One plate (n = 6) was subjected to irradiation with a 660 nm diode laser with an output power of 35 mW distributed through the well cross section yielding an energy dosage of 28 J/cm^2, and one was not irradiated. The absorption spectrum of the samples presented maximum bands at 330 and 620 nm, and when irradiated with these wavelengths showed high fluorescence intensity. At photochemical assay, the 1.3DPBF photodegradation was significantly enhanced in the presence of crude extract and isolated alkaloid indicating the production of singlet oxygen. The biological assays suggest inhibition of the growth of microorganisms tested in the presence of methanol extract and isolated alkaloid as photosensitizers in PDI. Laser irradiation alone or crude extracts or alkaloid at sub-inhibitory concentration are not bioactive. Further investigations are necessary to confirm the potential of these natural products as photosensitizers in PDI.

PI319 Action of constituents of *Solidago chilensis* DC (Brazilian arnica) in the mechanisms of wound healing

Gastaldo B[1], Hatanaka E[2], Bortolon JR[2], Murata GM[2], Bacchi EM[1]
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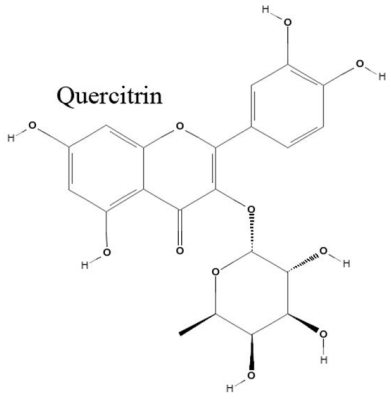
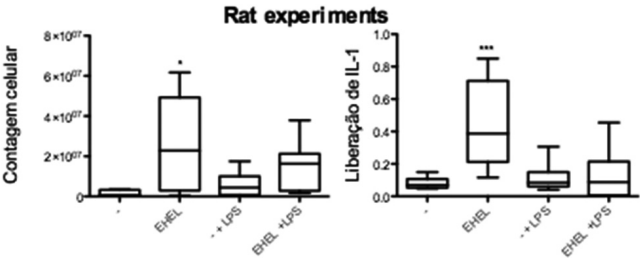
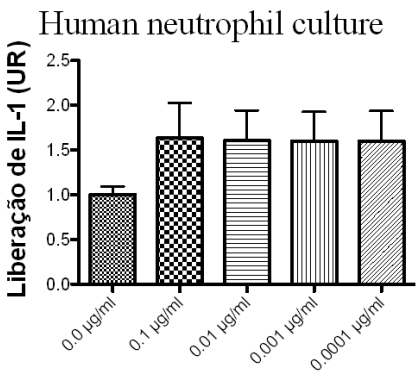
Ersatz of the Arnica montana, *Solidago chilensis* possess a lagged systematic scientific investigation. To solve the questions about its mechanism of action and considering that neutrophils are involved in inflammatory phase of the wound healing, we investigated the effects of *S. chilensis* in wound healing process. A wound in the dorsal surface of rats was performed and the lyophilized 70% hydroethanolic extract of *S. chilensis* (LHEE) were topically administered. On the 5th day a reduction (52%) of the wound area was verified. In air pouches experiments neutrophil influx was raised 18 times when compared to control and induced a marked increase of IL-1, L-selectin, and IL-6 release. In contrast, it strongly inhibited fMLP-stimulated migration of neutrophils and the amount of cytokines IL-6, L-selectin, IL-1, TNF. In human culture an increase of cytokines liberation was also noticed. We identified the presence of phenolic compounds in the extract, mainly quercitrin which possesses anti-inflammatory activity. Also terpenoids were present. Our results suggest that *S. chilensis* can regulate accumulation and depletion of neutrophils in the inflammatory foci and may modulate the inflammatory phase of wound healing.







PI320 Effect of light-emitting diode (LeD) on contents of lignans and anthocyanins in *Schizandra chinensis*

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Light-emitting diode (LED) is a semiconductor light source and it is known to enable plants to grow quickly and increase the secondary metabolites because it provides plants with light of specific wavelength, necessary for growth of plants. Fruits of *Schizandra chinensis* have been used for reduction of blood pressure and fatigue and protection of brain in Korea. Its bioactive constituents are known to be a series of lignans and anthocyanin pigments. In this study, we tried to investigate how LED has an influence on contents of four major lignans (schizandrin, deoxyschizandrin, gomisin A, and gomisin N) and anthocyanin in this medicine. The fruits of *S. chinensis* were placed under the LED wavelengths at 440 nm, 470 nm, 660 nm, white LED, and dark (control) for 1, 3, 7, 14, 21, 28 days, respectively. The contents of four lignans were investigated based on peak area of HPLC chromatograms under UV detection at 254 nm, while those of anthocyanin were measured based on UV absorption at 400 – 600 nm. In result, contents of the lignans increased under the 440 nm and dark conditions by 21 days, even though there is some fluctuations under other wavelengths. In addition, those of anthocyanin were the most at 440 nm and white LED. Detailed results and discussion on contents of lignans and anthocyanin by wavelengths will be presented.

PI321 Isolation of neuroprotective compounds from *Phlomis umbrosa*

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Phlomis umbrosa Turcz (Labiatae) is a perennial herb growing in North China. Roots of *P. umbrosa* has been used in traditional Chinese medicine for reduction of swelling, staunch bleeding, eliminating phlegm and detoxicating for thousands of years. Our phytochemical investigation to find natural compounds with protective activity against degeneration of retinal ganglion cells led to the isolation of ten compounds, ursolic acid (**1**), oleanolic acid-3-*O*-α-L-rhamnopyranoside (**2**), daucosterol (**3**), hederagenin-3-*O*-α-L-arabinopyranoside (**4**), sweroside (**5**), caffeic acid (**6**), esculetine (**7**), 3,4-dihydroxy bezaldehyde (**8**), loganin (**9**), and vanilic acid (**10**) from ethyl acetate fraction of methanol extract of *Phlomis umbrosa*. The structures of **1-10** were elucidated on the basis of NMR data and comparison with the literature. Their structure determination and biological activities will be presented.

PI322 Essential oil from the dried and fresh leaves of *Annona cacans* (Anonnaceae): GC/MS analyses and anti-leishmanial potential

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Annona cacans (Annonaceae) is known as "cortição" and the fruit is used as cathartic. In this study the chemical composition of essential oil from dried and fresh leaves of A. cacans and anti-leishmanial activity were evaluated. The leaves of A.cacans were separated into two batches of equal mass (266 g), and the lot n°.1 was submitted to drying in an oven (25 °C/12 h) and lot n°.2 is formed by fresh leaves. The samples of lot n°.1 and 2 were submitted to essential oil extraction by hydrodistillation in a Clevenger apparatus. The chemical composition of the essential oils was determined by GC/MS. The anti-leishmanial activity against Leishmania infantum promastigotes was determined. The essential oils were incubated to the highest concentration of 150 µg/mL for 24 h at 24 °C. Parasite viability was determined by MTT assay at 550 nm. Pentamidine served as positive control and DMSO 0.5% as negative control. Each assay was performed in triplicate and the concentration necessary to 50% inhibitory concentration (IC_{50}) was calculated in µg/mL. Twenty compounds were identified in the essential oils of dried and fresh leaves. The germacrene B was the major constituent of both oils. The oils shows in vitro anti-leishmanial activity with IC_{50}= 38.71 and 26.50 µg/mL for essential oils of dried and fresh leaves, respectively. Further investigations are necessary to understand the possible mechanism of action in parasites as well as their systemic adverse effects, toxicity and efficacy toward a clinical employment.

PI323 Isolation and structure determination of four new neolignans from Trogopterorum faeces

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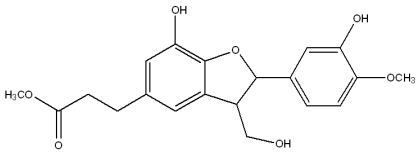
Trogopterorum faeces is the dry stool of *Trogopterus xanthipes, Pteropus pselaphon,* or *Pteromys volans*. It is known to have characteristic properties of invigorating blood, removal of extravasated blood, hemostasis, relieving pain, and deintoxication, and has been used for treatment of abdominal pain, menstrual pain, hypermenorrhea, amenorrhea, and snakebite in traditional oriental medicine. It was reported that it consists of diterpenoids, flavonoids, and fatty acid esters. Our phytochemical study to investigate bioactive compounds from Trogopterorum faeces resulted in the isolation of four new neolignans (**1 – 4**), which were isolated from the ethyl acetate fraction of methanol extract of Trogopterorum faeces. To the best of our knowledge, lignan or neolignan was isolated from this traditional medicine for the first time. Detailed structure determination of **1-4** on the basis of spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC, and HMBC including absolute stereochemistry will be presented.



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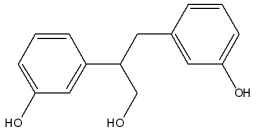

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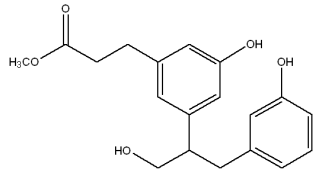

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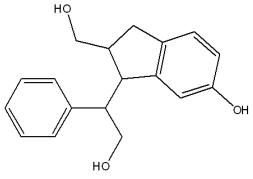

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PI324 Synergistic tyronsinase inhibitors from the roots of *Smilax china*

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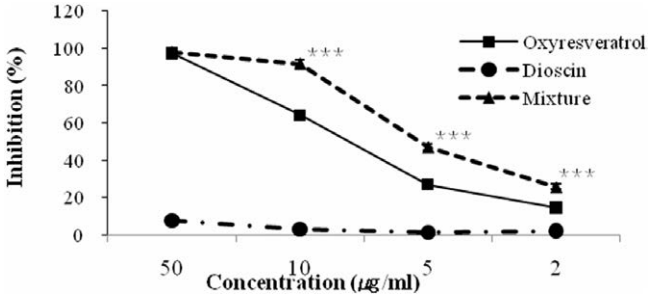
We investigated the inhibition of mushroom tyrosinase in *Smilax china*. A methanol (MeOH) extract of *S. china* was partitioned into hexane, ethyl acetate (EtOAc) and water. Of the 3 fractions, EtOAc extract showed the strongest inhibition of tyrosinase activity with L-tyrosine

or L-DOPA as a substrate. Two compounds were isolated from a final active fraction by activity-guided column chromatography. These compounds were identified as dioscin and oxyresveratrol. We discovered that a mixture of oxyresveratrol and dioscin (IC 50 = 5.1, 5.7 μg/ml) highly increased the inhibition of tyrosinase activity with L-tyrosine or L-DOPA as the substrate as compared to either oxyresveratrol (IC 50 = 7.8, 10.9 μg/ml) or dioscin (IC 50 > 100, 100 μg/ml) alone.



PI325 Neuroprotective alkaloids isolated from tubers of *Corydalis ternata*

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The tuber of *Corydalis ternata* (Papaveraceae) has been used not only as analgesic but also for the treatment of inflammatory, allergic diseases, and dysfunction in the traditional Korean medicine. Previous pharmacological studies of *Corydalis* species found that its extracts exhibited anti-gastric ulcer effect and showed significant inhibitory effects on acetlycholinesterase. The genus *Corydalis* is known to contain a number of isoquinoline alkaloids including protoberberine alkaloids and aporphine alkaloids. Our phytochemical study of neuroprotective compoundsproduced by *Corydalis ternata* led to the isolation of seven alkaloids including tetrahydrocoptisine (1), corydaline (2), tetrahydropalmatine (3), corybulbine (4), dehydrocorydaline (5), *N*-methyltetrahydroberbinium (6) and one unconfirmed compound (7). The structures of the isolated compounds were elucidated on the basis of spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC, HMBC and comparison with the literature. Their structure determination and neuroprotective effects on retinal ganglion cells will be presented.

PI326 Dammarane-type glycosides from *Gynostemma pentapyllum*

Lee C[1], Lee JW[1], Park DH[1], Bae JY[1], Hong JT[1], Lee MK[1], Lee MK[1], Hwang BY[1,]
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Gynostemma pentapyllum (Thunb.) Makino, a perennial creeping herb belonging to the Cucurbitaceae, is widely distributed in Korea, China, and Japan. The genus *Gynostemma* is a rich source of dammarane-type glycosides which are structurally related to the ginseng saponin. Many of these saponins were reported to exert a wide spectrum of pharmacological effects, including hypolipidermic, anti-inflammatory, immunomodulatory, and anticarcinogenic activities. In our continuing search for bioactive components from medicinal plant, two new dammarane-type glycosides together with twelve known compounds were isolated from the aerial parts of *G. pentapyllum* using column chromatographic separation techniques. The structures of these compounds were determined by 1D- (^{1}H, ^{13}C and DEPT), 2D- (HSQC, HMBC, COSY, NOESY) NMR and HRESI-MS spectrum. We report herein the isolation and structure determination of these two new dammarane-type glycosides.

PI327 Isolation characterization and anti-inflammatory activity of *Rhaphiostylis beninensis* Planch. Ex Benth (Icacinaceae)

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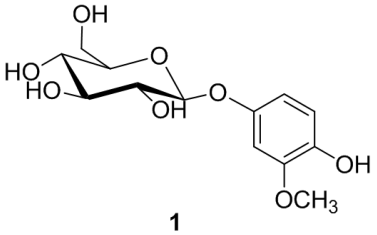
Rhaphiostylis beninensis is used in traditional medicine in Nigeria as an anti-inflammatory crude drug. This claim was investigated through bioactivity- guided fractionation chromatography using the Carageenan induced Rat Paw inflammation model. A sulphonyl derivative: N,N -Bis [(4-methoxyphenyl) methyl]thiourea was isolated and it's structure was elucidated using Mass Spectrometr and Nuclear Magnetic Resonance (HNMR and ^{13}CNMR). The anti-inflammatory activities were enhanced with fractionation and subsequent chromatographic exercises. The occurance and anti-inflammatory activity of the compound are reported for the first time in *Rhaphiostylis beninensis*. The results confirm the traditional use of the plant.

PI328 Inhibitors of adipogenesis in 3T3-L1 cells isolated from wheat bran

Jeong W[1], Hong SS[1], Kim JK[1], Kwon JG[1], Choi YH[1], Seo C[1], Lee JA[1], Ahn EK[1], Oh JS[1,2]
[1]Natural Products Research Institute, Gyeonggi Institute of Science & Technology Promotion, Suwon 443 – 270, Korea; [2]College of Pharmacy, Dankook University, Cheonan 330 – 714, Korea

As a part of our ongoing program on finding biologically active components from natural source we found three known constituents from the EtOH extract of the wheat bran. The known compounds were identified as tachioside (1), pinellic acid (2) and tryptophan (3). The structure and relative stereochemistry were determined from 1D, extensive 2D NMR, and MS techniques as well as by comparison of their data with the published values. All isolates were tested their inhibitory effects on the adipogenesis in 3T3-L1 cells. The effect of compounds from wheat bran on 3T3-L1 adipocyte differentiation were measured by Oil Red O staining. These results demonstrate that tachioside (1) and pinellic acid (2) decreased lipid content in 3T3-L1 adipocytes by inhibiting lipogenesis. These compounds had shown antiobesity activities. This work was supported by a grant from the Next-Grneration BioGreen 21 Program (No. 20120401 – 305 – 524 – 011 – 03 – 00), Rural Development Administration, Republic of Korea.



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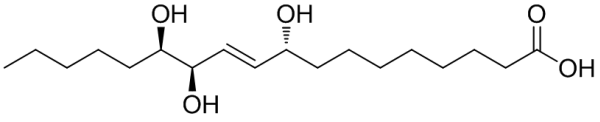

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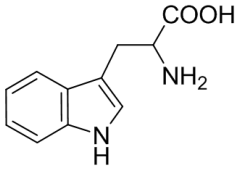

3

PI329 Phenolic content of species from leguminosae family and their antifungal activity

Morais CB[1], Silva FEK[2], Lana AD[2], Tonello ML[1], Luciano SC[2], Fuentefria AM[2], Zuanazzi JÂS[1]
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The phytochemical of Leguminosae family is well documented. Various types of flavonoids, isoflavones, coumarins and phenylpropanoids have been described in this family, most of them proceeding as defense chemicals or signal compounds. Phenolic compounds, including flavonoids, were found in large quantities in plants of this family, and exhibit a wide range of pharmacological properties. Thus, this study aimed to investigate the presence of phenolic compounds in extracts of 60 plants of the Leguminosae family by high performance liquid chromatography (HPLC) and evaluate the antifungal activity of 36 of them. Compounds that showed the highest frequency were flavonoids isovitexin (23%), vitexin (20%) and luteolin (16%). In the antifungal study, none of the 36 plants showed activity against yeasts. The species *Eriosema heterophyllum*, *Chamaecrista nictitans* and *Mimosa pigra* had a wider spectrum of action against the dermatophytes *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum gypseum* and *Epidermophyton floccosum*. The species *M. pigra* showed low values of minimum inhibitory concentration (MIC), around 1.9 μg/mL. After fractionation it was found that the dichloromethane fraction is the most active. *M. pigra* could be a promising species for treatment of dermatomycoses.

PI330 Triterpene acids from euscaphis and assessment of their cytotoxic and anti-no activities

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Six new triterpenoids, euscaphic acids G-L (**1**-**6**), along with nine known triterpene acids, and two known lignans were isolated from the ethanolic extract of the twigs of Euscaphis japonica. This is the first report concerning 1α,3β-dihydroxy-12-oleanen-28-oic acid isolated from a natural source. The structures of the new compounds were established by spectroscopic analysis. The cytotoxic and anti-NO production activities for the isolates are also evaluated and discussed, compound **1**, hederagenin (**11**), and arjunic acid (**12**) showed significant cytotoxicity against NCI-H460 cells, HT-29 cells, and CEM cells (IC_{50} = 1.64 ± 0.8, 2.11 ± 1.5, 1.73 ± 0.6 μM, respectively). Some of isolated triterpenoids showed marginal inhibitions on NO production induced by LPS.

PI331 Bioassay-guided isolation of anti-*Leishmania major* compounds from *Micromelum minutum*

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Anti-*Leishmania major* assay-guided isolation of *Micromelum minutum* leaves gave two new monoterpene coumarins, named minutin A and minutin B, together with four known coumarins, 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′diol, 8-hydroxyisocapnolactone-2′,3′- diol, 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol, and clauslactone E. Minutin A, minutin B, 8-hydroxyisocapnolactone-2′,3′-diol and clauslactone E showed satisfactory anti-*L. major* activity with IC_{50} of 26.2, 20.2, 12.1, and 9.8 μM, respectively, while 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol and 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol were not active.

PI332 Bioactive constituents from the stem bark of *Zanthoxylum avicennae*

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[1]Graduate Institute of Pharmaceutical Technology & Department of Pharmacy, Tajen University, Pingtung 907, Taiwan, [2]Faculty of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan. [3]Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan

Zanthoxylum avicennae (Lam.) DC. (Rutaceae) is an evergreen shrub distributed in Vietnam, Philippines, southern China, and Taiwan. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for *in vitro* anti-inflammatory activity, and *Z. avicennae* has been found to be one of the active species. In our search for compounds with anti-inflammatory activities, two new coumarin derivatives, 8-formylalloxanthoxyletin (**1**) and avicennone (**2**) and 14 known compounds (**3**-**16**) have been isolated and identified from the stem bark of *Z. avicennae*. The structures of all isolates were determined through spectral analyses and comparison of their physical and spectral data with literatures. Among the isolated compounds **1**, **5**, **10**, **13**, and **16** exhibited inhibition (IC_{50} values ≤ 7.65 μg/mL) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leuckyl-L-phenylalanine/cytochalasin B (fMLP/CB).

PI333 Chemical analysis of selected *Penstemon* species and their antimicrobial activity

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[1]Medical University of Lublin, Faculty of Pharmacy, Dept. of Pharmacognosy with Medicinal Plant Unit, Chodźki 1 str., 20 – 093, Lublin, Poland; [2]Dept. of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, University of Athens, Zografou, 15771, Athens, Greece

Penstemons are endemic plant species belonging to *Penstemon* botanical genus, which is considered as the biggest of North America's flora. They were widely used by Native Americans especially in Mexico in ethnomedicine as remedies for several diseases, such as inflammation, cold and cough, gastrointestinal and gynecological disorders. In the framework of our continuing research on several *Penstemon* species, our research project addresses the isolation and structural determination of twenty three (23) secondary metabolites from four different species: *Penstemon campanulatus*, *P. fruticosus var. frticosus*, *P. palmeri* and *P. venustus*. Iridoid glucoside (12 compounds) appeared as the most characteristic chemical group of these species, including plantarenaloside, geniposidic acid, aucubin, as well as a new compound – 10-isovaleroyldihydropenstemide. Moreover flavonoids, phenylpropanoid glucosides, acetophenone derivatives, monoterpene glucosides and lactones were also isolated and identified through modern spectral data. All the extracts and the isolated compounds were assayed for their antibacterial and antifungal activity, showing an interesting profile.

PI334 Hydroxycinnamoyl 1,2-dihydro-furo[3,2-e]tryptamine derivatives from defatted safflower seeds

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Defatted safflower seeds are known to contain hydroxycinnamoyl serotonine derivatives, which have been reported to possess strong antioxidative activities *in vitro* and exert various biological effects. For this reason, polyphenols prepared from defatted safflower seeds are used for health care product. We have been searching secondary metabolites useful for health care from medicinal plants. As part of this study, we have investigated defatted safflower seeds. The EtOAc-soluble fraction obtained from the MeOH extract of defatted safflower seeds (3.4 kg) was separated by repeated column chromatography to give seven new hydroxycinnamoyl serotonine derivatives, including four new hydroxycinnamoyl 1-(3-methoxy-4-hydroxyphenyl)-2-hydroxymethyl-1,2-dihydrofuro[3,2-e]tryptamine derivatives, together with five known serotonine derivatives. The structure elucidation and biological activities of these compounds will be presented.

PI335 The investigation of structural requirements for saponins to enable synergistic cytotoxicity with type-I-RIP/lectins

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Saponins and lectins, especially the naturally very low cytotoxic activity showing type-I-RIP (ribosome-inactivating proteins type I)/lectins exhibit a synergistic cytotoxicity when applied to cancer cells [1]. The preappliance of certain saponins can drastically amplify the cytotoxicity of the type-I-RIP [2, 3] and has become a promising strategy in anti-cancer research [2]. These saponins may also minimize the required effective dose of these very expensive (especially when linked to human antibodies and natural ligands) and time-consuming to purify/to create substances [5]. In our work we search for new saponins from plant-extracts capable of increasing the cytotoxicity of the naturally very low cytotoxic activity showing lectin saporin, considered as a standard type-I-RIP. The spotlight of our research is put on the plant-family of Caryophyllaceae, but saponins from other plant-families were also tested when fulfilling certain structural requirements. The investigation of these structural requirements that saponins have to fulfill to execute the synergistic cytotoxicity is in the focus of our work. All tests were performed in a cell culture model using ECV-304 cells. The cytotoxicity was measured by MTT assay and DNA quantification. **References:** [1] Hebestreit, P., Melzig, M.F. (2003) Planta Med. 69:921 – 925. [2] Hebestreit, P. et al. (2006) Toxicon 47:330 – 335. [3] Weng, A. et al. (2008) Chem. Biol. Int. 176:204 – 211. [4] Bachran, C. et al. (2009) J. Immunother. 32:713 – 725. [5] Bachran, C. et al. (2010) Brit. J. Pharmacol. 159:345 – 352.

PI336 Abietane diterpenoids from some salvia species: Chemical transformations and antiproliferative activity

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In this research the major secondary metabolites isolated from these species and their biological studies against five human cancer cell lines are described. A cold acetone extract of the aerial parts of *S. pachyphylla* was chromatographed on silica gel to give nine known natural products and the new diterpene identified on the basis of spectroscopic data analysis. From the aerial parts of *S. clevelandii,* seven known diterpenes were obtained and identified by comparison with the spectroscopic data found in the bibliography. The antiproliferative activity of the isolated metabolites was evaluated *in vitro* against A2780 ovarian cancer, SW1573 non-small-cell lung cancer, WiDr colon cancer, T-47D breast cancer, and HBL-100 breast cancer cells. Finally a series of β-amino alcohol analogs of sugiol were synthesized in a straightforward manner. The *in vitro* cytotoxic activities were examined, and the most potent analogs induced considerably growth inhibition in the range of 1.5 – 6.7 μM.

PI337 Anti-inflammatory and PPAR transactivational effects of oleanane-type triterpene saponins from the stem bark of *Kalopanax pictus*

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Eight new (**1 – 5** and **16 – 18**), and 15 known compounds (**6 – 15** and **19 – 23**) were isolated from the stem bark of *Kalopanax pictus*. The structures of new compounds were identified to be 16,23,29-trihydroxy-3-oxo-olean-12-en-28-oic acid (**1**), 4,23,29-trihydroxy-3,4-*seco*-olean-12-en-3-oate-28-oic acid (**2**), 3β,6β,23-trihydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranoside (**3**), 3-*O*-[2,3-di-*O*-acetyl-α-L-arabinopyranosyl]hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**4**), 3-*O*-[3,4-di-*O*-acetyl-α-L-arabinopyranosyl]hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**5**), 6β,16α-dihydroxy-hederagenin 3-*O*-β-D-glucuronopyranoside (**16**), 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranosyl-6β,16α-dihydroxy-oleanolic acid (**17**), and 3-*O*-β-D-galactopyranosyl(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**18**). Compounds **1 – 5**, **7 – 14**, **16**, **17**, **20**, and **23** inhibited TNFα-induced NF-κB transcriptional activity and decreased COX-2 and iNOS gene expression in HepG2 cells. Compounds **9**, **11**, **12**, **14**, **16 – 18**, **20**, and **23** upregulated PPARs transcriptional activity in HepG2 cells. Furthermore, the transactivational effects of the compounds on three individual PPAR subtypes, including PPARα, γ, and β(δ) were also evaluated.

PI338 Anti-carcinogenic and inhibitory effects on cytokine secretion of *Veronica lycica* E. Lehm. in human colon cancer cell line

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We studied anti-carcinogenic and anti-inflammatory activities of *Veronica lycica* plant. We observed such activities in two different extracts from the plant, methanolic and water, at 10 – 1000 μg/ml concentrations, in colon cancer cell line (Caco-2) and colorectal cancer cell line (CCL-221) using Trypan Blue Exclusion Test. We analyzed the anti-inflammatory activity of the extracts on interleukin 8 (IL-8) and interleukin 6 (IL-6) secretions after stimulating the cancer cell by tumor necrosis factor-α (TNF-α). We used different concentrations of the plant, ranging from 10 to 1000 μg/mL. We observed that the most effective anticancer activity was at 1000 μg/ml concentration. At 1000 μg/mL concentration, the methanolic extract showed anti-carcinogenic properties with maximum inhibition of 65% in CCL-221 cell, and maximum inhibition of 47% in Caco-2 cell. We observed no cytotoxic effect against normal cell line (Human fibroblast). Finally, we observed that IL-8 decreased from 519.07 pg/ml to 32.9 pg/ml with water extract of *V.lycica,* to 57.15 pg/ml with its methanol extract in ccl_221, IL-6 decreased from 63 pg/ml to 1pg/ml with the water extract, and to 2 pg/ml with methanol extract. These results showed that *V.lycica* could be used in pharmaceutical applications because of its remarkable anticancer and anti-inflammatory effects on CCL-221 and Caco-2 cancer cell lines.

PI339 Lignans from *in vitro* cultures of transgenic roots of *Taxus x media var. Hicksii*

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Recently, the *in vitro* cultures of yew transgenic roots have been demonstrated to be very potent as a source of paclitaxel and other taxanes. Lignans, dimeric phenylpropanoids, showed a great number of pharmacological effects such as antibacterial, antifungal, antiviral, antioxidant, anticancer, and anti-inflammatory. The presence of matairesinol, lariciresionol and pinoresinol has been investigated in three lines of *Taxus* transgenic roots. Two of them (ATMA and ATM) are carring additional taxadiene synthase gene (*txs*) while the third one – KT has been obtained after transformation with LBA 9402 *Agrobacterium rhizogenes* strain. The content of lariciresinol, pinoresinol and matairesinol has been examined in hairy roots cultivated in hormone free DCR-M medium or DCR-M medium supplemented with methyl jasmonate (MeJA) or with MeJA and L-phenylalanine (PHEN). The addition of MeJA to the medium resulted in the highest content of analyzed lignans. The highest yield of matairesinol (21 μg/g DW), pinoresinol (17 μg/g DW) and lariciresinol (1 μg/g DW) has been determined in hairy roots of KT line. Among two root lines carring *txs* gene, line ATM accumulated higher amounts of lignans: 19 μg/g DW, 13 μg/g DW and 2 μg/g DW, respectively.

PI340 Proteolytic activity in plant latices as a chemotaxonomic marker and a target for phylogentic studies

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More than 40 different plant families present proteolytic activity in latices. Plant proteases of latices are described for *Apocynaceae* (13), *Asclepiadaceae* (11), *Asteraceae* (1), *Caricaceae* (6), *Convulvalaceae* (1), *Euphorbiaceae (*64) and, *Moraceae* (62). Most of them belong to serine or cysteine endopeptidases family. In the order *Asterales* six species are known to present proteolytic activity, but the consideration refer to different plant parts. *Campanulaceae* are not yet investigated for presence of proteases. In our Investigation we verified the occurrence of proteolytic activity in latex of species of *Asteraceae* and *Campanulaceae.* We want to explore in particular the occurrence of serine proteases with the aim to establish a chemotaxonomic marker and a potential target for phylogenetic studies. Proteolytic activity were determined by fluorescence based assay using BODY PY® FL- cassein (invitrogen™) as a substrate and in the following distinguished by application specific protease inhibitors. In both families serine proteases were determine and the occurrence are compared with available informations of phylogenetic studies.

PI341 Antimalarial natural products from *Picrorhiza scrophulariiflora*

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As part of a program aimed at identifying antimalarial natural products, we screened traditional Chinese herbs that are alleged to have antimalarial activity and are used in primary health care. This study used selected herb extracts in a biological assay against *Plasmodium falciparum*. The crude CH_2Cl_2 and MeOH extract of the rhizome *Picrohiza scrophulariiflora* Pennell shows high potency and has been investigated in detail employing the same bioassay to guide the isolation. Chemical investigation of the activity fractions of *P. scrophulariiflora* have lead to the isolation of 12 natural products, two of them are novel compounds. The structures of the 12 compounds have been fully characterized based on their NMR and MS spectroscopic data. The evaluation of their antimalarial activity is currently underway.

PI342 Phenolic compounds from *in vitro* cultures of *Rindera gareca* Boiss. & Heldr.

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Rindera graeca (Boraginaceae) is an endemic plant of South-East Europe and Mediterranean Basin, growing in rocky places of Greece. The presence of phenolic compounds has been analyzed in established *in vitro* cultures of shoots, natural and transgenic roots of *R.graeca*. The content of caffeic acid (CA), rosmarinic acid (RA), lithospermic (LA) and lithospermic B (LAB) acids has been investigated using HPLC-DAD method. RA has a number of interesting biological activities, e.g. antiviral, antibacterial, antiinflammatory and antioxidant. LAB, a tetrametric derivative of caffeic acid, exhibits the endothelium-dependent vasodilator and hypotensive. LA showed antioxidant effects. CA has not been detected in examined plant material while LAB (0.09 mg/g DW) has been found only in shoots. The highest content of RA (15 mg/g DW) has been also determined in shoots. Among investigated two lines of natural roots (RgKN and RgKN-NOA) and two lines of transgenic roots (RgKT7 and RgKT17) the highest yield of RA and LA have been noted in natural roots derived from growing *in vitro* seedlings (RgKN), 13 mg/g DW and 1 mg/g DW, respectively. In transgenic roots the RA and LA content was 9 mg/g DW and 0.66 mg/g DW, respectively.

PI343 *In vitro* anticariogenic effects of aerial parts of *Drymocallis rupestris* and its phytochemical profile

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Dental caries is an infectious disease which is widely distributed throughout the world and is the most prevalent chronic oral disease in humans. In this study, for the first time, we investigated *in vitro* inhibitory effects of *D. rupestris* extracts and fractions obtained with solvents of different polarity (aqueous, 50% ethanolic, diethyl ether, ethyl acetate and *n*-butanolic) against cariogenic *Streptococcus* spp. strains. It was found that diethyl ether fraction exhibited bacteriostatic and bactericidal activity against all the test bacteria at concentrations of 0.75 or 1.5 mg/mL. The diethyl ether fraction also inhibited the viability of mutans streptococci in biofilm. The inhibitory effects of all tested extracts of *D. rupestris* on water-insoluble and water-soluble glucan synthesis by cell-free GTFs from *Streptococcus* spp. were examined. Both, aqueous and diethyl ether preparations showed the highest anti-GTFs activity. Additionally, the phytochemical profile were analyzed where the high polyphenolics (total phenol, phenolic acids, tannins, proantocyanidins, flavonoids) content were found. The results demonstrate that *D. rupestris* could become a useful supplements for pharmaceutical products as a new anticariogenic agent in a wide range of oral care products.

PI344 Characterization of alkamide isomers as potential partial PPARγ agonists from the roots of purple coneflower

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Partial PPARγ agonists are believed not to promote the same magnitude of undesirable side effects as the insulin sensitizing drugs prescribed for the treatment of insulin resistance e.g. thiazolidinediones. Recent investigations of a *n*-hexane extract of the flowers of purple coneflower (*Echinacea purpurea*) led to the isolation of a new C_{16}-alkamide able to activate PPARγ with no concurrent stimulation of adipocyte differentiation, and the ability to increase insulin-stimulated glucose uptake in adipocytes [1]. In our search for further bioactive alkamides, the dichloromethane extract of the roots of *E. purpurea*, previously found to contain potential partial PPARγ agonists, was investigated. A bioassay-guided fractionation, based on insulin-stimulated glucose uptake of the extract, by flash CC and semi-prep HPLC yielded the known isomeric dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid 2-methylbutylamides that showed PPARγ activity in a dose-dependent manner from 1 to 8 μg/mL. Due to these promising results, testing of these alkamide isomers is in progress in insulin-stimulated glucose uptake and adipocyte differentiation bioassays. Other alkamides from the active fractions warrant further investigation for their bioactive properties. References: 1. Christensen KB et al. *J. Nat. Prod.* 2009; 72: 933 – 937.

PI345 Stimulating effects of cheonggukjang extract on macrophage-mediated immune responses

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Cheonggukjang is soybean paste fermented by *Bacillus subtilis* and a traditional healthy food in Korea. Here, we investigated the regulatory effects of Cheonggukjang extract on macrophage-mediated immune responses. When Cheonggukjang extract treatment was used in combination with interferon-γ (IFN-γ), there was a marked cooperative induction of nitric oxide (NO) and tumor necrosis factor-α (TNF-α) production in macrophages. Cheonggukjang extract increased the expression of inducible NO synthase mRNA, protein and TNF-α mRNA in macrophages.

These alterations of Cheonggukjang extract-treated cells were associated with the activation of nuclear factor-κB (NF-κB). Cheonggukjang extract increased the phosphorylation and transcriptional activity of p65 in macrophages. These results suggest that Cheonggukjang extract increased NO and TNF-α production through phosphorylation of p65 following IκBα degradation and NF-κB activation. Treating macrophages with pyrrolidine dithiocarbamate and BAY11 – 7082, an inhibitor of NF-κB, decreased the synergistic effects of Cheonggukjang extract. In conclusion, our results demonstrate that Cheonggukjang extract can effectively promote the activation of macrophages, suggesting that Cheonggukjang extract may possess the potential to regulate immune responses.

PI346 Effect of polyphenols from *Aronia melanocarpa* on the total oxidative and antioxidative bone status of cadmium-exposed rats

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Bone damage belongs to the main health effects of chronic exposure to cadmium and oxidative stress is involved in its pathogenesis. It was investigated whether polyphenolic compounds, possessing antioxidative properties, may improve the bone oxidative/antioxidative status under exposure to cadmium. Total oxidative (TOS) and antioxidative (TAS) status, and the level of oxidative stress (OSI = TOS/TAS) of the bone tissue at the distal femur (trabecular bone region) of the female Wistar rats administered as the only drinking fluid 0.1% water extract of polyphenolic compounds from the berries of *Aronia melanocarpa* or/and cadmium (1 and 5 mg/kg diet) for 3 and 10 months were estimated. The exposure to cadmium alone, dose and duration dependently, decreased the bone tissue antioxidative capacity and/or increased its oxidative status leading to the development of oxidative stress. The administration of polyphenolic compounds during cadmium exposure improved the oxidative/antioxidative balance of the bone tissue preventing oxidative stress. It can be concluded that consumption of polyphenol-rich products such as berries of *Aronia melanocarpa* under chronic exposure to cadmium may have beneficial impact on the skeleton via improving the oxidative/antioxidative balance of the bone tissue. This study was financially supported by the Grant (No. N N405 051140) from the National Science Centre (Poland).

PI347 HPLC-DAD/ESI-MS analyses of aqueous preparations from *Stachys thirkei* K. Koch and quantification of major phenolic components

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The purpose of the study was to screen phenolic constituents present in aqueous and ethanolic extracts prepared from the aerial parts of *Stachys thirkei* K. Koch, one of the 83 species growing wild in Turkey and which are used with similar purposes as sage in traditional medicines. A previously developed and validated RP-HPLC method for *Stachys* species has been used for decoction, infusion and ethanolic extract of *S. thirkei*. Qualitative analyses showed the presence of three different classes of phenolic constituents: caffeoylquinic acids (mainly chlorogenic acid), phenylethyl glycosides (acteoside and lavandulifolioside) and flavone glycosides (isoscutellarein derivatives). HPLC-ESI-MS analyses of the ethanolic extract led to the identification of 19 components. Their structure identification was realised by comparing their retention times, UV and MS spectra with those of previously isolated compounds from *S. cretica* and/or literature data. Phenylethyl glycosides are expressed as acteoside, whereas flavonoids as isoscutellarein-7-*O*-[6'''-*O*-acetyl-β-allopyranosyl-(1→2)]-β-glucopyranoside. The most abundant component in all three extracts have been found to be acteoside constituting 7.08 percent of the infusion.

PI348 Anti-inflammatory compounds isolated from *Diospyros bipindensis*

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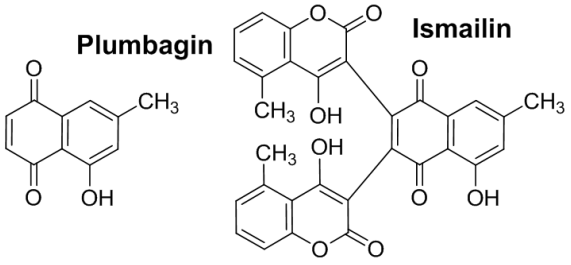
Diospyros bipindensis (Gürke) stem barks are used in Cameroon by pygmies Baka for the treatment of pulmonary diseases. A common process in all of these diseases is inflammation. Thus, we used the inhibition of NF-κB as a target. Bioassay-guided fractionation was performed on the active dichloromethane extract. A C_{18} reverse solid phase column was used to fractionate the extract in 5 fractions. Active fractions 3 and 4 were further separated by semi-prep HPLC to afford 4-hydroxy-5-methylcoumarin, plumbagin, canaliculatin, ismailin and betulinic acid as the main constituents. Plumbagin and ismailin inhibited NF-κB with an IC_{50} of 0.9 and 29.5 μM, respectively. Together with minor compounds, they could contribute to the anti-inflammatory activity of the extract. These results may support the traditional use of *Diospyros bipindebsis* for the treatment of pulmonary diseases.



PI349 Polyacetylenes from carrots with potential anti-diabetic effects

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In our continuous search for bioactive compounds with anti-diabetic effects, plant extracts were tested in a screening platform consisting of a series of bioassays: PPARγ transactivation, adipocyte differentiation, and glucose uptake. One promising extract was the dichloromethane extract of carrots (*Daucus carota*) that significantly activated PPARγ with a fold activation of 11.6% at 100 mg/mL compared to the vehicle (DMSO) with no or little effect on adipocyte differentiation and stimulated insulin-dependent glucose uptake. Carrots contain highly bioactive aliphatic polyacetylenes, which are also present in related vegetables of the Apiaceae family. These polyacetylenes are very similar in chemical structure to endogenous ligands for PPARγ and hence, may have important PPARγ activating properties. A bioassay-guided fractionation of the extract yielded several polyacetylene-rich fractions, which were all tested in the screening platform and found to be active. Individual polyacetylenes from the active fractions were isolated by a combination of flash CC and semi-preparative HPLC, and characterized by LC-PDA-APCI-MS/MS and NMR spectroscopy. The most bioactive polyacetylenes were falcarinol and falcarindiol, which were able to explain most of the observed bioactivity of the carrot extract.

PI350 Secondary metabolites from the aerial parts of *Centaurea pannonica*

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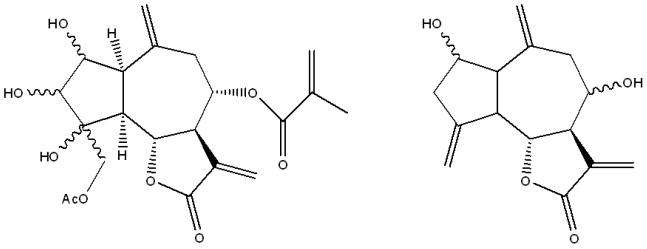
Further investigation of *Centaurea pannonica* (Heuffel) Simonkai [collected in Šumadija region-Serbia, on September 2008], afforded the new sesquiterpene lactone (**1**), compound **2**, previously described, as well as eleven known guaianolides, namely, babylin A, babylin B, chlorohyssopifolin C, repin, repdiolide, epoxy-repdiolide, chlororepdiolide, cebellin J, janerin, 19-deoxyjanerin, rhaposerine, three known lignans arctigenin, matairesinol, arctiin, three known flavonoids, apigenin, hispidulin, diosmetin and one known phenyl propanoid glucoside, syringine. The presence of guaianolides is characteristic feature of *Centaurea* taxa belonging to the section Jacea, where is classified *C. pannonica* [2, 3].



PI351 Use of water-soluble polysaccharides from mango (*Mangifera indica* L.) fruits as alternative source of mutanase inductors

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The fruits of mango have been suggested as a new alternative to streptococcal mutan for the mutanase induction in *Trichoderma harzianum*. The water-soluble polysaccharide was obtained from the mango fruits with the yield of 16.3%. Structural analyses revealed that this polymer contained of (1→4)-α-, (1→3)-α-linked Glc*p*. When the strain *T. harzianum* CCM F-340 was grown on glucan preparation from mango, maximal mutanase productivity obtained after 3 days' cultivation was 33.7 mU/mL. Additionally, using "real-time" RT-PCR, a 6-fold higher expression for mutanase was determined in the presence of this stimulus. Mutanase in a mixture with commercial dextranase showed a high hydrolytic potential in decomposition of native mutan, where maximal degrees of saccharification and solubilization of this biopolymer (60 and 90%, respectively) were reached in 6 h at 45°C. The mutanase preparation was also effective in mutan removal from oral biofilms, especially in a mixture with dextranase (100% hydrolysis in 6 h).

PI352 Towards the biomimetic synthesis of bipleiophylline

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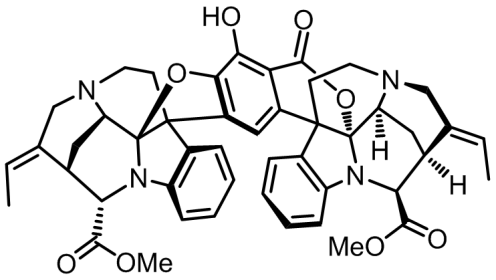
Bipleiophylline is a cytotoxic indolomonoterpenic alkaloid recently isolated from the bark of *Alstonia angustifolia* (Apocynaceae).[1] This is probably one of the most complex indole structures that can be encountered in nature. Of particular interest for us is the assembly of the aromatic central core of bipleiophylline that we wish to mimic in a biomimetic way. The progress towards the total synthesis of this intriguing natural substance in which we are currently engaged will be presented.



bipleiophylline

[1] T-S. Kam, S.-J. Tan, S.-W. Ng, K. Komiyama, *Org. Lett*, **2008**, *10*, 3749 – 3752.

PI353 Secondary metabolites from *Scutellaria rupestris* subsp. *adenotricha* (Boiss. & Heldr.) Greuter & Burdet

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Scutellaria rupestris subsp. *adenotricha* (Boiss. & Heldr.) Greuter & Burdet (Lamiaceae) is an herbaceous perennial plant, endemic to Albania and Greece [1]. The methanol extract of the aerial parts afforded eight iridoids, namely catalpol, albidoside, scutellarioside II, dihydrocatalpogenine (C-1) α-epimer/β-epimer, globularin, globularidin, mussaenosidic acid, two known phenylethanoid glycosides, acteoside, martynoside, and one known phenolic derivative, *E-p*-coumaroylglucoside. The chemical profile of this taxon shows many similarities to that of previously investigated *S. albida* subsp. *albida* [2,3], *S. albida* subsp. *colchica* [4] and *S. goulimii* [5], of the *S. albida* group. **References:** 1. Bothmer, R. (1985) Nord. J. Bot. 5: 421 – 439. 2. Gousiadou, Ch. et al. (2007) Phytochemistry, 68: 1799 – 1804. 3. Gousiadou, C. et al. (2012) J. Enzyme. Inhib. Med. Chem. doi:10.3109/14756366. 4. Çalis, I. et al. (1993) Phytochemistry 32: 1213 – 1217. 5. Gousiadou, C. (2012) BSE. 43:139 – 141.

PI354 Antioxidant capacity of fruits elderberries (*Sambucus nigra* L.) and black chokeberry (*Aronia melanocarpa* Wild.)

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Increased accumulation of free radicals involves in the pathogenesis of many civilization diseases (e.g. cancers). Intake of antioxidants can provide protection against the harmful effects of free radicals. In order to maintain health is therefore very important to ensure efficient supply of the body with specific antioxidants. It seems that antioxidants can protect biomolecules before oxidative damage, therefore are a lower risk of cardiovascular disease and cancer. The antioxidant capacity of fruits elderberries (*Sambucus nigra* L.) and black chokeberry (*Aronia melanocarpa* Wild.) was determined by the $DPPH^+$ method. The samples were diluted with methanol by the method of Šeršeň and Grančai (2008). Reduction of free radicals in the presence of $DPPH^+$ radical sample is spectrophotometrically manifested by increasing of absorbance at a wavelength 515 nm. The absorbance values of samples depending on the dilution and followed the dynamics of addiction and the absorbance of the antiradical capacity were measured in this study. The value of SC_{50} was calculated on the basis of observed values. The value of SC_{50} was

17.74 in the fruits of elderberry (*Sambucus nigra* L.) and 26.65 in black chokeberry (*Aronia melanocarpa* Wild.).

PI355 A new diterpenoid from *Salvia adenophora* Fernald (Lamiaceae)

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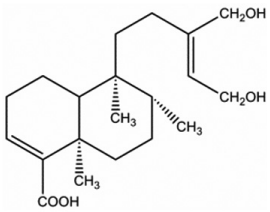
The surface exudate obtained by rinsing the aerial parts of *Salvia adenophora* with CH_2Cl_2 was subjected to repeated column chromatography on Sephadex LH-20 and silica gel and to HPLC-MS followed by semi-preparative RP-HPLC. Some known diterpenes have been isolated together with a new clerodane diterpenoid **1**, identified by IR, NMR, including TOCSY, COSY, HSQC, HMBC and ROESY experiments, ESI-TRAP-MS and HR-MS analysis.



1

PI356 The chemical profile on pyrrolizidine alkaloids of selected endemic Greek boraginaceae plants of *Onosma* genus

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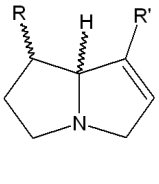
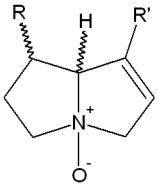
In the framework of our studies on Greek Boraginaceae plants, we report here the phytochemical analyses on the extracts of three endemic plants of *Onosma* genus (*Onosma leptantha* Heldr., *O. erecta* Sibth.& Sm., and *O. kaheirei* Teppner). Chemical isolations were focused on pyrrolizidine alkaloids (PAs) content, as their corresponding N-oxides (PANOs) or as free bases of the acyclic mono- and di-esteric type. The PANOs were structurally determined through modern spectral data (NMR, MS) and five new compounds were identified while the free bases PAs were detected by GC-MS on the basis of their RI values, molecular ion peaks and MS fragmentation pattern.



PAs



PANOs

PI357 UHPLC-LTQ-ORBITRAP based identification and HSCCC isolation of antifungal components from *Platanus SP.* (Platanaceae)

Thai QD[1,2], Tchoumtchoua J[1], Mitakou S[1], Michel S[2], Deguin B[2], Halabalaki M[1], Skaltsounis AL[1]
[1]Department of Pharmacognosy and Natural Products Chemistry, University of Athens, 15771, Athens, Greece; [2]Laboratoire de Pharmacognosie UMR 8638 CNRS-Université Paris Descartes, 4 rue de l'Observatoire, Paris, France

Platanus is a small genus of trees belonging to Platanaceae family, mostly found to urban settings and especially in riparian or wetland regions. *Platanus* species, and especially *Platanus orientalis* (Oriental plane) are known to be severely attack by plant pathogens such as *Ceratocystis fimbriata f. sp. Platani* for canker stain, *Apiognomonia veneta* for anthracnose and *Microsphaera platini* for powdery mildew. However *Platanus acerifolia* (London plane), a hybrid between the *P. occidentalis* and *P. orientalis*, have found to be resistant to these pathogenic fungi. Therefore, this work aimed to the phytochemical screening of *P. acerifolia* and *P. orientalis* and the identification of the components potentially responsible for the resistance of *P. acerifolia* using a UHPLC-PDA system hyphenated to a LTQ-Orbitrap MS. Secondly, a target isolation of these components, along with the major compounds of both plant extracts were performed using a HSCCC system and various chromatographic techniques (MPLC, CC, Sephadex LH-20, prep-TLC). Two new compounds namely isobraylin and platadihydrochacone as well as five known coumarins, six flavonoids and two triterpenoids were isolated from these plant extracts. Their structure was confirmed using 1D & 2D-NMR techniques. Both plants found to be rich in coumarins, with significant quantitative alternations and seem to be responsible for the antifungal activity.

PI358 Bioactivity of *Kalopanax septemlobus* extracts for functional materials

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Kalopanax septemlobus are used to medical purposes in Easton Asia. In a survey of biological activities natural products, the acetone extract from stem of *K. septemlobus* (SK), leaves of *K. septemlobus* (LK), stem of thornless cultivar of *K. septemlobus* (SCK), leaves of thornless cultivar of *K. septemlobus* (LCK), were investigated for the activities of anti-oxidation, anti-inflammatory, whitening and anti-wrinkle effect to apply as a functional natural ingredient. The electron donating ability of LCK was over 92% at 1000ug/ml. the superoxide anion radical scavenging inhibition effect of SCK was over 92% at 1000ug/ml. The ABTS radical cation decolorization activities of SK, LK, SCK and LCK were over 98% at 500ug/ml. The hydrogen peroxide scavenging activities of SK, LK, SCK and LCK were over 99% at 1000ug/ml. The Nitrite scavenging ability of LCK-E was over 58% at 1000ug/ml. The collagenase inhibition activity of LCK-E was over 82% at 1000ug/ml.

PI359 Lipid peroxidation and inflammatory cytokines inhibition effect of *Rhus verniciflua* extract *in vitro*

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[1]Department of Physical Education, Kyungpook National University, Deagu 702 – 701, Korea; [2]Department of Forest genetic resources, Korea Forest Research Institute, Suwon 441 – 350, Korea,

For this study, Sprague-Dawley rats were divided into 4 groups; sedentary (SED), exercise training (TRA) RVS extract ingestion (RVE) and RVS extract ingestion and exercise training (RVE-TRA). In order to analyze antioxidant function, blood SOD, GSH-Px, and MDA were examined. And, analysis of inflammatory cytokines sere examined using IL-6, TNF-a, CRP, and NO. SOD in TRA was significantly higher than SED and RVE ($p < 0.05$), and RVE-TRA was highest among the groups ($p < 0.05$). The MDA content of TRA, RVE and RVE-TRA were significantly lower than SED and RVE-TRA were significantly lower than other groups ($p < 0.05$). IL-6 and TNF-a content lower among group ($p < 0.05$). Finally, NO concentration of SED and TRA were higher than RVE and RVE-TRA ($p < 0.05$).

PI360 Secondary metabolites from the aerial parts of *Stachys tetragona*, a Greek endemic species

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Continuing our research on the chemical constituents of *Stachys* sp., we report here the results of the investigation of *Stachys tetragona* Boiss. & Heldr., a Greek endemic species growing wild to Evia island (Aegean Sea), belonging to the subsection Olisia [1]. Greece is an area particularly rich in taxa [2]. The dichloromethane extract of the aerial parts yielded oleanolic acid, stigmasterol and β-sitosterol; while the methanol extract afforded isoscutellarein 7-*O*-[6'''-O-acetyl-β-D-allopyranosyl (1→2)-β-D-glucopyranoside, isoscutellarein 7-*O*-[6'''-O-acetyl-β-D-allopyranosyl (1→2)-6''-O-acetyl-β-D-glucopyranoside, kaempferol, caffeic acid, acteoside, leucosceptoside A, forsythoside B, stachysoside D, lamiophlomiside, betonyoside F, (7*S*, 8*R*)-urolignoside, 8-acetyl-harpagide, 5-*O*-β-allopyranosyloxy-monomelittoside. The structure of the compounds was elucidated by NMR spectroscopy. Concerning the flavonoid content, *S. tetragona* revealed to possess similar profile to *S. ionica* Halácsy, a species wholly confined to the islands of the Ionian Sea [3]. **References:** 1. Bhattacharjee, R. (1980) Taxonomic studies in *Stachys*: II. A new infrageneric classification of *Stachys* L. Notes from the Royal Botanic Garden Edinburgh 38: 65 – 96. 2. Greuter, W. Burdet, H. M., Long, G. (1986) Med-Checklist, Vol. 3. Editions des Conservatoire et Jardin botaniques de la Ville de Genève, Genève. 3. Meremeti, A. et al. (2004) BSE, 32: 139 – 151.

PI361 Phytoestrogens from Genista halacsyi (Leguminosae)

Fokialakis N[1], *Aligiannis N*[1], *Alexi X*[2], *Alexis MN*[2], *Pratsinis H*[3], *Kalpoutzakis E*[1], *Miatkou S*[1]
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The genus *Genista* is remarkably rich in isoflavones, which consider to be the most well studied phytoestrogens. In continuation of our research for new phytoestrogens, the aerial parts of *Genista halacsyi* Heldr., an endemic plant of Greece, was studied. Firstly, by classical separation methods several isoflavones were isolated namely: genistein, daidzein, biochanin, 8Meo-formononetin, isoprunetin, 3'OH-isoprunetin, genistin, 8C-glucopyranosylgenistein, 8C-glucopyranosylorobol and 8C-glucopyranosyl-4'glucopyranosylgenistein. In continuation, high-speed countercurrent chromatography (HSCCC), a chromatographic technique that eliminates irreversible absorption of the sample onto solid support with an excellent sample recovery was used to isolate larger amounts. The estrogenic activity of all isolated compounds was tested using estrogen receptor-positive MCF7 and estrogen receptor-negative MDA-MB-231 cell lines. Their affinity for binding to estrogen receptors α (ERα) and β (ERβ), was also tested. In addition, we assessed their ability to promote the proliferation of estrogen receptor positive MCF7 breast carcinoma cells and the induction of Alkaline phosphatase activity in Ishikawa endometrial carcinoma cells, as well as, their ability to regulate the transcription of ERE-dependent reporter genes. We concluded that the tested compounds exhibit substantial estrogenic activity at concentrations of 1 – 10 μM.

PI362 Pharmacological screening of medicinal plants for anti cancer, anti inflammatory and anti diabetic activities

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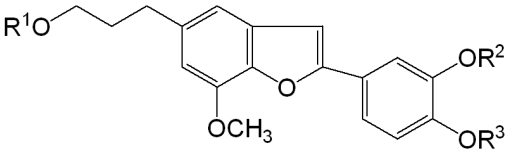
We report a detailed Pharmacological Screening of 11 Medicinal plants (36 samples) growing in Tabuk region of Saudi Arabia for Cytotoxicity against HeLa S3 Cells, Anti-inflammatory activity (Raw cells) and Adipogenesis. Three plant samples were found Cytotoxic against HeLa S3 cells namely Commiphora Africana (16.9%), Raetam ratam plant (8.2%), Raetam ratam root (3.8%). Six plant samples were found active as Anti-infammatory namely Artemisa seberi (28 – 0%), Commiphora africana (6 – 3%), Raetam ratam (20 – 6%), Citrullus colocynthsis plant (33.7%), Citrullus colocynthsis seed (40 – 1%), Ocimum bacillum (45.4%). Three plant samples were found with inhibitory effect on Adipogenesis namely Citrullus colocynthsis, Raetam ratam and Fagonia indica. We have also isolated several biomolecules responsible for all the three activities mentioned above, which will be presented.

PI363 Evaluation of the schistosomicidal activity of *nor*-neolignans from *Styrax pohlii*

Soares E[1], *Bertanha CS*[1], *de Oliveira PF*[1], *Gonçalves UO*[1], *Magalhães LG*[1], *Rodrigues V*[2], *Silva MLA*[1], *Tavares DC*[1], *Januário AH*[1], *Pauletti PM*[1]
[1]*Universidade de Franca, Av. Dr. Armando Salles de Oliveira, 201, 14404 – 600, Franca, São Paulo, Brazil;* [2]*Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14049 – 900, Ribeirão Preto, São Paulo, Brazil*

Styrax pohlii A. DC belongs to the family Styracaceae, which is known in Brazil as 'benjoeiro', 'estoraqueiro', 'árvore-de-bálsamo', 'pindaíba', 'pindaubuna' or 'pindauvuna'. This specie is commonly employed in folk medicine to relieve fever. The present study investigated the chemical composition of the bioactive EtOAc fraction of *S. pohlii* aerial parts, as well as schistosomicidal and cytotoxicity activities. The purification procedure resulted in the isolation of compounds **1**-**5**. The bioassay results indicated that **1**, **3**, **4** and **5** are able to separate coupled *S. mansoni* adult worms at 100 μM. Although, assayed compounds did not kill the adult schistosomes *in vitro*. Furthermore, in assayed concentration **1**, **3**, **4** and **5** were not cytotoxic in the cell line GM07492A (Human lung fibroblast).



	R^1	R^2	R^3
1	H	-CH_2-	
2	H	CH_3	CH_3
3	6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl	CH_3	CH_3
4	β-D-glucopyranosyl	CH_3	CH_3
5	6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosyl	-CH_2-	

PI364 Phenolic compounds from Taraxacum bessarabicum (Hornem.) Hand.-Mazz. subsp. bessarabicum

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The genus *Taraxacum* is a member of the family Asteraceae, subfamily Cichorioideae, tribe Lactuceae. The total number of *Taraxacum* in Turkey at present is 43 species. In folk medicine, this genus has been utilized for the treatment of various diseases such as dyspepsia, heartburn, spleen and liver complaints, hepatitis and anorexia. *Taraxacum bessarabicum* is a perennial herb growing Eastern Turkey. The isolation of sesquiterpene lactones and two phenolics has previously been reported from the roots of this species. In this work coumarins (esculetin, cichorin), flavonoids (luteolin, luteolin- 7-O-glucoside, gossypetin) and phenolic acids (p-coumaric acid, caffeic acid, ferulic acid, chlorogenic acid methyl ester, 3,5-dicaffeoylquinic acid, 3,5- dicaffeoilquinic acid methyl ester) have been isolated from the EtOAc and $CHCl_3$ fractions of MeOH extract from the aerial parts of *Taraxacum bessarabicum* subsp. *bessarabicum* collected from Erzincan, East Anatolia. Column chromatography and preparative thin layer chromatography were used for separation of these compounds. Their structures were established conclusively by UV, ESI-MS, 1-D and 2-D NMR spectra analyses and comparison with literature data. The presence of these compounds has been shown for the first time from this species. Gossypetin, chlorogenic acid methyl ester, 3,5-dicaffeoilquinic acid methyl ester are new for the genus *Taraxacum*.

PI365 Flavonoids from the aerial parts of *Satureja khuzestanica*

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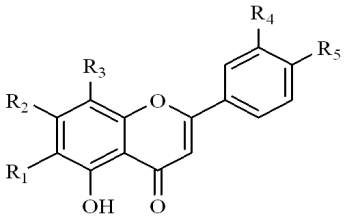
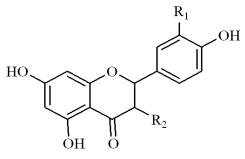
[1]Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; [2]iMed.UL, Faculty of Pharmacy, University of Lisbon, Av. Professor Gama Pinto, 1649 – 019 Lisbon, Portugal

Satureja khuzestanica Jamzad is an endemic plant, widely distributed in the southern part of Iran. Decoction of the aerial part of this species is a well-known herbal traditional medicine used as analgesic, anti-inflammatory, antidiabetic and antimicrobial agents. In this study, we reported isolation, purification and structural elucidation of the main flavonoids from the above mentioned medicinal plant. Xanthomicrol (1), cirsilineol (2), 6-hydroxyluteolin 7,3'-dimethyl ether (3), cirsimaritin (4), diosmetin (5), acacetin (6), apigenin (7), naringenin (8), aromadendrine (9) and taxifolin (10) were the main flavonoids which have not been previously reported in this species.





No	R1	R2	R3	R4	R5
OMe	OMe	OMe	H	OH	
2	OMe	OMe	H	OMe	OH
3	OH	OMe	H	OMe	OH
4	OMe	OMe	H	H	OH
5	H	OH	H	OH	OMe
6	H	OH	H		
7	H	OH	H	H	OH
8	H	OH			

No	R1	R21
9	H	OH
10	OH	OH

PI366 Evaluation of natural products as potential agrochemical agents with insecticide, fungicide and herbicide activities

Dumontet V[1], Litaudon M[1], Olivon F[1], Poullain C[2], Rasoanaivo P[3], Stien D[1], Eparvier V[1], Houël E[4], Fokialakis N[5], Halabalaki M[5], Skaltsounis AL[5], Espinosa A[6], Olmedo D[6], Gupta M[6], Fouche G[7], Hamburger M[8], Sorgenfrei O[9], Breuninger D[9], Guéritte F[1]
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The present work aims to identify new promising plant sources, which could be exploited for their agrochemical properties. A total of 484 natural products from academic libraries were selected for screening against four fungal pathogens, five insects and two plants. On the basis of the hits founded and a literature survey, the flora of source countries (New Caledonia, French Guiana, Madagascar, Panama, South Africa and Greece) was analysed for plants containing the desired scaffolds. Lists of 1800 plant part samples were thus established. The plant parts collected generated 3600 extracts that are being evaluated.

PI367 Estrogenic and anti-estrogenic compounds from Thai medicinal plant, *Cleome gynandra*

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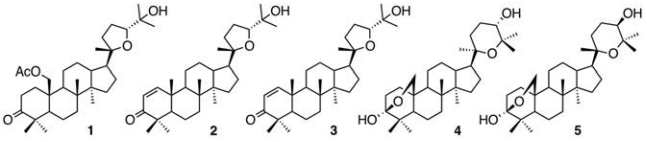
An edible Thai plant, *Cleome gynandra* L. (Capparaceae) was investigated for its estrogenic and antiestrogenic compounds as it has been traditionally used for improvement of menstrual dysfunctions in Thailand. The dried aerial parts of *C. gynandra* (1.7 kg) were extracted with methanol. The extract (150 g) was applied for bioassay-guided isolation by using estrogen-responsive cancer cells MCF-7 and T47D. From the extract, novel dammarane triterpenes (**1** – **5**) were isolated with some known compounds. Their structural characterization will be presented with their estrogenic and anti-estrogenic activities.



PI368 Exploration of the mechanism of action of alkaloids with antiparasitic activities from *Muntafara sessilifolia*

Girardot M[1 – 2], Deregnaucourt C[1], Imbert C[2], Rasoanaivo P[3], Mambu L[1]
[1]UMR 7245 CNRS-MNHN National Museum of Natural History, 75005 Paris, France; [2]UMR CNRS 7267 Faculty of Medicine Pharmacy, 86034 Poitiers, France; [3]LPAMIP, Malagasy Institute of Applied Research, 101 Antananarivo, Madagascar

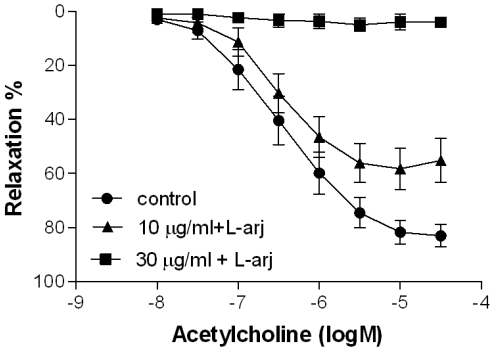
Muntafara sessilifolia (Baker) Pichon is an endemic plant of Madagascar whose stem-bark is traditionally used for the treatment of fevers. It was selected based on its inhibitory activity against the chloroquine-resistant strain FcB1 of *Plasmodium falciparum*. Bioassay-guided fractionation on *P. falciparum* led to the isolation and identification of 26 indole alkaloïds among which 11 were new compounds. Their antifungal activities were also evaluated. A strategy to clarify the modalities of the antiplasmodial action of products with noticeable activities was established. Thus, their hemolytic properties against healthy red blood cells, their inhibitory properties against geographical strains of *P. falciparum* with different levels of chloroquine sensitivity and the effect of human plasma on their antiplasmodial activity were evaluated. Also, the inhibitory effect of some compounds on the early intraerythrocytic development of *P. falciparum* was evaluated by optical examination of stained smears. As a result, two compounds, tabernaemontanine acetate and 3'oxotabernaelegantine A, showed interesting biological properties, in particular strain selectivity and late stage targeting. Those preliminary tests can thus be considered as an approach to the mechanism of action of compounds of interest.

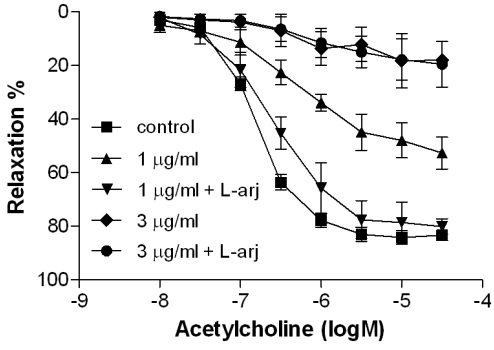
PI369 Inhibitory activity of *Verbascum latisepalum* Hub.-Mor. on endothelial nitric oxide synthase in rat thoracic aorta

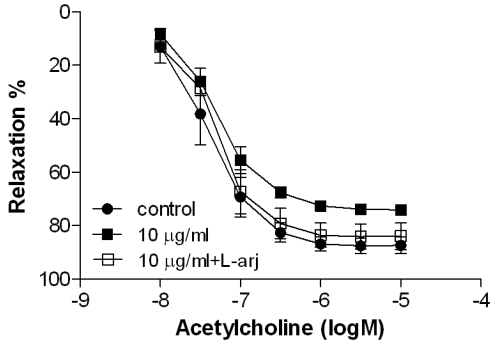
Tatli II[1], Bozkurt TE[2], Kahraman C[3], Sahin-Erdemli I[2], Akdemir ZS[3]
[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, Hacettepe University 06100, Ankara, Turkey; [2]Department of Pharmacology, Faculty of Pharmacy, Hacettepe University 06100, Ankara, Turkey; [3]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University 06100, Ankara, Turkey

The effect of the aerial parts of *Verbascum latisepalum* on nitric oxide synthase (NOS) enzyme in isolated rat thoracic aorta rings were evaluated in the present study. The inhibition of acetylcholine-induced relaxation responses in phenylephrine-precontracted tissues were examined in the absence and presence of the methanolic extract as well as the presence of L-arginine. The methanolic extract inhibited acetylcholine-induced relaxation responses in rat thoracic aorta which is attenuated by L-arginine. Through bioassay-guided fractionation and isolation

procedures phenylethanoid fraction and finally verbascoside was found to exert the same effect. This finding suggests that this inhibitory effect occurs through a NOS dependent mechanism.







PI370 Physicochemical and antioxidant properties of spray dried preparations from *Psidium guajava* L

Fernandes MRV, Azzolini AECS, Martinez MLL, Souza CRF, Lucisano-Valim YM, Oliveira WP
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This work evaluated the antioxidant activity and physicochemical properties of spray dried preparations (SDP) from leaves of *Psidium guajava* L. Different drying carriers, namely: maltodextrin, colloidal silicon dioxide, Arabic gum and β-cyclodextrin at concentrations of 40 and 80% relative to solids content were added to drying composition. SDP were characterized through determination of the total phenolic, tannins and flavonoid content. Antioxidant activity of the SDP was assessed by two assays: cellular test that measure the luminol-enhanced chemiluminescence (LumCL) produced by neutrophils stimulated with phorbol myristate acetate (PMA) and the DPPH radical scavenging. SDP containing 40% of maltodextrin showed the best result with respect to total phenolics (18.09% w/w), tannins (10.21% w/w) and flavonoid contents (16.56% w/w). SDP were effective inhibitors of the PMA-stimulated neutrophil function [IC_{50} (in μg/10^6cells) ranging from 5.42 to 6.50 μg/mL. LumCL (IC_{50} of quercetin = 1.67 μg/mL]. SDP showed antioxidant activities (IC_{50}) ranging from 6.16 to 10.31 μg/mL by the DPPH method (IC_{50} of quercetin = 0.96 μg/mL). The neutrophil reactive oxygen species generation, triggered by PMA and assessed by luminol was inhibited by SDP in a concentration-dependent manner, with insignificant toxicity to the cells under the tested conditions. Two chemical markers, catechin and quercetin which could be used in the monitoring of the quality of SDP were identified. Structural information of the compounds was obtained from the retention times, UV and mass spectra. In conclusion, SDP from *Psidium guajava* presented significant antioxidant activity with high potential as an active phytopharmaceutical ingredient for herbal medicine.

PI371 Cytotoxic and antimicrobial activity of Cynoglossum columnae Ten. *in vitro* roots

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Cynoglossum columnae Ten. (Boraginaceae) *in vitro* root cultures were established as producing naphthoquinone metabolites and investigated for cytotoxic and antimicrobial activity. The crude extracts of postcultured media showed cytotoxic activity against three cancer cell lines (HeLa, HL-60, HCT-116). The highest antimicrobial activity was observed against *Staphylococcus* spp., *Stenotrophomonas maltophilia* and *Candida* spp. strains. Two secondary metabolites of naphthoquinone type, present in the majority of tissues and postculture media, have been isolated and biologically investigated under the same conditions. The comparison of results showed 5O,6-(isohex-1-en-1,2-diyl)-2-methoxynaphthazarin as the most potent in cytotoxic activity towards all three investigated cancer cell lines (IC_{50} – 2,0 μg/ml for HL-60). The structure elucidation of second isolated naphthoquinone derivative is in progress.

PI372 Approaches to antiproliferatve agents from *Metaporana sericosepala* from the Madagascar dry forest

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The plant *Metaporana sericosepala* (Convolvulaceae) is endemic to Madagascar, and no phytochemical studies have previously been made on any members of the genus. In our ongoing research as part of the Madagascar International Cooperative Biodiversity Group (ICBG) program, a sample of *M. sericosepala* was collected in 2007 in the Diana region of northeast Madagascar. The plant was a climbing vine with white flowers, growing in the dry deciduous Amboahangibe Forest. An ethanol extract of its stems was found to exhibit anti-proliferative activity against the A2780 human ovarian cancer cell line, with an IC_{50} value of 20 μg/mL. Bioassay guided liquid-liquid partition, followed by C-18 solid phase extraction of the dichloromethane fraction (IC_{50} 14 μg/mL) and reverse phase C-18 HPLC led to the isolation of near-homogeneous fractions with moderate antiproliferative activity against the A2780 human ovarian cancer cell line. Final purification of these active fractions and structure elucidation of the purified bioactive compounds by 1D- and 2D-NMR spectroscopy are in progress, and the results of these studies will be reported.

PI373 Anti-ages activity screening of molecules isolated from Tanzanian clusiaceous species

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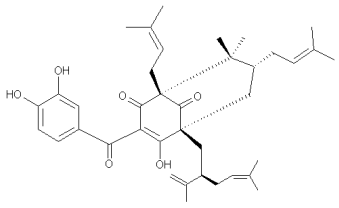
Advanced glycation end-products (AGEs) are involved in the progression of numerous pathologies such as diabetes or atherosclerosis. With the

objective of finding molecules with inhibitory effects on AGEs formation, we have conducted a phytochemical investigation on three Tanzanian Clusiaceous species: *Garcinia semseii, Garcinia volkensii* and *Allanblackia uluguruensis*. Several polyphenolic compounds exhibiting phloroglucinol moieties [*e.g.* polyprenylated benzophenones, such as guttiferone F (1) or biflavonoïds, such as morelloflavone (2)] were identified and their anti-AGEs activities were evaluated using a recently developed automated assay.



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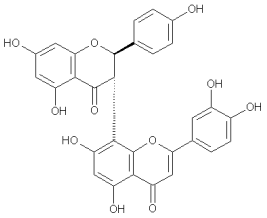

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PI374 MAO-A inhibitory activities of some benzophenones from *Gentiana* Spec

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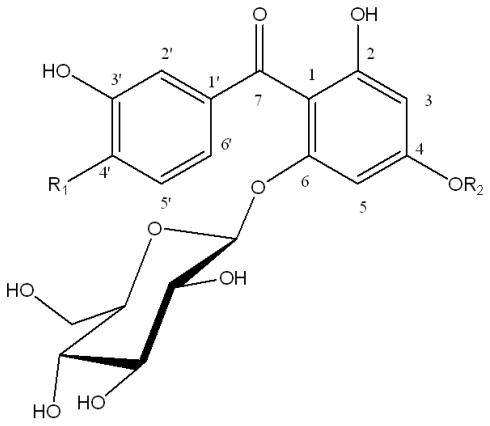
Inhibition of MAO-A predominantly affects neurotransmitters considered to be important in depression and anxiety disorders. Therefore, MAO inhibitors are expected to be useful in the therapy of psychosis, depression, schizophrenia, and so on (1). To date, MAO inhibitors such as coumarins, xanthones, flavonoids, piperine, and isoquinoline alkaloids have been isolated from natural sources or synthesized for the development of medicine (1 – 4). In this study MAO-A inhibition of benzophenone glucosides **1**, **2** and **3** isolated from *G. verna* L. subsp. *pontica* (Soltok.) Hayek. was evaluated. Compounds **1-3** showed significant inhibition on MAO-A with IC_{50}-values 1.2 – 34 μM.

1	R_1= H,	R_2= CH_3
2	R_1= OH,	R_2= H
3	R_1= H,	R_2= H



References: 1. Ohishi N, Suzuki T, et al. (2000) *J Molecular Catalysis B: Enzymatic* 10: 291 – 294. 2. Lee SA, Hong SS, et al. (2005). *Chem Pharm Bull* 53:832 – 835. 3. Hwang. J-S, Lee SA, et al (2005). *Archives of Pharmacal Research* 28: 190 – 194. 4. Han XH, Hong SS, et al (2007). *Arch Pharm Res* 30: 13 – 17.

PI375 Antioxidant and α-glucosidase inhibitory activities of Maple (*Acer* Spp.) bark extracts

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Maple (*Acer*) species have long been used as a source for maple syrup. In recent years, there has been a strong interest in investigating maple plant parts as a source of biologically active natural products. Recent phytochemical studies of sugar and red maple bark extracts have revealed the presence of a number of phenolics, including the new gallotannins, maplexins A-I, which exhibit strong α-glucosidase inhibitory activities. The present study focused on the polyphenolic content, antioxidant and α-glucosidase inhibitory activities of bark extracts of five maple species growing in the north eastern region of the United States: *A. saccharum* (sugar maple), *A. rubrum* (red maple), *A. platanoides* (Norway maple), *A. saccharinum* (silver maple), and *A. pseudoplatanus* (sycamore maple). There was a direct correlation between polyphenol content and antioxidant activity, with the red maple bark extract showing the strongest antioxidant activity and sycamore maple showing the weakest activity. In the α-glucosidase inhibitory assay, the red maple bark extract showed the best activity, consistent with the presence of maplexins.

PI376 Activities of extracts from saponin-containing plants on sheep erythrocytes, *Tetrahymena pyriformis* and Rumen protozoa

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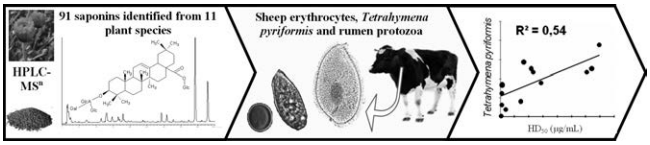
As the effects of saponins in the rumen are due to their membrane-disrupting ability on protozoa, the activities of extracts from saponin containing plants were determined on erythrocytes, *Tetrahymena pyriformis* and rumen protozoa. Inhibition of *Tetrahymena pyriformis* were found to be correlated (R^2= 0.54) with 50% hemolysis. The extracts supplemented to a standard feed, showed null to remarkable *in vitro* activity on rumen protozoa. With -51% and -41% protozoa inhibition, *Primula veris* and *Chenopodium quinoa* might have the potential to improve ammonia utilization in ruminants, meaning less excreted nitrogen and less environmental impact.



PI377 Screening for acetylcholinesterase inhibition of medicinal plants from Croatia

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Owing to their richness in secondary metabolites exhibiting remarkable diversity of both chemical structures and biological activities, medicinal plants are being recognized as promising sources of lead compounds for new drugs targeting neurodegenerative diseases. Alzheimer's disease is the most common age-related dementia with a steadily increasing prevalence, but its treatment still remains an area of significant unmet need, with therapies based largely on the acetylcholinesterase (AChE) inhibitors. Therefore, the present study aimed to evaluate selected Croatian medicinal plants belonging to the Lamiaceae family as a potential source of natural inhibitors of this target enzyme. The ethanolic extracts of 27 plant species were screened for their antiacetylcholinesterase activity by *in vitro* Ellman method at concentration range of 0.25 – 1 mg/

mL. Among them, remarkable inhibitory activity above 80% inhibition rate on AChE at 1 mg/mL showed the extracts of *Mentha x piperita* L., *M. longifolia* (L.) Huds. *Salvia officinalis* L., *Teucrium chamaedrys* L., *T. montanum* L., *T. polium* L. and *Thymus longicaulis* C. Presl. On the other hand, seven tested plant extracts (*Acinos arvensis* (Lam.) Dandy, *Calamintha sylvatica* Bromf., *Clinopodium vulgare* L., *Marrubium incanum* Desr., *Mentha pulegium* L., *Micromeria juliana* (L.) Benth. ex Rchb. and *Origanum vulgare* L.) did not reach the 50% inhibition of enzyme activity. The results of this study indicated that there is a great potential of Lamiaceae species growing in Croatia to provide novel drug leads for the treatment of Alzheimer's and other neurological disorders.

PI378 UPLC-HRMS based profiling of *Micromelum falcatum* extracts and isolation of coumarin derivatives

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Micromelum falcatum (Rutaceae) is a small tree growing in West Asian regions[1] used against cold and rheumatoid arthritis according to the traditional medicine of China[2]. The leaves of the plant, collected in Cambodia, were extracted following two extraction protocols and six different extracts were obtained. The analytical profiling of all extracts was performed using HPLC-DAD and LC-MS techniques leading to the detection of coumarin derivatives in the majority of the extracts. In parallel, a UPLC-ESI(+)-HRMS method using an Orbitrap analyzer was developed for the detection and characterization of coumarin derivatives in all extracts. All the compounds of interest were further isolated with semi-Preparative HPLC leading to the identification of seven coumarins among them three new natural products. The structure elucidation of all isolated compounds was performed via NMR and HRMS spectrometry and representative compounds have been evaluated for their anti-inflammatory activity. [1]Zhang Dianxiang, Thomas G. Hartley, Micromelum Blume Bijdr., Flora of China, 2008, 11, 79 – 80 [2]Xi-long Zheng, Fuwu Xing, Ethnobotanical study on medicinal plants around Mt.Yinggeling, Hainan Island, China, Journal of Ethnopharmacology 124, 2009, 197 – 210.

PI379 Evaluation of the safety of a tradicional herbal formulation with *Anacardium occidentale* bark

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Anacardium occidentale stem bark, commonly known as cashew bark, is a medicinal plant traditionally used in the Community of Portuguese Language Speaking Countries (CPLP) to treat different diseases including diabetes. The toxicity of a traditional recipe of this herbal medicine was evaluated through a repeated dose toxicity test. Three doses (40.2, 127, 402 mg/kg) of a traditional recipe of two types of *A. occidentale* (red and white), and water as control, where administered by gavage for a period of 14 days to mice. Daily evaluation of animal behaviour, body weight, feed and water intake was performed before sacrifice. No signs of degeneration, necrosis and inflammation were observed when the animals were sacrificed. Body weight variations were observed for both types of *A. occidentale*. No blood glucose level differences were observed at the end of the study. Kidney weight gain (8 – 10%) with red type of *A. occidentale* with all tested doses and significant liver weight loss (20%) for intermediate and higher dose of both types of *A. occidentale* was observed.

PI380 Abietane diterpenoids as butyrylcholinesterase inhibitors from *Salvia* species

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Salvia (sage) species have been used as medicinal plants since ancient times in the treatment of cold, sore throat, tuberculosis, angina pectoris, stomache ache, and menstrual disorders and they have been known as sedative, anti-depressant and memory enhancer agents since antiquity. In Europe, sage plants (namely *S. officinalis, S. lavandulifolia, S. fruticosa*) have traditional reputations that justify investigation for a potential role in reducing cognitive decline in the elderly. *Salvia* species (Lamiaceae family) are rich in abietane diterpenes and flavonoids and other phenolics which have antioxidant, antibacterial, antiviral, cytotoxic and antitumor properties. In this presentation, the anticholinesterase activity results of 50 abietane diterpenoids will be given which were isolated from a number of Anatolian *Salvia* species.They were investigated at 200 μM concentration against AChE and BChE enzymes by the Ellman method *in vitro*. The diterpenoids exhibited about 50% inhibition at least against one of the two cholinesterase enzymes, they were then subjected for the same test at the five different doses (12.5, 25, 50,100 and 200 μM) to find their IC_{50} values. Interestingly, most of them exhibited more or less activity against butyryl cholinesterase (BChE) enzyme, and among them, bractealine, 6-hydroxysalvinolone, royleanone 12-methyl ether, ferruginol and taxodione showed high activity with the IC_{50} values of 3.43, 17.21, 56.48, 17,49 and 7.73 μM, respectively, while their inhibition on AChE was found to be fairly weak. Hence, it was not observed a real correlation between the results against the two enzymes. Rationalization of the differences in the enzyme active sites has been performed using docking studies. Thus, abietane diterpenoids were found to be potent BChE inhibitors which might verify the ethnobotanical uses of *Salvia* extracts as memory enhancers.

PI381 Phytochemical and biological analysis of *Mallotus oppositifolius* (Euphorbiaceae)

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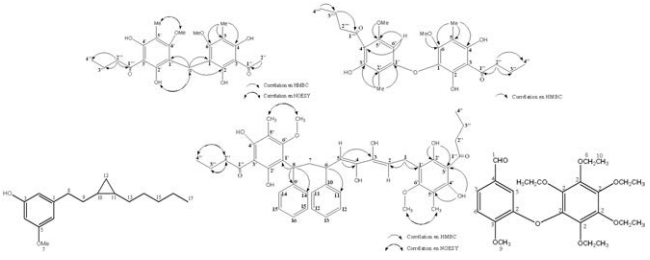
[1]UMR 8076 CNRS, Faculty of Pharmacy, University Paris-Sud, France; [2]Laboratoire de Chimie Organique Biologique, UFR Sciences des Structures de la Matière et de Technologie, Abidjan, Côte d'Ivoire

Mallotus oppositifolius (Geiseler) Müll. Arg., (Euphorbiaceae), is an endemic shrub from tropical Africa forests and savannas. It is widely used in popular medicine against infections, intestinal worms an malaria. Extracts of different parts of the plant have been shown to have anti-inflammatory, antioxidant, antidiarrheic, antibacterial, antifungal and antitrypanosomal properties. The present work reports the isolation of 32 compounds from the leaves, stem barks and roots. Among these compounds, six phenolic compounds are newly described. Biological assessment of these isolated compounds on *Trypanosoma brucei* and *Leishmania donovani* are described.



PI382 Biphenyl-type neolignans from *Magnolia officinalis*

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Reactive oxygen species (ROS) and granule proteases produced by human neutrophils contribute to the pathogenesis of inflammatory diseases. The MeOH extract of the stem bark of Magnolia officinalis showed potent inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Three new biphenyl-type neolignan derivatives, 5-allyl-5′-(1-hydroxyallyloxy)- biphenyl-2,2′-diol (1), 5,5′-diallyl-2′-(allyloxy)biphenyl-2-ol (2), and 5,5′-diallyl-2′-(3-methylbut- 2-enyloxy)biphenyl-2-ol (3), have been isolated from the stem bark of M. officinalis, together with 12 known compounds (4 – 15). Compounds 5 and 11 exhibited inhibition (IC 50 values ≤ 10.7 μM) of superoxide anion generation by human neutrophils in response to fMLP/CB.

PI383 Phytochemical investigations of *Euphorbia piscatoria*

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Euphorbia species are commonly named as spurge due to the use of the plant latex as purgative in traditional medicine. Additionally, they are also used to treat tumors and warts. Plants from this genus are able to synthetize a unique profile of diterpenes. Many of these compounds have shown interesting biological activities, making them promising leads for drug discovery. Compounds with tigliane, ingenane and daphnane skeletons have been associated with toxicity and skin-tumor promotion. On the other hand, jatrophane and lathyrane-type macrocycle diterpenes are non-irritant, being effective as anti-tumor agents by multidrug resistance reversion and apoptosis induction. Moreover, the polycyclic diterpenes with the ent-abietane skeleton showed to have antiproliferative activity against resistant cancer cells. The aerial parts of *Euphorbia piscatoria* Ait., an endemic species from Madeira archipelago, have been investigated. From the methanolic extract, the following diterpenes were isolated: four with the lathyrane skeleton, five ent-abietane lactones and one with the ent-atisane skeleton. Furthermore, one triterpene and several phenolic compounds were isolated. The structures of the compounds were characterized by spectroscopic methods mainly1D NMR (^{1}H, ^{13}C, DEPT) and 2D NMR (COSY, HMBC, HMQC, NOESY).

PI384 Bioactive constituents of Sacoglottis gabonensis, a tropical forest tree consumed by monkeys, apes and humans

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Sacoglottis gabonensis is a red brown tree, and only African representative of the Humiriaceae. The tree grows in wet, swampy areas of humid forest along the coastal region of west-central Africa. Tree products are used as food, building materials, and in traditional medicine by local peoples. For example, bark extract is used to treat ailments such as gonorrhea and as an emetic while other ingredients are used in the fermentation of palm wine. The major active compound from the stem bark is known to be bergenin which suppresses lipid peroxidation. Even though some phytochemical research has been performed on the bark of this tree, the fruit is not yet studied. The hexane and/or chloroform extracts of the fruit of *S. gabonensis* exhibited significant activity in the MTP, NF-κB, ROS, and Sema 3B assays. In this research, biological active compounds from the fruit of *S. gabonensis* were isolated, characterized, and identified.

PI385 Structural characterization of steroidal saponins from *Dioscorea* species using UHPLC-QTOF-MS

Avula B[1], Wang YH[1], Wang M[1], Ali Z[1], Smillie TJ[1], Zweigenbaum J[2], Khan IA[1,3]
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Yam (*Dioscorea* spp.) is an important tuber plant for edible and medicinal use to promote health and longevity in chinese tradition. In this work, the structural characteristics of the steroidal saponins from the dried rhizomes of *Dioscorea* species have been identified using UHPLC/TOF-MS in both negative and positive ion modes. The fragmentation patterns of reference standards were investigated and the steroidal saponins in the extracts were identified or tentatively characterized according to the retention times, and MS data. It also provides an excellent approach for rapid screening of steroidal saponins from plant extracts. Protodioscin was used as an example to discuss the fragmentation patterns in detail. In (-)-ESI-MS, gave $[M-H]^-$ ions at *m/z* 1047.53 and in (+)-ESI-MS, the mass spectrum gave $[M-H_2O+H]^+$ ions at *m/z* 1031.54 and showed major fragment ions at *m/z* 885.48, 739.44, 577.37, 415.32. The fragment ion at *m/z* 415.3 $[aglycone+H]^+$ was corresponding to the loss of two hexoses and two deoxyhexose. Twenty saponins were identified or tentatively characterized from the crude extracts of *Dioscorea* species.

PI386 Inhibitory effects of sumac (*Rhus* SPP.) fruit extracts and pentagalloyl glucose on mushroom tyrosinase enzyme

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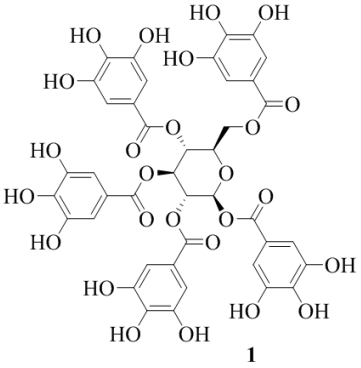
Several plant phenolics inhibit the tyrosinase enzyme which is involved in the biosynthesis of melanin. We recently reported that winged sumac (*Rhus coriaria*) fruit contains polyphenols including pentagalloyl glucose (PGG) (1) and the new galloyl derivative, galloyl malic acid dimethyl ester (2). Here, two sumac fruit extracts (*R. coriaria* and *R. copallinum*) were evaluated for anti-tyrosinase and anti-melanogenesis properties. The total polyphenolic content for the *R. coriaria* and *R. copallinum* extracts were 20.94 and 18.18% gallic acid equivalents, respectively. In the tyrosinase enzyme inhibitory assay, PGG (IC_{50}= 570.3 μM) was comparable to the positive controls, kojic acid and arbutin (IC_{50}= 440 μM and 1.7 mM, respectively). Our results demonstrate the inhibitory effects of sumac fruit extracts and PGG on tyrosinase enzyme, showing their antimelanin formation potential.



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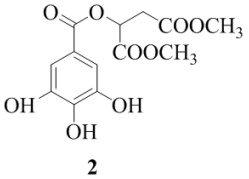

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PI387 Anti-allergic effect of *Cinnamomum cassia* extract in mice

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Cinnamomum cassia has been traditionally used to treat allergic disease in East Asian countries. Previous studies have demonstrated that *C. cassia* inhibits the development of mite antigen-induced skin lesions in NC/Nga mice by suppressing the T helper 2 cell response. The purpose of this study is to determine whether *C. cassia* has an inhibitory effect on systemic anaphylaxis and contact hypersensitivity in mice. Systemic anaphylaxis was induced by intraperitoneal injection of compound 48/80. Mortality was monitored for 1 h after the injection. Contact hypersensitivity response was induced by topical application of 2,4-dinitrofluorobenzene (DNFB) to the ears of mice. Ear thickness was measured 0, 24 and 48 h after DNFB challenge. We found that *C. cassia* significantly inhibited compound 48/80-induced anaphylaxis and DNFB-induced ear swelling. Compound 48/80-induced anaphylaxis was inhibited 100% at the dose of 60 mg/kg. When *C. cassia* was administered at the dose of 200 mg/kg, the change in ear thickness 24 h after DNFB challenge was significantly less than that in the control group (0.16 ± 0.03 vs. 0.32 ± 0.03 mm, $p < 0.01$). These results suggest that *C. cassia* suppresses allergic reactions, thus has potential for mitigation of atopic dermatitis.

PI388 Flavanoids of *Dalea searlsiae*: A plant under investigation for reintroduction to native habitats of the great basin

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The plant genus *Dalea* (Fabaceae) has been a prolific source of phenolic metabolites. We report here an investigation of *Dalea searlsiae*, an herbaceous member of the genus, native to the Great Basin areas of the Western United States. *D. searlsiae* is the subject of current studies on the reintroduction of native flora, via seed distribution, to rangelands. Cattle that graze on these species avoid 'bloat,' which may be attributed to the high phenolic content of the plants. Methanolic extracts of aerial and root portions of the plants have undergone fractionation using standard open-column techniques of silica gel vacuum liquid chromatography, Sephadex LH-20, and linear gradient chromatographic techniques. To date, these studies have afforded a suite of isoflavones and rotenoids from the aerial portions of the plant and related phenolic materials from the roots. Structure determination of pure compounds was accomplished primarily by extensive 1D and 2D NMR spectroscopy, and mass spectrometry. Antimicrobial bioassays have revealed significant activity concentrated in the root portions of the plant. *D. searlsiae* is active against wild-type and penicillin-induced resistant strains of *Staphylococcus aureus*, and toward *Streptococcus mutans* and *Bacillis subtilis*. The results of antiinsectan assays of the rotenoid-containing aerial portions will also be presented.

PI389 Antioxidant and anticancer activities of methanolic, ethyl acetate and chloroform extracts of *Arum Palaestinum*

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Phenolic and flavonoid contents, antioxidant and anticancer activities of the methanolic, chloroform and ethyl acetate extracts of *Arum Palaestinum* were evaluated. The highest phenolic (mg gallic acid equiv./g dry extract) and flavonoid (mg quercetin equiv./g dry extract) contents were observed in the methanolic (30. 9 ± 0.2; 14.4 ± 0.8), followed by chloroform (13.7 ± 0.6; 11.9 ± 0.9) and ethyl acetate (5.3 ± 0.6; 8.5 ± 0.8) extracts, respectively. The FRAP and DPPH antioxidant activities were highly correlated with the phenolic's and flavonoid's contents. The antiproliferative effects of the extracts were evaluated against the T cell lymphoblastic leukemia, Jurkat cells at 24 and 48 hr. Results showed a dose dependent reduction in cell proliferation, with more significance at 48 hrs. The IC_{50} values were (17.5 ± 2.1 µg/mL), (19.7 ± 2.8 µg/mL) and (23.3 ± 2.8 µg/mL) for ethyl acetate, methanol and chloroform extracts, respectively at non-cytotoxic concentrations. The present data suggests a lack of correlation between antioxidant and anticancer activities and further investigation is needed to elucidate the mechanism of action involved and to identify the active components.

PI390 Characterization of flavonoids and naphthopyranones in the extract *of Paepalanthus giganteus* by high-performance liquid chromatography coupled with electrospray ionization mass spectrometry

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The HPLC-ESI-MS^n method, based on high-performance liquid chromatography coupled to electrospray negative ionization multistage ion trap mass spectrometry, was developed to rapidly identify 23 compounds of type flavanonol, flavonol, and naphthopyranone. Some common features, such as CH_3·, H_2O, CO_2, hexose, pentose together with Retro-Diels-Alder fragmentations, were observed in flavonoids in *Paepalanthus giganteus*. The HPLC-UV-DAD and HPLC-MS/MS^n analyses of compounds present in the methanol extract of scape from *Paepalanthus giganteus* allowed the detection of flavonol and flavanonol derivatives, while methanol extract of capitulate, besides these flavonoids, showed the majority presence of naphthopyranone derivatives.The present study provided an approach to rapidly characterize bioactive constituents in *Paepalanthus giganteus* and reinforces the fact that such compounds can be considered taxonomic markers of *Paepalanthus* species belonging to the subgenus *Platycaulon.*

PI391 Structure-activity relationships study of new microtubule stabilizing taccalonolides

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Microtubule stabilizing drugs have an excellent utility in the treatment of adult solid malignancies. In the effort to find new microtubule stabilizing agents we evaluated tropical plants and identified a new class of microtubule stabilizers, the taccalonolides, from *Tacca chantrieri*. Recently, a number of new natural taccalonolides were isolated including one, designated taccalonolide **AF**, that retains microtubule stabilizing activity and has an IC50 value of 23 nM in Hela cells. This compound was the first to contain an epoxide group bridging C22 – 23, the only difference between **AF** and the major plant component taccalonolide **A**. This epoxide group results in a 230-fold increase in potency. A one-step epoxidation reaction was used to synthesize **AF** from **A** and **AJ** from **B**. **AJ**

is highly potent with an IC50 value of 4.2 nM. To generate sufficient **AJ** for *in vivo* antitumor efficacy studies seven different hydrolysis reactions were evaluated to produce **B** from **A**. The reaction conditions were optimized and an 80% yield of **B** from **A** was achieved with one method. Additionally, a new taccalonolide **AO** was also obtained. This optimized hydrolysis reaction was applied to synthesize **N** from **E** and yielded an additional 4 new taccalonolides, **AK**, **AL**, **AM**, and **AN**. They are all microtubule active and provide additional information for SAR.

PI392

Nematocidal activity of the extract of *Solanum lycocarpum* A. St.-Hil (Solanaceae) fruits on *Strongyloides Venezuelensis* in vitro

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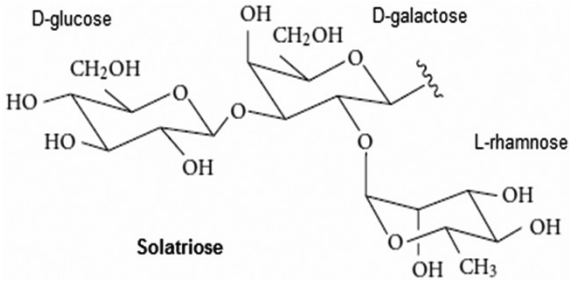
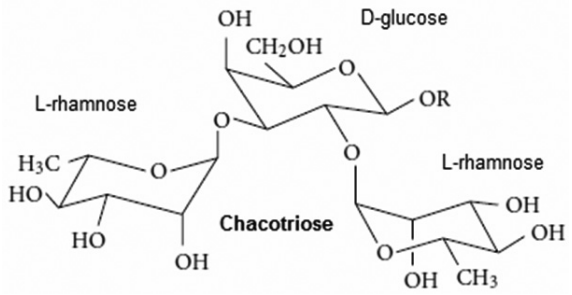
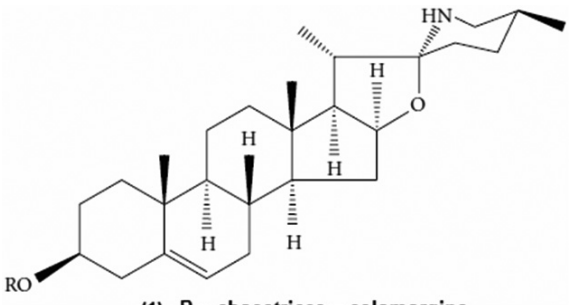
Solanum lycocarpum (Solanaceae) is a typical plant species of Brazilian savanna (cerrado) popularly known as 'lobeira' (wolf-fruit). These fruits contain two major alkaloids heterosides (solamargine (**1**) and solasonine (**2**) bearing both the same aglycone, solasodine (**3**). To evaluate the antihelminthic activity, the alkaloidic extract (AE) was obtained from the dried and grounded fruits using acid-base extraction. **1** and **2** were isolated by preparative TLC. The compound **3** was obtained by acid hydrolyses of the alkaloidic extract, and it was purified on silica gel column chromatography using gradient elution. The AE displayed potential antiparasitic activity against *Strongyloides venezuelensis*, a neglected disease.







(1) R = chacotriose = solamargine
(2) R = solatriose = solasonine
(3) R = H = solasodine

PI393

Simultaneous determination of sesquiterpenes and pyrrolizidine alkaloids from rhizomes of *Petasites hybridus* and dietary supplements using UPLC-MS

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UPLC-UV method has been developed for the analysis of major sesquiterpenes and pyrrolizidine alkaloids from rhizomes of *Petasites hybridus* (L.) G.M. et Sch. (Family, Asteracea) and dietary supplements claiming to contain *P. hybridus*. The best results were obtained with Acquity UPLC™ HSS T3 (100 mm x 2.1 mm, i.d., 1.8 µm) column system using gradient elution with a mobile phase consisting of ammonium formate (50 mM) and acetonitrile (0.05% formic acid) at a constant flow rate of 0.25 mL/min using UPLC-UV method. The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy and precision. The method was successfully used to analyze different *P. hybridus* market products, as well as to distinguish between other two *Petasites* species. The total content of petasins was found to be in the range from 0.02 – 11.6 mg/dosage form for 21 dietary supplements analyzed and petasins were not detected in six dietary supplements. The pyrrolizidine alkaloids were detected in seven dietary supplements.

PI394

Phenolic compounds as possible targets for quality control of HPLC fingerprint

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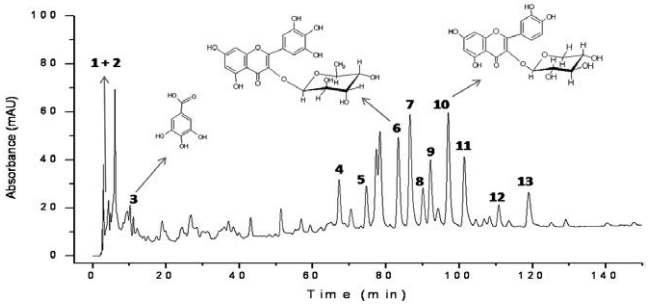
Hydroalcoholic extract of *Myrcia bella* Cambess. leaves was evaluated by HPCL-ESI-MS/MS, ESI-MS/MS^n, HPLC-PDA and streptozotocin-induced diabetes model. The fingerprint revealed as major flavonoids **6** and **10**, with known inhibition activity on diabetes related enzymes. The extract presents hypolipidemic and hypoglycemic activity. Therefore **6** and **10** could be active principles and possible targets for the quality control of *M. bella* extract.

A



B

Peak	Compound	µg/ml^{-1}
4	myricetin-O-hexoside	10,41 ± 0,96*
5	myricetin rhamnoside-galloyl	6,24 ± 0,87*
6	myricetin-O-deoxyhexoside	21,89 ± 1,90*
7	myricetin-7-O-rhamnoside	11,53 ± 1,06*
8	quercetin-O-pentoside	7,59 ± 0,87*
9	quercetin-O-pentoside	14,41 ± 1,29*
10	quercetin-O-pentoside	29,77 ± 3,01*
11	isorhamnetin-O-pentoside	16,09 ± 1,42*
12	quercetin-O-pentoside-galloyl	2,50 ± 0,66*
13	quercetin	3,10 ± 1,03*

C

	CTL SAL	CTL EXT	STZ SAL	STZ EXT
Fasting blood glucose (mg/dL)	126±6,8*#	125,5±16,7*#	507±37,3	338,1±56,6
Triglycerides (mg/dL)	105,1±12,5	79±17,88*	128,9±11,9	70,2±8,18*
Cholesterol (mg/dL)	142,9±12#	83,6±14,8*&	172,4±16,1	88,5±11,3*
Food intake (g/Kg)	22,6±0,94*#	19,66±1,2*#	51,1±3,3	31,45±2,0*
Water intake (mL/animal)	12,1±1,1*#	10,5±0,7*#	45,2±2,4	26,2±0,9*

A HPLC-PDA chromatogram at 254 nm. Peak: **1** cafeic acid, **2** quinic acid, **3** gallic acid, **4** myricetin-O-hexoside, **5** myricetin rhamnoside-galloyl, **6** myricetin-O-deoxyhexoside, **7** myricetin-7-O-rhamnoside, **8** quercetin-O-pentoside, **9** quercetin-O-pentoside, **10** quercetin-O-pentoside, **11**

isorhamnetin-O-pentoside, **12** quercetin-O-pentoside-galloyl, **13** quercetin. **B** Flavonoids quantification based on quercetin calibration curves ($R^2 > 0.9995$) at 360 nm. *The values are means ±SD, n = 9. **C** Metabolic parameters of normal and diabetic mice treated with extract and saline ($p < 0{,}005$), n = 8.

PI395 Simultaneous determination of tannins and triterpene saponins from the fruits of various species of *Terminalia* using UPLC-UV-MS

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Terminalia species are a rich source of tannins. Many preparations of these species are used in traditional medicine and have many different ethno-botanical applications. A simple UPLC method was developed for the simultaneous analysis of such hydrolysable tannins and triterpene saponins from the fruit rinds of *Terminalia* species. A separation by LC was achieved using a reversed phase column, a water/acetonitrile mobile phase, both containing formic acid using a gradient system and a temperature of 40 °C. Eight hydrolysable tannins (gallic acid, gallic acid methyl ester, corilagin, chebulagic acid, 1,2,3,6-tetra-*O*-galloyl-β-D-glucose, ellagic acid, chebulinic acid, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose) and six triterpene saponins (arjunglucoside-I, arjunglucoside-III, chebuloside II, bellericoside, arjunetin and arjunglucoside-II) could be separated within 20 minutes. The wavelength used for detection with the diode array detector was 254 and 275 nm for tannins and 205 nm for triterpene saponins. The method was validated for linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The developed method is economical, fast and especially suitable for quality control (QC) analysis of tannins and triterpene saponins from various commercial products.

PI396 Triterpenoid saponins from Albizia (Mimosaceae) decrease brain tumor cells proliferation

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Avicin D, a natural triterpenoid saponin isolated from Acacia victoriae found in Autralia's deserts, inhibits tumor cell growth and induces apoptosis in transformed tumor cell lines in vitro and mouse skin carcinogenesis models in vivo. In order to discover potential new bioactive avicin analogues, ten African Mimosaceae including Albizia, Acacia, and Entada genera were selected to screen their saponins content. Finally, three Albizia species were chosen for their HPLC-DAD, LC-NMR and LC-MS profiles. Saponin enriched-fractions were evaluated for their inhibitory effect on the metabolism of high grade human brain tumor cells. These include the U87 human glioblastoma cell line and glioblastoma cancer stem cells isolated from patients and known to be particularly resistant to chemotherapies. The isolated avicins induce a decrease in the ATP level in the different cell types tested with EC 50 values in the range of 3 to 6 μg/mL. For some avicins, a differenzial effect could be observed between the stem and non stem cancer cells, the compounds showing higher activity on the cancer cells with stem properties. The specificity of avicins compared to other saponosides has also been evaluated. This study unveils the presence of highly active avicins in African plants and suggests a possible use of these compounds as tools to investigate the pathophysiology of cancer cells and as efficient antitumor agents.

PI397 Cytotoxic peptide obtained by enzymatic hydrolysis from Ditaxis heterantha seeds

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Ditaxis heterantha is a plant from *Euphorbiacea* originally from semiarid zones in Mexico. Its seeds are composed of 20% of protein. The protein was fractionated by solubility in albumins globulins and glutelins. Antitumoral activity was evaluated for each fraction in mice BALB/c administrated by oral via, where glutelins fraction showed antitumoral activity against lymphoma L5178Y of 59%. Glutelins fraction was hydrolyzate using gastrointestinal proteases sequentially obtaining a hydrolyzate. This hydrolyzate was separated and purified by FPLC and to each purified peptide fraction was evaluated by cytotoxic activity against lymphoma L5178Y cells *in vitro*. A peptide was selected with a molecular weight of approximately 6.5 kDa and pI close to 4. This peptide showed cytotoxic activity of 54 ± 7% in Annexin V (+) cells at 0.04 μg/mL on lymphoma L5178Y in control of healthy untreated splenocyte cells obtained 29 ± 0.0%. For the propidium iodide (IP) test the murine lymphoma at 0.04 μg/mL was obtained 72 ± 1% of IP (+) and for healthy untreated cells it showed 25 ± 0.0% cyclophosphamide positive control at 10 μg/mL was used. The cytotoxic activity of this peptide can be attributed to an alteration on cell cycle in lymphoma L5178Y cells. An N-terminal assay showed that the amino acids analysis of peptides shared similarity with the chain E sequence of the trypsin-binding domain of Bowman-Birk type protease inhibitor and its interaction with trypsin.

PI398 Chemical screening of mimosaceae from Senegal for the identification of avicins analogues

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Avicins are complex electrophile triterpene glycosides with high anticancer potential, and until now only isolated from an Australian desert tree, *Acacia victoriae*. In order to discover new avicins analogues, nine Mimosaceae (Leguminosea) from three different genera (*Albizia*, *Acacia* and *Dischrostachys*) collected in Senegal were screened for their saponin content. Chemical analysis of the saponin-enriched fractions using HPTLC-MS, LC-UV, and NMR (1D and 2D) experiments, pointed out the presence of avicins analogues exclusively in *Albizia* species. These findings confirm that *Albizia* can be considered as the most reliable genus for the isolation and identification of new acacic acid derivatives from African Mimosaceae.

PI399 Three cycloartane saponins from *Astragalus tmoleus var. tmoleus*

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Astragalus tmoleus Boiss var. *tmoleus* is an endemic plant from Turkey [1]. Our phytochemical investigation on the CH_2Cl_2 extract of this plant led to the isolation and characterization of three known cycloartane-type saponins, astragaloside IV **(1)** [2], cyclocephaloside II **(2)** [3] and astrasieversianin I **(3)** [4] by using various solid/liquid chromatographic techniques. The identification of these compounds was mainly achieved by 1D and 2D NMR spectroscopic techniques (^1H-^1H COSY, NOESY, HSQC, HMBC), FABMS and by comparison of their spectral data with those previously reported. Our results confirm that triterpene saponins belonging to the cycloartane-type skeleton might be chemotaxonomically significant to the genus *Astragalus*. Although **1**, **2** and **3** have previously been isolated from several species including *A. membranaceus*

Bunge., *A. microcephalus* Willd and *A. gilvus* Boiss. [2 – 4], it is the first time that these compounds have been isolated from *A. tmoleus var. tmoleus.* References: 1. Davis, P.H. (1989) Flora of Turkey and East Agean Islands, University of Edinburgh University Press, 7, 857 – 861. 2. Kitagawa, I. et al. (1983) *Chem. Pharm. Bull.* 31: 698 – 708. 3. Bedir, E. et al. (1998) *J. Nat. Prod.* 61: 1469 – 1472. 4. Tabanca, N. et al. (2005) *Biochem. Syst. Ecol.* 33: 1067 – 1070.

PI400 Biological and phytochemical studies of *Huperzia selago* (L.) Bernh. ex Schrank et Mart. (Huperziaceae)

Szypuła WJ[1], Mistrzak P[1], Olszowska O[1], Kiss AK[1], Adamczyk A[2], Czapski G[2], Kania M[3], Wileńska B[3], Danikiewicz W[3]
[1]The Medical University of Warsaw, Faculty of Pharmacy, ul. Banacha 1, 02 – 097 Warsaw, Poland; [2]Mossakowski Medical Research Center, ul. Pawinskiego 5, 02 – 106 Warsaw, Poland; [3]Institute of Organic Chemistry, Polish Academy of Sciences, ul. Kasprzaka 44/52, 01 – 224 Warsaw, Poland

Fir clubmoss *Huperzia selago* growing in Europe is a rich source of Huperzine A (selagine, HupA)(1.59 mg g^{-1} d.w.) an effective, reversible and selective acetylcholinesterase (AChE) inhibitor, undergoing clinical trials as a potential medicine in Alzheimer disease and schizophrenia. Studies performed with modified Ellman's method have shown that apart from Hup A, other compounds with AChE and butyrylcholinesteraze (BuChE) inhibitory activity are present in the alkaloid extract of *H. selago*. Percentage inhibition range of the alkaloid extract fractions was 0 – 100% for AChE and 0 – 16.5% for BuChE, respectively. Several alkaloid fractions have shown antioxidative and cytoprotective properties. Significant reduction of macromolecules oxidation in condition of oxidative/nitrosative stress *in vitro* was observed. Concomitantly, the fractions prevented PC12 cells death evoked by sodium nitroprusside (SNP). Cytoprotective effect of these alkaloids might provide useful clues for developing novel therapeutic strategies for neurodegenerative disorders. However, further study will be needed to understand the precise molecular mechanisms of cytoprotection. To identify some active compounds of selected alkaloid extract fractions HPLC-ESI-MS/MS analysis are performed.

PI401 The effect of benzophenone on HIV-1 infection

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Benzophenones represent a potentially useful alternative to the current chemotherapeutic strategies to treat and/or prevent infectious diseases.Objective: This *in vitro* study evaluated the toxicity and the anti-HIV-1 activity of 7-epiclusianone. **Methods:** The benzophenone 7-epiclusianone was isolated from a hexane extract of *Rheedia gardeniana* fruit pericarp. The purity level was > 98% as determined by HPLC. Toxicity was evaluated using flurometric quantification of cellular viability.The anti-HIV activity on TZM cells, HeLa cells expressing CD4 and both HIV-1 co-receptors CXCR4 and CCR5, was determined using a luciferase reporter. To elucidate the molecular mechanisms of action of these agents we isolated the total RNA from TZM cells and performed Illumina whole genome expression profiling and Pathway-focused qRT-PCR array. The data management, enrichment analysis and pathway analysis were determined with MetaCore software (Thomson Reuters). **Results:** 7-epiclusianone was toxic above 25 μM. The anti-HIV activity was dose-dependent and inhibited 50% of HIV infection at 1.5 μM. The expression of both CCR5 and CD4 receptors for HIV were downregulated by benzophenone. Conclusion: 7-epiclusianone is a promising naturally occurring agent displaying anti-HIV activity. The putative pathway by which 7-epiclusianone affect HIV-1 infection may involve the downregulation of chemokine receptor.

PI402 Phytochemical analysis of alkaloids from the icelandic club moss *Diphasiastrum alpinum* (L.) Holub

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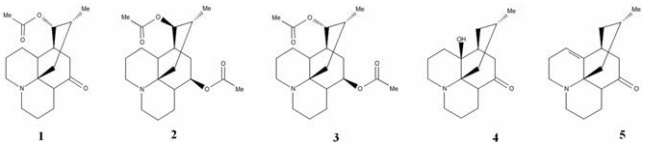
A new alkaloid, *O*-acetylepiclavolonine (**1**), has been isolated from the Icelandic club moss *Diphasiastrum alpinum* and the structure has been determined using a combination of mass spectrometry, NMR and optical rotation. The previously described alkaloids *O*-acetylfawcettiine (**2**), *O*-acetyllofoline (**3**), lycodoline (**4**) and anhydrolycodoline (**5**) were found in *D. alpinum* for the first time together with the expected lycopodine and clavolonine. The isolated alkaloids (**1**-**5**) did not show significant inhibition of acetylcholinesterase *in vitro*. Two alkaloids found in European alpine club moss, lycoclavine and *des*-*N*-methyl-α-obscurine, were not detected in the Icelandic plant material indicating an influence of different environmental conditions on the alkaloid profiles.



PI403 New 3,4-secocycloartane and lupane triterpenes from the leaves of the tropical rain forest tree *Hopea odorata* Roxb

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This poster is dedicated to the memory of the late Professor François Tillequin. Chromatographic fractionation of the cytotoxic *n*-hexane extract of *Hopea odorata* Roxb. leaves led to the isolation of 2 new 3,4-secocycloartanes bearing a unusual trihydroxylated side chain esterified with a fatty acid, together with 15 known other triterpenes (8 lupanes, 2 friedelanes, 2 oleananes, 1 cycloartane (*i.e.* mangiferonic acid), β-sitosterol and its palmitic acid ester) and 1 sesquiterpene (*i.e.* β-caryophyllene oxide). Among lupanes, 3,30-dioxolup-20(29)-en-28-oic acid was isolated for the first time from a natural source. Cytotoxic activities of lupane-type triterpenes against four human cell lines (PC3, MDA-MB-231, HT-29 and PC-3) are also reported.

PI404 Activities of xanthones against intramacrophage amastigotes of Leishmania (Leishmania) Amazonensis

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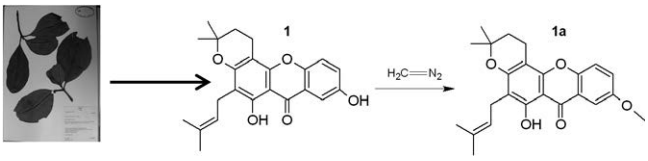
The current treatment of cutaneous leishmaniasis is hampered by factors such as toxicological effects, low adherence due long term administration and drug resistance (The Lancet, 2010, 376, 1363). Therefore, we have isolated a previously unreported xanthone (**1**), 6,9-dihydroxy-3,3-dimethyl-5-(3-methylbut-2-enyl)pyrano[2,3-*c*]xanthen-7(3*H*)-one, from ethyl acetate extract of *Clusia pernambucensis* G. Mariz (Clusiaceae) stem bark. In addition, a derivative of the same compound (**1a**) was obtained to compare and enhance the activity against the intramacroph-

age amastigote forms of *Leishmania* (*Leishmania*) *amazonensis*. The IC_{50} observed for both compounds were 25.2 and 22.5 μg/mL, respectively. In murine macrophages, the CC50 values were 17.22 μg/mL for **1** and 32.56 μg/mL for **1a.**



PI405 Potent new microtubule stabilizers with unique biochemical and cellular effects show promise for cancer treatment

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The taccalonolides are a new class of microtubule stabilizing agents isolated from plants of the genus *Tacca*. Similar to the effects of the taxanes, the taccalonolides cause microtubule stabilization, leading to the mitotic arrest and death of cancer cells. However, there are several properties of these molecules that suggest they work through a novel mechanism of action, including their ability to form distinct mitotic spindle structures and their propensity to affect interphase microtubules at much lower relative concentrations than the taxanes. The latter finding is of great interest given recent studies suggesting that the anticancer effects of microtubule targeting agents may be due in large part to their interphase effects. Our recent isolation of taccalonolides with potency in the low nanomolar range provided the first indication that these drugs interact directly with tubulin. Intriguingly, the kinetic profile of tubulin polymerization observed in the presence of these potent taccalonolides is unlike that observed with other stabilizers, further suggesting that the taccalonolides interact with tubulin in a manner that is markedly distinct from other classes of microtubule targeting agents. The unique biochemical and cell biological properties of these potent taccalonolides, together with the excellent *in vivo* antitumor activity observed for this class of agents in drug resistant tumor models, reveal the potential of the taccalonolides as a new class of anticancer drugs.

PI406 Further secondary metabolites from *Pimpinella kotschyana*

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Pimpinella (Apiaceae) is represented in Turkey by 23 species (5 endemic), 2 subspecies, and 2 varieties. *Pimpinella anisum* L. is a medicinal herb known for its expectorant, antispasmodic, carminative, and diuretic properties properties and it is an important agricultural crop in Turkey. *Pimpinella* species have used as carminative, expectorant, sedative, antidepressant, antiseptic, insecticidal, antiviral, antispasmodic, nematocidal, mutagenic, analgesic, antifungal and antibacterial agent. It was found that the existence of terpenoids, lipids, alkaloids, coumarins, flavonoids and phenylpropanoids in *Pimpinella species.* In our earlier studies of isolated compounds from *Pimpinella kotschyana,* we reported 2 new and 4 known flavonol glycosides. In our further research on this plant, *n*-BuOH phase prepared from methanol extract was fractionated on a silica gel column. The subfractions were rechromatographed over silica gel and Sephadex LH-20 to give two new acylated flavonol glycosides (Quercetin 3-*O*-α-L-(3''-*trans*-coumaroil) rhamnopyranoside and Quercetin 3-*O*-α-L-(2'',3''-di-*trans*-coumaroil) rhamnopyranoside), a new triterpene glycoside (saikogenin F 3-*O* {β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-β-D-fucopyranoside}), one erithritol derivative (2-C-methylerithritol) and one sterol (α-spinasterol-β-D-glucopyranoside). The structures of isolated compounds were determined by 1D and 2D NMR analysis.This is the first report describing of these isolated compounds from the aerial parts of *Pimpinella kotschyana.*

PI407 The inhibitory effects of Turkish endemic *Prangos* species against some food borne pathogens commonly found in milk and cheese

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The species of this genus is known as "Çaşır otu" in Turkey and used in making herbed cheese in order to give the aroma and taste in Van region and also used as folk medicine in various regions. Therefore, the aim of the study was to determine the inhibitory effects of endemic *Prangos* Lindl. (Umbelliferae) species on some food borne pathogen bacteria found in milk and cheese. The antimicrobial effect of methanol, acetone and water extracts of leafs and fruits from endemic *Prangos* species were tested by agar well diffusion method. The acetone and the methanol extracts exhibited remarkable antimicrobial activity in all species. In general, among the tested bacteria, *Salmonella typhimurium* was the most resistant bacterium, while *Bacillus cereus* was the most sensitive. *Listeria monocytogenes* ve *Staphylococcus aureus* showed moderate resistance against extracts. The highest inhibitory effect was determined against *B. cereus* RSKK 863 (11.9 ± 0.3 mm, inhibition zone diameter) by *Prangos denticulata* Fisch. & Mey. fruit acetone extract. *Prangos platychloena* Boiss ex Tchih. subsp. *engizekensis* H.Duman & M.F.Watson and *P. denticulata* fruit extracts demonstrated an inhibitory effect against all of the tested food borne bacteria. This active extracts for all test bacteria will be helpful devising antimicrobial formulations with which to protect foods especially cheese against infection by multiple pathogens.

PI408 Evaluation of carajurin content and stability of spray-dried *Arrabidaea chica* extract using different gum as wall material

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Arrabidaea chica (bignoniaceae) populary Known as Carajuru is widely distributed in South America tropical forest. Anti-inflammatory, astringent agents, among other uses have been described in traditional medicine (Devia et al.2002) for this species. Previous reports demonstrated that Carajurin (6,7-dihydroxy-5,4-dimethoxy-flavylium) and other anthocyanins are involved with the pharmacological activity. These compounds are easily decomposed with high temperatures, humidity and oxygen. Therefore this study evaluated the stability of spray-dried *A.chica* extract employing different core materials. Three different homogeinizaded wall material (arabic gum, cashew gum and mixture Arabic gum/maltodextrin 1/1) were tested. The extracts were processed in Spray Drier Büchi B 290 temperature 160 °C, N_2 flow rate at 35 m^3/h. 2,2 mm-diameter. The samples were stored in different types of packages (glass, plastic, aluminum) in stability chambers with 75% humidity and 40 °C during 90 days. The aglycone ratio was maintained in the Arabic gum wall material, whereas for 1:1 Arabic gum/maltodextrin wall material, 90% of the aglycone were lost as determined by HPLC-DAD and ESI-MS with direct infusion, Phenomenex Gemini C-18 column (4,6 mm x 250 mm i.d., 3 μm), flow rate 1 mL/min, mobile phase methanol: aqueous phosphoric acid (pH 2.00) gradient elution.

PI409 Coumarins from the aerial parts of *Lomatopodium staurophyllum*

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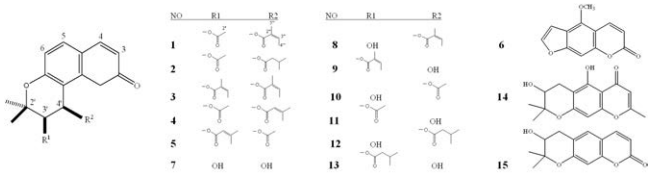
The genus *Lomatopodium* Fisch.& C.A.Mey, well-known as Pa-pahn in Persian language, includes one endemic species spread throughout North-East and Northern parts of Iran. Here, we focused on the isolation and identification of the main coumarins from the aerial parts of *L. staurophyllum* which has not been previously reported. Three new com-

pounds, 4'-acetyl-*cis*-khellactone (10), 3'-acetyl-*cis*-khellactone (11) and 3'-isovaleroyl-*cis*-khellactone (13), together with Pteryxin (1), Corymbocoumarin (2), Anomalin (3), Isosamidin (4), Samidin (5), Bergapten (6), *cis*-Khellactone (7), *d*-Laserpitin (8), 3'-angeloyl-*cis*-khellactone (9), 4'-isovaleroyl-*cis*-khellactone (12), Hamaudol (14) and Decursinol (15) were the main coumarins identified.



PI410 The influence of total phenol, β-carotene, and lycopene contents on antioxidant capacity in *Stachys obliqua* extracts

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This study is designed to examine *in vitro* antioxidant capacity of the extracts obtained from *Stachys obliqua* Waldst. & Kit. and to compare this capacity with the values of total phenol, β-carotene and lycopene contents. Antioxidant capacities of the samples were investigated by three different test systems namely β-carotene/linoleic acid bleaching, DPPH radical scavenging, and metal chelating effect. The phenolic contents of *S. obliqua* extracts were determined using Folin-Ciocalteu's phenol reagent. The methanol extract showed higher radical scavenging ability (IC_{50}= 0.011 ± 0.000 mg/ml) than the water extract (IC_{50} = 0.018 ± 0.001 mg/ml). The extracts have more effective scavenging ability on DPPH radicals than synthetic antioxidant BHT (IC_{50}= 0.023 ± 0.001 mg/ml). Moreover, both of the extracts exhibited the high chelating ability and showed a notable capacity to suppress lipid peroxidation. The methanol (230.78 ± 3.95 μg/mg) and the water (171.65 ± 4.17 μg/mg) extracts were found to be rich-in phenolics. However, β-carotene and lycopene were only found in small amounts in the methanol extract and not found in the water extract. A significant relationship between the antioxidative effects and total phenolic contents were found ($p< 0.05$). Therefore, further studies need to be directed to isolate and characterize antioxidant active compounds from the extracts which could be responsible for the high antioxidant activities.

PI411 Double blind clinical trial of the Ageratina pichinchensis extract in the topical treatment of onychomycosis

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Onychomycosis is a high-prevalence infectious disease that is usually treated with drugs administered chronically via the oral pathway, which produce important side effects. *Ageratina pichinchensis,* used in traditional medicine to treat dermatophytosis, has shown antifungal activity against *Thrichophyton rubrum,* and *T. mentagrophytes*. Clinically, a nail lacquer elaborated with the extract obtained from this plant showed similar rates of therapeutic effectiveness than 8% ciclopirox in patients with onichomycosis. OBJECTIVE: Evaluate two different concentrations of the standardized extract of *A. pichinchensis* in patients with mild to moderate onychomycosis. METHODOLOGY: By means of a randomly and comparative double blind clinical trial, 122 patients of either sex, within 19 and 65 years were included. A toenail sample was taken and submitted to direct microscopic identification before and after treatment. Two experimental groups were organized; group 1 was administered with a cosmetic nail lacquer formulation containing a 12.6% *A. pichinchenis* extract, while group 2 received an identical product containing 16.8% *A. pichinchenis* extract. All patients were treated and followed clinically for 6 months by means of monthly appointments. ANOVA and X^2 test were employed to identify differences between groups. Values of $p < 0.05$ were considered significant. **Results:** 103 patients concluded the treatment. The therapeutic effectiveness exhibited by the 12.6% formulation was 67.2% while that of the 16.8% was 79.1%. Regarding clinical evolution analysis of results at the end of the treatment evidenced that the 16.8% concentration possesses higher therapeutic effectiveness ($p = 0.01$).

PI412 Quantitative determination of casticin in Turkish samples of Vitex agnus-castus

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Vitex agnus-castus (Verbenaceae) is a widely distributed small tree in Turkish coastal areas. The fruits of this plant have long been used as a traditional medicine for the treatment of gynecological problems in Anatolia. In our previous study, the essential oil compositions of *Vitex agnus-castus* fruits collected from five different regions were identified by GC/GC-MS. In this study, quantitative analyses of casticin in the methanolic extracts of fruits of the same five samples of *Vitex agnus castus* were determined by HPLC according to the European Pharmacopoeia 2007. The results have shown that the amount of casticin determined in the fruits of Turkish samples of *Vitex agnus-castus* is compatible with the European Pharmacopoeia standards.

PI413 *in vivo* anthelmintic activity of *Syzygium cumini* leaves against *Haemonchus contortus* in sheep

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The gastrointestinal parasitism is currently considered the biggest health problem faced by producers worldwide. *Haemonchus contortus* is a parasite of small ruminants that is responsible for major losses. Many plants are known as having anthelmintic activity, however, their effects requiring verification. In this study, "Santa Inês" breed sheep were fed with leaves powder of *Syzygium cumini*, which was dried at 37 °C. This powder was added to animal feed (1,2 g/kg). The sheep weight, fecal egg counts per gram (EPG) and blood test were analyzed in the 1th, 7th and 14th day. Chemical and bromatology evaluation of powder of leaves was done. A reduction of 76,67% in EPG was observed in the 14th day. Seven days after treatment, EPG redution of 17,8% was yet observed. The amount of total phenolics compounds was 156,13 g tannic acid/kg of dry matter. The levels of protein and minerals contained on leaves was 9,1% and 45,8 g/Kg, respectively, in contrast with 21,61% and 57,36 g/Kg of feed. After 14 days, moderate weight gain was observed (3%) and the blood test showed decrease on eosinophils levels in animal treated. It is concluded that the *S. cumini* leaves showed anthelmintic activity when administered together with feed and a discrete weight gain was observed.

PI414 New anxiolytic phytopharmaceutical elaborated with the standardized extract of Galphimia glauca

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A new phytopharmaceutical with anxiolytic effectiveness has been developed. This product was elaborated with the standardized extract obtained from the plant species *Galphimia glauca* Cav. (Malpighiaceae). *Galphimia glauca* has for many years been used in Mexican traditional medicine as a "tranquilizer". Different *in vivo* and *in vitro* pharmacological studies have demonstrated the anxiolytic activity of crude extract obtained from *G. glauca*. The pharmacological effect produced by the *G. glauca* extract has been attributed to the nor-seco,triterpene galphimine-B (G-B). This compound has exhibited an innovative action mechanism, selective of dopaminergic neurons on ventral tegmental area.- The therapeutic effectiveness, safety, and tolerability of the new phytopharmaceutical was compared with lorazepam on patients with Generalized Anxiety Disorder (GAD). By means of a controlled, randomized, double-blind clinical trial, outpatients of either sex who matched the DSM-IV's diagnostic criteria with a score of ≥ 19 points on the Hamilton Anxiety Scale (HAM-A) were included. The experimental group was treated orally with the phytopharmaceutical in capsules twice a day for 4 weeks. The control group received lorazepam (1 mg) under the same conditions and presentation. A total of 152 patients were included

in the trial (72 in the experimental group). From the first week of treatment, the phytopharmaceutical showed important anxiolytic effectiveness, very similar to that produced with lorazepam. Both treatments showed therapeutic safety, nevertheless, concerning side effects the phytopharmaceutical evidenced considerably higher tolerability than lorazepam.

PI415 Phytochemical Investigation of Roman and German chamomile

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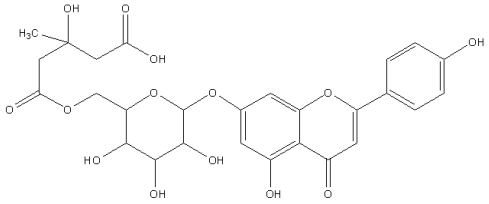
Roman chamomile (*Anthemis nobilis* L., syn. *Chamaemeleum nobile* L.) and German chamomile (*Matricaria recutita* L. syn. *Chamomilla recutita* L. or *Matricaria chamomilla* L.) are both used in a similar way for a variety of health conditions such as sleeplessness, anxiety, and gastrointestinal conditions. Both herbs belong to the family Asteraceae, and have the same common name as chamomile. The flowering tops of the chamomile plants are used to make teas, liquid extracts, capsules, or tablets. They are also often used topically for skin conditions and for mouth ulcers resulting from cancer treatment due to their anti-inflammatory properties. The two herbs are not only morphologically differentiable, but their contained secondary metabolites are also different. The aim of this project is to investigate the chemical profiles of both German and Roman chamomile. We report here the isolation and identification of the compounds that are characteristic for each herb.



PI416 Antileishymanicidal acvity of Gochnatia pulchra

Lucarini R, Salloum AIO, Rezende KCS, Esperandim VR, Ferreira DS, Magalhães LG, e Silva MLA, Cunha WR, Vinholis AHC, Martins CHG
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Plants are potential alternative sources for the research of new and selective therapeutic agents for the treatment of leishmaniasis. However, exploration by chemical agents from natural products, especially of plant origin, has the intention to find new molecules with lower toxicity and lower cost especially in situations of parasitic resistance. In this context, this report aims to investigate the leishmanicidal activity of the crude extract and hexane, diclchloromethane, ethyl acetate and water fractions of plant species *Gochnatia pulchra* against *Leishmania amazonensis*, seeking to identify the correlation between the major compounds and leishmanicidal activity. In assessing characterize the phytochemical profile of hexane fraction (HF), which identified four substances majority, β-amirina triterpenes, lupeol, lupeol acetate and ethyl taraxerol, since the ethyl acetate fraction was identified the compound 3,5-O- dicaffeoylquinic, the other fractions (dichloromethane and water) are being analyzed in order to elucidate their chemical constituents. Our results showed that the fraction FH, where triterpenes appear in high proportion (59.4% and 50.2%), showed better anti-promastigote activity. On the other hand, the other fractions did not show promising activity when compared with hexane fraction. Due to increased activity of anti-promastigote FH phytochemical study was performed to identify which were the majority in this fraction compounds and correlate with data from the scientific literature of these compounds against leishmanicidal activity. Our results indicate that the FH exerts a strong anti-promastigote activity at concentrations of 400 and 200 μg/mL, chemical profile with potential for future development of therapy against leishmaniasis.

PI417 Biological activity of *Alpinia galanga* root tubers

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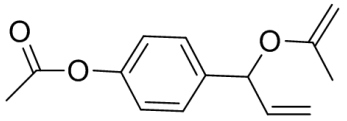
In our search for safe antileishmanial and antimicrobial agents from natural sources we undertook an investigation of root tubers of *Alpinia galanga* (L) Willd, a popular condiment used in Southeast Asia. Primary screening of the methylene chloride extract of the root tubers of *A. galanga* showed moderate in vitro activities against *Leishmania donovani* promastigotes, *Aspergillus fumigatus, Cryptococcus neoformans* and *Mycobacterium intracellulare*. Activity-guided fractionation of the above extract led to the isolation of previously identified 1'-actoxychavicol acetate (1) as the active compound. This compound showed good activity against *L. donovani* (IC_{50} 2.33 μg/mL) and *A. fumigatus* (IC_{50} 2.79 μg/mL) and strong activity (IC_{50} 0.97 μg/mL) against *M. intracellulare*. In the literature, it is reported to have a number of bioactivities including anti-tuberculosis, anti-allergic, antitumor, anti-HIV, anti-inflammatory, antifungal and unspecified antibacterial activities.[1]



1

PI418 Isolation of *1, 2, 3, 4, 11, 12*-hexadehydrositost-*5*-en-*3*β-ol with mild hepatoprotective effect from *Landolphia owariensis* P. Beauv.

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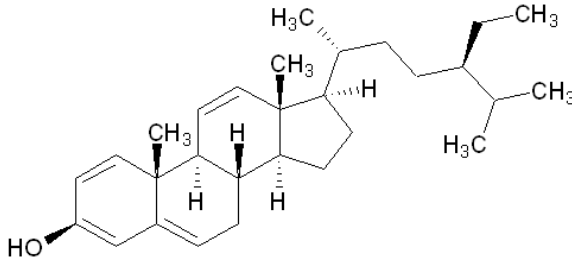
1, 2, 3, 4, 11, 12-hexadehydrositost-*5*-en-3β-ol (hexadehydro-3β-sitosterol) has been isolated from the n-hexane extract of *Landolphia owariensis* P. Beauv. stringy seed pulp. The chemical structure was confirmed by GC-MS and H-nmr spectroscopy. This is the first report of isolation of this compound from the plant. The isolated compound marginally reversed thioacetamide (800 mg/kg, i.p.) induced elevation of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP), with respect to normal control and silimarin (300 mg/kg) positive control rats; as well as reduced the hepatotoxin induced rise of serum albumin (ALB), total protein (TP), total bilirubin (TB), direct and indirect bilirubin (DB and IDB, respectively). Hepato-histology of the treated and control animals, further confirmed the mild hepatoprotective effect of *1, 2, 3, 4, 11, 12*-hexadehydrositost-*5*-en-3β-ol. Taken together, *1, 2, 3, 4, 11, 12*-hexadehydrositost-*5*-en-3β-ol is minor contributor to the confirmed hepatoprotective properties of *Landolphia owariensis* P. Beauv stringy seed pulp; and is suspected to possess cholesterol lowering effect in animal models.



1, 2, 3, 4, 11, 12-Hexadehydrositost-5-en-3β-ol
(Hexadehydro-3β-sitosterol)

PI419 Chemical and antifungal investigations of six *Lippia* species (Verbenaceae) from Brazil

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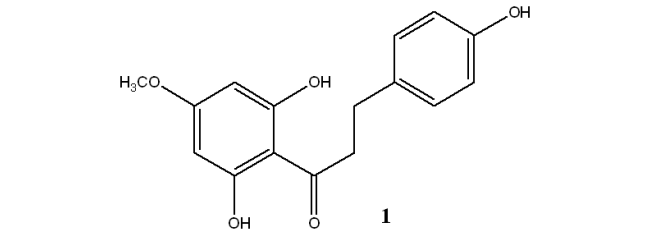
Lippia genus is used in ethnobotany as food, beverages, seasoning and remedies for antiseptic and anti-inflammatory purposes, among others. Chemical compositions of fifteen extracts of six *Lippia* species were investigated comparatively by HPLC-PDA. To avoid data replication, *L. lupulina* Cham. roots EtOH extract was selected for isolation procedures based on PCA analyses of previous works on this genus. Seven compounds previously unreported in this genus were isolated. The bioactivity of extracts, fractions and pure compounds were investigated towards *Candida albicans, C. krusei, C. parapsilosis* and *Cryptococcus neoformans* strains. Fractions from the EtOH extract of *L. salviaefolia* leaves showed marked inhibition of fungal growth, in addition to asebogenin (**1**) and verbascoside (**2**) which showed MIC values lower than 15,6 µg/mL and might be considered as promising prototypes for the development of new antifungal agents, especially against *C. neoformans*. *Acknowledgements: FAPESP, CAPES and CNPq.*



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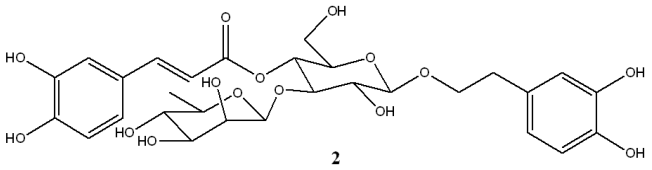

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PI420 *Haemonchus contortus*: *in vivo* anthelmintic activity of *Genipa Americana* L. leaves in sheep

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Intense occurrence of resistant strains of parasites to conventional anthelmintic has been observed for sheep producers in many countries. The use of new products against these parasites is still limited; however, numerous reports have showed plants that inhibit the larvae development or eggs hatch. In this work, "Santa Inês" breed sheep were fed with leaves powder of *Genipa Americana* L. (jenipapo), which was dried at 37 °C. This powder was added to animal feed (1,2 g/kg). The sheep weight, the fecal egg counts per gram (EPG) and blood test were measured in the 1th, 7th and 14th day. Chemical and bromatology analysis of powder of dried leaves was done. A reduction of 81,75% in EPG was observed in the 14th day. Seven days after treatment, EPG reduction of 47,6% was yet observed. The amount of total phenolics compounds was 6,59 g tannic acid/kg of dry matter. The levels of protein and matter minerals contained on leaves was 15,05% and 50,65 g/Kg, respectively, against 21,61% and 57,36 g/Kg of feed. After 14 days, weight gain was observed (11%) and the blood test showed considerable decrease on eosinophils levels in animal treated. In conclusion, the *Genipa americana* leaves could represent an anthelmintic natural alternative to sheep producers.

PI421 Anti-phytopathogen potential of diterpenes against *Colletotrichum gloeosporioides* and *Fusarium verticilloides*

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Many diterpenes from plants and fungi are being reported with significant antimicrobial activity. Herein the anti-phytopathogen activity of twenty four diterpenes obtained from several botanical sources and semi-synthetic methodologies were tested against the fungi *Colletotrichum gloeosporioides*, one of the responsible for anthracnose in many fruits and *Fusarium verticilloides* which infects mainly maize. The minimum inhibitory concentration (MIC) and the half maximal inhibitory concentration (IC_{50}) of these metabolites were determined. The best activity analyzed by MIC and IC_{50} against *C. gloeosporioides* and *F. verticilloides* were detected with *ent*-copalic acid, (-)-hydroxycopalic acid methyl ester, *ent*-agathic acid methyl ester, sodium salt of *ent*-pimara-8(14),15-dien-19-oic acid, dehydroabietic acid and esclareol. In conclusion, ours results pointed out diterpenes as an important class of natural products in the search for novel anti-phytopathogenic agents.

PI422 Double blind clinical trial of the ageratina pichinchensis extract in the topical treatment of onychomycosis

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Onychomycosis is a high-prevalence infectious disease that is usually treated with drugs administered chronically via the oral pathway, which produce important side effects. *Ageratina pichinchensis,* used in traditional medicine to treat dermatophytosis, has shown antifungal activity against *Thrichophyton rubrum,* and *T. mentagrophytes*. Clinically, a nail lacquer elaborated with the extract obtained from this plant showed similar rates of therapeutic effectiveness than 8% ciclopirox in patients with onichomycosis. OBJECTIVE: Evaluate two different concentrations of the standardized extract of *A. pichinchensis* in patients with mild to moderate onychomycosis. METHODOLOGY: By means of a randomly and comparative double blind clinical trial, 122 patients of either sex, within 19 and 65 years were included. A toenail sample was taken and submitted to direct microscopic identification before and after treatment. Two experimental groups were organized; group 1 was administered with a cosmetic nail lacquer formulation containing a 12.6% *A. pichinchenis* extract, while group 2 received an identical product containing 16.8% *A. pichinchenis* extract. All patients were treated and followed clinically for 6 months by means of monthly appointments. ANOVA and X^2 test were employed to identify differences between groups. Values of $p < 0.05$ were considered significant. **Results:** 103 patients concluded the treatment. The therapeutic effectiveness exhibited by the 12.6% formulation was 67.2% while that of the 16.8% was 79.1%. Regarding clinical evolution analysis of results at the end of the treatment evidenced that the 16.8% concentration possesses higher therapeutic effectiveness ($p = 0.01$).

PI423 Epoxidation at C22-C23 significantly increases the potency of microtubule stabilizing taccalonolides

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Microtubules remain an important target for anticancer drug discovery. Paclitaxel, a plant-derived microtubule stabilizer, is one of the most successful anticancer drugs currently used. A second class of microtubule stabilizers, the taccalonolides, were isolated from the tropical plant *Tacca chantrieri*. Recently, we isolated a number of known and new taccalonolides from *Tacca* sp. and obtained preliminary structure and activity relationship information. A wide range of antiproliferative potencies was obtained with the natural taccalonolides with IC_{50} values ranging from 23 nM to > 50 µM in HeLa cells. Taccalonolide **AF** with an

epoxy group at C22 – 23 showed the most potent activity. A simple and efficient method was developed to convert the taccalonolides **A** and **B** to taccalonolides **AF** and **AJ**, respectively. [2] **AJ** has an IC_{50} value of 4.2 nM, 734-fold more potent than the parent molecule, **B**. Based on this result, each natural taccalonolide was subject to the epoxidation reaction. All the new compounds possessing 22,23-epoxy group exhibited significant increased potency as compared to their natural precursor, with the most potent having an IC_{50} value of 0.73 nM. The new compounds also retained microtubule stabilizing activities. These results demonstrate that the C22 – 23 epoxy group facilitates optimal potency for these microtubule stabilizers.

PI424 Essential oil content of rosemary, golden sage, and spearmint treated with salicylic acid

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Studies on a wide range of plant secondary metabolites as natural products show that these bioactive compounds can act as potent anti-inflammatory, antioxidant, or anticancer agents. In general, herbs can be used to counteract various health problems in humans. Rosemary (*Rosemarinus officinalis*), golden sage (*Salvia officinalis*) and spearmint (*Mentha spicata*) are known from ancient times as important medicinal, aromatic and seasoning herbs. Salicylic acid is an endogenous plant signal molecule involved in many growth responses and disease resistance. It can also contribute to stress tolerance by stimulating highly-branched metabolic responses. In this study, plants fall into two groups: (1) plants treated with hougland solution + 2 mM salicylic acid, (2) plants with hougland solution. To evaluate total essential oil during all plants were harvested after five day, and then essential oils are extracted using steam distillatory and rotary evaporator respectively. Our data showed significant difference between total essential oil of treated and untreated plants. The result suggests that the salicylic acid may change the essential oil level by controlling metabolic pathways.

PI425 Investigation onto the mechanism behind the hepatoprotective effect of cucurbitacin compounds

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Cucurbitacins are natural triterpenoids known for their potent anticancer and anti-inflammatory activities. Recent studies showed that cucurbitacins protect HepG2 cell line against CCl_4 induced toxicity. The mechanism behind this cytoprotection is unknown. The hepatoprotective effect of cucurbitacin compounds might be due to the inhibition of tumor necrosis factor-alpha (TNF-α), an important inflammatory factor that connects inflammation and cancer. Previous reports demonstrated the role of TNF-α in tumor proliferation, migration, invasion and angiogenesis. TNF-α is produced due to the activation of IKK/NF-kB pathway in liver cells. Identification of a cucurbitacin molecular target was achieved using in Silico drug design approaches. Molecular docking of 300 natural and virtual cucurbitacin analogs over IKKβ and IKKαβ crystal structures was conducted. Docking data revealed approximately 100 potential cucurbitacin analogs with higher binding affinity to the hydrophobic pocket of IKKβ and IKKαβ compared to standard IKK inhibitor (BMS-345541). Cucurbitacin B, D, and iso-D were isolated from *Cucurbita texana* and characterized using spectroscopic techniques. In vitro cytotoxicity assay was conducted to measure the cytoprotective concentration of cucurbitacins on BRL-3A rat liver cell line. The result of the ELISA assays of TNF-α and IL-6 from pretreated liver cell line with natural cucurbitacins compounds will be presented.

PI426 Production of honokiol and magnolol in suspension cultures of Magnolia dealbata Zucc.

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Honokiol and magnolol, important anxiolytic and anti-cancer agents, have been produced in cell-suspension cultures of the eendangered Mexican plant *Magnolia dealbata* Zucc. *In vitro* cultures of the plant were established, and the accumulation of honokiol and magnolol in callus and cell-suspension cultures was measured. Leaf samples were the best explants for callus establishment and metabolite production, and FA1 line yielded 2.3 mg/g of honokiol and 5.9 mg/g of magnolol. Oxidation was inhibited with 1 g/L activated charcoal. Cell-suspension batch cultures derived from friable callus obtained from leaves of this species were grown for 30days in shaker flasks. Throughout the growth cycle, honokiol and magnolol levels, fresh and dry weight, and sucrose uptake were determined. The effects of carbon source concentration on biomassa accumulation and the synthesis of bioactive compounds were studied. By using 3 mL of inocula supplemented with 3% (w/v) sucrose, maximum yields of honokiol (8.1 mg/g) and magnolol (13.4 mg/g) were obtained after 25 days. These yields were 300% and 382%, respectively, of the yields of honokiol and magnolol obtained from field-grown plants

PI427 Antifungal compounds isolated from Diospyros bipindensis

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Diospyros bipindensis (Gürke) stem barks are used in Cameroon by pygmies Baka for the treatment of pulmonary diseases. One hypothesis was that this type of diseases could be caused by fungi. Thus the antifungal activity of the traditional preparation as well as that of different extracts was tested against Candida albicans. Some extracts presented a significant antifungal activity. The bioguided isolation was undertaken using HPLC-microfractionation in 96 well plates combined with bioautography in order to localize the active compounds in the HPLC profiling of the extracts. In a second step, medium pressure chromatography was used to isolate the active compounds. Using this approach seventeen compounds were isolated, nine of them are new natural products. The structures of the isolated compounds were elucidated by classical spectroscopic methods including UV, NMR and HR-MS.

PI428 Phytochemistry of *Dioscorea villosa*

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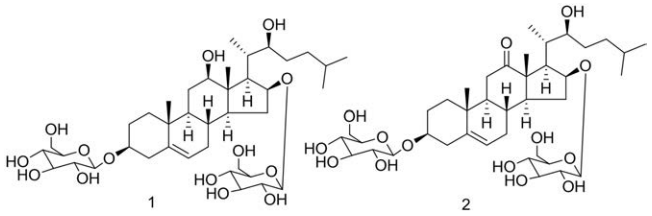
Dioscorea plants are mostly known for their spirostane and furostane steroid glycosides, particularly for diosgenin-base glycosides. *Dioscorea villosa* root, a wild yam, was investigated for new chemical constituents and two new cholestane (**1** and **2**) and several known spirostane and furostane steroid glycosides were isolated. The structures of the isolated compounds were established using spectroscopic techniques and chemical methods.



Acknowledgement: The work was supported by NIH (NIH Prime award number 1P50AT006268 – 01) and FDA (Specific Cooperative Research Agreement Number U01 FD 004246 – 01).

PI429 Haemonchus contortus: in vivo anthelmintic activity of leaves of anacardium humile

Oliveira LDR[1], Miranda JPHV[2], Curado GS[2], Costa Neto JP[2], Santos BF[2], Barros EEL[2], Louvandini H[3], Melo FR[2]
[1]UNB, Brazil; [2]UPIS, Brazil; [3]USP, CENA, Brazil

Helminthiasis represent the major health problem for sheep. The parasites reduce weight gain and reproductive capacity. The use of medicinal plants for helminthiases control has been studied. Here, we did *in vivo* evaluate of anthelmintic activity of *Anacardium humile* leaves in "Santa Inês" breed sheep, which were feed with leaves powder dried at 37 °C. This powder was added to animal feed (1,2 g/kg). The sheep weight, fecal egg counts per gram (EPG) and blood test were procedure in the 1th, 7th and 14th days. Chemical and bromatology studies of leaves were done. A reduction of 68,23% in EPG was observed in the 14th day. This result contrasted with Nery *et al.* 2010, which showed that aqueous extract of *A. humile* leaves had efficacy of 97,3% when *in vitro* assay. The amount of total tannins was 141,84 g tannic acid/kg of dry matter. The levels of protein and minerals on leaves was 9,25% and 33,87 g/Kg, respectively, against 21,61% and 57,36 g/Kg of feed. After 14 days, moderate weight gain was observed (5%) and the blood test showed decrease on eosinophils levels. It is concluded that the *A. humile* leaves could be a potential alternative for treating helminthiasis in ruminants. Reference: Nery, P.S., *et al.*/*Veterinary Parasitology* **171**, 361 – 364 (2010).

PI430 Two new glycosides of spirostane-fatty acid conjugate from *Dioscorea cayenensis*

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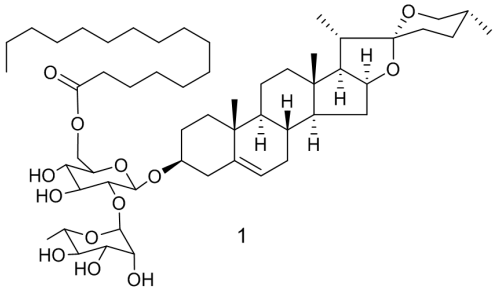
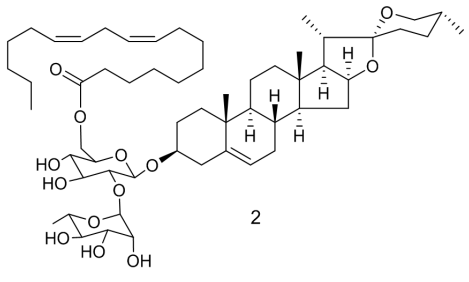
Two new glycosides of spirostane-fatty acid conjugate, progenin III-palmitate (**1**) and progenin III-linoleate (**2**), together with the several known steroid saponins were isolated from *Dioscorea cayenensis* tubers and identified by spectroscopic techniques and chemical methods.





Acknowledgement: The work was supported by NIH (NIH Prime award number 1P50AT006268 – 01) and FDA (Specific Cooperative Research Agreement Number U01 FD 004246 – 01).

PI431 Polyacetylenes and sesquiterpenoids from *Matricaria recutita* L. extract

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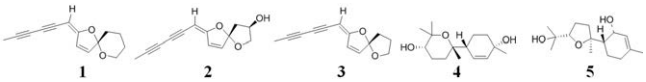
Chamomile is widely used in traditional Western medicine for the treatment of several diseases. Traditional uses mainly involve two species, viz., *Matricaria recutita* L. (German chamomile) and *Chamaemelum nobilis* (L.) All. (Roman chamomile). There are several chemotypes of *M. recutita*, mainly differing in the type and content of bicyclic sesquiterpenoids (bisabolol, bisabolol oxide A and B and chamazulene) and spyroether polyacetylenes (thongaosu, **3**). The plethora of biological activities is related to several bioactive compounds, occurring in both the essential oil and in the polar fractions of *M. recutita* extracts. Therefore, in order to find new potential biological active compounds a deeper investigation of the methanolic extract of *M. recutita* was performed. This effort resulted in the isolation and identification of several compounds that have not been reported in Chamomile before. Two of these compounds (**1**, **2**) are structurally related to the polyacetylene (**3**), and they have been reported previously in some *Chrysanthemum* species. Compounds **4** and **5** show spectroscopic similarity to bisabolol oxides A and B respectively, but there exist no evidence of their occurrence in other plants.



PI432 Spirostane and cholestane steroid glycosides from *Dioscorea caucasica*

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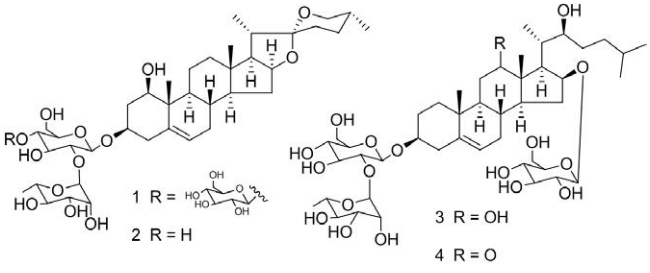
[2]Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS 38677, USA

Four new steroid glycosides of spirostane-type, 1β-hydroxydeltonin (**1**) and 1β-hydroxyprogenin III (**2**) and cholestane-type, dioscoreavillosides C and D (**3** and **4**), along with already reported steroid glycosides were isolated from the tubers of *Dioscorea caucasica*. The structures of new compounds were elucidated by spectroscopic and chemical methods.



Acknowledgement: The work was supported by NIH (NIH Prime award number 1P50AT006268 – 01) and FDA (Specific Cooperative Research Agreement Number U01 FD 004246 – 01).

PI433 Anticancer metabolistes from California walnuts (*Juglans* sp.)

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Walnuts are known to be a rich source of polyphenols and omega-3 fatty acids. Previous studies have indicated that walnut extracts contain substances that have anticancer properties. In our study, we prepared extracts of California walnuts (*Juglans sp.*), and tested them for activity

against the human breast cancer cell line MDA-MB 231. The cells were treated with walnut extracts and chromatographic fractions and counted for percent viability using Trypan blue staining. The crude walnut extract was partitioned with hexane, ethyl acetate and aqueous methanol; these fractions showed 87.6%, 3.9%, and 3.7% cell viability, respectively. Of these, the strongest activity was found in the ethyl acetate fraction (based on material recovered). This fraction was further purified using Sephadex LH20 chromatography and subjected to bioassay followed by LCMS analysis. Thus far, among the compounds identified are quercetin, catechin, and *epi*-cathechin, all of which have been previously reported from walnuts. Current progress to isolate and identify quantities of the anticancer metabolite(s) is in progress and current status will be reported.

PI434

Systematic naming system for *Actaea* triterpenes and the demand for dreiding models

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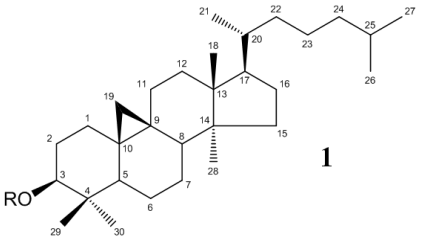
The genus *Actaea*, recently expanded to include the now obsolete genera *Cimicifuga* and *Souliea*, has been the source of ~200 structurally defined triterpenes (TTs). Except for a few ubiquitous TTs, all are endemic to *Actaea* and derivatives of the simple cycloartanol, **1**. Despite this one common denominator, the TTs comprise chemically diverse substructures, formed by oxidation, ring closure and ring cleavage. Extracts of many *Actaea* species are ethno-medicinal and CAM products. During their discovery, the TTs were given a wide variety of names, derived haphazardly from names of the species, the traditional medicine, or occasionally from a previously described structure. Often, there is little or no chemical logic to these names. We have developed a systematic naming system based on "actanol" for **1**, with all modifications following standard chemical nomenclature. The standard α and β TT stereo designation breaks down in several substructural types with spiro ring systems, and the Cahn-Ingold-Prelog system is used exclusively for defining the skeleton. In the more complex structures, this created a demand for stereo models, and the inability to purchase our favored Dreiding models, led us to establish the *Dreiding Model Exchange* (http://tigger.uic.edu/~gfp/dreiding/dreidingexchange.htm), which currently has over 3,000 pieces and is currently actively buying and selling these models.



PI435

In vivo anthelmintic activity of *Solanum lycocarpum* leaves against *Haemonchus contortus* in sheep

Oliveira LDR[1], Miranda JPHV[2], Curado GS[2], Costa Neto JP[2], Santos BF[2], Barros EEL[2], Louvandini H[3], Melo FR[2]
[1]UNB, Brazil; [2]UPIS, Brazil; [3]USP, CENA, Brazil

The favourite plant of maned wolf is the tomato-like fruit of Lobeira (*Solanum lycocarpum*). This animal works hard to eat a constant amount throughout the year, suggesting that this fruit is of some significant value. Brazilian researches correlated the fruit consumption to helminthiasis control. Here, we did *in vivo* assays with "Santa Inês" breed sheep infested by *Haemonchus contortus*. Dried leaves powder (37 °C) of *S.lycocarpum* was added to animal feed (1,2 g/kg). The sheep weight, fecal egg counts per gram (EPG) and blood test were done in the 1th, 7th and 14th day. Chemical and bromatology evaluation of powder leaves was done. A reduction of 59,3% EPG was observed in the 14th day. Seven days after treatment, EPG reduction of 48,8% was yet observed. The amount of total phenolics compounds was 30,37 g tannic acid/kg of dry matter. The levels of protein and minerals contained on leaves was 21,5% and 55,4 g/Kg, respectively, in contrast with 21,61% and 57,36 g/Kg of feed. After 14 days, weight gain was observed (11%) and the blood test showed decrease on eosinophils levels in animal treated. It is concluded that the *S. lycocarpum* leaves showed anthelmintic activity to sheep, as observed with maned wolf eating fruits of the same plant.

PI436

A new spirostane steroid glycoside from *Dioscorea nipponica*

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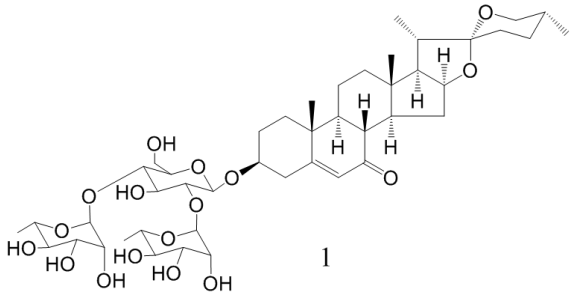
Phytochemical study of the rhizomes of *Dioscorea nipponica* resulted in the isolation of nine spirostane and furostane steroidal glycosides, including a new spirostane glycoside (**1**). The structure elucidation was achieved by spectroscopic and chemical methods.



Acknowledgement: The work was supported by NIH (NIH Prime award number 1P50AT006268 – 01) and FDA (Specific Cooperative Research Agreement Number U01 FD004246 – 01).

PI437

Ethnobotanical survey and antibacterial activity of plants of the Mexican altiplane used for the treatment of oral cavity infections

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The inhibitory effects on the growth of *Streptococcus mutans* and *Phorphyromonas gingivalis* of the aqueous and ethanolic extracts of 47 medicinal plants used in the Altiplane region of Mexico for the treatment of dental diseases such as toothache, dental caries, periodontal disease and gingivitis were determined during an ethnobotanical survey, as their minimum inhibitory and minimum bactericidal concentrations (MIC and MBC); this last determined from the wells of microplate with no visible bacterial growth. Interviews were performed to get information and inhibitory effects of the extracts were tested by the microdilution method. Values of the most active extracts (MIC and MBC) ranged from 10.5 to 67.5 μg/mL in the ethanolic extracts of *Haematoxylon brasiletto*, *Punica granatum*, *Iostephane heterophyla*, *Bursera simaruba*, *Cedrela odorata* and *Rhus standleyi*, and from 10.5 to 78.0 μg/mL for the aqueous extracts of *Haematoxylon brasiletto*, *Punica granatum*, *Iostephane heterophyla*, *Amphipterygium adstringens, Argemone mexicana*, *Cedrela odorata*, *Eysenhardtia polystachya*, *Persea americana*, *Syzygium aromaticum*, *Cinnamomun zeylanicum*, *Cnidoscolus multilobus* and *Rhus standleyi*, showing these last the highest inhibitory effect against *Streptococcus mutans* and *Porphyromonas gingivalis*. Most of the medicinal plants showed an antibacterial effect *in vitro*.

PI438 Pharmacognostic profile of root of *Cryptolepis sanguinolenta* (Lindl.) Schlechter

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Medicinal plants are traditionally found to be useful for many ailments. The present study highlights the pharmacognostical as well as phytochemical studies including parameters such as macroscopic, microscopic characters, physicochemical evaluation, chemomicroscopy and preliminary phytochemical studies of the root of *Cryptolepis sanguinolenta*. The morphological studies shows a root light to medium brown in color with hard and brittle texture, prisms of calcium oxalate crystals, sclereids and parenchyma cells. The transverse section showed parenchyma cells, vascular cambium, ray lines and phelloderm. Physico-chemical standards and their percentage values w/w were found to be: total ash (14.02 ± 0.12), acid insoluble ash (5.08 ± 0.18, %), water soluble ash (4.02 ± 0.27%), sulphated ash (4.26 ± 0.11%), alcohol soluble extractive (6.20 ± 0.45%), water soluble extractive (28.40 ± 0.75%) and moisture content (6.80 ± 0.25%). Chemomicroscopical investigation revealed presence of lignin, tannin, oils, cellulose and calcium oxalate. Phytochemical analysis of the root revealed the presence of carbohydrates, alkaloids, glycosides, saponins, resins, proteins, steroids and terpenoids. These findings will help in identification, standardization of the root of *Cryptolepis sanguinolenta* (Lindl.) Schlechter and also distinguish it from its adulterants.

PI439 Pentasaccharides from *Ipomoea alba* seeds

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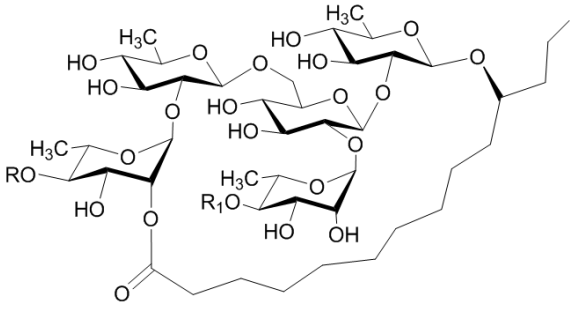
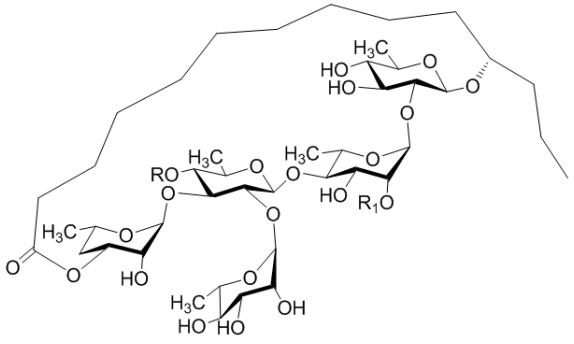
Departamento de Farmacia. Facultad de Química. Universidad Nacional Autónoma de México, Ciudad Universitaria. Mexico City 04510, Mexico

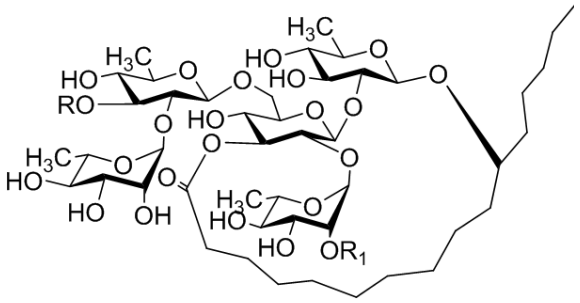
Three new resin glycosides, albinosides I and III (**1** and **3**), were isolated and purified by preparative-scale recycling HPLC from a chloroform-soluble extract of *Ipomoea alba* seeds. Their structures were established by NMR spectroscopy and mass spectrometry. Compounds **1**-**3** are partially acylated branched pentasaccharides derived from three new glycosidic acids (albinosinic acids A-C).



	R	R_1
1	nla	ace



	R	R_1
2	tga	tga



	R	R_1
3	tga	tga

PI440 Antioxidant and anabolic activities of wheat bran

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Wheat bran is a by-product of the wheat milling process and it contains various antioxidants that impart beneficial effects on human health. In wheat, *phenolic* compounds are mainly found in the form of insoluble bound *ferulic acid* and be relevant to resistance to wheat fungal diseases. *Alkylresorcinols* are phenolic lipids present in high amounts in the bran layer of wheat and rye (0.1 – 0.3% of dry weight). These constituents may contribute to the lowering of the plasma levels of the various parameters of the lipid profile. In this study, Four different fractions – Total ex. (EtOH ex.), Hexane fr., EtOAc fr. and BuOH fr. were tested for their antioxidant activities and the anabolic activity in C2C12 cells in the presence of BMP-2 by ALP staining and its activity assay and TRAP activity with RANKL staining. As a result, EtOAc fr. and BuOH fr. showed higher radical scavenging activities than others. The nitrite scavenging abilities of EtOAc and BuOH fr. were 78.0% and 80.5% respectively (10 mg/ml). Also, the TRAP activity result, 100μg/Ml concentration of Total Ex., Hexane fr. and BuOH fr. showed excellent effects. These results suggest that the wheat bran extract have inhibited osteoclast cells activities. Keywords: antioxidant, anabolic activity, C2C12 cells, TRAP activity, RANKL staining.

PI441 Anti-inflammatoty constituents from the stems of Aglaia odorata Lour.

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Bioactivity-guided fractionation on the stems of *Aglaia odorata* resulted in the isolation of four compounds including two flavonoids, one lignan and one triterpenoid. These structures were elucidated on the basis of extensive spectroscopic analysis and by comparison of their 1D- and 2D-NMR spectroscopic data with those reported in the literature. The inhibitory effects of all the compounds against lipopolysaccharide induced-nitric oxide (NO) production in RAW264.7 cell line were evaluated. Of the isolates, two flavonoids were found to active in the present assay.

PI442 Edible Passiflora (banana passion) fruits: a source of bioactive C-glycoside flavonoids obtained by HSCCC and HPLC-DAD-ESI/MS/MS

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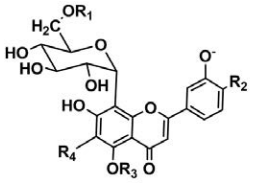
Tumbo fruits are edible fruits consumed raw and widely used in Ecuador, Peru and northern Chile to prepare an aromatic and acidic fruit juice. We have analyzed ethanolic extracts from peel and pulp of fruits of this plant by HPLC- DAD and HPLC-MS, and quantified their antioxidant as well as their flavonoid and phenolic content by spectroscopic methods. The fast fingerprint analysis allowed the detection of a family

of twenty eight C-glycoside and three O-glycoside flavonol derivatives which were characterized by UV and ESI-MS-MS. Several C-glycosides are structurally related to the new orientin derivative 4'-methoxy-luteolin-8-C-(6"acetyl)-β-D-glucopyranoside, **1** which was isolated from the peel along with other derivatives (**2 – 5**) by HSCCC and fully elucidated by spectroscopic methods. The pulp of the fruits showed good antioxidant activity, but the peel presented the highest content of flavonoids, which is related to the highest antioxidant and antiradical activity.



	R1	R2	R3	R4	[M-H]⁻
1.	Ac	OMe	H	H	503
2.	H	OMe	H	Glucose	623
3.	H	OMe	H	H	461
4.	Ac	OH	H	H	489
5.	H	OH	Glucose	H	609

PI443 Extract of Aneilema keisak inhibits TGF-β dependet signaling by inducing Smad2 downregulation

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It was observed that the *n*-hexane extract of *Aneilema keisak* (AKH) significantly inhibited TGF-β-dependent signalling, lowering Smad2 protein through in-house natural product screening. AKH treatment also inhibited a number of pathologic responses of keloid fibroblasts such as hyperplastic cell growth, migration and collagen production. Unlike bleomycin, a chemotherapeutic agent used for keloids, AKH did not induce DNA damage. We also provide evidence that miR-155 induction and alteration of Smad2 protein translation by AKH may contribute to the downregulation of Smad2 protein. Accordingly, we propose that AKH is a promising therapeutic candidate for keloid treatment once the active compound is further identified. These results suggest that AKH inhibits the pathological responses of keloid through down regulating Smad2 protein at the translational level, suggesting that identification of active compound in AKH is important for development of potential novel therapeutics for keloids.

PI444 IMAO-A activity from *Elaphoglossum erinaceum* extracts obtained by SFE, and the isolation of prenylated acylphloroglucinols

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The occurrence of phloroglucinols is restricted to ferns to which the genera *Elaphoglossum* and *Dryopteris* belong. Phloroglucinols display interesting biological activities such as antidepressant, as has been reported for hyperforin. The aim of the present study is the identification of new sources of phloroglucinols from ferns. The hexanic extract from rhizomes of *E. erinaceum* displayed an IC_{50} of 105.4 ± 2 μg/ml for the MAO-A isoform. In order to have a more simplified extract, an enriched fraction containing phloroglucinol compounds was obtained by supercritical fluid extraction (SFE). For this process, 100 g of material were extracted with CO_2 at 40 °C using a pressure gradient of 90 – 300 bars, affording a colored orange crude extract. The HPLC purification on a C-18 column resulted in the isolation of four peaks, which were collected by the technique of heart cutting, and independently reinjected in the apparatus operating in the recycle mode to achieve total homogeneity after consecutive cycles. As preliminary results, the ^{1}H NMR spectra from a purified compound indicated the dimeric structure of a prenylated acylphloroglucinol with characteristic signals for the -OH protons at δ 18.4, 16.1, 12.9 and 10.1, as well as the signals at δ 4.47 (br. t) and 4.76 (br. s) corresponding to the preny group. Thanks to CONACYT (Mexico) and CNPq (Brazil) to support the bilateral program approved for 2009 – 2012, and CONACYT project N° 80980 and 156276.

PI445 Protective effects of *Chenopodium album* (L.) on ethanol – mediated hepatotoxicity and oxidative stress

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Chenopodium album Linn. (CA, Chenopodiaceae), commonly known as 'bathua' or 'lamb's quarters', has been widely used in traditional Indian medicine for various ailments including hepatic disorders. The purpose of present study was to investigate the *in vitro* and *in vivo* protective effects of CA leaves against ethanol-induced hepatotoxicity. In the *in vitro* studies, different extracts and fractions of CA leaves were evaluated on ethanol toxicity (96 μl/ml) in primary cultured rat hepatocytes. Silymarin was used as a reference drug. *In vitro* antioxidant activity was also determined. *In vivo* hepatoprotective activity was assessed in wistar rats intoxicated with ethanol (5 g/kg, p.o.). The biochemical and histological changes caused by ethanol intake were monitored. In the *in vitro* evaluation, ethanol extract of CA (CAEE) was found to be more active than other screened extract/fractions. Moreover, CAEE was determined to be rich in phenolic and flavonoids and showed significant scavenging activity against free radicals i.e. DPPH and superoxide ion, when compared with other extracts/fractions. In the *in vivo* evaluation, CAEE (100, 200 and 400 mg/kg, p.o.) and silymarin (50 mg/kg, p.o.) exhibited a remarkable hepatoprotective activity as evident by the decreased levels of serum marker enzymes and lipid peroxidation and elevated levels of reduced glutathione, superoxide dismutase and catalase. Biochemical results were supplemented with histological observation of rat liver tissues. Collectively, the results of present study concluded that CAEE exhibited significant hepatoprotective activity on ethanol-induced liver toxicity and this activity seems mediated through antioxidant properties.

PI446 Polyphenolics from two Brazilian species of the arecaceae family

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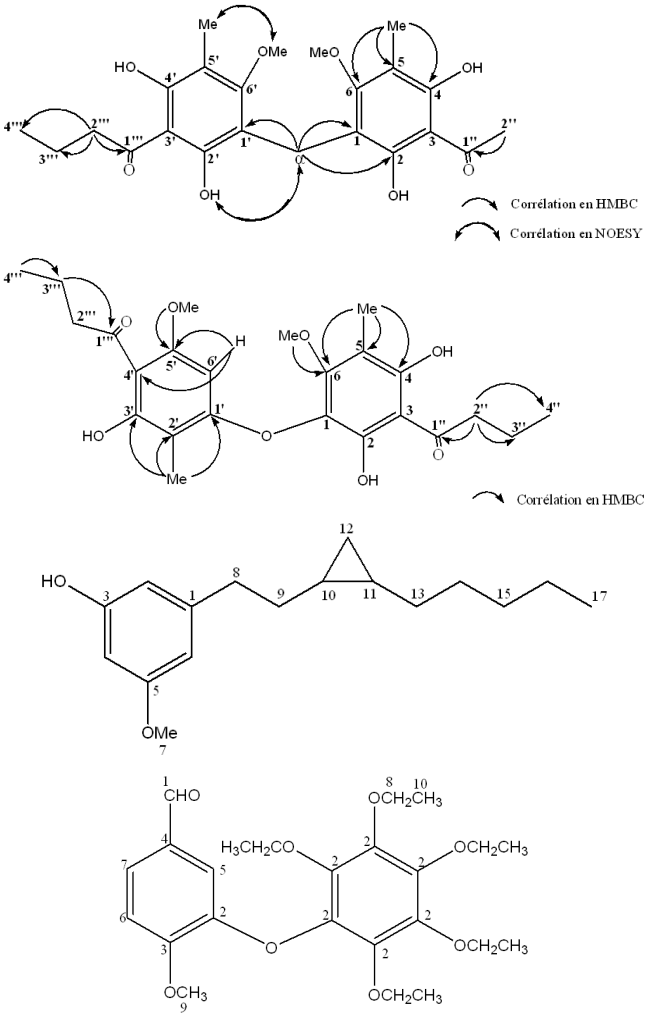
The Arecaceae family has around 270 species ocurring in Brazil, some of them valued as traditional medicines, food, and as raw materials for handicrafts like dendê (*Elaeis guineensis*), babaçu (*Orbignya speciosa*), piaçava (*Attalea fungifera*) and carnaúba (*Copernicia prunifera*). Coco-Cravo (one variety of *Cocos nucifera*) and açaí (*Euterpe oleraceae*) are two species from this family with interesting pharmacological activities (J. of Ethnopharmacol. 2004, 92, 269; Food and Chem. Toxicol., 2011, 49, 855 and Phytomedicine 2012, 19, 262). In this report we present our results on the phytochemistry of both species. HPLC/DAD and HPLC/ESIMS analysis of *C. nucifera* husk fiber extracts allowed a preliminary characterization of B-type condensed tannins of oligomeric/polymeric nature (Research in Microbiology 2002, 153, 647). After isolation, we identified catechin, epicatechin, two dimeric B-type proanthocyanidins, caffeoylshikimic acid, gallic and ellagic acids from the EtOAc fraction. *E. oleraceae* stone extract was analyzed by HPLC/DAD and the UV spectra observed were strongly indicative of the presence of condensed polymers. Positive Electrospray FTMS of the sample displayed peaks indicative of sodium aducts for proanthocyanidin oligomers from 2 to six catechin and gallocatechin units. Further work is now in progress to elucidate the composition of the polyphenolics.

PI447 Phytochemical and biological analysis of *Mallotus oppositifolius* (Euphorbiaceae)

Kabran FA[1,2], Maciuk A[1], Okpekon TA[2], Leblanc K[1], Seon-Meniel B[1], Bories C[1], Champy P[1], Djakouré LA[2], Figadère B[1]
[1]UMR 8076 CNRS, Faculty of Pharmacy, University Paris-Sud, France; [2]Laboratoire de Chimie Organique Biologique, UFR Sciences des Structures de la Matière et de Technologie, Abidjan, Côte d'Ivoire

Mallotus oppositifolius (Geiseler) Müll. Arg., (Euphorbiaceae), is an endemic shrub from tropical Africa forests and savannas. It is widely used in popular medicine against infections, intestinal worms an malaria.

Extracts of different parts of the plant have been shown to have anti-inflammatory, antioxidant, antidiarrheic, antibacterial, antifungal and antitrypanosomal properties. The present work reports the isolation of 32 compounds from the leaves, stem barks and roots. Among these compounds, six phenolic compounds are newly described. Biological assessment of these isolated compounds on *Trypanosoma brucei* and *Leishmania donovani* are described.



PI448 Evaluation of the gastroprotective activity of *Calliandra haematocephala* extracts

de Paula Barbosa A[1], Pereira da Silva B[2], Parente JP[3]
[1]Laboratory of Medicinal Plant Chemistry; [2]Natural Products Research Nucleus; [3]Health Sciences Centre, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Calliandra haematocephala (Leguminosae) is native from Tropical Americas, usually cultivated in gardens for ornamental purposes. Previous studies with other species of this genus confirmed some pharmacological properties, such as antiinflammatory, anticonvulsant, immunomodulatory, and mainly antiulcerogenic activity. Phytochemical investigations have been carried out on the constituents of this genus and demonstrated the presence of tannins, flavonoids and saponins. In order to confirm the ethnopharmacological use of this species, a phytochemical screening was realized with a butanolic extract and its gastroprotective effects were evaluated by measuring acute gastric lesions induced by acidified ethanol, using cimetidine as reference compound. The butanolic extract exhibited a moderate control of gastric lesions (35% of inhibition at 100 mg/kg), provoking an inhibition of the development of the hemorrhage and necrotic aspects of tissue injury, however showing lesser activity than the reference compound at the same dosage (50% of inhibition at 100 mg/kg). The intensity of gastric ulcers was quantified by the percentage of the injury area in relation to the control group. The results obtained confirm the gastroprotective activity of the butanolic extract of *Calliandra haematocephala*, which probably interfere with the ulcerogenic mechanism, showing a cytoprotective property, justifying the use of this plant in the traditional medicine.

PI449 Nepeta menthoides as memory enhancer

Kiyani N[1], Kiyani A[1], Khademizadeh M[1], Ranjbar Pazuki M[1], Aliasl Mamghani F[1], Mozaffari Dehshiri A[2]
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Nepeta menthoides belongs to the family Lamiaceae and known as Staechus in Iranian traditional medicine. In Traditional text, used as brain cleaner and memory enhancer[2]. So we decided to examine this claim. NMRI male mice were handled according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals (NIH US publication 86 – 23 revised 1985). The L-NAME and scopolamine used in this study were purchased from Sigma (St. Louis, USA), Nepeta. M water soluble and hydroalcoholic soluble extracts and traditional medication which was made by tradition recipe were produced by colleagues participating in this study. (Tehran, Iran). All drugs were freshly diluted in physiological saline and administrated intraperitoneally. (I.P) And Continuous spontaneous alternation (Y-maze) task used for evaluation. Mice were administered Nepeta water and hydroalcoholic extracts (50,100,150 mg/kg) 60 min and scopolamine (1 mg/kg) 30 min before training trials. Retention was performed according to the following schema in the presence and absence of scopolamine. In the second set of experiments, L-NAME (5 mg/kg) (Allami et al., 2011) was administered 30 min before training sessions. The results of each experiment were expressed as: In Y-maze: 1) exploration time (seconds) for each arm visits; 2) percentage of number of arm entries during an 8 min session; 3) total number of arm entries reflected as locomotor activity index. Data were expressed as mean ± SEM and analyzed using SPSS statistical software package. Results show N.menthoides has memory enhancing effect in mice.

PI450 Identification of novel phytotoxins from Australian range plants linked to severe mammalian digestive toxicity

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We have used a digestive developmental *in vivo* zebrafish model to examine Australian rangeland plants for their involvement in specific digestive disorders in livestock. The model allows us to discover causative phytotoxins linked to a syndrome. Here, we report the isolation and structural elucidation of five isoflavonoid compounds **1-5** with specific biological activity. From the active fraction, we identified the causative phytotoxins as two novel isoflavonoids (**1**, **4**), both with an unusual skeleton. In addition, we isolated an additional novel isoflavonoid (**3**), a novel pterocarpan derivative (**5**), and a known isoflavone, betavulgarin (**2**). Compound **4** contributed to the specific biological damage in the zebrafish. The compound is highly reactive and conjugates spontaneously with common nucleophilic biological molecules, indicating a strong electrophilic nature, suggesting a possible mechanism of action that explains death in the animals. The isolation and structural elucidation of **1-5,** together with their biological data, will be presented.

PI451 Phytochemical and pharmacological analysis of clematis species

Monschein M[1], Pferschy-Wenzig EM[1], Binder M[2], Bulusu M[2], Bochkov V[2], Zhao YM[3], Miao J[3], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria; [2]Medical University, Vienna, Department of Vascular Biology and Thrombosis Research, Schwarzspanierstr. 17, 1090 Vienna; [3]Guangxi Botanical Garden of Medicinal Plants, 189 Changgang Road, Nanning, China

Clematis L. (Ranunculaceae) is a genus of about 300 species widespread throughout the world. Many Clematis species are traditionally used for

various ailments, among them many diseases related to inflammation. As a part of a phytochemical and pharmacological study of Clematis species, extracts of the upper parts of *C. mandshurica* Ruprecht, *C. vitalba* L. and *C. integrifolia* L. were tested for anti-inflammatory activity *in vitro*. At a concentration of 50 µg/ml, the ethanol extracts in general showed much better inhibition on LPS-induced IL-8 expression in HUVEC-Tert cells than the dichloromethane extracts, with 78.2 ± 3.1 % (*C. man*), 80.8 ± 9.0 % (*C. vit*) and 80.6 ± 2.9 % (*C. int*) inhibition. LC-DAD-MS^n analysis was performed for phytochemical profiling of the tested extracts. The chemical profiles showed big variation between species, e. g. major differences were observed regarding phenolic constituents and triterpenes. Differences were also observed in the phytochemical and pharmacological analysis of leaves and stems of the three species.

PI452 Cucurbitacin glycosides from *Datisca glomerata* with antiplasmodial activity

Graziose R[1], Grace MH[2], Rathinasabapathy T[1], Rojas-Silva P[1], Dekock C[3], Poulev A[1], Lila MA[2], Smith P[3], Raskin I[1]
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The traditionally used antimalarial plant, *Datisca glomerata* (C.Presl) Baill., was subjected to antiplasmodial assay guided fractionation. This led to the isolation of seven new cucurbitacin glycosides, datiscosides I-O, along with the two known compounds, datiscoside and datiscoside B, from the aerial parts of *D. glomerata*. Their structures and relative stereochemistry were determined on the basis of mass spectrometry, 1D and 2D NMR. Antiplasmodial IC_{50} values were determined for all isolated compounds, which were also evaluated *in vitro* for their ability to inhibit *Leishmania tarentoleae*. The antiplasmodial activity of the compounds was moderate, and ranged from 33.3 µM to 7.7 µM. None of the compounds showed appreciable antileishmanial activity.

PI453 Analysis and presevation of endemic plants in the Cockpit Country region of Jamaica

Lamm AS
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The Cockpit Country region of Jamaica is a dry limestone forest with extensive, *Karst* topography. It is characterized by numerous round-topped, conical hills and sinks. Much of the region has retained its natural vegetation with a high level of endemic species. The unusual landscape creates vastly different, isolated microhabitats which supports many unique organisms. Most of which have unexplored phytochemical and medicinal properties. The Natural Products Research Laboratory (NPRL) was recently established through grant funding from the Global Environmental Facility/Small Grants Programme/United Nations Development Fund (GEF-SGP-UNDP) and the Environmental Foundation of Jamaica (EFJ). The laboratory's objectives are: 1) to conduct basic pharmacological, biomedical and phytochemical investigations of endemic and endangered plants of the Cockpit Country; 2) to expose the human value of these species thereby promoting conservation; 3) to foster discovery and scientific interests among high school students through active engagement; 4) to train undergraduate and graduate students to the highest international levels within the field; and 5) to extend local and foreign collaborations for enhanced success. The NPRL is also creating a repository of endemic plant extracts for the further investigation and development. This presentation will highlight the novelty of the region while showcasing the diversity of species, ethnomedicine, and bioactivities.

PI454 Human osteosarcoma proliferation inhibitors from royal jelly

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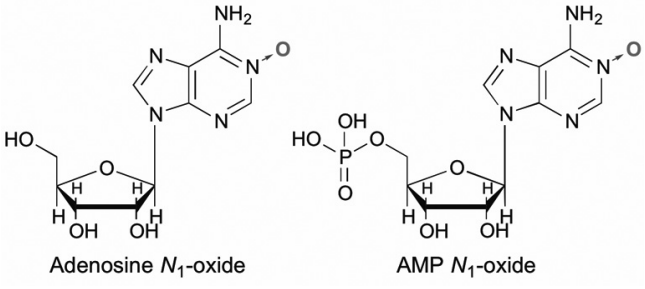
We found that royal jelly acted at the early stages of the G_1 phase and the S phase of a cell cycle and controlled multiplication of human osteosarcoma cell line, MG-63 cell. Separation of the active water extract by a dialysis membrane and a solid phase extraction suggested that active substances were high polar low molecular compounds. Furthermore, the existence of nitrogen-containing compounds having acidic groups was suggested by LC/MS (ESI^-) analyses. Further isolation procedure identified that the main active component was AMP N_1-oxide. Continuous examination revealed also the existence of adenosine N_1-oxide, ADP N_1-oxide, ATP N_1-oxide, and NAD N_1-oxide as active ingredients. AMP N_1-oxide and adenosine N_1-oxide inhibited multiplication of MG-63 cell strongly, and their control of the G_1 to S phase comparing with AMP as 1/100 low concentration was found. From these facts, AMP N_1-oxide, adenosine N_1-oxide, and other N_1-oxide are considered to be the main ingredients that contribute at MG-63 cell-growth control of royal jelly. The isolations, preparations and analyses of these N_1-oxides together with their additional biological data will be presented.



PI455 Inhibitory effect of biochanin a on cAMP level in HEK293 cells

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Biochanin A is a flavonoid in red clover, soy, and peanuts. Although biochanin A exert various biological functions such as antihyperglycemic, apoptotic, and anti-inflammatory activities, little is known about the roles of biochanin A on cAMP level in vitro system. As adenylyl cyclase (AC) and cAMP signal transduction plays crucial roles in proliferation, perception, and contraction of muscle in response to various stimuli, searching for regulators of AC-cAMP pathway is a promising tactic for clinical applications. AC is activated by binding of ligands to their G protein-coupled receptors and leads to changes of cAMP and Ca^{2+} levels. PKA, one of cAMP target, is then activated and stimulates several transcription factors influencing gene expressions. In addition, Ca^{2+} in cytosol changes plasma membrane potential and subsequently transmission of electric signals to the brain. Before investigating biological functions in AC-cAMP pathway, screening molecules regulating AC-cAMP pathway is first step. Here, we demonstrated that biochanin A synergistically increase the cAMP level and the phosphorylation of PKA, a downstream target of cAMP in HEK cells pretreated with forskolin, a stimulator of AC. Conversely, chloroquine suppresses the forskolin-induced increase of cAMP and phosphorylation of PKA. These results suggest that some molecules found in natural products play biological functions and might be applicable in clinical use.

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

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

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

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

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

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

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

Vicente Vilas V[1], Vega Bello J[1], Jiménez AMP[2], Hernández-Andreu JM[1]
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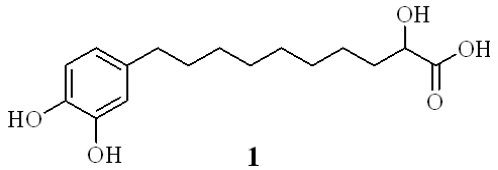
Lichens have demonstrated cytotoxic activity against many human cancer lines. In this work extracts of the Antarctic lichen, Usnea Aurantiaco-Atra, isolated with n-hexane, diethyl ether and methanol using a Soxhlet process and purified with solid phase extraction, were evaluated in vitro using two different human cancer lines (HeLa: human cervical cancer and HT-29: human colon adenocarcinoma). The MTT assay revealed significant cytotoxicity in all the fractions after purification and elution with acetonitrile. Since Usnea Auriantiaco-Atra grows in the Antarctic region, a highly UV-exposed area, antioxidant activity has been also evaluated for its potential therapeutic utilization. Antioxidant activities (AA), reducing powers (RP) and total phenolic contents (TPC) have been also determined.

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

Pedroso RCN[1], Pimenta LP[1], Lima WC[2], Soares MA[3], Magalhães LG[1], Crotti AEM[1], Silva MLA[1], Cunha WR[1], Pauletti PM[1], Januário AH[1]
[1]Núcleo de Pesquisas em Ciências Exatas e Tecnológicas, Universidade de Franca, CP 82, 14404 – 600 Franca-SP, Brazil; [2]Instituto de Ciências Exatas e da Terra; [3]Instituto de Biociências, Universidade Federal de Mato Grosso, Av. Fernando Corrêa da Costa, 2367, 78060 – 900, Cuiabá-MT, Brazil

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



1

PI461 Antimicrobial activity of *Cladonia incrassata* acetone extract

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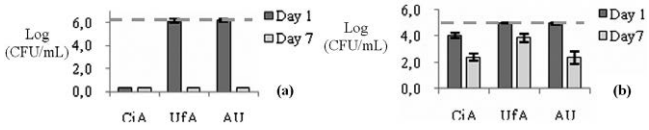
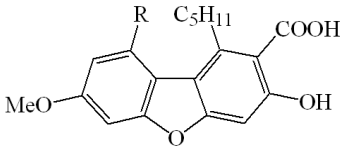
Lichens of the genera *Cladonia* and *Usnea* biosynthesize usnic acid, a widely spread dibenzofuran derivative endowed with antimicrobial activity. Usnic acid contents of *Cladonia incrassata* and *Usnea florida* acetone extracts were assessed by HPLC. Evaluation of antimicrobial activities against *Staphylococcus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



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



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

PI462 Unusual sesterterpenes from the lichen *Leprocaulon microscopicum*

Millot M[1], de Lassalle MM[1], Champavier Y[2], Chulia JA[1], Lacaille-Dubois MA[3]
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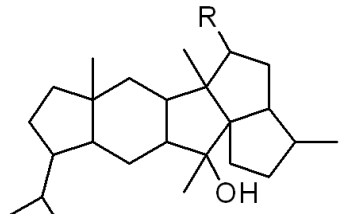
Leprocaulon microscopicum is a lichen belonging to anamorphic Lecanorales, growing in various countries and widely spread in humide areas of Limousin, France. Its chemical composition is partially described in the literature and publications still mention some unknown substances[1]. A phytochemical study of acetonic and hydro-methanolic extracts led to the isolation of (-)-usnic acid, dibenzofuran derivatives and terpenes. Among terpenoids, the common triterpene zeorin as well as two new sesterterpenoids (**1**) and (**2**) have been characterized in *L. microscopicum*. Structures were established on the basis of mass spectrometry and 2D NMR experiments. With molecular formulae $C_{25}H_{42}O$ and $C_{25}H_{42}O_2$, the new compounds featured a rare pentacyclic skeleton, closely related to retigeranic acid, the only sesterterpene isolated from lichens[2]. Thus, the present work notably extends the knowledge of the genus *Leprocaulon* and lichen chemistry. References: 1. Lamb and Ward (1974) Journ. Hattori Bot. Lab. 38: 499 – 553. 2. Kaneda et al. (1972) Tet. Let. 13: 4609 – 4611.



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

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

Froufe HJC, Abreu RMV, Barros L, Ferreira ICFR
CIMO-ESA, Polytechnic Institute of Bragança, Portugal

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

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

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

PI465 Qualitative analysis and biological evaluation of selected propolis samples all over the world

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Propolis (bee glue) is a resinous material that bees collect from plants to use it as a protective agent to prevent the spread of microbial in their hives and as construction material. It has been observed that the wide range of biological activities (antimicrobial, antiseptic, antitumor, anti-inflammatory, etc) of propolis depends on its chemical composition, more than 300 compounds have been identified in it, which in turn depends on geographical diversity. Due to its very complex chemical constitution and its pharmaceutical and nutraceutical use in market worldwide, it is necessary to characterize the quality of propolis and to guarantee a reproducible quality, in order to ensure a safe use. As a part of a systematic research on different propolis especially from Mediterranean area, we report in this study the chemical analysis of 20 selected propolis samples from all over the world: Europe (Greece-Cyprus-Bulgaria-Croatia-Germany-Poland), Africa (Algeria-Cameroon), Asia (India-China- Kirgizstan) and South America (Brazil-Uruguay). Their antibacterial, antifungal and antioxidant potentials were also evaluated showing a very interesting profile.

PI466 Physicochemical profile of selected Greek honeys – Biological activities

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As a part of a systematic research on the chemical composition of Greek bee-honeys, and as it has been observed an increasing interest in the use of honey for the treatment of bacterial infections as well as in traditional Mediterranean food, we report in this study the chemical analyses, the antimicrobial evaluation and the pollinic spectrum of twelve characteristic honey samples produced in different regions of Greece (from forests, islands, of different % of thyme, pine, fir, orange, etc). In the framework of typification of Greek honeys based on their pollen; pollinic spectra (which include the identification of the kind of pollen and their percentages) were obtained by Louveaux's quantitative microscopical analyses of our samples. The honey-samples have been studied for their physicochemical profile, by determination of pH, free acidity, humidity, conductivity, quantitative analysis of HMF (hydroxymethylfurfural) and sugar content. Also their chemical profiles were studied through GC-MS and classical isolation procedures. All of them showed a strong and broad spectrum of antimicrobial activities against all assayed microorganisms. **Acknowledgment:** This study has been partially financially supported by the project 70/3/10786

PI467 Chemical analysis of Greek royal jelly – Its influence of the long-term administration on spatial memory in aged rats

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Royal jelly (RJ) is a secretion of *Apis mellifera* bees, used to feed the queen bee. It possesses several pharmacological activities; therefore it has been widely used mainly as food supplement. In our study on beekeeping products, our research project addresses the chemical profile of Greek RJ by analytical methods and classical isolation procedures and its effect of chronic RJ pre-treatment on rat performance in the Morris water maze task (WM). Through the analysis the quality of the sample was determined containing as the most abundant compounds the aliphatic acids: 10-hydroxy-2-decenoic acid and 10-hydroxydecanoic acid and 3,10-dihydroxydecanoic acid. Also, it is provided a behavioral analysis of the effects of RJ on WM performance in rats, indicating that its long-term oral administration (50 mg/kg) improves a wide variety of behaviors while chronic pre-treatment causes cognitive and motor improvement such as: increase in the number of crossings; increase in swimming speed and prolongation of swimming distance. **Acknowledgment:** The Company "Apipharm S.A." for the kind offer of the studied royal jelly.

PI468 Phytochemical analysis and cosmetic applications of the tropical orchid Brassocattleya marcella Koss

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[1]Pharmacognosy and Bioactive Natural Products, UMR 7200, University of Strasbourg, BP 60024, 67401 Illkirch, France; [2]Service Commun d'Analyse, Faculty of pharmacy, University of Strasbourg, BP 60024, 67401 Illkirch, France, [3]LVMH Recherche, 185 avenue de Verdun, 45800 Saint-Jean de Braye, France

Brassocattleya marcella Koss is an orchid with remarkably effective depigmentation properties. The stem and leaves extracts were tested on human normal melanocyte and keratinocyte cultures and has proven its ability to act on the expression of key genes involved in each stage of the skin's pigmentation process and luminosity. The phytochemical analysis of this active extract reveals the presence of phenanthrene derivatives, such as eucomic acid and 3'4-Dihydroxy-3,5'dimethoxydibenzyl. *Brassocattleya* extract inhibits also PGE2 release on keratinocytes, with and without UVB stimulation. In controlling pigmentation and in fighting pro-inflammatory pigmentation related disorders, this orchid extract appears to be particularly adapted to treat fragile and spot sensitive skin.

PI469 Antileishmanial activity of fire ant venom alkaloids

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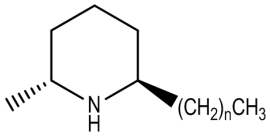
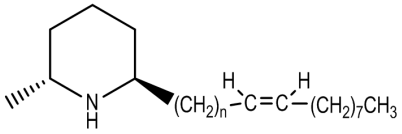
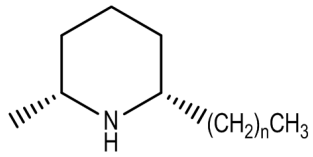
The imported fire ant *Solenopsis invicta* venom is mainly comprised of three *trans*-2-methyl-6-alkylpiperidines [solenopsins A (**1**), B (**2**), and C (**3**)] and two *trans*-2-methyl-6-alkenyl piperidines [dehydrosolenopsins B (**5**) and C (**5**)]. Varying amounts of three *cis*-2-methyl-6-alkylpiperidines [isosolenopsins A (**6**), B (**7**) and C (**8**)] are also found to be present in the venom of different types of ants. Previously, we described the stereospecific synthesis of these compounds and their antimicrobial activities. These compounds also exhibited good activity against *Leishmania donovani* (IC 50 = 0.68 – 3.5 µg/mL).



1. (2*R*,6*R*)-Solenopsin A n=10
2. (2*R*,6*R*)-Solenopsin B n=12
3. (2*R*,6*R*)-Solenopsin C n=14



4. (2*R*,6*R*)-Dehydrosolenopsin B n=3
5. (2*R*,6*R*)-Dehydrosolenopsin C n=5



6. (2*R*,6*S*)-Isosolenopsin A n=10
7. (2*R*,6*S*)-Isosolenopsin B n=12
8. (2*R*,6*S*)-Isosolenopsin C n=14

PI470 Biological and chemical evaluation of anti-TB coumarins from the polypore mushroom, *Fomitopsis officinalis*

Hwang CH[1,2], Jaki BU[1,2], Klein LL[1], Lankin DC[2], McAlpine J[2], Napolitano JG[2], Franzblau SG[1], Cho SH[1], Stamets PE[3], Pauli GF[1,2]
[1]Institute for Tuberculosis Research; [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St., Chicago, IL 60612, U.S.A; [3]Fungi Perfecti, P.O. Box 7634, Olympia, WA 98507, U.S.A.

Two naturally occurring chlorinated coumarins, 6-chloro-4-phenyl-2 H-chromen-2-one (**1**) and ethyl 6-chloro-2-oxo-4-phenyl-2 H-chromen-3-carboxylate (**2**), were isolated from the EtOH extract of the polypore mushroom, *Fomitopsis officinalis*. The structures of **1** and **2** were deduced spectroscopically and confirmed by chemical synthesis. In addition, analogues of the coumarins were synthesized as 7-chloro-4-phenyl-2 H-chromen-2-one (**3**) and ethyl 7-chloro-2-oxo-4-phenyl-2 H-chromen-3-carboxylate (**4**), and **1**-**4** were physicochemically characterized. An extensive assessment of their antimicrobial activities indicated that **2** - **4** display specific activity against both replicating and non-replicating *Mycobacterium tuberculosis* as well as *M. tuberculosis* isolates with mono-resistance to rifampin, isoniazid, streptomycin, kanamycin, or cycloserine, with MICs from 22 to 50 μg/ml.

PI471 Chemical constituents of Serbian propolis and their antimicrobial activity

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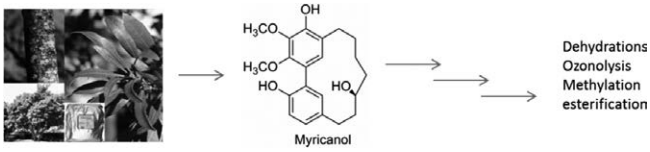
Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612

Propolis is a resinous mixture that honey bees collect from tree buds, sap flows, or other botanical sources. Samples of propolis from different geographical regions show great variety in their chemical composition and biological activities. The EtOH soluble extract of propolis collected in Serbia displayed potent activity against TB and a panel of microorganisms with MIC of 1.6 μg/ml. Dereplication using high resolution tandem mass spectrometry identified or tentatively identified approximately forty known constituents such as phenolic acids and their esters, flavonoids and prenylated flavonoids. In order to determine the active constituent(s) biological activities of the major constituents were also evaluated.

PI472 The diarylheptanoid(+)-S-myricanol from Myrica cerifera (bayberry) and its derivatives destabilize the microtubule-associated protein tau

Calcul L[1], Jinwal UK[2,3], Dickey CA[2,3], Baker BJ[1]
[1]Department of Chemistry and Center for Drug Discovery and innovation, University of South Florida; [2]Alzheimer's Institute, University of South Florida; [3]Department of Molecular Medicine, University of South Florida, Tampa, FL, 33620

Alzheimer's Disease (AD) is a neurodegenerative disorder and the most common form of dementia that affects approximately 5.1 million Americans and this number is rising in line with the aging population. The cause of the AD is not well known and to rationalize a target pathway the cholinergic, amyloid and tau hypotheses were proposed. Target-based drug discovery for AD based on the acetylcholine production and modulation of the amyloid plaques formation have not been effective. An overlooked approach that focuses on tau aggregation was used in our previous bioassay-guided fractionation study of a tau-reducing root bark extract of *Myrica cerifera* (bayberry) and led to the identification *S*-(+)-myricanol as a tau destabilizer agent. This diarylheptanoid compound may represent a novel scaffold for drug development efforts targeting tau turnover in AD. A larger scale extraction from the bayberry root bark provided enough pure *S*-(+)-myricanol to generate semi-synthetic derivatives which have been screened for their tau protein modulation and cytoxicity.



PI473 Gastroprotective effect of royal jelly in a model of acute gastric lesion and over gastrointestinal motility

Figueiredo IST[1], Queiroz NMS[1], Osório CBH[1], Olinda TM[2], Benevides FT[1], Oliveira RSB[1], Alencar NM[2], Aragão KS[1], Gonçalves DO[1]
[1]Estacio of Ceara Via Corpvs -Nutrition Faculty, Brazil; [2]Department of Physiology and Pharmacology, Brazil

Royal Jelly (RJ) is a highly nutritious food produced by worker bees. Particularly remarkable for its content of vitamin, minerals and unsaturated fatty acids. This study was performed to investigate the effects of RJ in the model of gastric ulcer induced by ethanol and over the gastrointestinal motility. Experimental protocols were registered on the Institutional Ethics Committee under number 104/09. Swiss male mice (n = 8) were used in the experiments after 18 hours of fasting period. Animals were treated by gavage with vehicle (NaCl 150mM/200 μL) or RJ (40, 100, 200 and 400 mg/kg) 1 h before absolute ethanol (200 μL/animal). The same treatment was applied before administration of 10% activated charcoal. After 30 min of ethanol administration, animals were sacrificed and the percentage of injured gastric area was determined by ImageJ software to evaluate the gastroprotective activity. After 60 minutes activated charcoal administration, the animals were sacrificed and removed the stomach, as well as small and large intestine to evaluate gastrointestinal motility by the distance traveled by activated charcoal suspension. RJ at doses of 100 and 200 mg/kg significantly reduced the percentage of ulcerated area and increased the gastrointestinal transit compared with the control group ($p < 0.05$). We can conclude that RJ showed gastroprotective activity in the model of gastric lesions induced by ethanol, probably due their antioxidant properties and increased the gastrointestinal motility by mechanisms as yet unknown. Additional studies are needed to better clarify the mechanisms involved in these effects.

Topic J: Natural Products Technology: Analytical Chemistry, Isolation/Chromatography, Structure Determination and other

PJ1 The spectro-electro array: A novel platform for the measurement of secondary metabolites in botanicals, supplements, and beverages

Acworth IN, Ullucci P, Thomas DH
Thermo Fisher Scientific, Chelmsford, MA USA

Plants contain an amazingly diverse range of secondary metabolites, many of which are purported to offer health benefits. The challenge for the analytical chemist for the measurement of these compounds is two-fold: first, to develop assays that can accurately discriminate between compounds that often have similar physicochemical characteristics; second, to analyze such compounds in complicated matrices including botanicals, supplements, and beverages. Gradient HPLC with diode array detection (DAD) is often used for the determination of natural products. However, this approach often suffers from a lack of specificity as compounds with similar structures may co-elute chromatographically and cannot be deconvoluted spectrally. Furthermore, this technique lacks sensitivity limiting its use for the study of natural product metabolism in animals and humans. Coulometric electrochemical array detection, on the other hand, is selective, being able to distinguish between subtle changes in chemical structure and, with sub pg LODs, can be used to

examine natural product absorption and metabolism. The combination of DAD and coulometric EC array detection extends the range of compounds measured by either technology alone. The technique has been used to resolve and quantify specific phytochemicals in crude extracts of a variety of natural products supplements, (e.g., ginseng, black cohosh and ginko) beverages (black and green tea), culinary herbs (oregano, rosemary) and spices (clove, nutmeg). Changes in the pattern of metabolites, when evaluated using chemometric modeling software, can be used to study: product authenticity, adulteration, contamination, and composition, and in the case of wine and fruit juice, the effect of growing region and differences between varietals.

PJ2 Novel, universal approach for the measurement of natural products in a variety of botanicals and supplements

Acworth IN[1], Ullucci P[1], Thomas DH[1], Roman MC[2]
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Botanicals contain a great diversity of compounds that exhibit wide variation in their physicochemical properties. Although no single analytical method is available to measure all potentially active components, HPLC with charged aerosol detection is a nearly universal approach that nonselectively measures any non-volatile and many semi-volatile compounds; that is, CAD does not require that analytes be ionizable (as required for mass spectrometry) or contain a chromophore (as required for UV spectrophotometry). A number of isocratic and gradient HPLC-charged aerosol detection methods were developed and evaluated for the measurement of phytochemicals extracted from a variety of botanicals including: flavonolignans from milk thistle (*Silybum marianum*); triterpene glycosides from black cohosh (*Cimicifuga racemosa*); ginkgolides and bilobalides from ginkgo (*Ginkgo biloba*); ginsenosides from ginseng (*Panax ginseng*); phytoestrogens from soy; ursane and oleanane triterpenes from gotu kola (*Centella asiatica*); and diterpene glycosides from stevia (*Stevia rebaudiana*). Analytes showed consistent response independent of chemical structure (typically < 10% variability between compounds corrected for gradient elution). All methods had a wide dynamic range (~four orders of magnitude), good sensitivity (typically low ng levels of detection), and excellent reproducibility (RSDs typically < 2%) even at low detection levels. Comparative data from ELSD and UV detection will also be discussed.

PJ3 Determination of isoxanthohumol, xanthohumol, bitter acids, and *trans* and *cis*-iso-alpha acids by HPLC with UV and EC detection

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Hops are the female flower clusters of a hop species, *Humulus lupulus.* They are used as a flavoring and stability agent in beer, and are also used in other beverages and as an herbal medicine. Hops contain a number of important phytochemicals including: Xanthohumol (a prenylated chalconoid), and alpha- and beta- acids. As part of the beer brewing process hops or hop extracts are added during the boiling of the wort. The virtually insoluble alpha-acids (humulones) are isomerized into the more soluble iso-acids, the main bittering substances in beer. Beta acids (lupulones) do not isomerize and have a negligible effect on beer taste. Instead, they contribute to beer's bitter aroma but unfortunately may oxidize into compounds that can give beer off-flavors. Many HPLC methods have been applied to the determination of phytochemicals in hops and beer. HPLC techniques using UV detection typically require a concentration step in the analysis to be able to determine low levels of the bitter acids. Presented here is a global HPLC method using serial UV and Electrochemical Detection and its application to the measurement of numerous phytochemicals in hops and beer. The chromatographic separation was performed on a Thermo Scientific Acclaim C30 column (3 µm, 3.0 x 150 mm) at 35 °C with gradient elution and simultaneous UV (270 nm) and EC detection (500 mV and 800 mV). Sample preparation involved extraction with acidified acetonitrile and centrifugation. All calibration curves showed good linear regression (r^2 > 0.996). RSD's over a twenty-hour run were as follows: isoxanthohumol and xantholhumol, 1.2%; alpha and beta bitter acids, 2.5%; and trans and cis-iso-alpha acids, 2.4%. LODs for most analytes were in the pg range.

PJ4 Composition of the essential oil of different populations of Ziziphora tenuior from Iran

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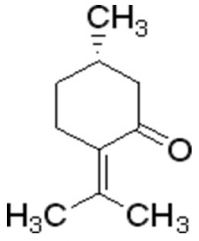
In Iranian folk medicine, *Ziziphora* species have been used as an infusion for various purposes such as for the anti-asthmatic, stomachic, carminative, activities etc [1 – 2]. The chemical compositions of the essential oil obtained from the aerial flowering parts of *Ziziphora tenuior* L. (Lamiaceae) by hydro-distillation using a Clevenger- type apparatus [3], from three different regions of Fars province, Iran. The essential oils were analyzed by GC and GC/MS. The major component of the oil was pulegone (51 – 87%). **Key words:** Essential Oil Composition, *Ziziphora tenuior* L., Pulegone



Pulegone

[1] Ghasemi Pirbalouti, A. 2009. Medicinal plants used in Chaharmahal and Bakhtyari districts, Iran. *Herba Polonica*., 55: 38 – 34. [2] Zargari, A. 1989 – 1992. Medicinal Plants. Vol. 1 – 6. University Publication, Tehran, Iran. [3] British Pharmacopoeia. 1988. Vol. 2, pp. 137 – 138. London: HMSO.

PJ5 Variations in carotenoid and anthocyanin compositions in sweet potato according to cultural varieties and home-processing methods

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A high-performance liquid chromatography method was applied to determine the content of carotenoid composition in nine cultural varieties of sweet potato (*Ipomoea batatas* Lam.) and home-processed one by most common cooking methods. The changes in carotenoid contents and composition were also observed during home-processing of an orange-fleshed cultivar showing the highest carotenoid content (529.7 µg/g DW as β-carotene). The loss of the carotenoids occurred through the all home-processing, and the baked or boiled or steamed sweet potato showed higher amount of β-carotene (245.8, 253.0 and 239.9 µg/g DW) than pressure-cooked, sautéed and fried ones (194.1, 200.6 and 110.9 µg/g DW). Interestingly, a *cis*-isomer of the all-*trans*-β-carotene, 13-*cis*-β-carotene was found in raised amounts in all processed samples, especially in boiled, steamed and pressure-cooked ones. The higher content of another isomer, 9-*cis*-β-carotene was detected in pressure-cooking and frying processed sweet potato than in the other samples. For a purple-fleshed cultivar, the variations in anthocyanin content during home-processing were also evaluated. *Acknowldgements: This work was supported by grants from the Next-Generation BioGreen 21 Program (SSAC, Grant # PJ008119), Rural Development Administration, Republic of Korea.*

PJ6 Validation of HPLC assay for the determination of oxyprenylated anthraquinones produced by *Rhamnus* L. species

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Rhamnus L. spp. (Rhamnaceae) is known to contain active anthraquinones secondary metabolites but the presence of oxyprenylated ones is still not reported. As continuation of our studies [1, 2], a new method [3] was validated to quantify these analytes in plant extracts after extraction with *n*-hexane and methanol using a ODS column, water and methanol (1% formic acid, v/v) as mobile phase at 0.7 mL min^{-1} in gradient elution. Quantitative analyses, at 435 nm, revealed LOQ of 0.5 μM and linearity up to 125 μM. Precision values ranged from 0.2% to 12.9% while trueness from 12.2% to 12.7%. References [1] Locatelli M. Current Drug Targets (2011) 12:366 – 380. [2] Kremer D. et al., Food Chemistry (2012) 131:1174 – 1180. [3] Locatelli M. et al., Journal of Chromatography A (2012) 1225:113 – 120.

PJ7 Quantification of 4'-geranyloxyferulic acid and boropinic acid by HPLC-DAD in grapefruit skin from different country origins

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Oxyprenylated natural products are a family of secondary metabolites featured by remarkable anti-cancer and anti-inflammatory effects. 4'-Geranyloxyferulic acid exerts valuable chemopreventive effects on several types of cancer, while its structural analogue boropinic acid avowed inhibitory effect against the growth of *Helicobacter pylori*. We wish to report herein, as a continuation of our studies [1], the validation of a new method for the selective determination of the title molecules in grapefruit skin extracts, after extraction with ethanol (1:3, *m/v*) using a ODS column at 10 °C, water and methanol as mobile phase at 1.2 mL min^{-1} flow rate in gradient elution mode. Quantitative analyses revealed LOQ of 0.5 μg/mL and linearity up to 50 μg/mL ($r^2 \geq 0.9960$). Precision values ranged from 0.80% to 11.7% while trueness ones from -10.5% to 14.2%. This method was successfully applied and significant differences were observed depending on the country of origin of the vegetable material.

PJ8 Chromatogaphic determination of molar absorptivity of natural prenyloxycinnamic acids in standardless analysis

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In recent years naturally occurring prenyloxycinnamic acids were shown to exert remarkable anti-cancer and anti-inflammatory effects. 4'-Geranyloxyferulic acid was found efficient as an active chemopreventive agent, and its structural analogue boropinic acid was shown to exert inhibitory effects against the growth of *Helicobacter pylori*. We report herein the comparison between traditional and HPLC-DAD methods for the molar absorptivity coefficient determination of 4-geranyloxyferulic acid (ε_{310}= 12,950 and ε_{288}= 11,910 $Lmol^{-1}cm^{-1}$) and boropinic acid (ε_{310}= 13,510 and ε_{288}= 12,350 $Lmol^{-1}cm^{-1}$). The data reported, as a continuation of previously reported work [1], will be useful for future studies aimed to better define the pharmacological profile, the mechanism of action of the title compounds, and to evaluate other natural products where standard powders are not available. **References [1]** Genovese S. *et al., Journal of Pharmaceutical and Biomedical Analysis* (**2010**) 53:212 – 214.

PJ9 Anthraquinone chemical fingerprint: Madagascin from the fruits of *Rhamnus* L. Spp.

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Prenylation is the chemical or enzymatic addition of a terpenyl moiety to an accepting molecule that occurs in nature in plant families like Rutaceae, Asteraceae, Apiaceae, Clusiaceae, Fabaceae. As a continuation of our studieswe report herein the determination of madagascin from the fruits of Rhamnus cathartica L., R. fallax Boiss., R. intermedia Steud. & Hochst., and R. rupestris Royle. The title compound is described for the first time as a component of fruit extracts and is noteworthy to underline that this compound was found only in Rhamnus cathartica L. and R. intermedia Steud. & Hochst., at 14.31 and 4.63 μg/g of fruit (dry weight), respectively.

PJ10 Robust standard procedures for plant metabolomics with NMR-improvements in the extraction and processing procedures

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In metabolomics comprehensive metabolic fingerprints are obtained and subsequently analyzed to find the metabolites which differentiate groups of samples. A new extraction method with a two-phase system of water and chloroform was evaluated and validated, by varying different variables in the extraction protocol (e.g. the extraction time and cycles of ultrasonic vibration). The effect of these variables was tested on the yield of the secondary metabolite eupatorin, a major secondary metabolite in plant material of *Baccharis trimera*. For preprocessing the NMR data before submission to multivariate data processing, a specific software has been developed for reference deconvolution. The signal from an internal standard is used for the determination of the experimental peak shape. Subsequently all signals in the spectrum are corrected for this peak shape, reducing the peaks to single lines. A great reduction in data points is obtained and furthermore small signals are preserved, even when they are not resolved from major peaks.

PJ11 Development of an immunochemical differentiation method for *Salvia divinorum*

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Salvinorin A (Sal A) which is the neoclerodane ditrepene and is an extremely potent and highly selective kappa opioid receptor agonist, is the main active constituent isolated from the leaves of *S. divinorum*. It is a powerful psychoactive perennial herb, belongs to Lamiaceae family. Sal A has been shown to have various pharmacological activities. We have successfully prepared monoclonal antibodies (MAb) against Sal A, and developed an enzyme-linked immunosorbent assay (ELISA) system for determination of Sal A. A single-chain variable fragment (scFv) which is a kind of recombinant antibodies for Sal A was prepared and used in an ELISA. The recombinant antibodies which are derived from a MAb against Sal A prepared by us was expressed by *Escherichia coli* cells and characterized regarding their reactivity and specificity.

PJ12 HPTLC-densitometry: A step further for routine quality control of cranberry (Vaccinium macrocarpon) extracts

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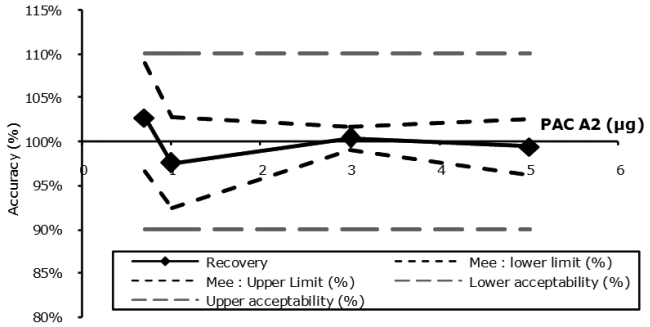
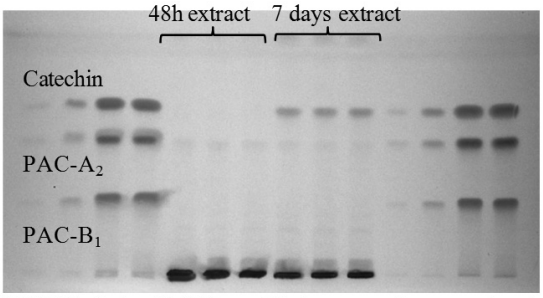
Cranberry (*Vaccinium macrocarpon*) focused the interest of scientist for years now, due to assessed biological activities such as anti infectious, anti inflammatory or anti cancer activity. Anti infectious activity is linked to Proanthocyanidins A (PAC A), major PACs in cranberry. To characterize PACs content of cranberry extracts, official method is called BL-DMAC which is a global quantitation method. A new routine analytical method of PAC content will be presented here based on HPTLC – densitometry. This protocol allows selective quantitation of catechin, PAC-A_2 and PAC B_1 (both dimeric PACs) on one plate.



Accuracy profile of PAC-A_2 quantitation on the range of 0.7 to 5 µg



HPTLC plate of 48 h and 7 days cranberry extracts (70 mg/ml), mobile phase CH_2Cl_2 /AcOEt/Formic acid (6:10:1, v/v), stained reagent 1% vanilin in HCl

PJ13 3-phase sample preparation chip hyphenated to nano-HPLC/ESI-MS for rapid miniaturised analysis of alkaloids

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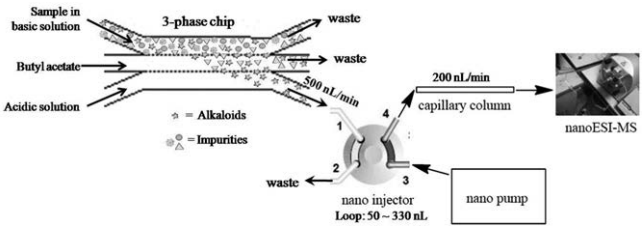
A 3-phase liquid-liquid extraction (LLE) chip was hyphenated with nano-HPLC/ESI-MS (Fig.). Thus the purification, separation and MS detection of alkaloids was accomplished on-line. The system is solvent-efficient with a nanoLC flow rate of 200 nL/min, and chip flow rate of 500 nL/min, sample-efficient (50-330 nL sample volume) and time-efficient (saving tedious off-line sample pretreatment steps. The transfer efficiency of strychnine was above 92% in 25 sec). The analyses of alkaloids in Semen strychni, *Cephaelis ipecacuanha*, *Atropa belladonna*, *Vinca minor*, and Coptidis rhizome were successfully performed. The limits of detection (LODs) of the alkaloids are lower than with conventional HPLC/ESI-MS and nano-HPLC-DAD, i.e. 5 ng/mL for emetine, palmatine, vincamine, strychnine and 10 ng/mL for atropine.



PJ14 withdrawn

PJ15 HPLC quantitative analysis of three major antioxidative components of *Moringa oleifera* leaf extracts

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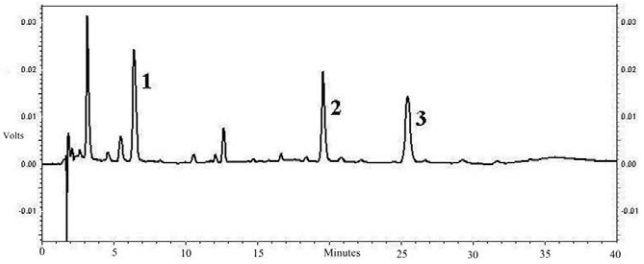
Moringa oleifera Lam. has been used as a medicinal plant in tropical and subtropical countries. Crypto-chlorogenic acid, isoquercetin and astragalin are main antioxidative components in the leaves of this plant. However, there has been no report on the contents of these substances in *Moringa oleifera* leaves. In the present study, HPLC was performed using a Hypersil BDS C18-column eluted with gradient methanol: 1% acetic acid with a flow rate of 1 mL/min and detection at 334 nm. The method was illustrated to be precise with RSD < 2%. The average recoveries of crypto-chlorogenic acid, isoquercetin and astragalin were 98.50, 98.47 and 98.59%, respectively. Fourteen samples of *M. oleifera* leaf extracts were analyzed and the average contents of crypto-chlorogenic acid, isoquercetin and astragalin were found to be 0.07, 0.06 and 0.13% w/w, respectively. This work would be useful as a guidance for standardisation of *M. oleifera* leaf extract raw materials and their pharmaceutical commercial products.



HPLC chromatogram of 70% ethanolic extract of M. oleifera leaves; 1 = crypto-chlorogenic acid, 2 = isoquercetin, 3 = astragalin

PJ16 A rapid validated UHPLC-PDA method for anthocyanins quantification from *Euterpe oleracea* fruits

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Commercialization of *Euterpe oleracea* fruit has increased because of its abundance in anthocyanins [1]. Characterizations of these compounds are important for the food industry. The aim is to validate an UHPLC-PDA method for major anthocyanins quantification in this fruit after fast extraction procedures and samples preparation. Fruits were harvested in Abaetetuba (Brazil) and extracted sequencially by EtOAc, MeOH and MeOH 50% all at 0.1% HCl. A HSS C18 column (1.8 µm) was used with a gradient elution of ACN and 5% HCOOH. Total error and accuracy profiles were used as validation criteria. A first EtOAc extraction re-

moves the lipophilic compounds and allows an easier extraction by MeOH and quantification of anthocyanins in this extract. It was found to be faster (17 min) that HPLC-UV methods [2]. Calibration in the matrix was found to be more accurate than calibration without matrix. Trueness (< 6.76% relative bias), repeatability (< 4.6% RSD), intermediate precision (< 5.3% RSD), selectivity (by UHPLC-ESI$^+$-HRMS), response function and linearity for cyanidin-3-glucoside and cyanidin-3-rutinoside were evaluated. The concentration range validated was 1 to 48 µg/mL for both compounds. [1] M. Heinrich *et al*., Phytochem. Lett. 4 (2011) 10. [2] L. A. Pacheco-Palencia *et al*., Food Chem. 115 (2009) 1199

PJ17 Optimization and validation of ursolic acid by HPLC in Ocimum sanctum

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Ocimum sanctum (Holy basil) (Lamiaceae) is regarded as one of the most important plant used in Ayurvedic medicine which constitute ursolic acid (3-β-hydroxy-12-ursen-28-ic acid, CAS# 77 – 52 – 1), a pentacyclic triterpene acid with anti-cancer and anti-stress activities. The primary aim was to develop a simple and rapid reverse phase HPLC assay method for estimation of ursolic acid in *Ocimum sanctum* herb powders and extract. A HPLC system comprising of RP-C 18 column (250 x 4.6 mm, 5 µm particle size) with solvent system of acetonitrile and water in isocratic mode and detection at 210 nm using PDA detector. The optimized method with run time of 18 minutes was validated for LOD, LOQ, linearity, accuracy, precision, sensitivity, system suitability and stability. The proposed method was found to be linear in the range of 0.25 – 500 µg/ml, with correlation coefficient of 0.9997. The LOD was 0.125 µg/ml and LOQ was 0.25 µg/ml. The developed HPLC method was found to be precise, sensitive, accurate and reproducible, and may be used for quantitative estimation of ursolic acid in *Ocimum sanctum* herb powders and extracts. .

PJ18 Total and inorganic arsenic contents in rhizomes of three herbal spices cultivated in Thailand

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Parts of plants can accumulate arsenic at different concentrations. Several concerns have been raised regarding the arsenic levels since inorganic arsenic is found in almost food and known as a human carcinogen. Arsenic is present in several forms with different toxicities. Inorganic arsenic species [As(III) and As(V)] are the most toxic form found in food. In this study, total and inorganic arsenic contents were determined in the rhizomes of three spices in the family Zingiberaceae cultivated in Thailand; *Alpinia galanga*, *Curcuma zedoaria* and *Zingiber cassumunar* which were collected from different parts of Thailand between January 2011 and March 2012. Total arsenic (expressed in ng/g, dry wt) in the three spices were 92.4 ± 9.19 (ranged from 74.7 to 107), 89.8 ± 17.5 (55 to 127), and 107 ± 19.5 (73.6 to 145), respectively, while inorganic arsenic were 48.8 ± 6.99 (39.3 to 65.1), 38.7 ± 4.71 (24.4 to 44.6), and 71.2 ± 11.6 (52.7 to 92.1), respectively. Percentages of inorganic arsenic relative to total arsenic were found to be 53.2 ± 8.18 (38.9 to 69.6), 44.5 ± 9.95 (28.5 to72.8), and 67.4 ± 7.46 (54.6 to 83.1), respectively. This study will be used for risk assessment of Thai people from dietary exposure of inorganic arsenic.

PJ19 Phytochemical analysis of the herbal 'legal high' kratom (Mitragyna speciosa)

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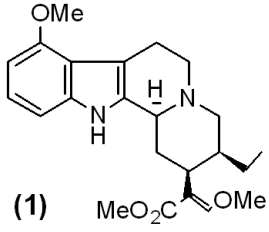
'Legal highs' are psychoactive synthetic or natural products that can be purchased from the internet or in retail stores without legal restriction. In 2011, the European Monitoring Center for Drugs and Drug Addiction reported a rapid growth in legal high online sales mostly from the US and UK. Products from the leaves of Mitragyna speciosa Korth (Kratom), a psychoactive plant from Southeast Asia, were the most common legal highs provided by internet suppliers. While the growing and selling of the plant are restricted in the source countries, products labeled as 'Kratom' are legally and widely distributed in western countries. In this study, ten commercial samples of Kratom were acquired from different UK-based websites. Phytochemical analyses of the samples were carried out using TLC, NMR, ESI-MS and HPLC techniques. Mitragynine (1), a psychoactive component possessing opiate-like effects was used as a standard marker, and was detected in all samples. Quantitative phytochemical analysis led to the isolation of other related indole alkaloids, demonstrating that the Kratom products were authentic and of plausible pharmacognostical origin. The presence of 7-hydroxy-mitragynine, the major µ-opioid agonist from this species was also confirmed, suggesting that these samples may have opiate-like harm potential.



PJ20 Characterization of the purple vacuolar pigment of *Zygogonium ericatorum* alga

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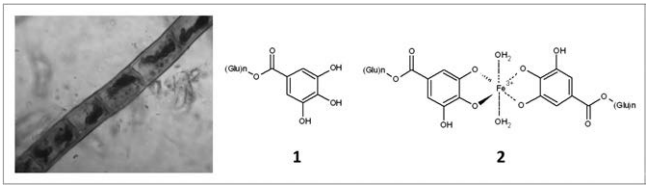
Zygogonium ericatorum is an acid and desiccation tolerant filamentous green algae that thrives in extreme habitats. A purple pigment which accumulates in the vacuoles of sun exposed layers of *Z. ericatorum* algae has remained unidentified for over fifty years. In this work, samples of the algae were collected from acid bogs in Yellowstone National Park, WY. The purple pigment was isolated and characterized by NMR, mass, IR, UV, EPR, X-ray, and ^{57}Fe Mössbauer spectroscopy. The purple pigment was found to be a highly branched polymer of glucose containing traces of ester linked polyphenolic moieties such as gallic acid (**1**). The purple color of the polysaccharide is due to complexation of the polyphenolic groups by ferric iron in a *bis* (L_2Fe^{3+}) configuration (**2**).



PJ21 Aloe vera: Quantification of key metabolites for identity and quality assessment

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Aloe vera is a medicinal plant with a wide range of uses from topical application to soothe burns to oral consumption to aid digestion. It is added to a wide range of health and beauty products. The quality and safety of dietary supplements has been emphasized since the 2007 FDA cGMP ruling, which states that manufacturers must ensure identity, purity, strength, and composition of their products. Botanical material is highly variable depending on species and growing conditions which make evaluation of these materials challenging. For analyzing *Aloe vera* extract in detail, components include glucose, acetylated mannose polymers, and malic acid. As the material ages, degradation products include acetic acid, lactic acid, formic acid, and fumaric acid. Common additives include the preservatives sodium benzoate and potassium sorbate. Depending on the formulation, other additives such as glycerol may be present. Nuclear magnetic resonance spectroscopy provides an effective

means of evaluating botanical material as a result of its ability to be used as (1) a fingerprinting tool and (2) for quantitative analysis. Presented here is the implementation of a ^{1}H-NMR spectroscopy-based method [1] to evaluate *Aloe vera* in the Assure-RMS software package to provide a fully automated analysis of *Aloe vera* samples. The automated analysis will be described, first presenting the readily quantitated components, emphasizing the features of the spectra of these components that lend themselves to robust analysis. Then more problematic components will be examined. Strategies to improve the quantitation, including additional data and more sophisticated analysis, will be discussed. **References:** [1] Jiao *et al.* (2010) J. of the AOAC International, Vol. 93, p 842 – 848.

PJ22 Anthocyanin analysis of *Acanthopanax* fruits using HPLC-ESI tandem MS

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Acanthopanax species are indigenous medicinal plant and the fruits of *Acanthopanax* species have been used as a remedy for "wipe out evil wind" in traditional medicine. Although there have been a few phytochemical studies on *Acanthopanax* fruits, no study on the comparison of anthocyanin contents and types between *Acanthopanax* species distributed in Korea was performed. In order to provide the phytochemical information about two major *Acanthopanax* species in Korea, *Acanthopanax senticosus* and *Acanthopanax divaricatus* var. *albeofructus*, HPLC-MS studies were performed. Cyanidin-3-lathyroside, a characteristic anthocyanin of Araliaceae family, was identified for the first time from the fruit of *A. senticosus* as a major anthocyanin. The relative content of cyaniding-3-lathynoside between *A. senticosus* and *A. divaricatus* var. *albeofructus,* and the information about some other minor anthocyanins were characterized.

PJ23 TLC-densitometric analysis of beta-sitosterol in agarwood leaf extracts

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The leaves of agarwood (*Aquilaria* spp.) of Thymelaeaceae family, have been used as a herbal tea for health promotion. A quantitative TLC-densitometric method was developed and validated for determining of beta-sitosterol in leaf extracts of the three agarwood species i.e., *Aquilaria crassna*, *A. malaccensis* and *A. subintegra*, cultivated in Thailand. The method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The linearity was found over the range of 125 – 1750 ng/spot with r^2= 0.9996. The relative standard deviations of intraday and interday precisions studies were less than 2%. The average recovery was 100.78% while LOD and LOQ were 25 and 112.5 ng, respectively. The contents of beta-sitosterol in the extracts of *A. crassna*, *A. malaccensis* and *A. subintegra* were 0.14, 0.12 and 0.12% w/w, respectively. The proposed TLC-densitometric method was simple, precise, specific, sensitive and accurate for routine quality control of raw materials of agarwood leaf extracts and their products.

PJ24 Microwave assisted glycosylation for determining the absolute configuration of carbohydrates in plant glycosides

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Glycosides are common natural products often isolated from plant sources. Rarely is a novel monosaccharide identified from a plant derived glycoside. However, numerous monosaccharides are found in natural products as their D or L enantiomers. A number of techniques have been developed to determine the absolute configuration of the monosaccharides found in glycosides. These procedures usually entail the derivatization of the free hydroxyl groups followed by an acid catalyzed methanolysis of the glycoside to give the individual O-methyl glycosides which are verified by applying GC-MS and comparison to known standards. Once the structural identity of the monosaccharide is determined the procedure is repeated with a chiral alcohol (e.g., R or S 2-butanol or 2-octanol) to produce a single enantiomer (or diasteromeric mixture) which is compared to independently synthesized standards. This presentation discusses our recent efforts to determine the absolute stereochemistry of monosaccharides using chiral 2-butanolysis of permethylated glycosides catalyzed by strong acid ion exchange resins with a microwave reactor. This procedure is run in a minimum amount of neat chiral alcohol with a small sample size and requires no reaction work-up. The experimental GC-MS results of both standard monosaccharides and intact glycosides will be presented.

PJ25 Multiresidue screening of organochlorine pesticides in herbal products marketed in Singapore

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In Singapore, there has been a significant and growing interest in herbal preparations/or health supplements from natural products. However, no quality control procedures in terms of pesticide contamination are required for the import of herbal materials at this moment. Therefore, a pesticide screening study of the local marketed herbal products is of particular interest. In the present study, Solid-Phase Extraction (SPE) combined with Gas Chromatography Mass Spectrometry (GC/MS) in selected-ion monitoring (SIM) mode for the simultaneous analysis of organochlorine pesticide residues (OCPs) in herbs or their formulations was developed. Nine selected herbal preparations in the different forms, such as raw material, powder, capsule, pill and tablet were purchased from the local market and screened for OCPs. Although OCPs were detected in most of the samples, their concentrations were found well below the Maximum Residue Level (MRL) set in the European Pharmacopoeia (2008). α-BHC, β-BHC, γ-BHC, γ-chlordane, p,p'-DDE and hexachlorobenzene were the most frequently detected contaminants in these samples. These widespread pesticide contaminations may pose potential health risks to consumers and significantly affect the export of herbal products to new markets, thus must be carefully monitored.

PJ26 Simultaneous determination of 12 bioactive compounds in 'Jaeumganghwa-tang' by HPLC-DAD

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Jaeumganghwatang is a traditional Korean herbal medicine for the treatment of chronic bronchitis, nephritis and diabetes mellitus. A high performance liquid chromatography-diode array detector (HPLC-DAD) method was developed for simultaneous determination of 13 major compounds, decursin, decursinol, nodakenin, paeoniflorin, 5-HMF, hesperidin, naringin, berberine, mangiferin, glycyrrhizin, catalpol and aucubin in Jaeumganghwa-tang. The separation use Shishedo C_{18} column and detection wavelength was set at 205, 250, 280 and 300 nm. The developed analyses had good linearity (R^2> 0.9997). The range of limit of detection (LOD) and limit of quantification (LOQ) were observed from 0.08 to 1.05 and from 0.3 to 3.17. The intra- and inter-day test was indicated less than 3%. The recoveries were between 95.22 - 101.01%. This HPLC-DAD method is successfully applied for simultaneous determination of 12 major compounds in Jaeumganghwa-tang samples.

PJ27 Development and validation of a rapid RP-HPLC method for analysis of (-)-copalic acid in Copaiba oleoresin

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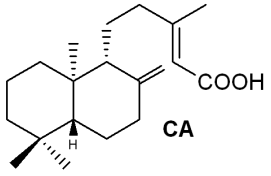
The *Copaifera* species (Leguminoseae) are popularly known as "copaíba" or "copaíva" are grown in the states of Amazonas, Pará, and Ceará in Northern Brazil, are also largely commercialized in Brazil and exported to Europe and North America. The oleoresins obtained from these species have been extensively used due their pharmacological potential and their application in cosmetic and pharmaceutical preparations. In the present study, the development and validation of a novel, rapid, and efficient RP-HPLC methodology for the analysis of the diterpene (-)-copalic acid (CA), pointed out as the only chemical marker of the *Copaifera genus*, is described. The results have led to the conclusion that the use of CA as the sole chemical marker of copaíba oleoresins only allows for the recognition of the authenticity of the samples. It is not possible to determine whether the product is adulterated or constituted by oleoresins from different *Copaifera* species.



PJ28 Bioreactors based on cathepsin D: A new analytical approach for inhibitors screening of natural products

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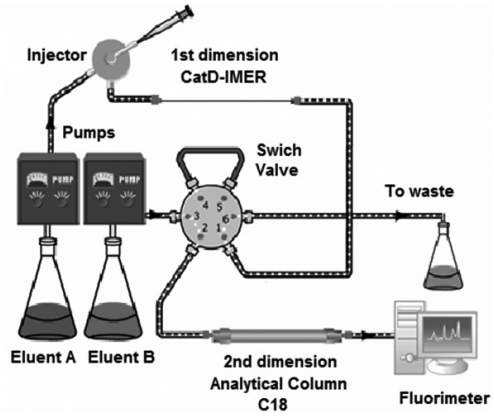
Cathepsin D (CatD) inhibitors could form the basis of novel anti-malarial and schistosomiasis chemotherapies. In this work, we report the preparation of bioreactors based on CatD (CatD-IMER) for rapid on-line screening of natural and combinatorial libraries. To monitoring the CatD-IMER activity, a multidimensional chromatography (Figure 1) method was developed and validated. The CatD-IMER retained the catalytic activity (K_M= 78.7 ± 6.85 μM) and remained stable for over one month, an important finding in proteases since they are susceptible to autoproteolysis. The adopted strategies for the method development, enzyme immobilization and kinetic studies will be presented. So far, the developed procedure has demonstrated to be a useful strategy in natural products analytical technologies. Figure 1. Scheme of the multidimensional chromatographic system. CatD-IMER inserted in the first dimension (50 mM phosphate buffer, pH 4.0, 50 μL/min) and in the second dimension an octadecyl column Luna-Phenomenex (100 Å, 5 μm, 10 x 0.46 cm) which separates the substrate and product obtained with CatD catalyzed reaction (H_2O with 0.1% TFA/ACN (61:39 v/v), 0.5 mL/min).



PJ29 Antioxidant activities and metabolite profiling of North American and neotropical blueberries using LC-TOF-MS and multivariate analyses

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North American blueberry species (*Vaccinium* spp.) have been studied extensively for their potential health benefits due to their high levels of polyphenolic antioxidants. There are many neotropical relatives of the blueberries and recent studies have shown that some have even stronger antioxidant activity than the well-known North American blueberry. Fourteen antioxidant marker compounds were successfully predicted by applying multivariate statistics to data from LC-TOF-MS analysis and antioxidant assays of three North American blueberry species and tweleve neotropical blueberry species. This application of multivariate analysis to bioactivity and mass data can be used for identification of markers contributing to the pharmacological activities of natural products. Also, the compositional differences between North American and neotropical blueberries were determined by chemometric analysis of LC-TOF-MS data. North American blueberries formed a distinct profile from the neotropical species, and 44 marker compounds contributing to these differences were detected.

PJ30 Quality control procedures for *Dysphania graveolens*: HPLC determination of the major flavonoids

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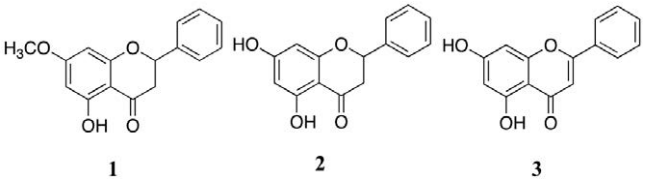
Dysphania graveolens is widely used in Mexican traditional medicine against gastrointestinal ailments. Previous investigations revealed that its flavonoids are important active principles; however, there is not a reliable and accurate analytical method for determining these compounds in the crude drug. Therefore, a validated HPLC method for quantifying the major active flavonoids [pinostrobin (**1**), pinocembrin (**2**), and chrysin (**3**)] of *D. graveolens* was developed. The method allows the quantification of the flavonoids and the determination of their total content. For each compound a linear response was evaluated within the range of 0.5 – 2.0 mg/mL for **1**, 0.25 – 1.25 mg/mL for **2**, and 0.05 – 0.5 mg/mL for **3**.



PJ31 HPLC-MS^3 identifying profiles of Astragalus membranaceus var. mongholicus for diabetic nephropathy and peripheral neuropathy

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The dried roots of *Astragalus membranaceus* or *A. membranaceus* (Fisch.) Bge var. *mongholicus*, Radix Astragali, was prescribed more than 80% in compound traditional Chinese medicines for diabetes mellitus. Current research found Radix Astragali demonstrates pharmacological effect on diabetic complications such as diabetic nephropathy (DN) and diabetic

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

PJ32 A simple HPLC method for detecting adulteration of ginkgo extracts with flavonol aglycones

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

PJ33 Facile determination of absolute configurations of α -hydroxy acids by the expansion of Marfey's method

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

PJ34 Ultratrace level voltammetric determination of total mercury and toxic metals in tea matrices

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

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

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

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

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

PJ37 Qualitative and quantitative analysis of flavonoids in Saba Senegalensis P. leaves by HPLC-DAD-ESI-MS/MS and HPTLC-UV

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

PJ38 Fractal dimension in mass spectra from herbal extracts: Hypothesis for a new method of phytocomplex characterization

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

PJ39 Analysis of phenolic, polysaccharidic and lipidic fractions of mushrooms from northeast Portugal

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

PJ40 Characterization of flavonoid glycosides in traveller's tree (Ravenala Madagascariensis S.) leaves by HPLC-DAD-ESI-MS^n

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

PJ41 From drupes to olive oil: How do bioactives variate during a single production procedure?

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

PJ42 A monoclonal antibody-based elisa for the hedgehog inhibitors cyclopamine and cyclopamine-KAAD

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

diseases associated with the Hh signaling pathway. A monoclonal antibody-based ELISA was developed to detect and measure cyclopamine and cyclopamine derivatives in biological samples. The limits of detection of the assay for cyclopamine and cyclopamine derivatives were < 3.0 pg. This assay was also useful for the detection and measurement of cyclopamine in sera from mice dosed with cyclopamine. The ELISA method described demonstrates the potential of using these techniques for the rapid screening of biological samples for the presence and levels of cyclopamine and cyclopamine derivatives that are Hh inhibitors with anticancer potential.

PJ43 Oleacin and oleocanthal: Two olive oil bioactives in multiple chemical forms

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Oleacin and oleocanthal are major secoiridoid derivatives of virgin olive oil. Although promising therapeutic agents, e.g. oleocanthal possess anti-inflammatory activities similar to ibuprofen [1], their analysis, detection and quantification in olive extracts and in biological fluids are still complicated. Indeed, their dialdehydic nature makes them extremely unstable, mainly due to their ability to form hemiacetals and to the keto-enolic tautomeric equilibrium that involves also ring-closing. Finally, in a single analysis there is always the question of which chemical form, bioactive compound or artefact, is really detected. To this aspect, this work involves (i) the determination of the basic derivatives of both molecules in protic and aprotic environment employing a hybrid ESI-(+/-)-LTQ-Orbitrap apparatus; (ii) the establishment of the best platform for the analysis and quantification using different stationary phases (e.g. C18, Normal Phase, Hilic, Chiral); (iii) the elucidation of their fragmentation mechanisms by HRMS/MS and (iv) the investigation of their reactivity with key biological agents such as amino acids and nucleosides. The overall will further contribute to the better conception of their chemical nature concerning biosynthesis and will result to new insides related to their pharmacological role. [1] Nature, 437 (2005), 45 – 46.

PJ44 Rapid identification of *Acronychia*-type acetophenones in *Acronychia pednculata* using an UPLC-ESI-MS/MS platform

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Utilization of *Acronychia* species in traditional medicine has caused great interest in their chemical investigation. Prenylated acetophenone dimers (*Acronychia*-type acetophenones), especially acrovestone and other derivatives, have been reported as chemotaxonomic markers of the genus and as significant cytotoxic principals. However, their structure elucidation using MS methodologies have not been reported up to now. In this study, a UPLC-HRMS/MS method using ESI and APCI ionization sources, in both modes, utilizing an Orbitap analyzer was developed for the initial detection and analysis of *Acronychia*-type acetophenones in the extracts of *Acronychia pedunculata*. Furthermore, a multi-level MS approach involving $HRMS^n$ and data dependent acquisitions was employed for the characterization of diagnostic ion peaks and the determination of fragmentation pathways of *Acronychia*-type acetophenones. The fragmentation mechanism of specific dimers is proposed using literature data and Mass Frontier software.

PJ45 On the possible structural differencies between molecules present in natural extracts and the synthetic ones

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Discrepancies are observed in biological activity of the same molecule present in natural substrates and those extracted from the phytocomplex or produced by synthesis. In the last two cases a lower activity is sometimes observed, e.g. as described for quercetin and hypericin in St. John's wort, artemisin in Artemisia annua, VitaminC and Flavonols in Citrus. This behavior can be ascribed to synergism with other molecular species, but a further hypothesis can be considered, i.e. that the different activity could originate from a different conformation of the active compound in the biological substrate, due to interactions with other molecular species and/or with oligoelements. The drug-receptor interaction is responsible for biological activity and consequently it is related to the drug conformation, which would be highly specific in the natural substrate. In the case of synthetic products the spatial structure at the lowest internal energy is expected to be different to that of the same molecule present in the natural substrate; however, molecular dynamics indicates that there is a non-zero probability that it assumes the right conformation for the interaction with the receptor site. This aspect could explain the partial maintenance of biological activity and give account for its lowering with respect to that of the natural product.

PJ46 Ion mobility spectrometry in metabolite profiling of complex plant extracts

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Plant extracts are composed of hundreds of compounds, which vary widely in structure, physicochemical properties and concentration. LC-MS systems that provide high performance separation and detection are routinely used to obtain detailed information on the composition of such complex extracts in the frame of metabolite profiling studies. There is however a need for better resolution in both LC and MS dimensions to better detect if possible 'all' metabolites in a given natural extract. Ion mobility spectrometry (IMS) represents an additional dimension to LC-MS, based on the separation of the ions in the gas phase according to their chemical and physical interactions with a drift gas [2]. Coupled with a TOF-MS, IMS offers a high speed (milliseconds) separation capable of resolving many of the isomers and stereoisomers not easily separated by LC. In this work, the potential of IMS for the deconvolution of isomeric flavonoids has been studied, and the capacities and complementarities of IMS-TOF and UHPLC-TOF-MS platforms for the metabolite profiling of the medicinal plant *Ginkgo biloba* have been compared.

PJ47 Gas chromatography of epoxide compounds from microencapsulated *Varronia verbenacea* essential oil after storage

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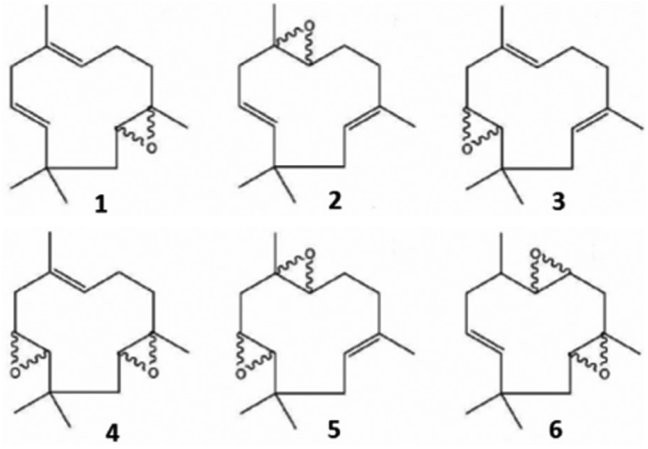
Varronia verbenacea (DC.) Borhidi (popular erva-baleeira) has anti-inflammatory activity attributed to α-humulene. The oil's activity can be lost during storage. Polimeric microparticles is an option to increase stability for pharmaceutical preparations. The profile of epoxides generated on storage was simulated by epoxidation reaction of the essential oil with meta-chloroperbenzoic acid. Erva-baleeira's essential oil was obtained by steam distillation and the respective microparticles by spray-drying technique with cashew gum as wall material. The epoxides were identification by GC/MS analysis. The fragmentation of some compounds showed mono and di-epoxides as compared with reported data (mono 1 – 3 and 4 – 6 di-epoxides) (Lam & Deinzer 1987 J Agric Food Chem.).



PJ48 Determination of theanin content in green tea samples – A challenge for NIR

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The amount of tea produced worldwide surpassed 4.4 million tons in 2010.To handle such vast amounts fast analytical techniques are needed. The currently used HPLC provides a lot of information about the analysed sample, but requires sample preparation and the use of solvents. It would be desirable to have fast technique with little sample preparation at hand. Here near infrared spectroscopy (NIR) could be the solution. We developed a method for the determination of theanine content in dried green tea on the basis of 43 different samples including flavoured ones. Theanine is an unusual amino acid with important impact on esp green tea flavour, biological activity and quality. Using the partial least square algorithm, we obtained a coefficient of correlation of 78% compared to the HPLC reference method. With NIR being well established in industrial analytical laboratories, we show that it also serves as a fast and cost effective method for the quality testing of green tea.

PJ49 New adaptive algorithm for gaussian fitting and color difference in HPTLC plant analysis

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Peak overlap is an important cause of erroneous quantification in HPTLC, and color differences of zones are not usually studied. A new adaptive algorithm was developed based on the assumption that the zone due to each component resembles the shape of a gaussian distribution. The chromatogram is the sum of the different gaussian peaks, and the algorithm is able to identify partially overlapped peaks and determine the parameters of every single peak, making a more accurate approximation. Determination of the relative area of each component is done by computing the area of zones. In order to perform substance finding, our algorithm calculates Rfs and uses Delta-E to calculate the color difference of zones. Delta E (ΔE) is a standard measure for color difference, defined by the CIELAB 1976 as the Euclidean distance between two points corresponding to two colors in the L*a*b* space. ΔE color differences below 1 are usually assumed to be not distinguishable by the human vision. The program was implemented in C++ using open-source libraries: *CImg* library and *tinyxml*, a free XML parser library, modified by us for supporting stylesheet attaching, since data are presented in a clear format readable by the user with a standard web browser. It was compiled under two platforms: Linux Ubuntu 10.10 GNU GCC compiler version 4.4.3 and Windows 7 Home Edition Microsoft Visual C++ Express 2010.

PJ50 *Leonurus cardiaca*, *L. Japonicus*, *Leonotis leonurus*: Quantitative HPLC and instrumental HPTLC determination of fourteen phenolics

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Leonurus cardiaca, *Leonurus japonicus*, and *Leonotis leonurus* are traditionally used for cardiovascular diseases in Europe, East Asia, and Africa. Still, only a single HPLC analytical study on potentially bioactive phenolics, solely for *L. cardiaca*, has been reported. Here, a novel RP-HPLC method is presented for quantification of 12 phenolics (chlorogenic, caffeic, ferulic, rosmarinic, cichoric acid, lavandulifolioside, verbascoside, hyperoside, isoquercitrin, rutoside, apigenin-7-O-D-glucoside, quercitrin) in 18 herbal and seed samples of the 3 species as well as in a *L. cardiaca* refined extract [1]. The theorized presence of leonoside A and B was refuted via HPTLC. Only ferulic acid was found in every sample, whereas rosmarinic acid and apigenin-7-O-D-glucoside were not detected in any sample. Chlorogenic, caffeic, cichoric acid and rutoside were detected in all 3 species. Lavandulifolioside and verbascoside were not present in any sample of *L. japonicus*, but in every sample of the aerial parts of *L. cardiaca*. Lavandulifolioside was found in this first ever HPLC analysis on phenolics of *L. leonurus*. Hyperoside was not found in *L. cardiaca* but in both *L. japoni- cus* and *L. leonurus*, whereas isoquercitrin was detected in *L. cardiaca* and *L. leonurus* but not in *L. japonicus*. This approach facilitates identification and quality control via HPLC/HPTLC fingerprints.

PJ51 Stability study of a α-methylene-γ-butyrolactone issued from lichens

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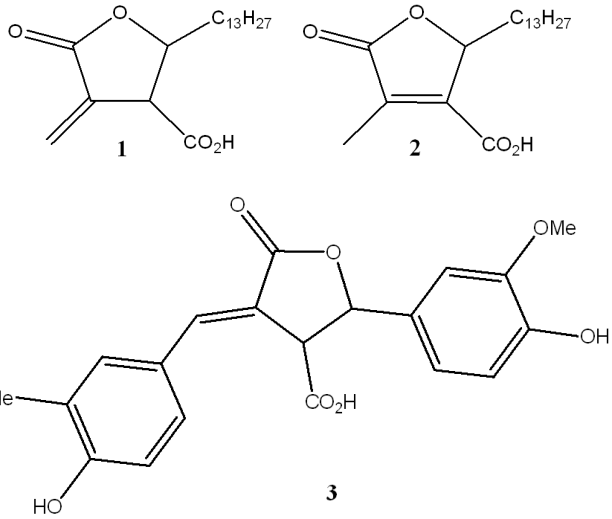
Protolichesterinic acid (PLA) **1** and lichesterinic acid (LA) **2**, isolated from lichens are described to have anti-inflammatory, anti-tumor and anti bacterial properties. LA can be readily obtained from PLA through isomerization thus raising the question of stability of the latter. So we studied the stability of **1** under different conditions of solvent, temperature and time. Solvents were chosen for their ability to improve the solubility of **1** which is low in aqueous media, thus of negative incidence in biological testing. Concentrations were determined by HPLC coupled with a photodiode array detector. The calibration curves have been established from synthesized products. In a second time, compound **3** was synthezised and submitted to temperature and solvent conditions, determined by the above study, to elucidate the role of the carboxylic function.



PJ52 A new concept for olive oil classification based on the oleocanthal and oleacein content through H-NMR quantitation

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Extra virgin olive oil contains multiple minor bioactive components, especially phenolic compounds. Among them, (-)- decarboxymethyl ligstroside aglycone, also known as oleocanthal, has been shown to possess strong anti-inflammatory and neuroprotective properties, while (-)- decarboxymethyl oleuropein aglycone, also known as oleacein is considered as the most powerful antioxidant of olive oil. We developed a method for direct measurement of the oleocanthal and oleacein levels by quantitative ^{1}H-NMR in $CDCl_3$ at 600 or 800 MHz in order to identify possible differences between extra virgin olive oils. The method was applied on > 200 monovarietal commercial olive oil samples from Greece and California. The main findings were: 1. There are olive varieties that independently from geographic origin and harvest time produce olive oil that contains both compounds in low levels 2. There are olive cultivars that are able to produce olive oil rich in these substances, depending on the harvest and milling conditions. 3. The oleacein to oleocanthal ratio seems to depend on the olive cultivar, whereas it is independent from the olive extraction procedure. We propose a new index to classify extra virgin olive oils as a combination of D1 = oleocanthal + oleacein and D2 = oleacein/oleocanthal. The significant differences between the samples regarding the D1 (0 – 451 mg/L) and D2 (0 – 1.3) could be used to discriminate the olive oils according to their possible health effects, organoleptic properties and varietal/geographic origin.

PJ53 Identification of coumarins in DCM bark, leaf and fruit extracts from *Mammea neurophylla* (Calophyllaceae) by LC-PDA-MS^n

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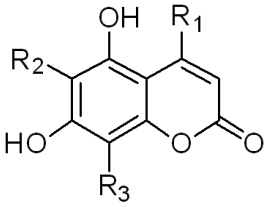
4-phenyl and 4-propylcoumarins display a wide variety of biological activities including anti-oxidant and anti-inflammatory effects, antiparasitical activities against Leishmania or Plasmodium as well as antibacterial, antiviral (HIV) and cytotoxic activities. Using LC-PDA-MS^n we have developed a specific protocol allowing the simultaneous and qualitative detection of 4-phenyl and 4-propylcoumarins in DCM bark, fruit and leaf extracts obtained from Mammea neurophylla. By comparison of their retention times, MS and UV data with that of authentic samples, nine, seven and five 4-phenylcoumarins could be directly identified in bark, leaf and fruit extracts respectively. On the other hand, interlocking UV spectra and ESI-MS^n data analysis allowed us to deduce plausible structures of five, eight and four other coumarins in bark, leaf and fruit respectively by comparison with their reported spectral data. During this study new Mammea A/AA 9-hydroxy-cyclo F and Mammea A/AB 9-hydroxy-cyclo F were identified. We believe that this protocol will be useful in case of dereplicative studies of Mammea and related species.



PJ54 LC-MS/MS quantitation of polyphenols and secoiridoids in edible olives

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Table olives are a traditional product and a very important component of the Mediterranean diet. Olive fruits are sources of hydroxytyrosol, oleuropein and many other biophenols or secoiridoid derivatives. The chemical consistency of the final olive products is influenced by the olive variety and the debittering method. Our study was focused on the impact of California style and the dry salt processing on the concentration of hydroxytyrosol (HT), oleuropein (OLE), hydroxytyrosol glucosides, saligstroside, luteolin glucosides, rutin, verbascoside, oleoside methyl ester, 2,6-dimethoxy-p-benzoquinone, phenolic acids (caffeic acid, chlorogenic acid, coumaric acid), oleuropein (OLE-A) and ligstroside aglycons. A rapid method for the extraction and quantitation by LC-MS/MS of the above described products in olive fruits was developed. The method was first applied in California style processed Manzanilla olives. The initial values of OLE was 7.4 mg/g wet flesh, for OLE-A 3.7 mg/g wet flesh and for HT 0.72 mg/g wet flesh. At the end of the debittering process only HT, OLE and OLE-A could be detected in levels ranging between 130 – 165 μg/g. All the other compounds presented concentration lower than 50 μg/g and 7 of them could not be detected. In contrast the Mission and Throuba Thassos olives processed by the dry salt method presented high amounts of almost all studied compounds especially OLE, HT, OLE-A and hydroxytyrosol glucosides (ranging from 312 to 1720 μg/g). In conclusion, the variety and processing style has a strong impact on the majority of the main olive bioactive compounds.

PJ55 Determination of in vitro CACO-2 permeability and oral pharmacokinetic profile of Platycodin D in rats

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Platycodin D (PD) was regarded to be major active components of the roots of *Platycodon grandiflorum* for the various bioactivities. However, there have been relatively few studies determining ADME and pharmacokinetic properties. High-performance liquid chromatography with evaporative light scattering detector method was developed and validated to determine the permeability of PD, deapio-platycodin D (De-PD) and platycoside F (PF). The P_{app} values of PD, De-PD, and PF (Concentration, 100 μM) in the absorptive direction were 29.1 34.9, and 25.2 at a level of 10^{-6} cm/s, respectively. The efflux ratio were between 0.45 – 0.53. The three compounds have high permeability in the Caco-2 cell monolayer without the effect of efflux transport. A simple, rapid and sensitive method for the determination of platycodin D in rat plasma was developed using liquid chromatography electrospray ionization mass spectrometry. This method was further applied to determine the pharmacokinetic study of PD after a single oral administration in rats. Quantification of rat plasma samples pretreated by protein precipitation was performed by LC-ESI/MS in negative selective ion monitoring mode. After a single oral dose of 10 mg/kg, the peak plasma concentration (C_{max}) of platycodin D was 28.9 ± 9.03. The time to reach C_{max} (T_{max}) was 60 (45 – 240) min. The terminal elimination half-life ($T_{1/2}$) was about 201 ± 80.6 min.

PJ56 Is orbitrap quantitative? Critical aspects of its potential using natural products and synthetic drugs as models

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Orbitrap analyzers represent a new exciting technology in the field of mass spectrometry as they offer unsurpassed mass accuracy (often in the sub-ppm area) along with high resolving power (up to 150.00). Its use is widespread including structure elucidation of natural products, proteomics, metabolism studies and metabolomics as well fragmentation and H/D exchange studies. Nevertheless quantitation studies using such instrumentation are rather scarce and are usually performed using

the XIC mode of full scan mass spectra. This study involves a detailed study on the quantitative abilities of the instrument investigating critical factors pertaining its operation, such as the scan range, the resolution, the acquisition mode (centroid or profile) as well as the MS/MS capabilities of the instrument. Five substances (four natural products – oleuropein, hydroxytyrosol, acrovestone and hermanioside B, one semi-synthetic – 6-bromoindirubin-3-oxime and one synthetic drug – clopidogrel) have been used as model compounds. The evaluation of the results has been based on the statistical comparison of calibration curves constructed under the aforementioned conditions using either the extra sum-of-squares F test or the Akaike's information criterion methodologies.

PJ57 Structure-oriented UHPLC-LTQ-orbitrap-based approach for the identification of isoflavonoids from *Amphimas pterocarpoides*

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Hyphenated techniques and especially UHPLC-MS methods are nowadays widely employed in natural products research. However, the complex nature of plant extracts complicates considerably the analysis and the identification of their constituents. Nevertheless, new MS analyzers with increased resolving power and accuracy such as the orbital trap (Orbitrap) could facilitate this process. The objective of this study is the development of a new structure-oriented approach based on fast UHPLC-HRMS/MS methodologies for the identification of flavonoids in crude extracts. Additionally, aims to assist dereplication procedures and orient the focused isolation of natural products. As a proof of concept, *Amphimas pterocarpoides* methanolic extract was selected. Based on a chromatographic and spectrometric features (Rt, UV, accurate *m/z*, proposed EC, RDBeq., RIA) as well as HRMS/MS spectra, several isoflavonoids were identified. In order to verify the proposed structures 11 isoflavonoids were selectively isolated and unambiguously identified using 1&2D NMR techniques. A further study these isolated isoflavonoids was carry out in HRMS/MS level, employing ESI and APCI sources, in both modes and useful information regarding their fragmentation patterns as well as their diagnostic ions were obtained. The proposed Orbitrap-based dereplication strategy could comprise a novel approach for the analysis of crude extracts.

PJ58 Qualitative and quantitative determination of bioactive marker compounds from *Gastrodia elata* by HPLC-DAD-MS

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Qualitative and quantitative determination of four bioactive compounds including gastrodin, gastrodigenin, *p*-hydroxybenzaldehyde, bis(4-hydroxybenzyl)ether isolated from *Gastrodia elata* has been developed using high performance liquid chromatography-diode array detector coupled with electrospray ionization/mass spectrometry (HPLC-DAD-MS). The analysis of four standard compounds showed good linearity, intra- and inter-day precisions, and accuracy of them. In addition, metabolite profiling of 23 different *Gastrodia elata* extracts was carried out to identify their origins. Total analyzed metabolites were applied to multivariate statistical analysis including principal component analysis and hierarchical clustering analysis for pattern analysis.

PJ59 Age differentiation of *Panax ginseng* and structure analysis of key constituents using metabolomics technique

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For the age differentiation of *Panax ginseng*, non-targeted analysis of hairy root of ginseng was performed using ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-Tof MS) technique followed by multivariate analyses. Various classification methods were applied to find an optimal method which best describes the ginseng ages by selecting the influential metabolites of different ages. Through the metabolite selection process, several age-dependent key constituents having potential as biomarker candidates were determined, and their structures were identified by tandem mass and accurate mass with the comparison of the in-house ginsenosides library and literature data. This proposed method applied to the hairy root of *P. ginseng* showed the improved efficacy compared to our previous result using main roots for age differentiation, and the identified key metabolites can be used as biomarker candidates for the quality assurance in ginseng.

PJ60 Metabolomic approach for origin discrimination of *Anemarrhena asphodeloides* bunge using UPLC/Q-TOF MS

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An ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-Tof MS) analytical method has been developed for metabolite profiling and pattern analysis of *Anemarrhena asphodeloides* Bunge. Total 21 extracts of *A.asphodeloides* bunge from different origins were analyzed by UPLC/Q-Tof MS. In addition, quality control samples were also analyzed to validate the analytical method and to ensure more reliable and accurate results. Subsequently, multivariate statistical analyses were performed to compare the patterns among tested samples. Furthermore, statistic methods, *t*-test and significance analysis of microarrays, were applied to extract influential metabolites for the efficient comparison of two groups. As a result, PCA and HCA with the selected metabolites clearly showed origin discrimination of *A.asphodeloides* bunge.

PJ61 Chemical composition of *Cladonia sylvatica* (L.) Hoffm.

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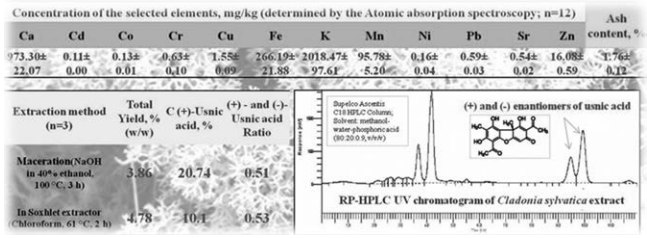
Cladonia sylvatica (L.) Hoffm. has been used in traditional medicine for many years and was included in the Pharmacopeia monograph "Lichens" registered in USSR in 1973. Here it is recommended as the natural source of usnic acid to produce sodium usniate, an antibacterial remedy for external use to treat infected wounds, trophic ulcers and burns (*PhA 42 – 766 – 73*). Recently a renewed interest in usnic acid has been noticed. The chemical composition of lichen *Cladonia sylvatica* collected in Mari El Republic of Russian Federation in 2011 has been analyzed in this study:



PJ62 Normal phase HPLC profiling of the acetylcholinesterase activity in apolar plant extracts

Landreau A[1], Bertrand S[2], Simoes-Pires C[2], Marcourt L[2], Bach TD[1], Litaudon M[3], Guilet D[1], Richomme P[1], Carrupt PA[2], Wolfender JL[2]
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Among nineteen evaluated Clusiaceous species, one stem bark CH_2Cl_2 crude extract was selected based on a significant inhibition of acetylcholinesterase (AChE) using the micro-dilution Ellman's method [1]. A normal phase HPLC profiling with micro-fractionation of this extract provided discrete fractions every 20 seconds. In order to obtain a comprehensive profiling of AChE activity all microfractions were tested [2] in dilution assay (Ellman) as well as by bioautography (the Fast Blue B salt method). Furthermore the potency of inhibition was evaluated both by keeping the genuine concentration within the extract and after normalisation to a standard concentration level. From the active fractions five pure compounds were isolated and identified. The different methods of sample preparation and biological evaluation associated with normal-phase micro-fractionation of plant extracts are critically discussed. [1] Brühlmann *et al*., *Chem. Biodivers*., **2004**, 1, 819; [2] Wolfender *et al*., *Chimia*, **2011**, 65, 400

PJ63 High resolution antifungal HPLC profiling for the early identification of compounds in plants from French Polynesia

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In order to rapidly localize antifungal compounds in crude plant extracts, micro-dilution assays with the opportunistic yeast *Candida albicans* were used in combination with semi preparative HPLC micro-fractionation [1]. Using this approach, HPLC antifungal profiles of selected active crude methanolic extracts of plants from French Polynesia were efficiently recorded. The leaf extract of the tree *Alphitonia zizyphoides*, a plant traditionally used to treat dermatomycoses [2], appeared to be the most active. The combination of HPLC antifungal profiling of *A. zizyphoides* together with dereplication by LC-MS and microflow NMR [3] leaded to the rapid dereplication of the bioactive compounds. The method presented is generic and applicable for the screening of extracts containing polar to medium polar antifungal agents. *Acknowledgements:* This work was supported by Swiss National Science Foundation Sinergia Grant CRSII3_127187 (to J.-L. W. and M. M.) [1] Wolfender, J.-L., *et al*., *Chimia*, **2011**, 65, 400; [2] Pétard P, Plantes utiles de Polynésie, **1986**; [3] Wolfender, J. L., *et al*., Curr. Org. Chem., 2010, 14, 1808.

PJ64 LC-ESI-MS/MS analysis of *Quassia amara* leaves tea. Is antiplasmodial activity of the tea is due to quassinoids?

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Université de Toulouse, UPS, UMR152 (Pharmacochimie et Pharmacologie pour le Développement-PHARMA DEV), Toulouse, France

Quassia amara L. (Simaroubaceae) leaves tea is a widely used remedy against malaria all over the north-west amazon. Our group has isolated different quassinoids from the leaves and shown that the most active compounds against *Plasmodium falciparum* were simalikalactones D and E (IC_{50} 10 and 24 nM respectively). In order to evaluate if the antiplasmodial activity of the traditional preparation is due to these quassinoids, a LC-ESI-MS/MS analysis was performed on teas prepared from both mature fresh and dry leaves of *Q. amara*. The results showed the presence of phenolic compounds, mainly gallic acid (GA), methyl gallate (MG), a new apiosyl gallate and vitexin (VT). Simalikalactone D was not detected and simalikalactone E was only detected at very low concentration (0.0072% *m/m*). The quantification (% *m/m*) of the 3 main compounds revealed concentrations of 15, 4.4 and 10% for GA, MG and VT respectively in the tea prepared from dry leaves and 7.9, 2.2 and 1.7% form GA, MG and VT in the fresh leaves tea. Antiplasmodial IC_{50} were measured to be 9 µg/ml for fresh leaves tea and 1, 17 and 80 µg/ml for GA, MG and VT respectively. So, the antiplasmodial activity of *Quassia* tea is due to gallic acid derivatives.

PJ65 A rapid qualitative analysis of natural products by atmospheric solids analysis probe mass spectrometry (ASAP-MS)

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A number of techniques are used for qualitative analysis of natural products, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), and even DNA analysis. Many of these techniques require sample preparation that can become very tedious and time-consuming. The application of the atmospheric solids analysis probe coupled with a mass spectrometer (ASAP-MS) allows for qualitative data to be acquired very rapidly (within seconds), eliminates the need for standards, and also requires minimal preparatory work. Various plant and fungi samples with application to food, drugs and cosmetics were gathered from the University of the Sciences herbarium collection and wild populations and were scanned for detection of specific indicative compounds for identification. For example, a crude opium (*Papaver somniferum*) sample produced molecular ions indicating the presence of morphine, codeine, papaverine and noscapine. Preliminary experiments have also shown the application of ASAP-MS to identification of major active components in commercially available food, drug and cosmetic products.

PJ66 Determination of biogenic amines in *Acacia rigidula* and its dietary supplements

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Acacia rigidula (Leguminosae) is a shrub that grows in the southern part of Texas, USA. In an earlier published investigation, the presence of several toxic amines, alkaloids and many unexpected components as analyzed by GC-MS was reported. Currently, the leaf extract of this plant is used in several weight-loss and body-building products in the US market without published data on its biological effects. Further, there is lack of conclusive information on the identity and quantity of amines in the plant material and the products. In our chemical investigations on *A. rigidula*, we have established an LC-MS-MS method for the quantitative determination of several phenethylamine, tyramine and tryptamine derivatives. The separation of these amines was achieved on a reversed phase pentafluorphenyl (PFP) column in 15 minutes. To enrich these

components and to reduce the matrix induced ion suppression during LC-MS, the extracts were subjected to cationic exchange clean-up. Heptylamine and isotope labeled compounds were used as internal standards for the quantitative analysis. The new method is rapid and sensitive and it enables simultaneous analysis of several amines in *A. rigidula* plant material. The content of biogenic amine of several dietary supplement products containing *A.rigidula* was also established.

PJ67 A comprehensive metabolomic study of wine from the Vaud Switzerland vineyard

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In recent years, terroir characteristics have become increasingly important for the wine industry. A recent study conducted in the Vaud viticultural area has shown that vine nitrogen content appeared to be one of the most important parameter that influence the vine-fruit-wine continuum. In order to confirm these results, a large scale study on several sites during five seasons (2006 – 2010) in the Vaud vineyards has been undertaken on soil presenting the same characteristics. A given concentration of assimilable nitrogen has been supplied on leaves during the grape-growing to simulate the vine nitrogen content. To unravel the subtle biochemical changes induced by nitrogen supply on wine composition, a combined metabolomic approach based on reverse phase and hydrophilic interaction liquid chromatography TOF-MS and proton NMR fingerprints has been undertaken. Several biomarkers in close relation to nitrogen supply could be highlighted by supervised data mining and identified by means of their accurate mass, fragmentation pattern and proton NMR spectra.

PJ68 De-inventing the wheel: Dereplication tools for natural products research

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In ongoing studies to explore filamentous fungi for anticancer drug leads, over 140 compounds have been isolated and characterized over the past four years, with approximately 25 to 35 compounds being discovered annually. Approximately 30% of these represent new chemical entities. To expedite the discovery of new leads, and to avoid re-isolation of previously known compounds, a UPLC-PDA-HRMS method was developed for dereplication of fungal secondary metabolites in crude culture extracts. A database was constructed by recording HRMS and MS/MS spectra of fungal metabolites isolated to date, utilizing both positive and negative ionization modes. Additional information, such as UV-absorption maxima and retention times, were also recorded. Screener cultures that showed cytotoxic activities were dereplicated before engaging in the isolation/purification process. Examples will be presented that demonstrate the speed and efficiencies with these procedures, particularly in conjunction with software programs that help collate the data. Utilizing this work flow, a promising sample can be dereplicated in 45 min.

PJ69 Annonaceous acetogenins within extracts: dereplication by HPLC-ESI-LTQ-Orbitrap® using post-column lithium infusion

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Annonaceous acetogenins (AAGs) are a homogenous class of polyketides proposed as environmental neurotoxins. Previous dereplication studies of AAGs were limited by the use of low resolution mass spectrometers. Only poor information in terms of structures was provided due to the limited fragmentation of protonated or sodiated species. An innovative approach, using reversed-phase high-performance liquid chromatography coupled to a hybrid linear ion trap/orbitrap mass spectrometer (LTQ-Orbitrap®), was therefore performed. Sensitivity was enhanced by post-column infusion of lithium, as AAGs have a high affinity for this cation. High level of structural information was obtained from low-energy-CID fragmentation experiments of lithium-cationized AAGs ($[M+Li]^+$ ions). The method was then applied to a total ethyl-acetate extract of soursop nectar (*Annona muricata* L.), giving surprising insights on the number, structural types and diversity of AAGs within a single extract.

PJ70 Mexican propolis research: HPLC determination of the major flavonoids

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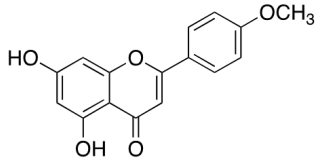
Propolis is a natural resinous substance collected by bees from aerial parts of the plants, buds or exudates. The chemical composition of the plant source its related with the composition of bee glue. Combined with the knowledge of the active principles, it gives clues to standardization and quality control procedures, allowing the specification of propolis types that have different chemical composition. Therefore, a validated HPLC method for quantifying acacetin (**1**), 4,7-dimethyl naringenin (**2**), and 4′,7-dimethyl apigenin (**3**), main flavonoids present in the Mexican propolis, was developed. For each compound a linear response was evaluated within the range of 0.5 – 2.0 mg/mL for **1**, 0.25 – 1.25 mg/mL for **2**, and 0.05 – 0.5 mg/mL for **3**.



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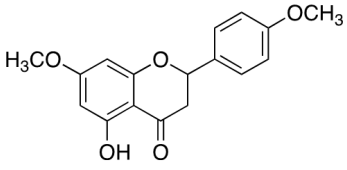

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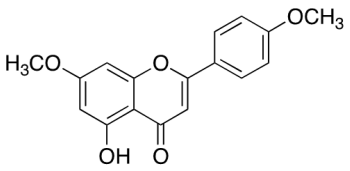

3

PJ71 Analytical investigation of geranium oils from *Pelargonium graveolenes*

Wang M[1], Chittiboyina A[1], Avula B[1], Zhao J[1], Tabanca N[1], Wang YH[1], Weerasooriya A[1], Khan IA[1,2]
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Pelargonium graveolens L'Hér (Geraniaceae family), also known as rose-scented geranium, is a highly valued aromatic plant native to South Africa. It is the main specie cultivated specifically for its essential oil. The oils also possess insect repellant, antifungal and antibacterial properties, making it useful in the medicinal and pharmaceutical fields. The goals of the proposed research were: i) to develop a gas chromatography-mass spectroscopy method that could be used to differentiate *Pelargonium graveolens* chemotypes of plant materials and commercial Geranium oil products; ii) to determine the enantiomeric ratios of Geranium oils using chiral GC/MS; iii) and to detect possible adulteration of commercial Geranium oils. A series of authenticated, fresh *Pelargonium graveolens* plant materials, commercial Geranium oils, and samples obtained from foreign collaborators were analyzed by GC/MS and chiral GC/MS. Fingerprinting analysis allowed the identification of possible markers for differentiating *Pelargonium. graveolens* chemotypes. The presence or absence of the relevant sesquiterpene, i.e. 10-*epi*-γ-eudesmol and 6,9-guaiadiene can be used to distinguish Egyptian and Bourbon/China cultivars.

PJ72 Ultra-high pressure liquid chromatography in separation of botanicals and natural products: Retention mechanism studies

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[1]National Center for Natural Products Research, University of Mississippi, MS 38677, USA; [2]Department of Pharmacognosy, School of Pharmacy, University of Mississippi, MS 38677, USA

Liquid chromatography with C_{18}-bonded packings and aqueous-organic eluents is a mature method for the separation and analysis of botanicals and natural products. Recent improvements in pump technology (1,200 bar pressure) and silica chemistry (very stable, bridged-ethylene hybrids) have allowed the use of very small particles (< 2 μm) to achieve high efficiency separations. However, the role of the stationary phase has not been thoroughly investigated mostly due to the complexity of the commonly used chemically-bonded reversed phase liquid chromatography (RPLC) packings. The systems are physically complex and heterogeneous since in a RPLC column, the aqueous-organic components of the mobile phase interact with, and thus influence the chemical properties, of the stationary phase. Mass spectrometric tracer pulse chromatography is one of the few experimental techniques that is capable of accurately measuring the uptake of binary eluents by chemically bonded RPLC stationary phases. This experimental technique was used to measure the uptake of eluent and, at the same time, the retention volume of analytes as a function of eluent composition. In this way, the excess amount of eluent sorbed by the stationary phase can be determined. The effect of such eluent uptake on the separation of botanicals and natural products was investigated.

PJ73 An evaluation of thickness effect in glycyrrihizic acid inside glycyrrhiza glabra root with compartion in several locations

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Licorice is a self-propelling plant that has many applications in food, tobacco and pharmaceutical industries. Glycyrrhiza glabra is a kind of licorice whose main active constituents a triterpenoid saponin called glycyrrhizin. In this article we selected roots in three thickness sizes 0.4 to 1.5 cm as s (small), 1.5 to 2.5 cm as m (medium), 2.5 to 3.5 cm as l (large) then roots were extracted with pressurized hot water extraction method inside Lab scale extractor (5 Liters) then evaporated and dried to analyze Glycyrrhizic acid content with HPLC.The results showed roots from all of locations are following this rule: more thickness, more Glycyrrhizic acid content such as in Afghanis, Uzbekistan, Turkey, Azerbaijan And Iran (7 areas) but we did not see similar slope for increasing glycyrrhizic acid against of thickness.

PJ74 Quantitation of components in complex mixtures utilizing two dimensional NMR spectroscopy

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Nuclear Magnetic Resonance (NMR) spectroscopy is well documented to provide qualitative data for structural determination of chemical compounds. More recently, numerous applications of NMR for quantitative analyses of chemical mixtures have been utilized. The majority of these applications use integration of discrete 1D NMR spectral resonances to quantitate individual components of the mixtures. 1D ^{1}H NMR typically provides the highest sensitivity analyses with excellent linear response to component concentrations. Quantitative analyses of complex mixtures via 1D NMR however are often hindered by resonance overlap. Utilization of ^{1}H-^{13}C heteronuclear correlation (HSQC) and 2D J-resolved experiments provides dispersion in a second dimension, ^{13}C chemical shift and ^{1}H coupling constant respectively, for better identification and quantification of signals. The HSQC cross-peak volumes are influenced by uneven excitation, etc. and therefore the volumes for different resonances from the same molecule can vary based on these factors. Nevertheless, utilizing the same experimental parameters for any given cross-peak, the areas for the same peak should scale linearly with molar concentration. In this study, we have investigated the quantitative accuracy of these 2D spectra on a complex model system containing phenolics, alkaloids, sugars, terpenes, and polyketides and made comparisons to quantification using chemometric approaches. Applications to natural product extracts and formulations and ability to implement this into automated analysis methods will be presented.

PJ75 Comparison of mass spectrometric ionization techniques for analysis of bioactive loline alkaloids

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Loline alkaloids are a natural product of the endophyte complex *Epichloë*, which is commonly present in cool season grasses. These alkaloids, which may possess insect-deterrent and insecticidal activity, have previously been analyzed using gas chromatography-mass spectrometry GC-MS with electron ionization (EI). The purpose of this study was to develop an effective method for relative quantitation of loline alkaloids in endophyte-infected *Achnatherum robustum* (sleepygrass) samples. We compared three different analytical techniques, liquid chromatography coupled to electrospray ionization (UPLC-ESI-MS), liquid chromatography coupled to atmospheric-pressure chemical ionization (UPLC-APCI-MS), and GC-EI-MS. All three methods were effective for detecting the standard loline alkaloid N-acetylnorloline, but the best sensitivity and shortest analysis time was achieved using LC-MS techniques. UPLC-ESI and UPLC-APCI gave very similar detection limits, but greater fragmentation of the precursor ion was observed with APCI. Ultimately, our findings suggest that UPLC-APCI-MS may be a desirable alternative to

GC-MS for profiling loline alkaloid content in endophyte-infected grasses.

PJ76 Mass spectrometric detection of virulence factors from methecillin-resistant *Staphylococcus aureus* (MRSA)

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There is increasing interest in the use of anti-virulence based approaches to disarm drug-resistant bacterial pathogens. *Staphylococcus aureus* expresses virulence factors via the agr quorum-sensing system, which is activated by the autoinducing peptide AIP 1 (**Fig. 1A**). We employed quantitative LC-MS (using UPLC coupled to an LTQ-Orbitrap MS) to monitor AIP 1 directly from *S. aureus* spent media. AIP 1 was produced at μM concentrations, and was detectable as early as 4 hrs incubation time (**Fig. 1B**). AIP 1 production was suppressed six-fold by the known quorum quenching peptide AIP 2 (22.8 μM). These results demonstrate the potential of using LC-MS based AIP 1 detection as an assay to screen for quorum quenching natural products.

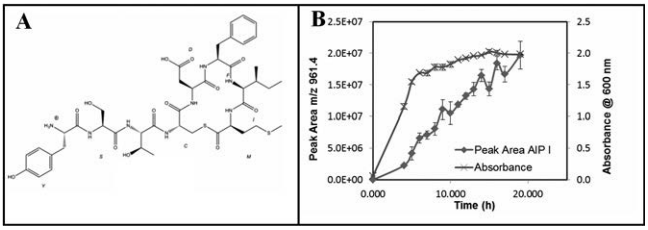

Fig. 1: AIP 1 structure (A), and time-dependent secretion of AIP 1 by MRSA (B).

PJ77 Rapid detection of clandestine 1,4-butanediol adulteration in a dietary supplement utilizing phytoforensic techniques

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Dietary Supplements (DS) are regulated in the United States under 21CFR part 111. By law a DS may not contain any pharmaceutical ingredient. Recently, there has been a substantial rise in the clandestine adulteration of DS with various pharmaceutical compounds and analogues. The discovery of drugs of abuse, especially drugs implicated in sexual abuse, is of great concern. In order to evade detection, clandestine manufacturers often substitute the desired drug with a prodrug. We present the application of phytoforensic techniques utilizing GCMS, LCMS and FTIR to rapidly detect and confirm the presence of 1,4-butanediol (BD), a pro-drug that converts to gamma-hydroxybutryic acid (GHB) upon ingestion in an off-the-shelf allegedly all-natural supplement. The concept of the phytoforensic approach and how it differs from routine analysis is discussed as well as the importance of these techniques for non-targeted analysis.

PJ78 Composition of the essential oil of different populations of *Myrtus communis* L. from Iran

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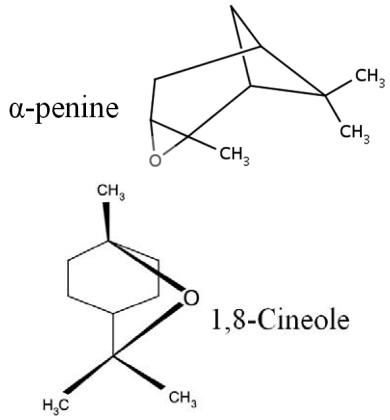
In Iranian folk medicine, *Myrtus communis* L. has been used as an infusion for various purposes such as for the Skin discords, digestive discords, astringent, good hair condition, bronchodilatotor, activities etc. The chemical compositions of the essential oil obtained from the leaves of *Myrtus communis* L. (Myrtaceae), from three different regions of Fars province, Iran by hydro-distillation using a Clevenger- type apparatus. The essential oils were analyzed by GC and GC/MS. The major components of the oil were α-penine (34 – 38%), 1,8-Cineole (21%), Linalool (8 – 12%) and Linalyl acetate (5.5 – 8%).



PJ79 Innovation of extraction and isolation technique of shikimic acid from Ginkgo biloba leaves utilizing an ionic liquid

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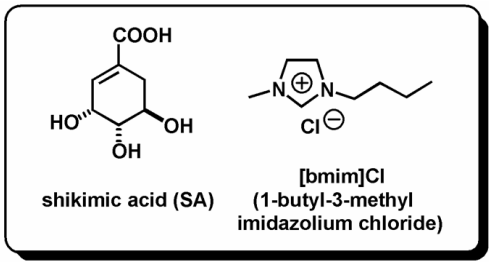
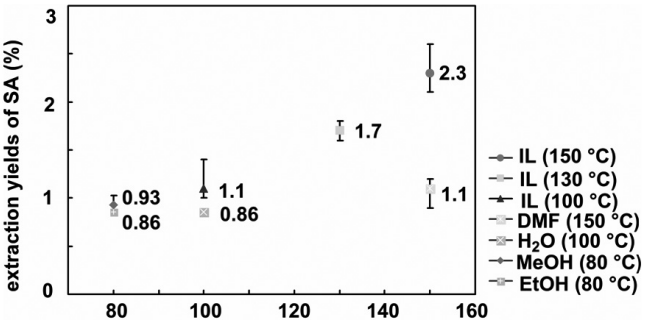
Shikimic acid is an important biosynthetic intermediate in plants, and is the starting material in the commercial synthesis of oseltamivir phosphate (Tamiflu®). Here, we report the efficient extraction and isolation technique of shikimic acid from the leaves of *Ginkgo biloba*, which is cultivated worldwide. Through the utilization of an ionic liquid 1-butyl-3-methylimidazolium chloride ([bmim]Cl), which dissolves cellulose, the procedure gave twice the amount of or more of shikimic acid than the normal method. The proposed technique is likely to be applicable to other plant leaves, allowing for isolation of greater quantities of other natural products as well as unknown natural compounds.





PJ80 Quantification of atractyloside in Fructus Xanthii using HILIC-ESI-MS/MS

Yang L[1], Wu J[1], Zeng X[1]
[1]Second Affiliated Hospital, Guangzhou University of Chinese Medicine, GZ 510120, China

Fructus Xanthii is thought to be toxic in traditional Chinese medicine. The bur is usually taken orally to treat certain diseases, as accordingly results in poisoning incidents happen occasionally in China. Atractyloside, a highly hydrophilic compound, is the main lethal component in Fructus Xanthii. Unfortunately, so far the quantitative method of this toxic compound is unavailable in Chinese Pharmacopoeia. In this study, a simple and rapid method using hydrophilic interaction liquid chromatography-tandem mass spectrometry has been developed to quantify the toxic component in Fructus Xanthii. The method was further validated from linearity, sensitivity, accuracy, and precision and extraction efficiency. As a result, the limits of detection and quantification were 0.0625 and 0.25 ng/mL, respectively, and the accuracy ranged from 99.37 to 103.97% with relative standard deviation less than 4.86%. The established method was then used to determine the toxin contained in 28 batches of commercial herbal drugs, as showed that the method was satisfying for determination of atractyloside contained in these samples over a large content range of 0.19 to 80.75 mg/g, which, together with time-saving, sensitive and accurate characteristics, makes this method very attractive for quantitative analysis of the toxic component in the crude drug and related Chinese medicine preparations.

PJ81 Combination of bioautography with HPTLC-MS/NMR: A fast identification of AChE inhibitors from Galbanum

Adhami HR[1], Scherer U[2], Kaehlig H[3], Hettich T[2], Schlotterbeck G[2], Reich E[4], Krenn L[1]
[1]Department of Pharmacognosy, University of Vienna, Vienna, Austria; [2]Institute for Chemistry and Bioanalytics, University of Applied Sciences Northwestern Switzerland, Muttenz, Switzerland; [3]Institute of Organic Chemistry, University of Vienna, Vienna, Austria; [4]CAMAG Co. Laboratory, Muttenz, Switzerland

Isolation of bio-active compounds from plant extracts is laborious and time-consuming. A dichloromethane extract of galbanum, the oleo gum-resin from *Ferula gummosa* Boiss., had shown AChE inhibitory activity (1). For fast identification of active compounds, HPTLC bioautography was combined with HPTLC-MS/NMR. After pre-fractionation of the extract by vacuum liquid chromatography, fractions were separated by automated HPTLC and active zones determined by bioautography. The mobile phase was individually optimized for each active fraction according to the CAMAG guideline and automatic detection of the compounds by UV absorbance was performed in a TLC scanner. A CAMAG TLC-MS interface was used to elute the single compounds from the plates directly into the mass spectrometer. In parallel, the most concentrated active zones were extracted from HPTLC plates for one and two-dimensional ^{1}H and ^{13}C NMR and QTOF-MS. The most abundant compound with AChE inhibitory effects was identified as auraptene. The structure elucidation of other compounds is in progress. This is the first report of auraptene in *Ferula gummosa*. HPLC analyses carried out to determine the concentration of auraptene in the extract. **Reference:** 1. Adhami HR, Farsam H, Krenn L. 2011. Phytother Res. 25: 1148 – 1152.

PJ82 Comparative metabolomic fractionation and characterization of licorice roots

Simmler C, Nikolic D, van Breemen RB, Lankin DC, Chen SN, Pauli GF
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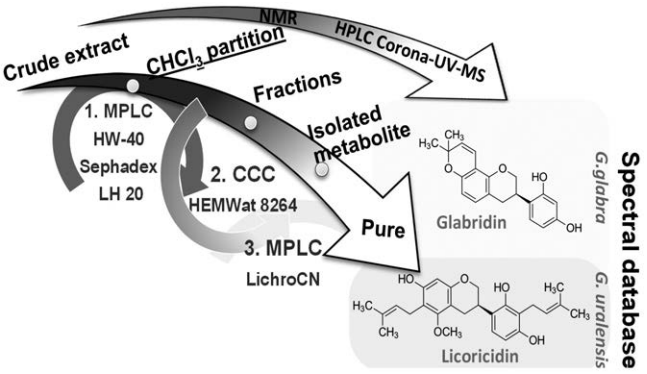
Glycyrrhiza glabra L. and *G. uralensis* Fisch. (Fabaceae) are the most widely used licorice species. Despite their chemical similarities, each species is defined by the presence of a broad range of secondary metabolites found in trace amounts. For our aim, which is directed toward their exhaustive metabolomic characterization, a 3-step fractionation map (see figure) has been developed, combining reproducible medium-pressure liquid and countercurrent chromatografies (MPLC and CCC). Each technique associated with a type of licorice marker, was selected for its high recovery. An in-house spectral database has been compiled to foster extended metabolomic studies of licorice samples.



PJ83 HPLC analysis and antileishmanial activity of supercritical fluids extracts from *Piper amalago* L

Cortez DAG[1], da Silva Carrara V[1], Serra LZ[1], Cardozo-Filho L[2], Cunha-Júnior EF[3], Torres-Santos EC[3], Cortez LER[4]
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Piper amalago L. leaves were extracted with supercritical carbon dioxide, compressed propane, and chloroform were compared in terms of alkaloid content, using the validated HPLC method. Supercritical carbon dioxide (SFE-CO_2) at 313 K and 12.55 MPa showed the highest selectivity for the alkaloid (600.53 mg/g of extract). All the extracts were tested against the promastigote and intracellular amastigote forms of *Leishmania amazonensis*. The cytotoxicity was also evaluated against J774A1 macrophages. The antileishmanial activity was evaluated in terms of inhibitory concentration for 50% of protozoa (IC_{50}). The cytotoxic concentrations for 50% of macrophages were obtained (CC_{50}). The SFE-CO_2 (313 K; 12.55 MPa) extract showed the highest antileishmanial activity with IC_{50} value of 16 μg/ml against the promastigotes, and an IC_{50} value of 7 μg/ml against the intracellular amastigotes forms. The extract showed cytotoxicity with CC_{50} value of 93 μg/ml.

PJ84 Separation of indole alkaloids from Aspidosperma rigidum by PH-zone-refining countercurrent chromatography

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Species of *Aspidosperma* genus (Apocynaceae) are, generally, trees found in Central and South America and the teas made from its barks are popularly used by Amazonian "quilombola's" communities to treat several diseases. Although this genus is characterized by the occurrence of indole alkaloids, no recent reports on *A. rigidum* chemical constituents were found. The present work shows the application of the modern technique of HSCCC in the pH-zone refining mode to the separation of the components present in a dichloromethane extract of the barks from *A. rigidum*. In this study, the separation of 200 mg dichloromethane extract was performed using the solvent system composed by M*t*BE-water (1:1, v/v) with different concentrations of the retainer triethylamine (TEA) in the organic stationary phase, and of the eluter formic acid or chloridric acid in the aqueous mobile phase. The overall results of our work demonstrated a good separation of the indole alkaloids, including stereoisomers. However, each mode showed to have specific characteristics. Three alkaloids were isolated and identified as 3α-aricine (**1**), isoreserpiline (**2**) and 3β-reserpiline (**3**) in a 2 hours one step fractionation in each experiment. Alkaloids (**1**) and (**2**) were identified for the first time in this species.

PJ85 Antibacterial activity of essential oil obtained from *Origanum majorana* L. by solvent-free microwave extraction

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With the increasing demand for the use of volatile oils in the pharmaceutical and food industries, an efficient "green" extraction method for isolation of essential oils (EOs) from plant materials has become important. Solvent-free microwave extraction (SFME) is a new technique which combines microwave heating with dry distillation at atmospheric pressure for the isolation and concentration of the EOs from plant materials[1,2]. The objective of this study was to compare the SFME and conventional hydrodistillation (HD) processes for *Origanum majorana* L. and to characterize the resulting EO for their chemical constituents using GC/MS and antibacterial properties against a marker organisms (Table 1). **Table 1.** Antimicrobial activity of *Origanum majorana* L. EOs. againts the bacterial strains tested by the disc diffusion method.

Bacterial species	SFME, mm	HD, mm	Antibiotic control, mm[a]	Negative contol
S.aureus ATCC 25923	28	29	35(P)	-
P.aeruginosa ATCC 27853	9	9	27(MEM)	-
E.coli ATCC 25922	29	30	19(AMC)	-
K.pneumoniae ATCC13883	21	21	24(CAZ)	-
S.marcescens ATCC 8100	18	19	32(MEM)	-
E.faecalis ATCC 29212	12	15	21(VA)	-

[a] P = Penicillin G (10 units); VA = vancomycin (30 µg); AMC = amoxicillin/clavulanic acid 2:1 (30 µg); CAZ = ceftazidime (30 µg);
MEM = meropenem (10 µg); and FEP = cefepime (30 µg).

[1] Sozmen. F, Uysal. B, Oksal. B.S, et al. (2011) J. AOAC Int., 94:243 – 250,
[2] Uysal. B, Sozmen. F, Aktas. O, Oksal. B.S, Kose, E.O (2011) Int. J. Food Sci. Tech., 46:1455 – 1461

PJ86 Fractionation of cocoa procyanidins according to the degree of polymerization by Centrifugal Partition Chromatography

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Procyanidins are present in a wide variety of foods such as cocoa, apples, and many berries. There is a growing interest in determining their pharmacological properties and significance as dietary antioxidants. We have designed a novel approach to the preparative fractionation of cocoa procyanidins according to degree of polymerization (DP) by applying Centrifugal Partition Chromatography (FCPC Kromaton). In an experiment optimized for the best separation of DP families between 5 and 10, we employed a bi-phasic solvent system, consisting of ethyl acetate – ethanol – water (6:1:5) in ascending-mode. The procyanidin oligomers (DP2 to DP10) eluted with the mobile phase in an order of increasing DP. The separation of bands between DP5 and DP10 was impressive in a context of the best preparative approaches reported so far. The fractionation was monitored by a NP HPLC analysis on a polyvinyl alcohol (PVA) column and by a C 18 RP column and by MALDI-TOF. The RP HPLC analysis revealed that the dominant peaks in each DP band were the linear epicatechin (4β-8) oligomers.The FCPC fractionation appears as an efficient approach to produce higher procyanidin oligomers in gram quantities required for evaluation of their biological properties.

PJ87 Isolation of bioactive diterpenes from *Tetradenia riparia* by HSCCC

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Tetradenia riparia (Hochst) Codd. (Lamiaceae) is a shrub originally from Africa where it is used in traditional medicine. In Brazil it is used in rituals and is commercialized in open markets. Literature reports diterpenes as constituents with interesting antimicrobial activity. Fractionation of the dichloromethane extract of leaves of *T. riparia* (687,4 mg) by HSCCC with the solvent system hexane-ethyl acetate-methanol- water (3:1.5:3:1.5) afforded the isolation of 7-α-hydroxyroyleanone, along with other 14 fractions, which were assayed against *Mycobacterium tuberculosis* by the microdilution method using resazurin as an indicator of cell viability. The best results were obtained for compound 1 and for the fraction containing a flavonoid derivative. Ibozol, obtained by further fractionation of fraction F4 with hexane-acetone-methanol-water in a second step (3:1.5:2.5:2) was also found to be active. Scale-up of this fractionation was performed with 1.5298 g of the extract and the same chromatographic profile was obtained.

PJ88 Ion exchange centrifugal partition chromatography: An innovative tool for bioactive peptide capture in complex protein hydrolyzate

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Alfalfa (Medicago sativa) is a common feed, with a high protein contain of good nutritional value like RuBisCO (Ribulose-1,5-biphosphatase). RuBisCO hydrolysis using Delvolase® leads to a complex mixture of peptides (average MW = 600 g.mol-1), which exhibits an opioid effect. Among this mixture, one dipeptide VW is an angiotensin-converting enzyme inhibitor. In order to develop nutraceutical anti-hypertensive product, VW content of 0.3% must be improved. Centrifugal Partition Chromatography (CPC) is a support free liquid/liquid chromatography process, which has demonstrated high interest for analyte capture inside complex matrix, typically natural product extracts or crude hydrolyzates, especially in ion exchange mode. Here is described a new ion exchange CPC process designed for peptide capture and applied to RuBisCO hydrolyzate. Enriched fractions were obtained, containing 11% of VW, with a high recovery rate of 97%.

PJ89 Fast and efficient process for recovery of naturally abundant chiral synthon: Iridoids from *Lonicera tatarica*

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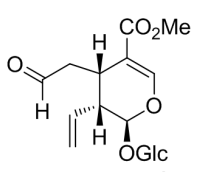
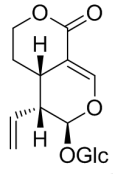
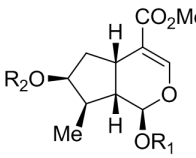
Dedicated to the memory of Pr François Tillequin The genus *Lonicera* (Caprifoliaceae) is a rich source of iridoids, such as secologanin **1** and sweroside **2**. These highly functionalized monoterpenes represent a new class of chiral synthons for semisynthesis. Therefore we developed a new and fast process to recover these products from the leaves of *Lonicera tatarica* by Centrifugal Partition Chromatography. Major compounds of the stems where also separated and identified as loganingenin **3**, loganin **4**, and periclymenoside **5**. In the context of green chemistry, this easy up-scalable process represent significant improvement as it is silica free and it replaces chlorinated solvents with water and small quantities of organic solvents.



Secologanin **1**



Sweroside **2**



3 loganingenin : R_1 = H, R_2 = H
4 loganin : R_1 = Glc, R_2 = H
5 periclymenoside : R_1 = Glc R_2 = 4-O-ß-D-glucopyranosyl-trans-ferulic acid

PJ90 Optimization of the microwave assisted-extraction of ricinine from Ricinus communis

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Ricinus communis (Euphorbiaceae) is distributed over many tropical areas. It is an exotic plant found in all regions of Brazil. It was described as a toxic plant to the leaf-cutting ants (*Atta sexdens rubropilosa*) due to the presence of the alkaloid ricinine found in its leaves and flowers. The microwave assisted-extraction (MAE) was used as an alternative to conventional methods used in the extraction of ricinine. To develop the process of extraction, an experimental design with some variables were studied: temperature, solvents (ethyl acetate, methanol and water), power: 500 – 1000W, solvent volume, sample amount and extraction time. The most influent variables on the extraction of ricinine by MAE were extraction temperature and solvent. The optimized extraction conditions for quantitative recoveries were: 1000W, 175 °C, 5 minutes and 10% ethyl acetate/methanol as extracting solvent. No degradation of ricinine was observed using the developed method.

PJ91 Isolation and identification of flavonoids from Annona mucosa

Utherdyany Bicalho K[1], Domingos dos Reis LA[1], das Graças MF, da Silva F[1], Vieira PC[1], Fernandes JB[1]
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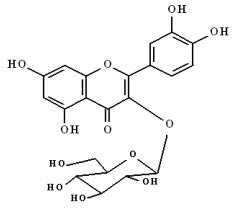
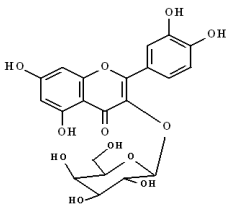
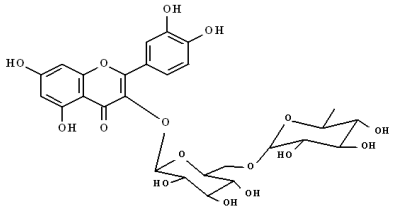
Annona mucosa, a typical tropical fruit tree, is widely distributed in Brazil. It is used in folk medicine as a therapeutic agent in the treatment of tumors and its phytochemical study revealed it as great source of secondary metabolites such as annonaceous acetogenins, alkaloids and lignans with several biological activities. The present study aimed to perform the phytochemical study of polar extracts of this plant in order to contribute to its phytochemical profile and find new polar compounds that can also be related to those biological activities. The phytochemical study of the ethyl acetate fraction of the partition of ethanolic extract of leaves of *A. mucosa* allowed the isolation of four flavonoids identified as isoquercetin (**1**), hyperin (**2**), rutin (**3**) and astragalin (**4**). These flavonoids were isolated by the first time from this plant. The isolation procedures and identification data (NMR and LC-MS) will be presented.



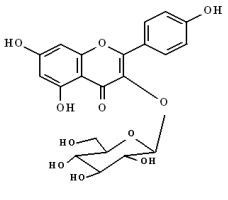
(**1**)



(**2**)



(**3**)



(**4**)

PJ92 Targeted purification of natural products by orthogonal *K*-based countercurrent separations

Qiu F[1], Friesen JB[1,2], McAlpine J[1], Lankin DC[1], Pauli GF[1]
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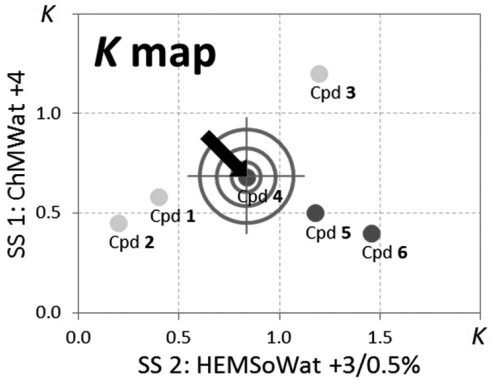
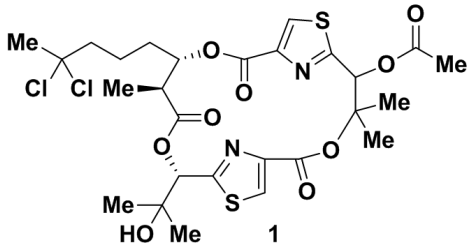
Countercurrent separation (CS) is based on the differences in partition coefficients (*K*) of analytes distributed between a liquid stationary phase and a liquid mobile phase. The *K* value is not only an important theoretical parameter in CS, but also has much practical value. *K* values can be used to predict the CS profiles of analytes, assist in the selection and optimization of CS solvent systems (SSs), guide targeted separations, and enhance the reproducibility of purifications. In the present study we developed a quantitative ^{1}H NMR (qHNMR)-based approach for simultaneous measurement of *K* values of multiple components directly from crude plant extracts. Furthermore, two-dimensional selectivity provided by a pair of orthogonal CS SSs was introduced in order to enhance the resolution of difficult-to-separate natural product congeners. Using the *K* values in optimized orthogonal SSs, a "*K* map" was created that guided the locating of the target compounds in a series of CS fractions (see figure). These methods were successfully employed in the targeted separation of marker compounds from *Ginkgo biloba*- and *Camellia sinensis*-based botanical dietary supplements.



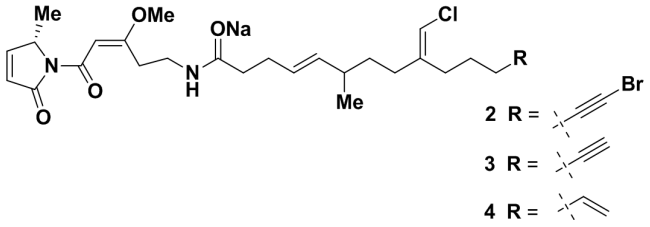
PJ93 Spectroscopic networking based dereplication applied to a strain of *Moorea producens* affords a fuller picture of its metabolome

Boudreau PD[1], Dorrestein PC[2,3], Gerwick WH[1,3]
[1]Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, United States; [2]Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California 92093, United States; [3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California 92093, United States

Moorea producens JHB is a well-studied strain of cyanobacteria from which our laboratory has isolated hectochlorin (**1**), and the jamacamides (**2 – 4**). Using spectroscopic networking an extract of this cyanobacterium was profiled based on the MS^2 fragmentation patterns of the metabolome. While the known compounds **1 – 4** were readily observed, a suite of unreported compounds related to **1** were also observed, along with another independent series of compounds related to **2 – 4**. These minor metabolites and the techniques used to discover them will be presented.



1



PJ94

Reversed phase centrifugal preparative chromatography for the isolation of triterpenene saponins glycosides from *Fagonia Cretica*

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Fagonia cretica (Zygophyllaceae), a shrub distributed in the Mediterranean regions, is used as a remedy for skin lesion. This plant contains saponins glycosides, which displayed anti-inflammatory, antimicrobial and antitumor activities. An EtOH extract of the aerial parts was subjected to Centrifugal Preparative Thin Layer Chromatography (CPTLC, Chromatotron®) using a silica gel rotor, which yielded olean-12-en-28-oic acid, 3-(α-L-arabinopyranosyloxy)-23-hydroxy-, β-D-glucopyranosyl ester **(1)** and a mixture of saponins **(2 + 3)** with identical R_f values. The mixture **2** and **3** was separated quickly and efficiently by a customized reversed phase CPTLC rotor. Compounds **2** and **3** were characterized using 1D and 2D NMR spectral data as olean-12-en-28-oic acid, 23-hydroxy-3-[(2-*O*-β-D-xylopyranosyl-α-L-arabinopyranosyl)oxy]-, β-D-glucopyranosyl ester **(2)** and Olean-12-en-28-oic acid, 3-[(2-*O*-α-L-arabinopyranosyl-α-L-arabinopyranosyl)oxy]-, β-D-glucopyrano- syl ester **(3)**. To our knowledge, this is the first report of RP ChromatoRotor in the centrifugal (or radial) chromatography for the isolation of polar compounds from natural and synthetic sources.

PJ95

New glycosides from *Stevia rebaudiana*

Wu C[1], Venkataraman SK[1], Nettles BJ[1], Jaksch F[1], Rodenburg DL[2], Alves KM[2], Ibrahim MA[2], McChesney JD[2]
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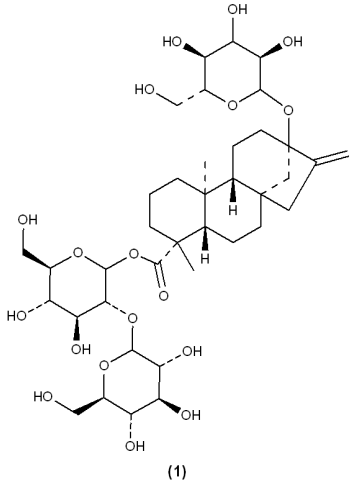
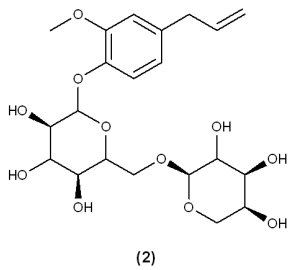
The approval of Stevia glycosides as GRAS and their expected widespread use in foods and beverages as non-caloric sweeteners has prompted a need for high purity reference standards. As part of the effort to prepare those standards, we have purified quantities of more than twenty glycosides from commercial *Stevia rebaudiana* extract. We are reporting the characterization of three, **1 – 3**. Compound **3** very nearly co-elutes with Rebaudioside D in the current JEFCA Stevia HPLC method. This is the first report of eugenol glycoside **2** in *Stevia rebaudiana.*



(1)



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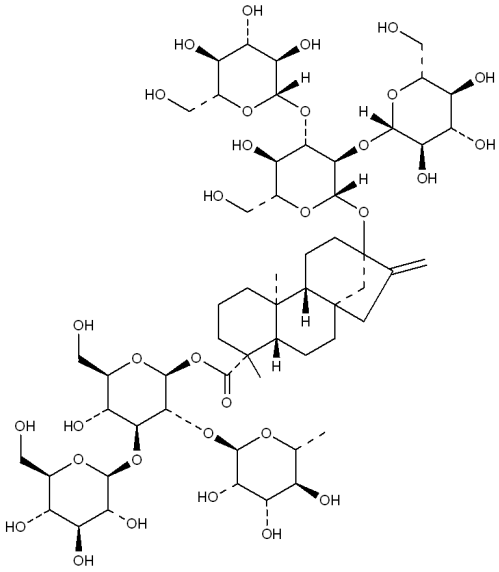

PJ96

Simultaneous determination of anthraquinone distribution in *Cassia tora* using high performance liquid chromatography

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A reversed-phase HPLC method has been developed and validated for the quantitative determination of four anthraquinones (AQs): aloe-emodin (1), emodin (2), chrysophanol (3) and physcion (4) in various part of *Cassia tora*. The method utilised a TSK-gel ODS-80Tm column (5 μm, 4.6 x 150 mm) at 25°C. The mobile phase composed of 2% aqueous acetic acid (A) and methanol (B) (gradient elution as follows: 0 – 10 min, 70% B – 80% B; 10 – 30 min, 80% B to 70% B), at a flow-rate of 1 mL/min, and UV detection at 266 nm. From the validation results, the HPLC method showed good linearity (r^2 > 0.9996), high precision (intra-day R.S.D. < 1%, inter-day R.S.D. < 2%) and high recovery rates (95.34 to 105.91%). Various parts of *C. tora* including leaves, roots, flowers, stems, young seeds and mature seeds were examined in order to compare the AQ content in each tissue. The mature seed extract was accumulated the highest amount of total AQs (30.53 mg/g DW), while the other tissues were consisted of only (2) in low amount (1.20 – 1.82 mg/g DW). Moreover, the mature seed extract was capable of producing of total AQ (30.53 mg/g DW) higher than the young seed extract (4.92 mg/g DW). Major AQs found in the seed extract were (3) (13.64 mg/g DW) and (4) (14.27 mg/g DW) whereas (2) was the minor one (2.62 mg/g DW).

PJ97

Establishment of assay specification in fennel

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Fennel is the fruit of *Foeniculum volgare* in the family Umbelliferae. And fruit is commonly used for its expectorant, antispamodic, carminative and diuretic properties. In this study, we determined the chemical composition of the volatile fractions obtained from fennel fruits. The main conponents were trans-anethole, p-anisaldehyde and estragole. The assay specifications have been suggested with trans-anethole and p-anisaldehyde as active compounds in *Foeniculum volgare*. We have anlayzed identification test and assay specification about 64 samples. In the result of analysis, we have established the content standard of trans-anethole for *Foeniculum volgare* in Korean Pharmacopoeia.

PJ98 Development and application of a methodology for the recovery of high added value products from peach industry waste

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Every year, in Greece, more than 300.000 tones of peach fruit (*Prunus persica*), are used to produce juices and canned products. Consequently, thousands tones of fruit waste are discarded, aggravating annually the environment by a total of 3 million m^3 of liquid and 23.000 tones of solid waste. Peach solid and liquid wastes are rich in phytochemicals like phenolics and carotenoids that have high economic added value, due to their remarkable biological activities that find many applications in food, cosmetic and pharmaceutical industries. Within this project we have developed and apply a method for the recovery of polyphenols with an eco friendly approach using mainly the Adsorption Resin Technology. The methodology has been applied in lab and in pilot scale. In pilot scale 200 kg peach pulp were treated with 25 kg resin XAD 4 type in order to recover polyphenolic compounds. Using a few simple steps of separations we have isolated and identified several phenolic compounds that belong to flavonoids (e.g.luteolin, prunin), phenolic acids (e.g. cafeic acid), lactones (e.g. D-decalactone) and triterpens (e.g.3-epi maslinic acid). In addition, the peach kernel that is another by-product of the industry has been submitted successfully to Super Critical Fluid extraction and we have obtained, in good yields, the kernel oil that is also known as "persic oil" and has commercially high added value in cosmetic industry.

PJ99 New hyphenated CPC-HPLC-DAD-MS strategy for simultaneous isolation, analysis and identification of phytochemicals

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We present here the development of a versatile tool for fast screening and rapid detection of bioactive natural products from plant extracts: the on-line coupling of centrifugal partition chromatography (CPC)-UV to HPLC-UV-MS, via a six position switching valve [1]. This strategy offers the possibility to get instantly HPLC fingerprint of fractions and structural information about separated molecules during the CPC fractionation step. This new approach was applied to the fractionation and purification of xanthones from *Garcinia mangostana* (Clusiaceae) pericarp. CPC was conducted using the biphasic solvent system heptane/ethyl acetate/methanol/water (2:1:2:1, v/v) and HPLC separation was done with a monolithic column under reversed phase conditions. The combined CPC-HPLC-DAD-MS allows the simultaneous fractionation, detection and characterization of sixteen molecules. Ten molecules of them were identified based on their UV and MS spectra. Furthermore, the methodology has led to isolation of pure α-mangostin and γ-mangostin in a very short time. [1] J. Chromatogr. A, 1218 (2011), 6173 – 6178.

PJ100 Isolation of *Geissospermum laeve* alkaloids by pH-zone refining centrifugal partition chromatography for metal corrosion studies

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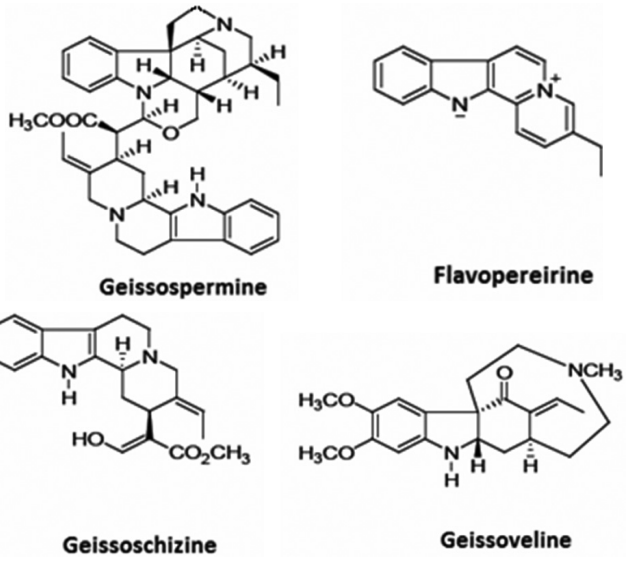
Control of metal corrosion is of technical, economical, environmental, and aesthetical importance. The use of corrosion inhibitors is one of the best options to protect metals and alloys from corrosion. The environmental toxicity of synthetic inhibitors has prompted the search for green compounds. Plant extracts have become important as an environmentally acceptable, readily available and renewable source for a wide range of biodegradable, heavy metals-free inhibitors. Among natural compounds, alkaloids have very high corrosion inhibition efficiency. An alkaloidic extract of *Geissospermum laeve* (Apocynaceae) has been fractionated by centrifugal partition chromatography using the pH-zone refining mode in order to investigate the effect of isolated alkaloids on C38 steel corrosion in HCl 1 M. Indole alkaloids were identified by LC-MS, ^1H and ^{13}C NMR.



PJ101 Quantification of the SERCA inhibitor thapsigargin in different samples of *Thapsia garganica* L. roots

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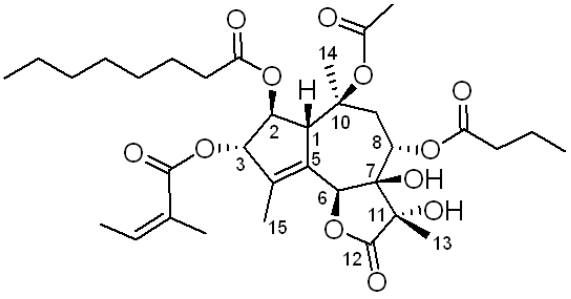
Dedicated to the memory of Pr François Tillequin. The nanomolar SERCA inhibitor Thapsigargin has been isolated in large scale from different samples of *Thapsia garganica* L. roots recolted in northern Algeria. Optimization of both extraction and purification steps have been performed using high pressure automatised extractor (Speed-Extractor Buchï) and Centrifugal partition chromatography (CPC Armen). Comparaison of Thapsigargin amounts in the different samples will be presented.



We thank the ANR program THASER ANR 2010 BLAN 722 3 for financial support

PJ102 Purification of phenolic flavanoids with flash chromatography

Silver JE, Drooby M, Lewis RL
Teledyne Isco, Lincoln, NE 68504

Phenolic flavonoids are common plant secondary metabolites. They occur as flavonoids, anthocyanidins, and anthocyanins. The interest in flavanoids is due to investigations into their anti-oxidant activity; they are also interesting as natural food colorants that may also possess nutraceutical benefits. As the compounds are chemically similar to each other, obtaining pure compounds is problematic. Advances in flash chromatography columns, systems, and detection techniques allow easier purification of phenolic flavonoids. Methanolic extract from *Camellia sinensis*, a rich source of phenolic flavonoids, is used as a model system for purification of these compounds.

PJ103

Centrifugal partition chromatography – ECO-friendly method for the multigram purification of aucubin and aucubigenin

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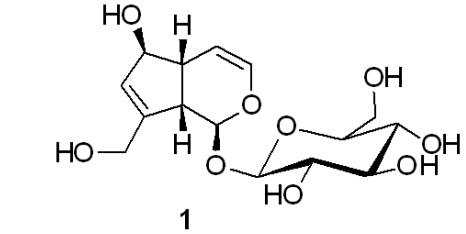
dedicated to the memory of Pr François Tillequin Aucubin **1**, isolated by Bourquelot and Herissey in 1902, is secondary plant metabolite which can be extracted in large amounts from the fresh aerial parts of *Aucuba japonica* Thunb.. We have showed that this iridoid serves as an excellent starting chiral material in semisynthetic purposes. In this respect we have been interested to develop efficient and fast method of its purification. Multigram water extract can be directly purified by centrifugal partition chromatography using green solvent system based on water and small amounts of non-halogenated solvents. In addition to aucubin **1**, biologically related iridoid aglycone, eucommiol **2** and unstable aucubigenin **3**, generated by enzymatic cleavage of **1**, were isolated in high-purity.



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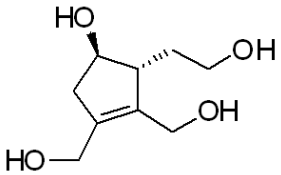

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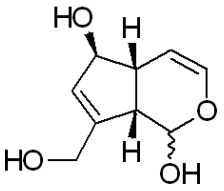

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We acknowledge financial support from the ANR (ANR-09-CP2D-09 – 01: IRNACHIR-2009).

PJ104

Rapid scaleup of high performance countercurrent chromatography from bench to kilogram

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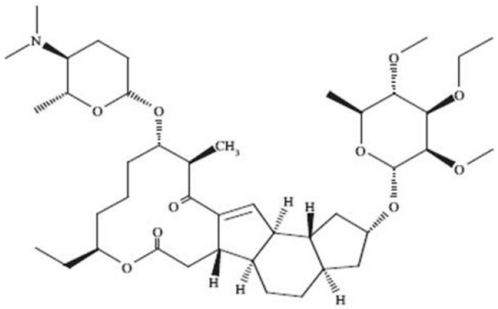
Countercurrent chromatography (CCC) has found broad application for natural product purification at the discovery scale but one limitation to the practical use of the technique has been rapid and predictable scale-up. This limitation has been overcome using HPCCC (HPCCC) instrumentation. As an example, method development and the kilogram scale purification of two semi-synthetic spinosyns, spinetoram-J (**1**) and -L (**2**), at the kilogram scale will be described.



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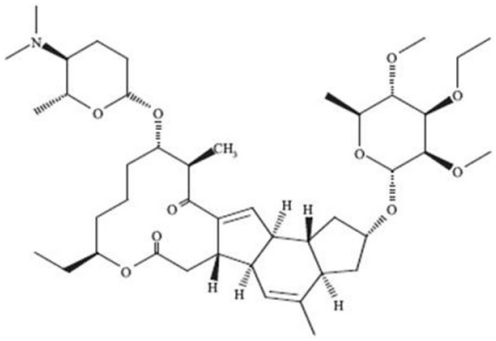

2

PJ105

Centrifugal partition chromatography for the isolation of tropical orchid constituents

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Orchidaceae is one of the largest botanical families and some species have been used as ingredients of traditional preparations with beneficial properties. Despite the high popularity of these plants, there are really few studies concerning their chemical composition. In the aim to acquire more basic knowledge about metabolites from different tropical orchids, several species were selected. The present study deals with the rapid isolation of orchid constituents by centrifugal partition chromatography (CPC). Dried aerial parts were extracted with CH_2Cl_2, and the obtained extract was fractionated by CPC using the solvent system *n*-heptane-ethyl acetate-methanol-0,1 % formic acid. Different proportions were tested to find convenient solvent conditions for the separation of potential chemotaxonomic markers.

PJ106

Rapid on-line dereplication by HPTLC-MS interface in orchid extracts

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Previous phytochemical analyses of crude extracts of *Vanda coerulea* Griff ex. Lindl (Orchidaceae) have led to the identification of stem-specific metabolites. The three most concentrated stilbenoids (imbricatin, methoxycoelonin and gigantol) displayed complementary biological activities in slowing down the skin cell senescence process and can thus be considered as biomarkers of this orchid species [1]. *V. coerulea* stem extract is now used in cosmetics for its anti skin ageing effect. In search for new bioactive compounds in orchids, other species from the same Vandeae tribe were explored. In order to accelerate the isolation of new molecules, a dereplication approach using HPTLC-MS interface was de-

veloped to quickly identify the three markers previously mentioned and to priority isolate other metabolites. Using this technique, the stem-specific biomarkers of *V. coerulea* were proven to be also present in other genera, along with other phenanthrene derivatives. This performing tool not only permits to provide a quick dereplication approach, but also to help us determine if these bioactive compounds could be considered as taxonomic markers of the Vandeae tribe.

PJ107 Using hyphenated HPTLC-MS for quality control of Brassocattleya marcella Koss orchid extracts

Cakova V[1,3], Wehrung P[2], Urbain A[1], André P[3], Bonté F[3], Lobstein A[1]
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Thanks to its pharmacological efficacy on human skin pigmentation disorders, a *Brassocattleya marcella* Koss orchid extract is now used as an active agent in dermocosmetics [1]. In the aim of quickly checking the conformity and the analytical quality of an industrial extract, we developed a new method using the hyphenated HPTLC-MS interface. We also compared raw materials from different geographical origins in order to rapidly identify 3 phenanthrene derivatives considered as molecular markers of the biologically active extract. The use of this rapid and low-cost technique allows the identification control of the presence of these three phytochemical markers in different batches or different suppliers.

PJ108 Chemical subtraction of bioactive prenylated phenols generates knock-out extracts of hops (*Humulus lupulus* L.)

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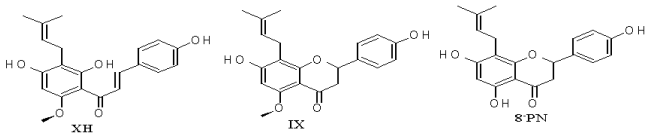
UIC/NIH Botanical Center, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, 833 S. Wood St., Chicago, IL, 60612

Recently, the generation of "knock-out (KO) extracts" has been reported, using affinity chromatography with monoclonal antibodies for removal of single phytoconstituents from plant extracts. Another approach, termed chemical subtraction, uses countercurrent chromatography (CCC) to deplete target(s) from a complex mixture, following the equation: extract (minuend) – target compound (subtrahend)= knock-out extract (difference). Chemical subtraction enables further chemical and biological characterization of both the otherwise intact KO extract as well as the residually complex subtrahend(s). This concept is applied to bioactive prenylated phenols in hops (xanthohumol [XH], isoxanthohumol [IX], and 8-prenylnaringenin [8-PN]), using HSCCC and HEMWat 0 as initial solvent system. Reflecting their different abundance in the extract and differences in CCC selectivity, the subtracted XH (33%), IX (3%), and 8-PN (0.35%) had qHNMR purities of 90, 54, and 12% w/w, respectively. The low 8-PN purity also reflects its co-elution with XH. Thus, a second CCC subtraction was performed using HEMWat -3 and orthogonal HterAcWat +3 systems. All HSCCC fractions other than those of the targets were recombined to provide the KO extracts. Quantitative LC-MS, qHNMR and UHPLC profiles will be presented for four hops KO extracts: XH-KO, IX-KO, 8-PN-KO and XH/IX/8-PN-KO.



PJ109 Scale-up of polyphenolic isolation by HPLC from *Uncaria guianensis* leaves by applying chromatographic models

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[1]Universidade Federal do Rio de Janeiro, Instituto de Química, Dep. Química Orgânica, C. T., Bloco A, Ilha do Fundão, 21941 – 909, Rio de Janeiro, RJ, Brazil; [2]Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos, R. Sizenando Nabuco 100, 21041 – 250, Rio de Janeiro, RJ, Brazil

Uncaria guianensis (UG) (Rubiaceae) is a species widely used in traditional medicine. The EtOH extract of their leaves has demonstrated anti-inflammatory and anti-allergic activities. In this work Semi-Prep-HPLC was employed to separate the polyphenolic compounds present in the UG EtOH leaf extract by applying chromatographic models. Polyphenolic enriched fractions previously obtained by RP-CC were analyzed in an Inertsil Prep-ODS column (250 x 6 mm, 10 µm), 30 °C, flow rate 1.4 mL/min, isocratic mode of CH_3CN:H_2O at five different compositions (3% to 20% v/v) and UV detection at 265 and 280 nm. From the retention times and the peak widths, the parameters *k* (capacity) and *h* (reduced plate height) were determined. From the application of the *k* data in four different models those based on linear and squared equations have shown correlation coefficient above 0.99 thus well representing the retention. Average *h* value was adopted. The prediction of the retention and efficiency of the compounds under unevaluated conditions was then possible. By applying two chromatographic models expressed by statistical moments, the simulation of the chromatograms under volume overload indicated the best conditions to isolate the compounds in each fraction. Kaempferitrin (~98% pure by ^{1}H NMR) and other minor polyphenolic compounds were separated and their structures are currently under characterization.

PJ110 Maitenin in cell line of Maytenus ilicifolia (Celastraceae) Reissek front different sources of carbon

da Silva Coppede J[1], Souza Pina E[1], Sarazete Pereira IV[1], Bertoni BW[1], de Castro França S[1], Soares Pereira AM[1]
University of Ribeirao Preto – Department of Plant Biotechnology – 2201, Costabile Romano, Ribeirao Preto/SP, Brazil

The species *Maytenus ilicifolia* ex Reissek which produces triterpenes quinonametideos exhibit cytotoxic activity, however these compounds are stored only in the bark of the roots of the plant and reduced concentrations it difficult to use of these active compounds on an industrial scale. The objective of the present work is to evaluate the production of maitenin in cell suspension cultures of the species *M. ilicifolia* cultivated in different carbon sources. Cell lines maintained for thirty-five days in culture medium. This cell was transferred (2 g) to 60mL of MS medium + 30 g/L sucrose + 1 mg/L 2,4-D and 0,5 mg/L kinetin. The cells were maintained in growth room with controlled temperature and photoperiod, next the cells were filtered and dried in circulating air oven. The drug plant was subjected to maceration with dichloromethane in a ultrasonic bath for 20 minutes three times. The extract generated was analyzed using HPLC C-18 column, phase mobile methanol: water 85:15 + 0.1% formic acid at a flow rate of 1mL/min for 25 minutes. The chromatograms generated showed that difference was found in the productivity of maitenin and cells cultivated in medium culture containing sucrose showed higher contents of triterpene production when compared to the same asset obtained from the cultivation on glucose.

PJ111 Production of quinonemethide triterpenes by *Peritassa laevigata* cell culture

Souza Pina E[1], Aparecida Lopes A[2], da Silva Coppede J[1], Bertoni BW[1], de Castro França S[1], Soares Pereira AM[1]
[1]University of Ribeirao Preto, 14100 – 000; [2]Faculty of Pharmaceutical Science of Ribeirao Preto, USP, 14040 – 903 Ribeirao Preto, SP, Brazil;

Peritassa laevigata (Hoffmanns. Ex. Link.) A. C. S., is a plant from the Celastraceae family native to the Cerrado biome. Quinonemethide triterpenes derived from the friedo-nor-oleanane skeleton are among a number of secondary metabolites produced by this species. Reports on the pharmacological activity of the quinonemethides evidenced their anti-tumor efficacy. The aim of this work was to investigate the production of

quinonemethide-type triterpenoids in root of *P. laevigata* cultivated *in vitro*. *Peritassa laevigata* roots growing easily and the presence of IBA regulators had positive effect on the cell culture. The dicloromethane extract from the roots of *Peritassa laevigata* was analyzed by HPLC. Separation carried out on a C-18 analytical column (25 x 4.6 mm 5 µm), mobile phase isocratic MeOH:H_2O (85:15), 1.0 mL/min and UV absorption was determined at 254 and 420 nm simultaneously using the PAD. Compound **1** was detected on the extract, showing that the terpene biosynthetic ability has been maintained in the cell culture. These studies will be used to further investigate quinonemethide triterpenes biosynthesis pathways.

PJ112 Flavonoid glycosides and phenolic compounds from *Potentilla supina*

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[1]School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do 440 – 746, South Korea; [2]Korea Research Institute of Chemical Technology, Taejeon 305 – 600, South Korea

Potentilla supina L., which belongs to the family Rosaceae, grows as a perennial herb, and is distributed in temperate regions of northern hemisphere. Its whole plants have been used as a Chinese herbal drug for the treatment of various bleeding, diarrhea, dysentery, enteritis and uterine cervical cancer. In our continuing studies to find bioactive compounds from natural products, we have found that the MeOH extract of *P. supina* whole plants has cytotoxic effects against human tumor cell lines. The MeOH extract was consecutively partitioned with organic solvents to give n-hexane, CH_2Cl_2, EtOAc, n-BuOH and H_2O fractions. Among these fractions, the EtOAc soluble which showed comparatively higher cytotoxic effects (IC_{50}: 17.0 µg/ml for SK-MEL-2) than the other fractions was subjected to column chromatographic separation. A new and twelve known compounds were isolated from the EtOAc fraction. Twelve known compounds were identified as protocatechuic acid, gallic acid, methyl gallate, astragalin, kaempferol 3-glucuronide, kaempferol 3-glucuronide 6"-methyl ester, tiliroside, isoquercitrin, quercetin 3-glucuronide 6"-methyl ester, luteolin 7-glucuronide 6"-methyl ester, 2-O-(*trans*-caffeoyl) malic acid 4-methyl ester, and ellagic acid by comparison of their spectral data with literature values, The structure of a new compound was established as 2-O-(*trans*-caffeoyl) malic acid 1,4-dimethyl ester by spectroscopic evidence. Cytotoxicity for isolated compounds were evaluated by the sulforhodamin B (SRB) assay against human tumor cell lines (A549, SK-OV-3, SK-MEL-2, HCT15).

PJ113 A rapid extraction technique for dry plant materials utilizing flash chromatography with integrated multi-detection

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Grace Davison Discovery Sciences, 2051 Waukegan Rd. Deerfield, IL 60015

Isolating single components for study from plant materials is challenging and often involves multiple steps using one or a combination of extraction techniques. Plants in their raw form may not solubilize easily in organic solvents and may require harsher extraction techniques incorporating pH changes or an application of heat to process. These techniques can pose risk of decomposition and be time-consuming. This work investigates the use of flash chromatography, frequently utilized to purify dry and liquid extracts or simplify complex botanical mixtures, to increase productivity of botanical isolations using on-line extraction and fractionation of pulverized dry plant materials. Goals of the study include decreasing extraction steps, solvent usage, and processing time.

PJ114 Comprehensive chromatographic and NMR methods for the separation and identification of glucosinolates from mustard seeds

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Glucosinolates are an important group of natural occurring thioglycosides which are the flavor precursors of cruciferous vegetables, condiments, relishes and other vegetables. Much effort has been devoted to developing methods for the efficient isolation and identification of glucosinolates due to their health benefits as well as oral care benefit, e.g., elimination of bad breath. In this study, we have isolated glucosinolates from 3 different commercially available mustard seeds, namely, black mustard seed, organic black mustard seed and yellow mustard seed by solvent extraction and followed by gel chromatographic separation. An ion-pair HPLC method was developed with a C-18 reverse column. We have observed that sinigrin and sinalbin are the main constituents in the organic black mustard seed and in the yellow mustard seed, respectively. In contrast to previous reports, we observed that gluconapin was the main constituent in the (non-organic) black mustard seed. All three glucosinolates were further purified by gel chromatograph. Their structures were analyzed by ^{1}H NMR.

PJ115 Absolute configuration of natural products through quantum chemical calculation of CD spectra-selected examples

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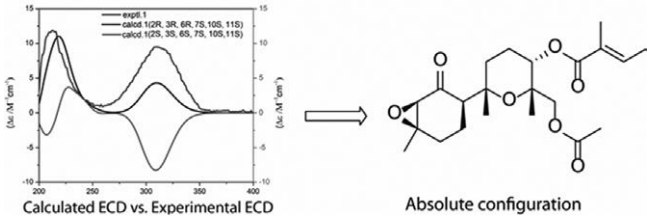
[1]Division of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, 4056-Basel, Switzerland; [2]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran; [3]Division of Molecular Modeling, University of Basel, Klingelbergstrasse 50, 4056-Basel

Chiroptical methods such as electronic circular dichroism (ECD) has been used for assignment of absolute configuration since several decades, but were limited in their applicability. Recent progress in the areas of conformational analysis and time dependent density function theory (TDTDF) calculation allows prediction of CD spectra by quantum chemical calculations. This is especially valuable for the analysis of new molecular entities when chemical synthesis of the reference compound is not an alternative. We successfully applied this methodology to establish the absolute configuration of structurally diverse and conformationally rigid or flexible molecules including monoterpenes, sesquiterpene coumarins, bisabolol sesquiterpenes, and complex isoprenoids with novel scaffolds. With a series of bisabolol oxide esters we demonstrate the assignment of absolute configuration by ECD calculation for compounds in which relative stereochemistry of two discrete parts of the molecule but not of its entirety could be established by NMR.



PJ116 Isolation, structure elucidation and antiplasmodial activity of novel bisindole alkaloids from *Gonioma malagasy*

Beniddir MA[1], Martin MT[1], Tran Huu Dau ME[1], Grellier P[2], Rasoanaivo P[3], Guéritte F[1], Litaudon M[1]
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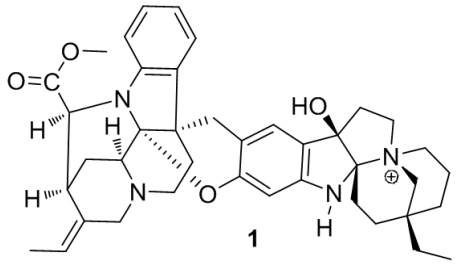
In the frame of the search for antiplasmodial compounds from the Madagascarian biodiversity, we chemically and biologically investigated the endemic species *G.malagasy* Boiteau (Apocynaceae). Five novel bisindole alkaloids possessing an unprecedented linkage between the two indole moieties have been isolated and characterized. The planar structure of the main compound (fig.**1**) was elucidated using usual spectroscopic methods, while its absolute configuration was deduced following the comparison of experimental and theoretically calculated ECD spectra, and through biogenetic consideration. We will report in this communication, their isolation, structure elucidation and antiplasmodial activities.



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PJ117 4-methylthio-butanyl derivatives from the seeds of *Raphanus sativus*

Kim CS[1], Kim KH[1], Moon E[2], Kim SY[2], Choi SU[3], Lee KR[1]
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Raphanus sativus L. (Brassicaceae) has long been cultivated as a food crop in Korea. The seeds have been used in Korean traditional medicine as a therapeutic agent for carminative, diuretic, expectorant, laxative and stomachic. Some glucosinolates have been isolated from the seeds of *R. sativus*. Glucosinolates and/or their breakdown products have recently attracted considerable interest because of their cancer-preventive properties. In our screening test, the MeOH extract of the seeds of *R. sativus* exhibited significant cytotoxicity against some human tumor cells and anti-inflammatory activity by inhibiting LPS-induced NO production. The purification of the MeOH extract from the seeds of *R. sativus* using repeated column chromatography afforded four new 4-methylthio-butanyl derivatives (**1 – 4**), together with four known ones (**5 – 8**). The structures of the new compounds were elucidated by analysis of 1D and 2D NMR data. We are studying the anti-neuroinflammatory effects of isolates for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells.

PJ118 Bioactive phenolic compounds from the leaves and twigs of *Euonymus alatus*

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Euonymus alatus (Thunb.) Sieb. (Celastraceae) is commonly known as winged euonymus in Korea and has been widely used in traditional medicine to regulate blood circulation, relieve pain, eliminate stagnant blood and treat dysmenorrhea. In addition, this tree has been clinically used to treat type 2 diabetes for many years as a folk medicine in China. The young leaves of this tree are edible as kitchen herbs, and the cork cambium on the twigs has been traditionally used to treat cancer. Recent pharmacological studies revealed the potential of *E. alatus* as an antitumor agent using a variety of *in vitro* and *in vivo* models, which is also confirmed by our group. In our screening test, the MeOH extract from the leaves and twigs of *E. alatus* exhibited significant cytotoxicity using a SRB bioassay. The purification of the MeOH extract using repeated column chromatography furnished five new phenolic compounds (**1 – 5**), together with seven known ones (**6 – 12**). The structures of the new compounds were elucidated by analysis 1D and 2D NMR data. The absolute configurations of **1 – 5** were determined by a modified Mosher's method. We are studying the antitumor activity of isolates (**1-12**).

PJ119 Sulfur compounds from the mushroom *Boletus pseudocalopus* and their bioactivity

Kim CS[1], Kim KH[1], Choi SU[2], Lee KR[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440 – 746, Korea; [2]Korea Research Institute of Chemical Technology, Daejeon 305 – 600, Korea

In our continuing search for structurally interesting and bioactive metabolites from Korean wild mushrooms, we have collected scores of endemic Korean mushroom species in the mountainous areas during the hot humid summer and prepared MeOH extracts of them for antitumor-activity screening test. Among the collected wild mushrooms, the extract of *Boletus pseudocalopus* (Boletaceae) showed significant cytotoxicity against three human tumor cell lines. This mushroom is an inedible mushroom with toxicity that is recognized by its yellow fruiting bodies and the blue staining when the fruit-bodies are cut or brushed. Recently, we reported the identification of three new fatty acid esters with cytotoxicity. In continuing study on this mushroom, two sulfur compounds (**1 – 2**) together with four known compounds (**3 – 6**) were isolated from the *n*-BuOH-soluble fraction. The chemical structures of the new compounds were determined on the basis of 1D and 2D NMR spectroscopic data. The isolated compounds were evaluated for cytotoxicity against some human tumor cell lines using a SRB assay.

PJ120 Two new lignans from the rhizomes of *Sinomenium acutum*

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Sinomenium acutum (Menispermaceae) is a deciduous shrub that grows in Asia, particularly Japan, China, and Korea. The stems and roots of the plant are widely used to treat various rheumatic diseases, allergy, dropsy, and dermatophytosis in the traditional Chinese prescriptions. Previous phytochemical investigations on *S. acutum* have reported to isolate various alkaloids, *e.g.* protoberberine-type, aporphine-type, isoaporphine-type, and morphine-type alkaloids and some of these components showed antiinflammatory, anticancer, immunosuppressive, and arthritis ameliorative activities. In our continuing investigation for bioactive constituents from Korean medicinal plant sources, we investigated the MeOH extract of the rhizomes of *S. acutum*, since the extract showed significant cytotoxic activity in our screening procedures. Column chromatographic isolation of the extract furnished two new lyoniresinol-type lignans (**1-2**), together with four known lignans (**3-6**). The identification and structural elucidation of these new compounds were based on 1D and 2D NMR, and HR-FABMS data. The absolute configurations of the new compounds (**1-2**) were clarified by CD study. We are studying the cytotoxic activities of isolates by determining their inhibitory effects on the human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) using a SRB assay.

PJ121 Cytotoxic lignans from the twigs of *Lindera glauca* (Sieb. & Zucc.) Blume var. *Glauca*

Woo KW[1], Kim KH[1], Kim HK[1], Choi SU[2], Lee KR[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440 – 746, Korea; [2]Korea Research Institute of Chemical Technology, Daejeon 305 – 600, Korea

Lindera glauca (Sieb.&Zucc.) Blume var. *glauca* (Lauraceae) is a small deciduous tree growing in the forests at low altitudes in Japan, China, and Korea. This tree has been used as Korean traditional medicine to treat cancer such as stomach, and lung, and uterine cancer without any side effect. Previous phytochemical studies from the leaves, roots, and stems of this plant have revealed the presence of alkaloids, terpenes, and butanolides. In our continuing search for bioactive constituents from Korean medicinal plants, we investigated the cytotoxic constituents of the twigs of this plant, since the MeOH extract of the source showed significant cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 using a sulforhodamine B (SRB) bioassay. A bioassay-guided fractionation and purification of the MeOH extract resulted in the isolation and identification of three new lignans (**1-3**), along with three known lignans (**4-6**). The structures of these new compounds were elucidated on

the basis of 1D and 2D NMR data as well as CD studies. The isolated compounds were evaluated for cytotoxicity against some human tumor cell lines using a SRB assay.

PJ122 Three new lignans from *Rudbeckia laciniata*

Lee SY[1], Cho HK[1], Han JY[1], Choi SU[2], Lee KR[1]
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Three *Rudbeckia* species, *R. bicolor*, *R. hirta*, and *R. laciniata*, are wide spread in Korea. Extracts from the plants have been used as traditional Chinese medicine in the treatment of the common cold, and urinary diseases. Various phytochemical constituents, sesquiterpenes, flavonoids, polyacetylenes, and carotenoids have been reported from the genus *Rudbeckia* and a wide range of biological activities, including antitumor, antioxidant, antibacterial, and antifungal activities were studied. In a continuing search for bioactive constituents from Korean Compositae medicinal plants, we performed a phytochemical investigation of a MeOH extract from the aerial parts of *R. laciniata*. By repeated column chromatographic separation of the extract three new furofuran lignans (**1-3**), together with one known lignin (**4**) were isolated. The identification and structural elucidation of these new compounds were based on 1D and 2D NMR and HR-FABMS data. The cytotoxic activities of the isolated compounds (**1-4**) were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) in vitro using the sulforhodamine B (SRB) assay.

PJ123 Quinone derivatives from the rhizomes of *Acorus gramineus*

Lee SY[1], Oh JY[1], Choi SU[2], Lee KR[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440 – 746, Korea; [2]Korea Research Institute of Chemical Technology, Daejeon 305 – 600, Korea

Acorus gramineus (Araceae) is widely distributed in Korea, Japan, and China. The rhizomes of the plant have long been used in the traditional Chinese medicine as a remedy for cognitive problem, sedation, and analgesia. In Korean traditional medicine, the herb has been also used for learning and memory improvement. We have recently reported the isolation of lignan derivatives and their inhibitory effects on NO production in lipopolysaccharide (LPS)-activated macrophages, and their cytotoxic activity. In a continuing research on the MeOH extract of *A. gramineus*, we have further isolated three new quinone derivaties (**1-3**), together with six known compounds (**4-9**). The identification and structural elucidation of these new compounds were based on 1D and 2D NMR, and HR-FABMS data. The absolute configurations were established on the basis of their CD data. We are studying the cytotoxic activities of isolates (**1-9**) by determining their inhibitory effects on the human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) using a SRB assay. We report herein the isolation, structural elucidation of the new compounds, and cytotoxicity of the isolated compounds.

PJ124 Absolute configuration and conformation of brevipolides, cytotoxic compounds from hyptis brevipes

Suárez-Ortiz GA[1], Cerda-García-Rojas CM[2], Pereda-Miranda R[1]
[1]Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, 04510 Mexico; [2]Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, A.P. 14 – 470, DF 07000 Mexico

The absolute configuration and conformational behavior of the highly flexible natural products brevipolides H and I were ascertained by circular dichroism and X-ray diffraction, in conjunction with a molecular modeling protocol which includes geometry optimization by DFT B3LYP/DGDZVP calculations and comparison between DFT and experimental ^1H-^1H NMR coupling constants. These results confirmed the biogenetic assumption that the stereogenic center in the final portion of the chain at C-6' of all natural 6-heptenyl-5,6-dihydro-2*H*-pyran-2-ones from the mint family (Lamiaceae) is always *S*. Financial Support: CONACyT (104887).

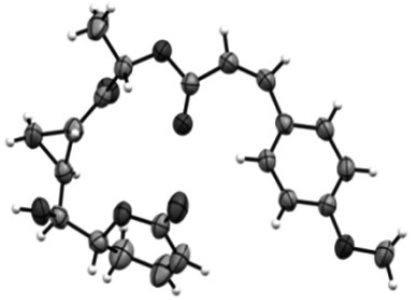
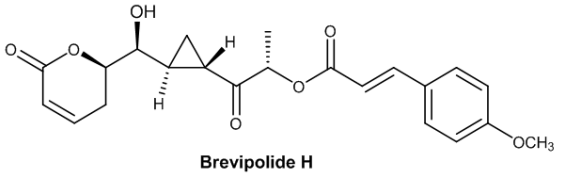
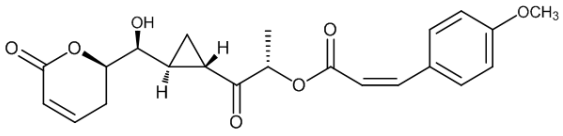


Fig. 1: X-ray of brevipolide



Brevipolide H



Brevipolide I

PJ125 New flavonol glycosides from the leaves of *Allium victorialis* var. *platyphyllum*

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[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440 – 746, Korea; [2]Graduate School of East-Weat Medical Science, Kyung Hee University Global Campus, Yongin 446 – 701, Korea

Allium victorialis var. *platyphyllum* (Liliaceae) is widely distributed in the northern part of Korea. The bulbs and leaves of this plant have been used as vegetable in Korea and also used as Korean traditional medicine for the treatment of gastritis and heart failures. Previous phytochemical studies on this plant reported the isolation of flavonoids, steroidal saponins and sulfur compounds. In the course of our continuing search for bioactive compounds from Korean medicinal plant sources, we investigated the 80% MeOH extract of the leaves of *A. victorialis* var. *platyphyllum*. Column chromatographic purification of the MeOH extract resulted in the isolation of eight new flavonol glycosides, named Allivictoside A-G (**1-8**), together with twelve known flavonoids (**9-20**). The structures of new ones were determined on the basis of 1D and 2D NMR data and chemical means. We are studying the anti-neuroinflammatory effects of the isolates for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells.

PJ126 Three new steroidal constituents from *Hosta longipes*

Kim CS[1], Lee SY[1], Woo KW[1], Moon E[2], Kim SY[2], Lee KR[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440 – 746, Korea; [2]Graduate School of East-West Medical Science, Kyung Hee University Global Campus, Yongin 446 – 701, Korea

Hosta longipes (FR. et SAV.) MATSUMURA (Liliaceae) is a perennial grass that is widely distributed throughout Korea. This plant has been used as a Korean traditional medicine for the treatment of ulcer, tuberculosis, melena and leukorrhea. In the previous phytochemical investigations, steroidal saponins were mainly isolated and some of them showed cytotoxic activity towards HeLa cells. In the course of our continuing search for biologically active compounds from Korean medicinal sources, we investigated the MeOH extract of the aerial parts of *H. longipes*. The column chromatographic separation of the MeOH extract re-

sulted in the isolation of three new steroidal compounds, longipenane (**1**), longipenane 26-*O*-β-D-glucopyranoside (**2**) and neogitogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside} (**3**), along with two known steroidal saponins. The structures of the compounds were determined on the basis of spectroscopic analyses, including extensive 2D NMR data. The stereochemistry of the compounds was clarified by *J* values and a modified Mosher's method. The isolated compounds are in progress for the test of inhibitory effects against the nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells.

PJ127 Quinic acid derivatives from *Pimpinella brachycarpa*

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[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440 – 746, Korea; [2]Graduate School of East-West Medical Science, Kyung Hee University Global Campus, Yongin 446 – 701, Korea

In our continuing search for bioactive constituents from Korean medicinal plants, we performed a phytochemical investigation on the MeOH extract of the aerial parts from *Pimpinella brachycarpa* (Umbelliferae). *P. brachycarpa* are widely distributed in Europe, Africa, and Asia (China and Korea). This species has been used in folk medicine for the treatment of gastrointestinal disturbances, bronchial asthma, insomnia, and persistent cough. In previous phytochomical studies, phenylpropanoids, polyacetylenes, and flavonoids were isolated from this source and some of them exhibited antifungal, antioxidant, antiinflammatory, and cytotoxic activities. A combination of preparative column chromatographic separation of the MeOH extract of the plant furnished five new quinic acid derivatives (**1-5**), together with nine known quinic acid compounds (**6-14**). Their structures have been established on the basis of spectroscopic analyses, including extensive 2D NMR studies. We are studying the anti-neuroinflammatory effects of isolates (**1-14**) for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells.

PJ128 Bioactive isoindolinone alkaloid from *Hericium erinaceum*

Noh HJ[1,2], Kim KH[1], Park HB[1], Kim GS[2], Lee SE[2], Kim SY[2], Choi SU[3], Lee KR[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440 – 746, Korea; [2]Department of Herbal Crop Research, National Institutes of Horticultural & Herbal Science, RDA, Eumseoung 369 – 873, Korea; [3]Korea Research Institute of Chemical Technology, Daejeon 305 – 600, Korea

Hericium erinaceum (Hericiaceae) is an edible mushroom which is widely distributed in China, Japan and Korea. Some phytochemical constituents, phenolics, fatty acids and sterols have been reported from this source and a wide range of biological activities, including antimicrobial, macrophage activation, NK cell activation, neuroprotective effects were studied. In a continuing search for bioactive constituents from Korean mushrooms, we performed a phytochemical investigation of the MeOH extract from the fruiting bodies of *H. erinaceum*. By repeated column chromatographic separation of the extract one new isoindolinone alkaloid (**1**), together with eleven known compounds (**2-12**) were isolated. The identification and structural elucidation of the compounds was based on 1D and 2D NMR spectral data. The cytotoxic activities of the isolated compounds (**1-12**) were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) in vitro using the sulforhodamine B (SRB) assay.

PJ129 Bisabololoxide derivatives from *Artemisia persica*, and determination of their absolute configurations by ECD

Ebrahimi SN[1,2], Moradi-Afrapoli F[3], Smiesko M[4], Raith M[1], Zimmermann S[1,5], Nadjafi F[2], Brun R[5], Hamburger M[1]
[1]Division of Pharmaceutical Biology, University of Basel, 4056 Basel, Switzerland; [2]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G. C., Evin, Tehran, Iran; [3]Department of Pharmacognosy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran; [4]Division of Molecular Modeling, University of Basel, 4056 Basel, Switzerland; [5]Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland

Five new antiplasmodial bisabololoxide sesquiterpene diesters were isolated from an EtOAc extract of the aerial parts of *Artemisia persica* following an HPLC-time-based activity profiling of the extract. Structure elucidation was achieved by 1D and 2D NMR experiments. Relative configurations of cyclohexenone/cyclohexene and tetrahydropyran moieties of **1 – 5** were established on the basis of $^3J_{H\text{-}H}$ coupling constants and NOE difference spectra. Stereochemical correlation of the two rings, and assignment of absolute configuration of **1 – 5** were achieved by comparison of experimental ECD spectra with simulated ECD data for possible stereoisomers, by using time dependent density function theory (TDDFT). Bisaboloids **1-4** exhibited *in vitro* antimalarial activity against *Plasmodium falciparum*, with IC_{50} values ranging from 2.8 to 20.1 μM, and selectivity indices (SI) in L-6 cells of 3.7 to 11.9.

PJ130 NMR vicinal $J_{H\text{-}H}$ from DFT theory as reliable tools for stereochemical analysis of 6-heptenyl-5,6-dihydro-2 H-pyran-2-ones

Juárez González FJ[1], Cerda-García-Rojas CM[2], Pereda-Miranda R[1]
[1]Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, 04510 Mexico; [2]Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, A.P. 14 – 470, DF 07000 Mexico

A protocol for stereochemical analysis, based on the systematic comparison between theoretical and experimental vicinal ^{1}H-^{1}H NMR coupling constants, was developed and applied to a series of flexible compounds derived from the 6-heptenyl-5,6-dihydro-2 H-pyran-2-one framework. The method included a broad conformational search, followed by geometry optimization at the DFT B3LYP/DGDZVP level, calculation of the vibrational frequencies, thermochemical parameters, magnetic shielding tensors, and the total NMR spin-spin coupling constants. The stereochemistry of natural and unnatural 6-heptenyl-5,6-dihydro-2 H-pyran-2-ones containing diverse functional groups in the heptenyl side chain was analyzed by application of the correlation between the theoretical (*J*pre) and experimental ^{1}H-^{1}H NMR (*J*exp). A remarkable correlation demonstrated the predictive value of this approach for the stereochemical assignment of highly flexible compounds containing multiple chiral centers.

PJ131 Batatins VII-X, glycolipid ester-type dimers from sweet potato (*Ipomoea batatas*)

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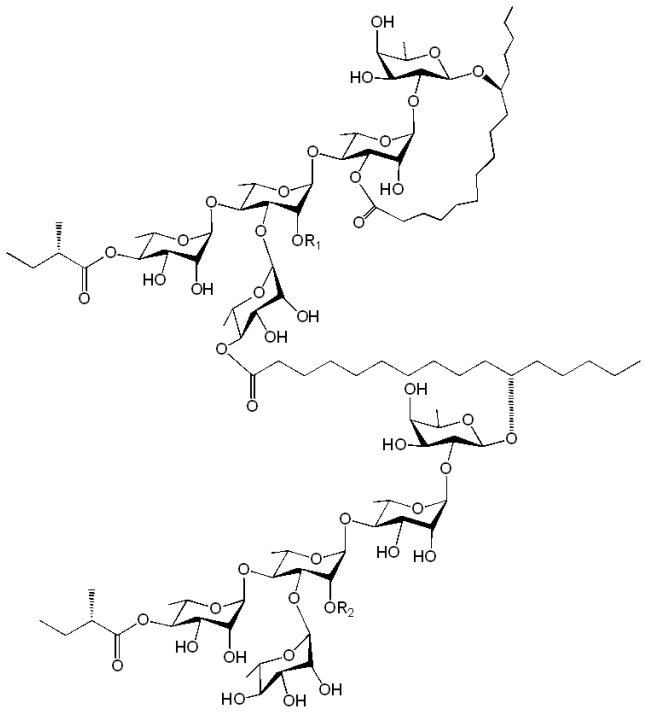
Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, 04510 Mexico

Batatins VII-X (**1-4**) were purified from the resin glycoside contents of the tuberous roots of three varieties of sweet potato, using different HPLC techniques. Their structures were characterized by means of several high-resolution NMR and MS techniques and the main structural differences were found in the site of lactonization to form the macrocyclic structure, the type of acylating residues, and the position of esterification to establish the ester-type dimer. Different types of acid residues esterified each unit: (2*S*)-methylbutanoic, cinnamic, decanoic and dodecanoic acids. Morning-glory resin glycosides have shown to modulate the multidrug resistance developed to therapeutic agents by microorganisms and tumor cells. Dimeric structures in the convolvulaceae family have been described previously, being of the specie of *Ipomoea batatas* which have been isolated and characterized more diversity, ba-

tatins I-X. Financial Support: DGAPA, UNAM (IN217310) and CONACyT (101380-Q).

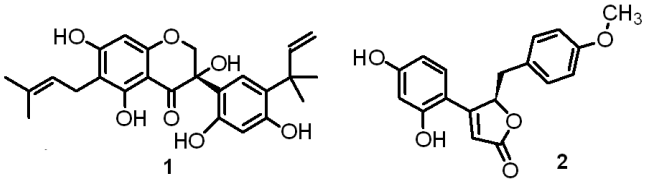


3. R1 and R2 = decanoic **4**. R1 and R2 = dodecanoic

PJ132 Absolute configuration of 3-hydroxyisoflavanones and conjugated 2-(5*H*)-furanones through electronic circular dichroism

Coleman CM[1], Zou Y[1], Eisenberg V[2], Belofsky G[2], Ferreira D[1]
[1]Department of Pharmacognosy and Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, U.S.A; [2]Department of Chemistry, Central Washington University, Ellensburg, WA 98926, U.S.A.

The 3-hydroxyisoflavanones and the 2-(5*H*)-furanones both constitute relatively small but chemically and biologically significant classes of natural products. Representatives of each class, including sedonan B (**1**, a 3-hydroxyisoflavanone) and 4'-*O*-methylpuerol (**2**, a 2-(5*H*)-furanone), were recently isolated from an extract of the dried roots of *Dalea formosa* Torr. (Fabaceae). As part of the structural elucidation of both compounds, their absolute configurations were determined by comparing experimental electronic circular dichroism (ECD) results with literature reports. While the absolute configurations of both compounds were established, a number of issues indicated that theoretical simulations of the ECD spectra for both compounds were warranted to further support these empirical assignments. Simulated ECD spectra were therefore generated using conformational analyses and geometric optimization followed by calculations of excitation energies and rotational energies using the TDDFT method at the B3LYP/6 – 31G(d,p) level. Molecular orbital analyses were also performed for 4'-*O*-methylpuerol. This presentation will discuss the issues that led to the need for simulated ECD spectra and the experimental and computational results for both compounds.



1 **2**

PJ133 Antiprotozoal isoflavan quinones from *Abrus precatorius*

Hata Y[1,4], Raith M[1], Ebrahimi SN[1,5], Zimmermann S[1,2], Mokoka T[3], Naidoo D[3], Maharaj V[3], Brun R[2], Kaiser M[2], Hamburger M[1]
[1]Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland, [2]Swiss TPH, Socinstrasse 57, 4002 Basel, Switzerland; [3]Biosciences, CSIR, P.O. Box 395, Pretoria, 0002, South Africa; [4]Pharmacy Department, National University of Colombia, 111321 Bogotá, Colombia, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University G.C., Evin, Tehran, Iran

A library of 309 extracts from selected South African plants was screened *in vitro* against a panel of protozoan parasites. A CH_2Cl_2/MeOH (1:1) extract of *Abrus precatorius* L. *ssp. africanus* Verdc. (Fabaceae) strongly inhibited *Plasmodium falciparum* (97.8%), *Trypanosoma brucei rhodesiense* (100%), and *Leishmania donovani* (75.5%) when tested at 4.8 mg/mL. Active constituents were tracked by HPLC-based activity profiling, and isolated by RP-HPLC. Structures were established by HRMS and NMR. The absolute configuration was determined by comparison of electronic circular dichroism (ECD) spectra with calculated ECD data. Five compounds were obtained and identified as isoflavan quinones and hydroquinones, among them two new natural products. (3*R*)-8-hydroxy-7,3',5'-trimethoxyisoflavan-1',4'-quinone and (3*R*)-6,7,8,2',3'-penta-methoxyisoflavan-1',4'-quinone showed strong *in vitro* activity against *T. brucei rhodesiense* (IC_{50}s of 0.30 µM ± 0.1 and 0.16 µM ± 0.1, respectively). Selectivity indices (SI) as calculated from cytotoxicity data in L-6 cells were 78.3 and 61.3.

PJ134 New insights on the structure of the anti-TB peptide H14: Crystal structure, ^{1}H NMR full spin analysis, and biosynthetic pathway

Gao W[1,2], Napolitano JG[1,2], Kim JY[3,4], Lee IA[3,4], Lee JE[3,4], Choi J[3,4], Rodríguez-Brasco MF[1], Jaki BU[1,2], Cho S[1,2], McAlpine J[1,2], Pauli GF[1,2], Kim J[5], Suh JW[3,4], Franzblau SG[1,2]
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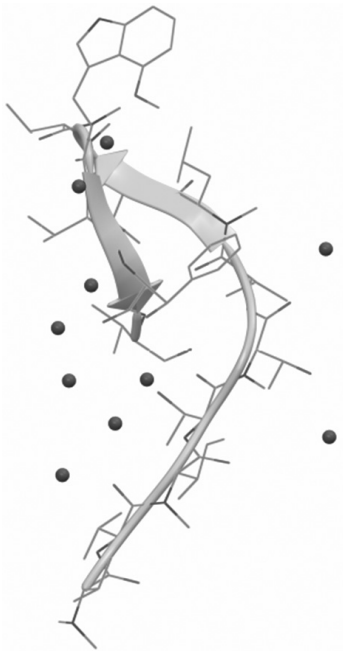
Previous anti-TB HTS of actinomycete extracts led to the extract of strain E5123, a *Nonomuraea sp.*, from which two cyclic depsipeptides, **H14** and **H16**, with potent and selective anti-TB activity were isolated. Three distinctive approaches to structural analysis of compound H14 are presented and discussed. An X-ray crystallographic analysis of H14 provided information on its tertiary structure and stereochemistry. From the X-ray structure, a ^{1}H NMR iterative full spin analysis (HiFSA) of H14 was carried out, giving all parameters of the spectrum (all δ_H and J_{HH}). The biosynthesis was investigated by genome sequencing. A portion of the presumed NRPS responsible for the four C-terminal amino acids in H14/H16 and several tailoring enzymes leading to the unusual amino acids, were identified. The lack of any epimerization domains in this sequence is strong presumptive evidence that at least 12 out of the 13 amino acids are L.



PJ135 Synergistic trail sensitizers from *Barleria alluaudii* and *Diospyros maritima*

Whitson EL[1], Sun H[2], Thomas CL[1], Henrich CJ[1,3], Sayers TJ[3,4], Gustafson KR[1], McMahon JB[1], Griesinger C[2], McKee TC[1]
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A high-throughput screen was developed to identify compounds that could sensitize tumor cells to the killing effects of tumor necrosis factor-alpha-related apoptosis-inducing ligand (TRAIL). Extracts from *Barleria alluaudii* and *Diospyros maritima* showed promising activity in the initial screen and were further investigated. As a result of this study, two naphthoquinone epoxides, 2,3-epoxy-2,3-dihydrolapachol (**1**) and 2,3-epoxy-2,3-dihydro-8-hydroxylapachol (**2**), both not previously isolated from natural sources, and the known 2-methyl anthraquinone (**3**) were identified from *B. alluaudii.* Time-dependent density functional theory (TD-DFT) calculations of electronic circular dichroism (ECD) spectra were utilized to establish the absolute configuration of **1** and **2.** Additionally, five known naphthoquinone derivatives, maritinone (**4**), elliptinone (**5**), plumbagin (**6**), (+)-*cis*-isoshinanolone (**7**), and ethylidene-6,6'-biplumbagin (**8**) were isolated from *D. maritima*. Compounds **1**, **2**, and **4-6** showed varying levels of synergy with TRAIL. Maritinone (**4**) and elliptinone (**5**) showed the highest synergistic effect, with more than a three-fold increase in activity observed with TRAIL than with compound alone.

PJ136 Some physical and chemical changes of pomegranate fruit characteristics during storage shelf-life conditions

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Pomegranate (*Punica granatum* L.) is commonly grown in different part of the world including Turkey for its high value of nutrients and its bioactive compounds such as phenolics and flavonoids in fresh pomegranate and juice. This study was conducted to determine changes in the physical and chemical characteristics after harvest up to sale during the shelf-life conditions of pomegranate grown in Antalya, Turkey. 'Hicaznar' (sour-sweet) and 'Finike' (sweet) pomegranate cultivars were chosen as the plant material. The fruits were harvested at optimal harvest time and placed in plastic boxes. The fruits were kept uncovered during the experiment. All fruit samples were stored at 20 ºC with 60 – 65% relative humidity (at shelf-life conditions). During storage the fruits were evaluated every 10 days for weight loss, 100 aril weight, juice yield, titratable acidity, total soluble solid, pH, total phenolic and flavonoid contents, skin thickness, skin and aril color. Weight loss of fruit increased and the color of the peel was dull and dry in both pomegranate cultivars parallel to the extended storage time. The value of titratable acidity decreased depending on the cultivars and during the storage time. Total phenolics significantly changed from 1411,50 to 2074,17 mg GAE/L and from 1664,00 to 2085,33 mg GAE/L in 'Finike' and 'Hicaznar' cultivars, respectively. Total flavonoids varied from 170,79 to 235,54 mg CE/L and from 231,33 to 348,87 mg CE/L in 'Finike' and 'Hicaznar' cultivars, respectively. Results of this study indicated that pomegranate fruit loose some physical and chemical properties under the shelf-life conditions.

PJ137 Facile methods to determine double-bond positions in long-chain olefins and absolute configurations of α-hydroxy acids

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Even with modern spectroscopic techniques and advanced knowledge in organic chemistry, structural determinations of some natural products require tedious and challenging processes. We developed new methods to facilitate the structural determinations of long-chain olefins (Kwon, Y.; Lee, S.; Oh, D.-C.; Kim, S. *Angew. Chem. Int. Ed.* **2011**, *50*, 8275) and α-hydroxy acid-bearing depsipeptides (Moon, K.; Lim, C.; Kim, S.; Oh, D.-C. *Chem. Comm.* in preparation). The positions of double bonds in unsaturated long-chain compounds can be easily determined by chemical derivatization using a cross-metathesis reaction and chromatography-mass spectrometry. The produced olefins have distinct physicochemical properties suitable for LC/MS or GC/MS analysis that depend on the cross-metathesis partner used. In addition, the absolute configurations of α-hydroxy acids can be established by a modified reaction with Marfey's reagent. This reaction derivatizes the α-hydroxy groups of α-hydroxy acids along with the amine groups of amino acids. This generates their diastereomeric derivatives, enabling simultaneous determination of the absolute configurations of α-hydroxy acids and α-amino acids in a depsipeptide by LC/MS analysis. We also show successful applications of these new methods to real natural products. Our new procedures are practically useful because of their simple application to natural products at a submilligram scale.

PJ138 C-glucosidic ellagitannins from Lythri herba (ph. eur.) and their microbial metabolites

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Lythri herba, a pharmacopoeial plant material (Ph. Eur.), is obtained from the flowering parts of purple loosestrife (*Lythrum salicaria* L.). Although extracts from this plant material were proven to possess some interesting biological activities and its pharmacopoeial standardization is based on total tannin content determination, the phytochemical characterization of this main group of compounds has not been yet fully conducted. Five main C-glucosidic ellagitannins – monomeric: vescalagin and castalagin together with new dimeric structures salicarinins A-C composed of: vescalagin and stachyurin, vescalagin and casuarinin, castalagin and casuarinin units connected via formation of valoneoyl group – were isolated using column chromatography and preparative HPLC. Structures were determined according to ^{1}H and ^{13}C NMR (1D and 2D), ESI-TOF, MS^{n} and circular dichroism spectra, together with acidic hydrolysis products analysis. Formation of potentially bioactive dibenzo[b,d]-pyran-6-one derivatives – urolithins A, B and C – by the human intestinal flora was established for Lythri herba extract and vescalagin.

PJ139 mMass as a software tool for the annotation of cyclic peptide tandem mass spectra

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Natural or synthetic cyclic peptides often possess pronounced bioactivity. Their mass spectrometric characterization is difficult due to the predominant occurrence of non-proteinogenic amino acid monomers and the complex fragmentation patterns observed. Even though several software tools for the annotation of cyclic peptide tandem mass spectra have been published, these tools are still unable to annotate a majority of the signals observed in experimentally obtained spectra. They are thus not suitable for extensive mass spectrometric characterization of these compounds. This lack of an advanced and user-friendly software tool has motivated us to extend the fragmentation module of a freely available open-source software, mMass (http://www.mmass.org), to allow for cyclic peptide tandem mass spectra annotation and interpretation. The resulting software has been tested on several cyanobacterial and other naturally occurring peptides and has been found to be superior to all other tools currently available with regard to both usability and annotation extensiveness. Thus it is highly useful for accelerating the structure confirmation and elucidation of cyclic as well as linear peptides and depsipeptides.

PJ140 Direct NMR analysis of sugars from glycosides

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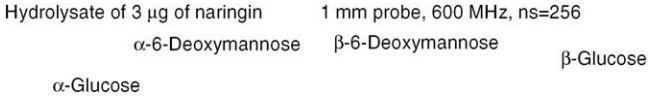
The structural identification of glycosides often poses great challenges. NMR is a powerful method, but overlapping signals complicate 2D NMR approaches. To identify the sugars in a glycoside, often a small sample is hydrolyzed and analyzed by GC after derivatizing the free sugars. We have developed a direct ^{1}H-NMR approach to sugar identification based on the anomeric signals that does not require derivatization. The chemical shifts of the anomeric signals and their α/β ratios will be presented for all $C_6H_{12}O_6$ aldoses as well as common pentoses and modified sugars. An approach to determine linkage patterns will be presented.



PJ141 Analysis of rotenone degradation products by electrospray mass spectrometry

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[1]Pharmacognosy Research Group, Louvain Drug Research Institute, Université Catholique de Louvain B1 7203, Av. E. Mounier 72, 1200 Brussels, Belgium; [2]Research Group of Bioorganic Mass Spectrometry, Dept. Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, BE-2610 Antwerp, Belgium

Rotenone is a natural phenolic compound inhibiting the mitochondrial respiratory chain that was formerly used for its insecticidal activity. Its toxicity linked with oxidative stress and dopaminergic neuronal degeneration causes many features of Parkinson's disease in animal models [1] and may also contribute to this disease in man. This compound is found in several plants among which seeds of yam bean (*Pachyrhizus* sp.) which are also rich in macro- and micronutrients [2]. As rotenone is somewhat unstable, we decided to decrease its initial content of the seeds by thermal degradation. HPLC-HR-(+)ESI-MS^n was used to analyse the mixtures formed upon thermal degradation of rotenone, and the MS fragmentation of rotenone and its major degradation products. First, based on HR-(+)ESI-MS, MS^2 and MS^3 spectra, we examined in detail the fragmentation behavior of rotenone and were able to propose structures for the seven main product ions of the protonated molecule. Then, based on the MS fragmentation behaviors, UV absorption spectra and abundance of the compounds according to pH, we identified the three main degradation products formed when rotenone is heated at 100 °C in methanol. The major degradation product was identified as a rotenone epimer and the epimerisation mechanism is also proposed.

PJ142 Walnut leaves against diabetes mellitus? Potential mechanism and active principle

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Walnut (*Juglans regia* – JR) is one of the medicinal plants used against diabetes mellitus in Austrian folk medicine. The anti-diabetic effect of Folium Juglandis has also been scientifically proven by several in vivo studies in mouse models. The present study is designed to scrutinize the active anti-diabetic principle of JR leaves and to achieve first hints of its physiological mechanism of action. The chlorophyll-free methanol extract of JR leaves (JR wC) enhanced the basal glucose uptake in C2C12 myocytes by 1.5 times at concentrations of 25 µg/mL compared to untreated cells. Compared with the solvent DMSO, this extract elicted more than doubled insulin-stimulated cellular glucose uptake rate. These effects could partly be explained due toby the inhibition of protein tyrosine phosphatase 1B (PTP 1B). JR wC inhibited PTP 1B by about 80% at a concentration of 25 µg/mL. Further separation of this fraction resulted in decrease and dispartment of activity, suggesting synergy between the individual components of JR wC. LC-MS analyses of the active JR wC extract led to the identification of chlorogenic acid, cumaroylquinic acid and naphthoquinone-hexoside, as well as eight flavonoids. Two of the flavonoids were identified as hyperoside and avicularin by comparison with reference compounds. The sugar moiety of three further flavonoids was determined by trimethylsilyl-derivatisation and GC-MS analyses leading to quercetin-L-arabinoside, quercetin-L-rhamnoside and kaempferol-L-arabinoside.

PJ143 Standardization and scavenging activity of Siamese neem flower extracts

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Siamese neem tree (*Azadirachta indica* A. Juss. var. *siamensis* Valeton) of the family Meliaceae is a medicinal plant found in every part of Thailand. Young leaves and flowers of this plant are commonly consumed as a bitter tonic vegetable. Decoction extract of Siamese neem leaves gave high DPPH scavenging activity. In this experiment, decoction extracts of Siamese neem young flowers collected from 14 different locations in Thailand were quantitative analyzed for the contents of active components, and determined loss on drying, heavy metals and pesticide residues, microbial contamination, solubility and chromatographic fingerprints. By HPLC analysis, the flower extracts contained rutin, and quercetin in the ranges from 388 to 1178 mg% dry weight (average 772 mg%), and 1 to 10 mg% dry weight (average 5 mg%), respectively. EC_{50} of scavenging activity of all extracts was found in the range from 27 to 133 µg/mL, classified as moderate activity. Loss on drying of the extracts was less than 7%w/w while HPLC fingerprints of all extracts showed the same pattern that rutin was a major active component. This study provides guidance for the specifications of aqueous extracts of Siamese neem flowers.

PJ144 Unusual chemical transformations of a germacrane ester from *Ferulago antiochia* Saya & Miski; A natural pro-drug in disguise?

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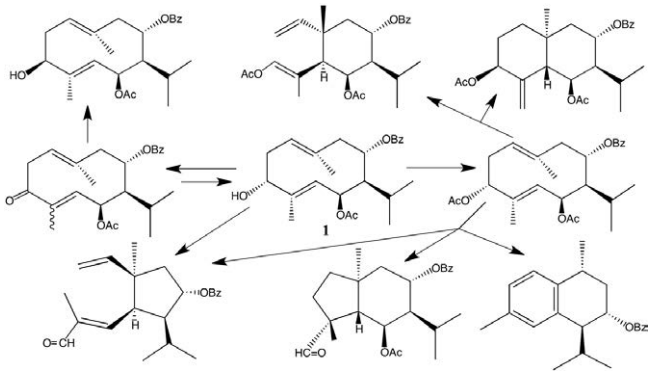
Conformation-dependent chemical rearrangements of the germacrane skeleton were examined by subjecting antakyatriol (1) derivatives to a number of a chemical transformations and/or thermal rearrangements. Although most of these reactions progressed in a predictable manner, some transformations have yielded unexpected novel rearrangement products.



PJ145

Pterodon pubescens Benth: Stability study of microencapsulated extract and isolated compounds monitored by antinociceptive assays

Servat L, Spindola HM, Rodrigues RAF, Sousa IMO, Ruiz ALTG, de Carvalho JE, Foglio MA
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We previously reported the antinociceptive properties 6α, 7β-dihydroxy-vouacapan-17β-oate methyl ester, isolated from *Pterodon pubescens* Benth seeds. This report describes the antinociceptive properties of isomers 6α-hydroxy-7β-acetoxy-vouacapan-17β-oate methyl ester and 6α-acetoxy-7β-hydroxy-vouacapan-17β-oate methyl ester (C1) isolated from *P. pubescens* crude extract, and furthermore the influence of microencapsulation process as a tool for maintaining stability of both the crystals and extracts. Therefore, microencapsulated, free samples of C1 and the crude extract on the antinociceptive assays were evaluated. The most relevant findings were that: sample C1 i) possess antinociceptive activity revealed by writhing test; ii) showed significantly anti-allodynic, but not antihyperalgesic effect; iii) the microencapsulation kept the activity and integrity of both sample C1 and Pp crude extract; iv) microencapsulation by spray drying is a useful alternative to increase shelf life time. The free and microencapsulated C1sample maintained stability even when stored in drastic temperature and humidity conditions. Whereas the crude *Pp* extract only maintained stability with the microencapsulation process, not only preserving the content, but also solving the inconvenience of low aqueous solubility.

PJ146

Searching for iridoids from tropical plant species: Detection, isolation and potential uses as raw chiral material

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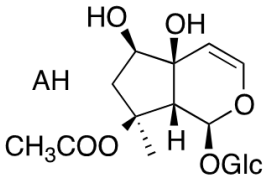
Iridoid glycosides, which form a large group of cyclopentane monoterpenoids, are biosynthesized by a large number of plant species belonging to approximately 20 important botanical families. They possess a highly functionalized aglycon, which may be regarded as starting material for the synthesis of a number of new chiral molecules. For this study, approximately 500 species were selected from iridoid-containing families. A methodology, based on the combination of different analytical and spectroscopic techniques such as LC/UV/DEDL, LC/MS and NMR, was developed in order to select plants of interest. Among these, we found that the New Caledonian species *Oxera coronata* (Lamiaceae) produced high level of 8-*O*-acetylharpagide (AH) in leaves, twigs and fruits. We will present the methodology used to detect and isolate iridoids, the quantitative evaluation of AH in *O. coronata*, and its potential uses as raw material for semi-synthesis. Acknowledgement: This 4-years project is funded by the French National Research Agency (ANR-09-CP2D-09; IRNACHIR 2009).



PJ147

Entrapment of *trans, trans*-farnesol in amorphous silica capsules and its release behaviour: On the route of futur applications

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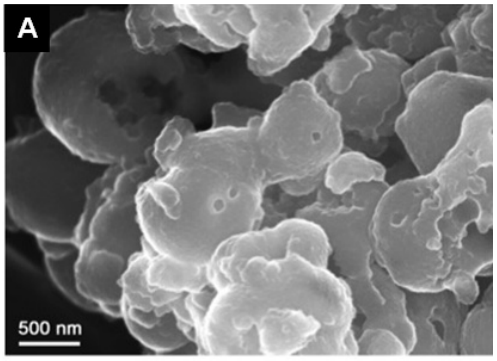
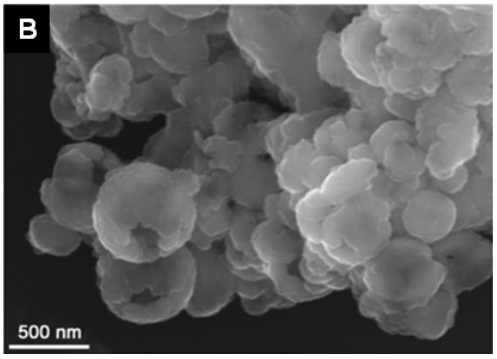
Farnesol, a compound widely found in several plant-related matrices, including agro-food by-products, is an important bioactive agent that can be exploited in cosmetics and pharmaceutics but the direct bioapplication of this compound is limited by its volatility. Here the entrapment of farnesol in silica capsules was investigated to control the release of this bioactive compound in the vapor phase and in ethanol solutions. The preparation of silica capsules with oil cores was obtained by employing a sol-gel method using O/W/O multiple emulsions. Two distinct chemical vehicles for farnesol have been investigated: retinol (A) and oleic acid (B). The release of farnesol from the prepared SiO_2 capsules was investigated. It is demonstrated that these capsules are efficient for the long controlled release of farnesol and that the respective profiles depend on the chemical parameters employed in the synthesis of the capsules. It is well known that terpene compounds (e.g. *trans,trans*-farnesol) are praised for their beneficial effects to human health, namely as anti-oxidant agents. The release profiles reported here are compatible with the needs of an anti-oxidant effect in fast stress oxidative situations or in cases of a preventive action for longer periods of time.





PJ148 Hyperspectral imaging: a visual tool for quality control of herbal raw material

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Hyperspectral imaging (HSI) integrates conventional spectroscopy and imaging to obtain spectral and spatial information from a sample. This non-destructive method can provide species identification in less than a (New York) minute; lightning fast compared to conventional analysis methods such as liquid chromatography. The potential of near infrared (NIR) hyperspectral imaging in combination with chemometric data analysis as a rapid quality control method for commercially important herbal medicines such as *Illicium* and *Echinacea* species was investigated. Hyperspectral images of authenticated botanical specimens were acquired using a sisuChema short wave infrared (SWIR) hyperspectral pushbroom imaging system with a spectral range of 920 – 2514 nm. Principal component analysis (PCA) was applied to the images to reduce the high dimensionality of the data, remove background pixels and to visualise the data. The score images and plots were used to interactively assign classes to the data. Classification models were developed using partial least squares discriminant analysis (PLS-DA) and the models were subsequently used to accurately predict the identity of the species introduced as an external dataset, producing a visually interpretable result. Clearly, hyperspectral imaging is ideally suited as a qualitative tool for the quality control of herbal raw material as it is a rapid, accurate, non-destructive method with high prediction ability.

PJ149 Antispasmodic activity of pimaradienoic acid derivatives obtained by microbial transformation

Severiano ME[2], Simão MR[1], Veneziani RC[1], dos Santos RA[1], Furtado NAJC[2], Said S[2], Ambrósio SR[1]
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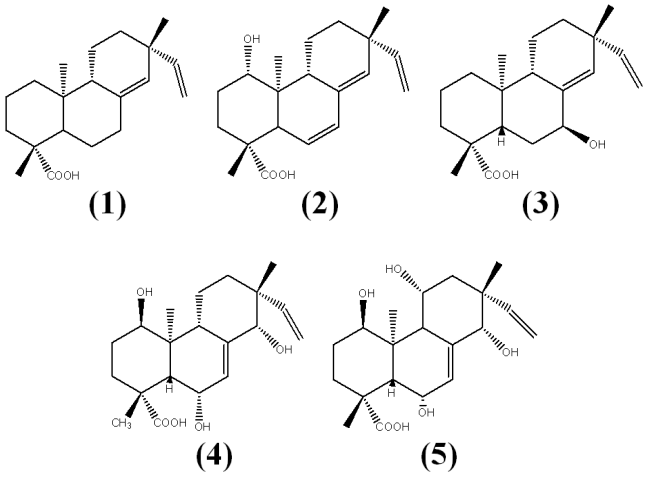
Pimaradienoic acid (PA, **1**) is a pimarane-type diterpene which is associated with a wide spectrum of biological activities, including its great ability to inhibit rat aorta contraction [1]. In the present work, the fungal transformation of PA (0.1 g/L) was performed using culture of *Aspergillus niger* (1.5×10^7 spores/mL) during 9 days of incubation. After this period, the culture was filtered and the aqueous layer was extracted with ethyl acetate to furnish the extract codified as AtPA. Chemical and NMR studies of AtPA allowed us to isolate and to identify four PA derivatives (**2** – **5**). The chemical structures of **2**, **4** and **5** are described for the first time in the scientific literature.



In addition to the microbial transformation studies, inhibitory action of these diterpenes on phenylephrine-induced rat aorta contraction was performed [2]. Ours results show that these metabolites were not able to inhibit the rat aorta contractile response, thus suggesting that minor structural differences on PA chemical skeleton significantly influences its antispasmodic activity.

PJ150 Content of selected phenolic compounds in wine from rondo grapes grown in denmark and effect of heat and cryomaceration

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Studies have shown that red wine contain several polyphenolic compounds, e.g., anthocyanins, catechins, flavonols and stilbenes, which are believed to have beneficial effects on human health. Pre-fermentation treatments are known to increase the content of these compounds in wine, resulting in changes of the sensory characteristics (e.g., flavor, astringency and color). In 2000, Denmark was approved as a wine producing country by the EU and one of the grape varieties cultivated in Denmark to produce red wine is Rondo. In this study we investigated the effect of cryomaceration (liquid nitrogen) and thermovinification (70°C) vs. a traditional pretreatment (30°C) on the content of phenolic compounds in the wine produced from Rondo grapes grown on the island of Funen, Denmark. Compared to traditional pretreatment, thermovinification increased the content of anthocyanins, catechin and resveratrol by 62%, 69% and 260%, respectively, but reduced the content of quercetin by 580%, while cryomaceration increased the content of quercetin by 6%, but reduced the content of anthocyanins, catechin and resveratrol by 11%, 9% and 60%, respectively. These results should incite Danish wine producers to implement thermovinification as a pre-fermentation treatment as it intensifies the color of the wine produced (anthocyanins, 313 mg/L).

PJ151 Hydroxylation of kaurenoic acid by *Aspergillus terreus*

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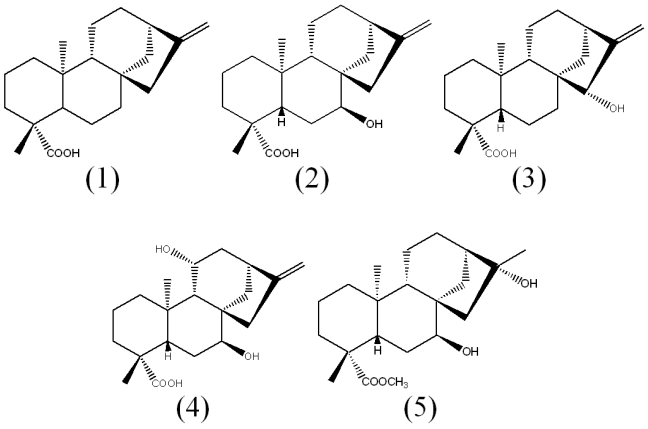
[1]University of Franca, Av. Dr. Armando Salles de Oliveira 201, 14404 – 600, Franca-SP, Brazil; [2]University of São Paulo, Av. Café s/n, 14040 – 903, Ribeirão Preto-SP, Brazil

In the present work the microbial transformation of kaurenoic acid (1) was performed using submerged shaken liquid culture of *Aspergillus terreus* (1.5×10^7 spores/mL). The microorganism was grown by a two-stage fermentation procedure. Compound 1 was added as a dimethylsulfoxide solution (0.1 g/L) and incubated for 14 days. The culture was filtered and the aqueous layer was extracted with ethyl acetate to furnish the extract codified as AtKA. Chemical and NMR studies of AtKA allowed us to isolate and to identify four kaurenoic acid hydroxylated derivatives (Compounds **2** – **5**).

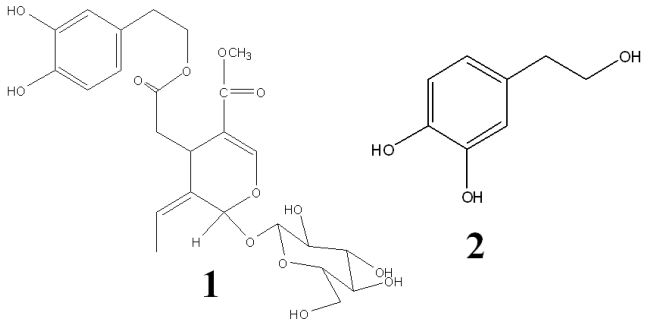


In addition to the microbial transformation studies, the *in vitro* effect of 1 on cell viability was assessed by the XTT assay using breast tumor cell line (SKBR-3). Compound 1 displayed significant cytotoxicity activity in all evaluated concentrations (12.5, 25.0 and 50.0 µM), thus denoting the importance of this kaurane-type diterpene in the search for novel antitumor agents.

PJ152 Production of extracts from olive by-products and evaluation of their cytotoxic/cytostatic activity

Xynos N[1], Argyropoulou A[1], Samara P[2], Aligiannis N[1], Tsitsilonis O[2], Skaltsounis AL[1]
[1]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis Zografou, 15771, Athens, Greece; [2]Department of Animal and Human Physiology, Faculty of Biology, University of Athens, Panepistimiopolis Zografou, 15784 Athens, Greece

Over the years, several studies have related olive to a low incidence of several diseases, including cancer. This may be due to the phenolic compounds encountered in olive products. Recent studies have shown that the wastes produced during olive processing are valuable sources of phenols. In the present work, extracts were obtained from various by-products generated during olive processing, specifically: olive fruit debittering, olive oil mill waste, olive paste and olive leaves, and were chemically characterized. Furthermore, their cytotoxic/cytostatic effects were evaluated against a panel of various cancer cell lines as determined by MTT assays. Our results show that these extracts are rich in phenolic compounds, mainly oleuropein **1** and its degradation molecule hydroxytyrosol **2**, and exhibit promising biological activities.



PJ153 Study on increasing the content of 6-gingerol from ginger rhizomes by supercritical fluid extraction and fractionation

Huang SC[1], Lin YS[2], Liu FI[1], Yu HM[1], Chang LC[1]
[1]Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan; [2]Kaiser pharmaceutical CO., LTD

The purpose of the study was to evaluate the optimum conditions applied by the supercritical fluid extraction and fractionation to maximize the content of 6-gingerol extracted from Ginger rhizomes. 3000 Kg of Ginger rhizomes were collected from Jia Shian in Taiwan. The rhizomes were dried and ground into three granule-size groups, 0.297~0.840 mm, 0.840~2.00 mm, and > 2.00 mm, respectively. The granules were extracted using supercritical carbon dioxide (SFE) under various combinations of pressures (80, 100, 120 bar) and temperatures (35, 45, 55°C). The extracts collected under different conditions were then analyzed by HPLC to determine the content of 6-gingerol. The results showed that the extracts collected from 0.840~2.00 mm group at 35°C and 200 bar produced the greatest content of 6-gingerol, 20.23%. In order to evaluate the optimal conditions to further increase the content of 6-gingerol from ginger oil, 30 Kg of SFE-obtained ginger oil by scaled-up SFE extraction was run by the supercritical fluid fractionation under different conditions. Selected pressures (80, 100, 120 bar), temperatures (35, 45, 55°C), CO_2 flow rates/volumes of ginger oil (5/1, 10/1, 15/1) and re-extracted times (0, 1, 2) of collected ginger oil from separator were tested to evaluate the best conditions that can help increase the content of 6-gingerol from the fractionation extracts. The results showed that the greatest content of 6-gingerol, 68.83%, was collected under one specific set of conditions: 120 bar, 35°C, 10/1 (CO_2 flow rate/volume of ginger oil) and re-extracted 2 times of collected ginger oil from the separator. The results may be utilized by the industry to enhance the 6-gingerol content during Ginger production.

PJ154 PBCA-mediated neurotrophin-3 and hypoxia response element delivery in the treatment of rats with hemorrhagic stroke

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[1]Department of Chemical Engineering, National Chung Cheng University, Chia-Yi, Taiwan; [2]Department of Neurosurgery, Chang Gung Memorial Hospital, Chia-Yi, Taiwan

Hypertensive ICH is a rapidly evolving process which causes necrotic cell death followed by apoptotic cell death, and alters some gene expression levels in the surrounding tissue area of brain injury. The blood-brain barrier (BBB) limits the penetration of substances into the brain, and the development of intravenously administered carriers for controlled release of gene vector has been an ambitious challenge. It has been concluded that polybutylcyanoacrylate (PBCA) nanoparticles (NPs) could successfully deliver protein across the BBB. In this study, we construct a Neurotrophin-3 (NT-3) expression plasmid containing the HRE and a CMV promotor. It is applied to activate the HIF-1/HRE system of gene regulation and used to produce NT-3 proteins after ICH. The data indicate that the PBCA NPs/NT-3-HRE complexes are very low toxicity. The PBCA NPs can protect cmvNT-3-HRE from degradation and sustain across the BBB in vitro, and enhance the NT-3 protein expression after hemorrhagic stroke with PD treatment. Moreover, the disrupted BBB and impaired neurons can be revived by PD treatment after ICH. In conclusion, the PBCA NPs justified as an appropriate system for gene delivery in the brain because of it possibly influences therapeutic outcome of ICH in the rats.

PJ155 Jasmonate-status and transcriptional regulation in *Catharanthus roseus* (L.) G. Don (Apocynaceae)

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Catharanthus roseus (L.) G. Don (Apocynaceae) is one of the most important medicinal plants worldwide given that the alkaloids naturally present in this species had been used for more than 40 years due to their potent anti-tumor activities. Consequently, it became one of the best-studied medicinal plants and it has served as a model system for biotechnological studies on plant secondary metabolism. To these days, the leaves of this species remain as the only natural source of 130 terpenoid-indole alkaloids (TIA's), such as the anticancer drugs vinblastine and vincristine, administered as single agents or in combination therapy for several neoplasms, and the antihypertensive compounds like serpentine and ajmalicine. Here we describe a targeted metabolomic approach for the elucidation of alkaloid, fatty acid and jasmonate derivatives status, as well as the gene expression, involved in the biosynthetical response after the elicitation of cell suspension cultures with jasmonic acid, a lipid-derived phytohormone. Fatty acids were separated and identified as methyl esters by GC-MS. This allowed the quantification of saturated and unsaturated species ranging from C 10:0 to C 24:0. The major fatty acids found were palmitic acid, linoleic acid and linolenic acid, being the saturated acids predominant over the unsaturated ones. The analysis of TIA's was performed by HPLC-DAD, which allowed the identification of 13 different TIA's like serpentine, tabersonine, strictosidine and catharanthine and were further confirmed by LC-MS.

PJ156 Orthogonal validation of analytical and quality systems for botanical products

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[1]Pharmanza Herbals Pvt. Ltd., Dharmaj, Gujarat, India. [2]Verdure Sciences, Noblesville, Indiana

The widespread human use of botanical products for health benefits depends on a solid scientific foundation to document their safety, efficacy, chemical composition and manufacturing control. US FDA current good manufacturing practices (cGMP) for dietary supplements require established specifications and testing methods to ensure identity, purity, strength and consistency of supplement ingredients, including botanicals. As complex mixtures prone to natural variation due to climate, location of origin, and harvest methods, botanicals present unique challenges for traditional quality assurance systems. Further, validation of reference standards, methods and processes for botanical quality assur-

ance are essential to conformance to GMPs. Using ICH, ISO and Total Quality Management principles, an orthogonal framework was established to address the quality issues often associated with commercial botanical extracts. This framework is essential to understanding the critical factors affecting botanical product quality, and provides valuable insights into the analytical methods and standards required to document and improve botanical product quality.

Topic K: Synthetic Approaches

PK1 Diastereoselective aldol reactions and applications to various natural product frameworks

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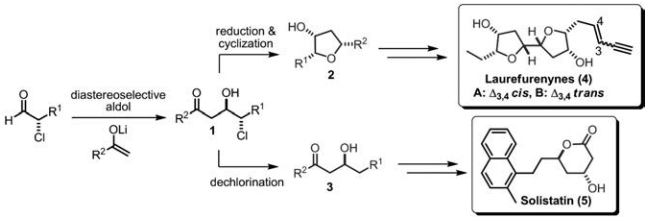
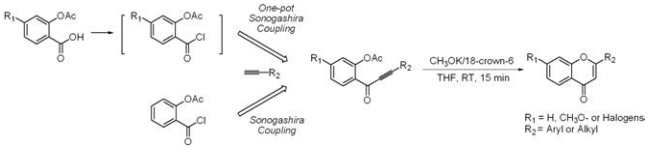
Research in our lab has shown that lithium enolate additions to chloroaldehydes occurs selectively to yield predominantly *anti*-ketochlorohydrins (**1**). These enantioenriched building blocks can be further functionalized to provide scaffolds for various natural products; this includes reduction and cyclization to yield substituted tetrahydrofuranols (**2**) or radical dehalogenation to produce asymmetric hydroxyketone moieties (**3**). Research presented will include enantioselective synthesis of natural products Laurefurenynes A and B (**4**) and Solistatin (**5**) using this recently developed methodology.



PK2 Synthesis of γ-benzopyranones using sonogashira coupling and 18-crown-6 ether mediated cyclization

Chuang DW[1], El-Shazly M[1,2], Barve Balaji D[1,3], Chung YM[1], Chang FR[1], Wu YC[4]
[1]Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan; [2]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, Ain-Shams University, Cairo 11566, Egypt; [3]Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan; [4]School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung 404, Taiwan

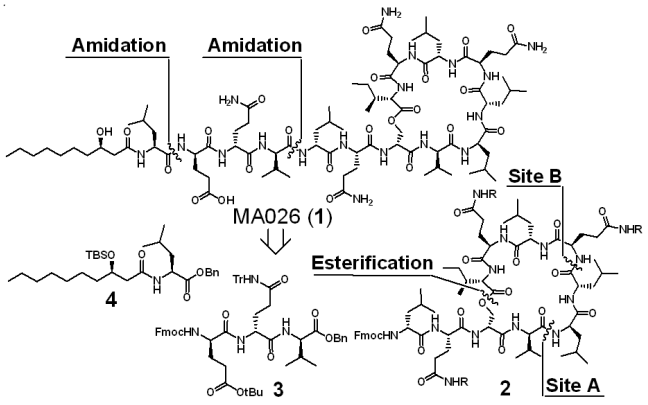
An efficient method for the synthesis of γ-benzopyranones and flavones utilizing mild Sonogashira coupling and 18-crown-6 ether mediated 6-*endo* cyclization of *o*-alkynoylphenol acetates has been developed. By using this strategy, γ-benzopyranones and flavones bearing electron-donating groups, halogens and simple alkyl substituents were synthesized with satisfactory yields.



PK3 Synthetic studies of MA026, A novel antiviral lipocyclodepsipeptide

Shimura S[1], Ishima M[1], Ota I[1], Tsutsui E[1], Kamisuki S[1], Murata H[1], Yamazaki T[1], Suzuki T[2], Kuramochi K[3], Takeuchi T[1], Watashi K[4], Kobayashi S[2], Sugawara F[1]
[1]Faculty of Science and Technology; [2]Faculty of Pharmaceutical Science, Tokyo University of Science, Noda, 278 – 8510, Japan; [3]Graduate School of Life and Environmental Science, Kyoto Prefectural University, Kyoto, 606 – 8522, Japan; [4]National Institute of Infectious Diseases, Tokyo, 162 – 8640, Japan

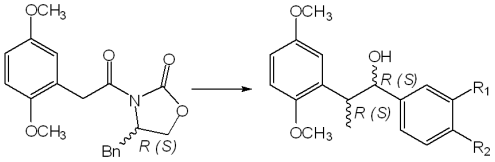
MA026, a novel lipocyclodepsipeptide, exhibits multiple antiviral activity, and its mode of action is unrevealed. MA026 has the potential to create a novel antiviral drug and thus more biological characterizations are required. To accomplish the biological investigation, a flexible chemical synthesis is essential. MA026 consists of (*R*)-3-hydroxydecanoic acid, linear peptide and cyclodepsipeptide. To maximize the convergency, MA026 was devided into three segments: branched cyclodepsipeptide **2**, tripeptide **3** and fatty acid moiety **4**. Macrocyclization of depsipeptide is a key step in the total synthesis, so we chose two macrocyclization sites (A) and (B). Here, we present synthetic studies of MA026.



PK4 Asymmetric synthesis of Pterolinus D derivatives

Wu SF[1], Chang FR[1], Wu YC[1,2], Lee KH[3]
[1]Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan; [2]School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan; [3]Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill

Pterolinus D was an active component of anti-inflammatory activity and cytotoxicity from *Pterocarpus santalinus*. Herein, an asymmetric synthesis of pterolinus D derivatives (7a-b, 7'a-7'b) was processed by Evans chiral auxiliary methodology to introduce a methyl stereogenic center, followed by Friedel-Crafts reaction and $LiAlH_4$ reduction to give an hydroxyl stereogenic center as a Cram isomer. The structures are confirmed by NMR, MS and CD-ORD spectroscopy methods.



7a: *R*-CH_3 *R*-OH R_1: OH R_2: OCH_3
7b: *S*-CH_3 *S*-OH R_1: OH R_2: OCH_3
7`a: *R*-CH_3 *R*-OH R_1:OCH_3 R_2: OH
7`b: *S*-CH_3 *S*-OH R_1:OCH_3 R_2: OH

PK5 Synthesis and determination of antimycobacterial activity of tryptanthrine, vasicine, vasicinone and derivatives thereof

Rudolph I[1], Ramm M[2], Moellmann U[2], Imming P[1]
[1]Institut fuer Pharmazie, Martin-Luther-Universitaet Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, 06120 Halle (Saale), Germany. [2]Leibniz-Institut fuer Naturstoff-Forschung und Infektionsbiologie, Hans-Knoell-Institut, Beutenbergstrasse 11a, 07745 Jena, Germany

We describe the first application of the Niementowski quinazoline synthesis for the synthesis of vasicinone **3** (Fig. 1), an alkaloid from *Adhatoda vasica*, a traditionally used plant in India for the treatment of respiratory diseases such as asthma and tuberculosis. This straightforward 2-step synthesis is an attractive basis for further medicinal chemistry work on vasicinone. Vasicine, vasicinone, deoxyvasicinone and deoxyvasicine were tested against Mycobacterium vaccae, a non-pathogenic mycobacterium closely related to Mycobacterium tuberculosis. Tryptanthrin, a related indoloquinazolinone from *Strobilanthes cusia,* showed antimycobacterial and antifungal activity as well as anti-proliferative activity.

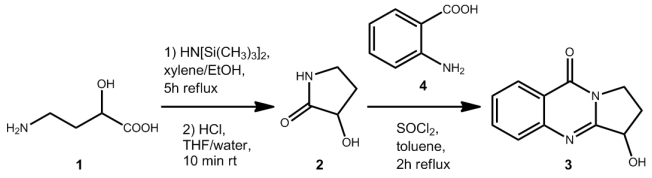


Fig. 1: Synthesis of vasicinone **3** via the Niementowski quinazoline synthesis.

PK6 Pyridomycin: Evaluating its antimycobacterial potential through the synthesis and testing of new analogues

Laqua K[1], Richter A[1], Ramm M[2], Moellmann U[2], Imming P[1]
[1]Martin-Luther-Universitaet Halle-Wittenberg, Institut fuer Pharmazie, Wolfgang-Langenbeck-Str. 4, 06120 Halle (Saale), Germany; [2]Hans-Knoell-Institut, Jena, Germany

Pyridomycin is an antimycobacterial depsipeptide produced by *Streptomyces pyridomyceticus.* Its constitution and absolute configuration were determined by X-ray crystallography. Pyridomycin is a twelve-membered ring with a semicyclic (*Z*)-*s*-butylidene group, 3-pyridylmethyl and hydroxypicolinoyl side chains and five stereocentres. It shows activity against *Mycobacterium tuberculosis* at 2 – 5 μg/ml and some Gram-negative bacteria. The poster will present the successful establishment of the synthesis of various twelve-membered ring derivatives related to pyridomycin. After assembling the peptide building blocks, the ring closure poses the crucial point of the whole synthetic procedure (possible polymerization, difficult work up etc). With regard to the difficult isolation, we found that direct substitution with the picolinic acid derivatives represented a viable alternative (Fig. 1). We report the first microbial evaluation using different mycobacterial species as well as Gram-negative and Gram-positive bacteria. In addition we determined the antiproliferative and cytotoxic effects of the new substances.

PK7 Synthesis of (S)-5,6-Dibromo-tryptophan derivatives as building blocks for peptide chemistry

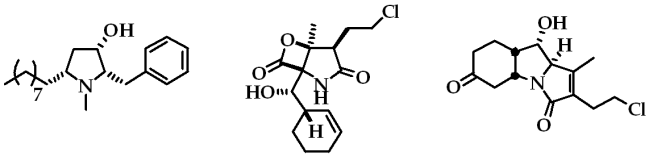
Mollica A[1], Stefanucci A[1], Costante R[1], Pinnen F[1], Locatelli M[1]
[1]Dipartimento di Scienze del Farmaco, Università degli Studi "G. d'Annunzio" Chieti-Pescara, Via dei Vestini 31, 66100 Chieti (CH), Italy

5,6-Dibromo-tryptophan and its derivatives are found in a variety of highly bioactive natural compounds. Notwithstanding its relevance, no synthetic pathway was found in the literature. Here, an efficient pathway for the synthesis of 5,6-dibromotryptophan derivatives is reported [1]. Selective bromination [2] at position 5 of 6-Br-isatin was followed by BH_3 reduction of the intermediate α-keto-amide and alkylation with Ser-OH in Ac_2O/AcOH. Optical resolution of the obtained racemic mixture was carried out by enzymatic de-acetylation [3]. Finally, an *in situ* N^{α}-Boc protection of the optically pure S form yielded the desired N^{α}-Boc-(S)-5,6-dibromo-tryptophan.

PK8 Total synthesis of pyrrolidine-containing natural products preussin and salinosporamide C

Jason D, Robert B
Simon Fraser University, 8888 University Drive, Burnaby, B.C., V5A 1S6, CANADA

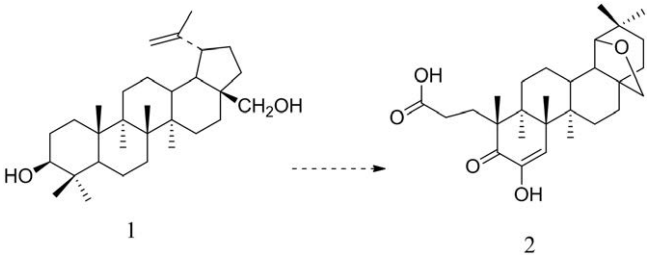
The flexible, efficient, and stereocontrolled syntheses of hydroxypyrrolidine-containing molecules remains an unsolved problem. Our work attempts to grant access to some of these valuable natural products and their potentially biologically relevant analogs. We report an unprecedentedly concise synthesis of the fungal metabolite (+)-preussin and related hydroxypyrrolidines, as well as application of our newly developed heterocycle-forming reaction in progress toward the total synthesis of salinosporamide C. The strategy to these molecules reported herein centers around the elaboration of ketochlorohydrins by introduction of nitrogen and subsequent cyclization by intramolecular chloride displacement.



PK9 Synthesis of unusual compounds from abundant betulin

Lugemwa FN
Department of Chemistry, Pennsylvania State University-York, PA 17403

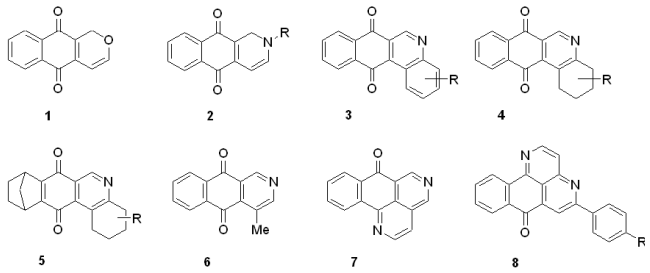
Modifications that included cleavage and oxidation of the 19-side chain of betulin 3β,28-diacetate; and the contraction of ring A of readily available betulin **1**, followed by cleavage and oxidation, yielded three unusual compounds with moderate microbial activity against a number of micro-organisms. Of the three compounds investigated, the most active was 18β,28-epoxy-6-hydroxy-5-oxo-3,5-seco-A[4], 23,24-trinor-18α-olean-6-en-3oic acid **2**, with minimum inhibitory concentration of 11 μg/ml against C. *albicans*. Further modification of this compound by methylation and acetylation did not increase the activity of the compound.



PK10 Synthesis of antimycobacterial benzo[*j*]phenanthridine-7,12-diones and related compounds

Claes P[1], Mbala BM[1], Jacobs J[1], Cappoen D[2], Jacobs J[1], Huygen K[2], Verschaeve L[2], Tuyen NV[1], De Kimpe N[1]
[1]Dept. of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, B-9000 Ghent, Belgium; [2]Scientific Institute of Public Health, Engelandstraat 642, B-1180 Brussels, Belgium

Structural modifications of the antibiotic pentalongin, isolated from the medicinal plant *Pentas longiflora*, towards N-containing analogues were envisaged.



Nitrogen-containing pentalongin analogues **2**, benzo[*j*]phenanthridine-7,12-diones **3**, the corresponding reduced derivatives **4** and **5**, and 2-azaanalogues of the natural products cleistopholin and sampangin (**6** and **7**) were synthesized by using Heck protocols and Pommeranz-Fritsch cyclizations while sampangin analogues **8** were obtained by MW-induced 2-azaanthraquinone synthesis utilizing pyridinium ylid chemistry. *In vitro* testing of this library of azaheterocyclic compounds for their activity against *Mycobacterium tuberculosis* showed promising results.

PK11

Hemisynthetic derivatives of oleuropein

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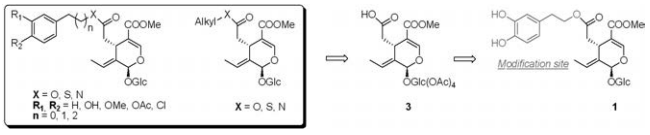
University of Athens, School of Pharmacy, Department of Pharmacognosy & Natural Products Chemistry, Panepistimioupolis, 15771 Zografou, Athens

Oleuropein (**1**), the major constituent of *Olea europea* leaves, has been found to possess a large range of pharmacological properties. In this context, because of the non-specificity of these effects, different analogs have been synthesized from **1** in order to study structure/activity relationships. Therefore, the hydroxylation degree of the hydroxytyrosol part has been modified along with the length of the connecting chain. New alkyl esters, amide and thioesters derivatives have also been synthesized. These compounds were prepared by esterification/amidation of **3** which was obtained by saponification of **1** followed by acetylation of the glucose part. The final selective deprotection of the acetate was carried out by diethylamine.



PK12

Methodology for the preparation of olive oil open ring secoiridoids

Vougogiannopoulou K[1], Lemus C[2], Michel S[1], Smith III AB[3], Skaltsounis L[2], Deguin B[1]
[1]Laboratoire de Pharmacognosie de l'Université Paris Descartes, Sorbonne Paris Cité, Faculté des Sciences Pharmaceutiques et Biologiques, U.M.R./C.N.R.S. 8638, 4, Avenue de l'Observatoire, F-75006 Paris; [2]Department of Pharmacognosy, Faculty of Pharmacy, University of Athens, Zografou, 15771, Greece; [3]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104 – 6323, USA

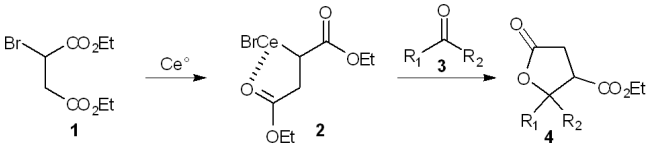
Dedicated to the memory of Prof. François Tillequin. Olive oil (OO) as an important component of the Mediterranean diet has been extensively studied and correlated with NSAID properties, simulating the ones of ibuprofen. The phenolic content of OO includes open ring secoiridoids such as oleacein (**2**), closely related to oleuropein (**1**). The latter is easily isolated from olive tree leaves and can be used as a natural synthon. Herein, it is proposed that the opening of the oleuropein secoiridoid ring can afford a series of secoiridoid dialdehydes similar to oleacein. This methodology can be expanded to various secoiridoid conjugates providing a tool for the synthesis of a number of analogues. Project was carried out under the scope of OLITEC (IAAP 230763).

PK13

Cerium-catalyzed reformatsky-type reaction with ethyl bromosuccinate for the synthesis of γ-lactones

Muniz Machado Rodrigues S, da Silva GVJ, Gomes Constantino M
University of São Paulo, Avenida Bandeirantes, 3900, 14040 – 901-Ribeirão Preto-SP, Brazil

The use of lanthanoid compounds in the realm of synthetic organic chemistry has been of great interest due to special characteristics of these compounds, such as their strong oxophilicity and nucleophilicity while showing reduced basicity towards carbonyl compounds. A Reformatsky-type reaction using cerium compounds prepared from 3-bromoesters is a convenient route to γ-lactones. In this work, we have been studying the use of ethyl bromosuccinate (1) instead of simple 3-bromoesters. The additional carbonyl group should enhance the stability of organolantanoid intermediate (2) while simultaneously providing a useful substitution in the lactone ring (4) (Scheme below). The reaction of (2) was carried out with a number of linear, cyclic and aromatic ketones producing the expected lactones in good yields. On the other hand, aldehydes such as benzaldehyde, isobutyraldehyde and crotonaldehyde, γ-lactones could not be furnished.



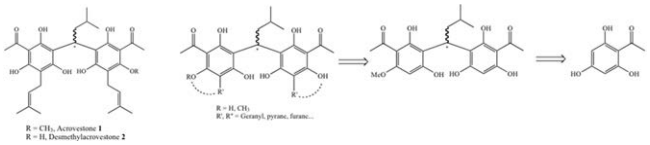
We concluded that the use of stabilized lantanoids from ethyl bromosuccinate (1) in Reformatsky-type reactions, under mild conditions, is viable and produces functionalized γ-lactones in good yields. The experimental results will be presented and discussed.

PK14

First synthesis of acrovestone, desmethylacrovestone and derivatives

Gaboriaud-Kolar N[1], Svouraki A[1], Halabalaki M[1], Skaltsounis AL[1]
[1]Department of Pharmacognosy and Natural Product Chemistry, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15771, Athens, GREECE

Acrovestone **1** and desmethylacrovestone **2** are natural products belonging to the family of bis-acetophenone. They were isolated from the leaves and fruits of *Acronychia penduculata* L. (Miq.). New derivatives[1] have been recently discovered and showed a substantial cytotoxic activity on A2058 melanoma cells. However, some structural features remain to be elucidated. To conclude on their structures we realized the first synthesis of acrovestone **1** and its natural and non-natural derivatives. This three steps synthesis involved the key dimerization between two acetoxyphloroglucinol monomers using a Friedel and Craft reaction. This dimer was then functionalized by e.g. prenyl, geranyl or furane moieties. The cytotoxic activities of the new compounds have been evaluated and are reported.



1: E. Kouloura et al, *J.Nat.Prod.*, **2012**, *on Press*.

PK15 Towards the biomimetic synthesis of lodopyridone

Barbahn N, Evanno L, Poupon E
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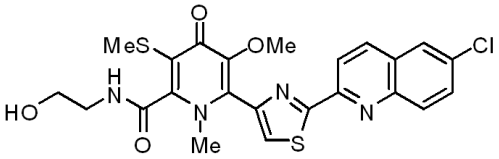
Lodopyridone is an alkaloid isolated in 2009 from the marine actinomycete *Saccharomonospora* sp. collected in sediments of the submarine canyon of La Jolla in the United States.[1] The molecule contains three nitrogen heterocycles: *i*- a chlorinated quinoline, *ii*- a thiazole and *iii*- a pyridone.



This polyheterocyclic structure rather surprising at first for a natural substance is actually probably a modified tetrapeptide. The 4-pyridone cycle found in lodopyridone is quite uncommon in natural substances, and to date, only about twenty molecules containing this framework have been described. To realize the total synthesis, a biomimetic approach is privileged based on our biogenesis hypothesis and will be presented. 1) K. N. Maloney, J. B. MacMillan, C. A. Kauffman, P. R. Jensen, A. G. DiPasquale, A. L. Rheingold, W. Fenical, *Org. Lett.*, **2009**, *11*, 5422 – 5424.

PK16 Natural product-like skeletons from curcuminoids under oxidative dearomatization conditions

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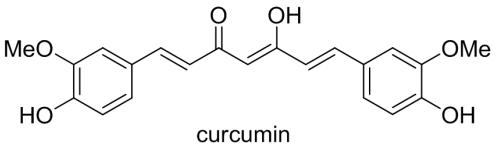
Curcumin, a natural diarylheptanoid polyphenol found for example in turmeric (*Curcuma longa*, Zingiberaceae), has recently gained a particular interest for an impressive number of biological activities and its putative therapeutic or chemopreventive effects on various health disorders [1]. Under oxidative dearomatization conditions, a complete remodeling of several natural analogs of curcumin is observed and leads to interesting new skeletons that will be presented.



[*Curcuma longa*, Zingiberaceae]

[1] S. C. Gupta, S. Prasad, J. H. Kim, S. Patchva, L. J. Webb, I. K. Priyadarsini, B. B. Aggarwal, *Nat. Prod. Rep.* **2011**, *28*, 1937 – 1955.

PK17 Synthesis of sterols with antileishmanial activity from *Pentalinon andrieuxii*

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Leishmaniasis is a parasitic disease transmitted through the bite of infected sandflies. Although therapeutics are currently available for the treatment of the disease, the emergence of drug resistance in the parasites suggests a continued need for the discovery of novel chemical entities that act via new or different mechanisms of action. In an effort to identify compounds which may be effective for the treatment of leishmaniasis, a multidisciplinary research effort involving natural product isolation, chemical synthesis, and biological evaluation was initiated. Several sterols were isolated from the roots of *Pentalinon andrieuxii*, a plant native to Mexico, that show potent antileishmanial activity both *in vitro* and *in vivo*. A structural survey of the active compounds indicated three distinct structural subtypes. Semi-synthetic approaches toward these structural classes from affordable, commercially available starting materials have been designed to prepare the molecules in quantities sufficient to enable detailed mechanistic studies and structure-activity relationship studies. Representative examples from two of the classes, pentalinonsterol and ergosta-4,24(28)-dien-3-one, have been synthesized from 5-pregnen-3β-ol-20-one and lithocholic acid, respectively. These routes have also facilitated the preparation of a number of structurally related analogues.

PK18 Oxidative rearrangements of a pelletierine-derived building block

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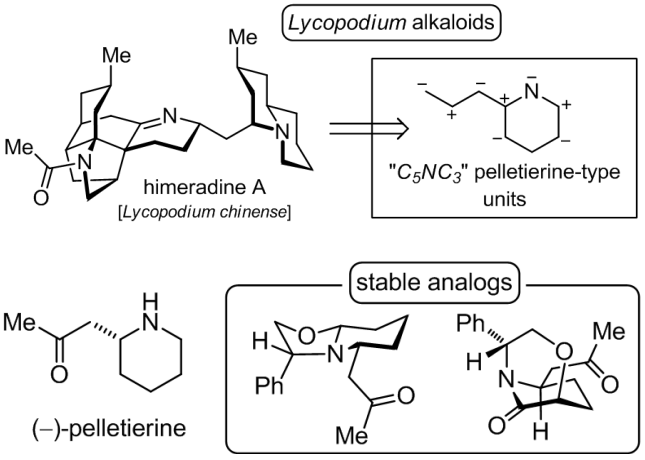
Propylpiperidine units ("C_5NC_3") closely related to pelletierine are involved in the biosynthesis of *Lycopodium* alkaloids [1]. Our interest in the biomimetic synthesis of this class of sometimes complex alkaloids (see the example of himeradine A below), led us to the conception and preparation of a stable (*R*)-phenylglycinol-based oxazolopiperidine analog of pelletierine. Among other reactivity studies, an original rearrangement in oxidative conditions will be presented leading to another useful analog.



[1] Review article on *Lycopodium* alkaloids: Hirasawa, Y.; Kobayashi, J.; H. Morita, H. *Heterocycles* **2009**, *77*, 679 – 729.

Topic L: Other

PL1 A practical guide to the safe use of natural products in consumer products

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Natural products have endless application possibilities in consumer products. Examples include: food or dietary supplement ingredients; preservatives; sweeteners; pre- and probiotics; functional additives such as emulsifiers, clarifying agents, processing aids in manufacturing, and "active" packaging; cosmetics; personal care products; drugs; and more. With use of a natural product comes a concomitant consumer exposure to the molecule, fraction, extract, or organism. This exposure is addressed by scientific means to ensure the safety of the substance for the end user. Natural products for use in consumer products will be subjected to a scientific safety assessment. A safety assessment of a natural product will typically consider the following issues: 1) identity and composition of the substance, 2) likely or probable contaminants/co-purifying molecules/reaction byproducts (for synthetically or semi-synthetically produced natural products), 3) consistency of composition

of the substance as demonstrated over time across non-consecutive lots, 4) dose, 5) route of exposure, 6) and anticipated duration of exposure to the substance. Pharmacognosists are well-equipped to play a key role in carrying out safety assessments of natural products. Many common scientific "data gaps" discovered during safety assessments can be filled by the application of pharmacognostic techniques or tools. Example data gaps will be presented, and the suggested means to fill them will be discussed. Recommendations for increasing the success of regulatory submissions will be highlighted as relevant.

PL2 Effect of *Ruta graveolens* hydro-alcoholic extract on pentylenetetrazole-induced seizure in male mice

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Aim &Introduction: Seizure is an important symptom of epilepsy and many neurogenic disorders. Despite a variety of current anti-convulsive drugs, research for discovering new drugs with more efficacy and unsuitable adverse effects has been continued. Herbal medicine has various natural substances and proper context for this type of research. This study was surveyed the anticonvulsant effects of hydro-alcoholic extract of rue (*Ruta graveolens*) in male mice. **Materials and methods:** This is an experimental study in which anticonvulsant effect of extract evaluated by PTZ induced seizure. 56 NMRI mice in the range of 25 – 35 gr were divided to 7 groups of 8 mice that included five cases, one positive control group and one negative control group. Case groups have been injected by 100, 300,500, 800, 1000 mg/kg of extracts, positive control group 40 ml/kg Phenobarbital and negative control group 10 ml/kg normal saline intra-peritoneal. All groups were injected by *pentilen-tetrazole* (80 mg/kg) intra-peritoneal, after 45minutes and initiation time of myocolonic and tonic-colonic seizures and percent of 24hours death were measured. **Results:** consequences of different doses of rue hydro-alcoholic extract increased delay in initiation of myocolonic and tonic-colonic seizures rather than control group dose-dependently and reduced 24hours seizure-induced mortality ($P < 0.05$). Delay in myocolonic in 1000, 800, 500, 300 and 100 mg/kg in comparison with negative control group was significant ($P < 0.001$). Initiation time of tonic-colonic seizures in dose 1000 mg/kg by $P < 0.001$ and doses 300,500 and 800 by $P < 0.05$ in comparison with negative control group were significant. **Conclusion:** Regarding to collected results, it seems that extract of this herb has decremental effect on PTZ-induced seizure in male adult mice.

PL3 Evaluation of antimicrobial effect of hydro-alcoholic extract of *Ruta graveolens* on *Enterococcus fecalis*

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Enterococci are the second most cause of nosocomial infection. *Enterococcus fecalis* is responsible for often 90% of enterococci infections that usually are transferred by hand of health care workers and instruments. It can causes bacteremia, urinary system ulcer, biliary and endocarditis in adult and meningitis and septicemia in pediatrics. In order to adverse effects of chemical and synthetic drugs, it has made a positive attitude toward alternating herbal medicine instead of chemical ones. *Ruta graveolens* is an ancient herb in Iranian traditional medicine and other nations and has a wide therapeutic application for various diseases. Aim of this study has been effect of this herb on *enterococcus fecalis* growth. In this investigation we used standard *Enterococcus fecalis* PTCC-1237 which prepared from collection of bacteria and fungi, scientific and industrial research organization. Effect of hydro-alcoholic extract of *Ruta graveolens* on growth of bacteria has been evaluated by disc diffusion and serial dilution method and compared with eight prevalent antibiotics. Extract in range of 10 to 200 μg/μl didn't avoid from growth of bacteria in both MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) but bacteria was susceptible to 6 antibiotics. In this study preventive effect on growth of bacteria were not seen. It seems that this result is from high resistance of bacteria to antibiotics and it recommended more studies.

PL4 Foliar morphoanatomical characterization of medicinal plant *Youngia japonica* (L.) D.C. (Asteraceae)

de Almeida Ribeiro da Silva LF[1], dos Santos Moreira N[1], dos Santos Nascimento LB[1], Leal-Costa MV[1], Schwartz Tavares E[1]
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Youngia japonica (L.) D.C. (Asteraceae) is a synanthropic herb native to Southeastern Asia, commonly known as asiatic or oriental [false] hawk's beard. It's a cosmopolitan weed with medicinal and edible uses and reported antioxidant, antiallergenic, antiviral and antitumor properties. In Brazil, this plant was recently introduced and is in frank spreading. The study aims to perform the contribution to morphoanatomical characterization of the species. For the characterization of morphology, anatomy and leaf venation pattern, fully expanded leaves from plants collected on the campus of the UFRJ, in Ilha do Fundão, RJ, Brazil were subjected to standard plant histology procedures. Conical uniseriate tector trichomes are present along the foliar blade and petiole epidermis. Petiole shows uniseriate epidermis whose cells have isodiametric to elliptic cross section and thickened periclinal walls. In the proximal region of the petiole it's observed a discontinuous layer of subepidermal lacunar collenchyma. Vascular system is composed of a flattened crescent of collateral bundles. In the central bundle are observed one or more layers of cells with angle-thickened walls, facing the abaxial side. Bordering the abaxial face of the bundles occur laticifers. Leaves are amphistomatic, with anomocytic stomata. Mesophyll is homogeneous formed by cells of variated sizes, with a slight tendecy to bilaterallity in some portions. In the margin of leaves there are projections associated with the leaf venation. This study is the first fully addressing *Y. japonica* foliar morphoanatomy, which is required, in Brazil, for registration as a plant drug.

PL5 Identification of botanicals: DNA fingerprinting advantages and limitations

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Botanicals often pass through many hands to become a raw material for finished goods, increasing the possibility of intentional or accidental substitution or modification that may impact the nature or benefits of the ingredient. The Code of Federal Register (Title 21, 111.70) makes obligatory GMPs that include at least one "scientifically valid" method to establish taxonomic identity of botanicals. We analyzed the genetic material of botanical samples from a wide variety of material types to establish the limits of DNA fingerprinting technology in determining botanical authenticity, purity, and quality. Results were compared against GenBank references and herbarium vouchers to create aligned matrices and phylogenies of the materials. Additionally, marker compounds and phytochemical fingerprints were correlated to genetic differences. The results from this study are presented here.

PL6 Preparation of a reconbinant fab against the anti-malarial drugs, artemisinin and artesunate and their application in an ELISA

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An antigen-binding fragment (Fab) was prepared against artemisinin (AM) and artesunate (AS) and was developed for use in an enzyme-

linked immunosorbent assay (ELISA). The FAb, which was derived from a monoclonal antibody against AM and AS (MAb 1C1) prepared by us, was expressed by *Escherichia coli* cells, and its reactivity and specificity were characterized. The specificity of the Fab was similar to that of MAb 1C1 in that it showed specific reactivity toward AM and AS only. The sensitivity of the icELISA (0.16 µg/mL – 40 µg/mL for AM, 8.0 ng/mL – 60 ng/mL for AS) was sufficient for analysis of anti-malarial drugs, and its utility for quality control of analysis of *Artemisia* spp. was validated. The Fab expression and refolding systems provided a good yield of high-quality antibodies. The recombinant antibody against AM and AS provides an essential component of an economically attractive immunoassay and will be useful in other immunochemical applications for the analysis and purification of anti-malarial drugs.

PL7 Inhibitory effect of grape seed extract (GSE) on cariogenic bacteria

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Aims: *Streptococcus mutans* plays an important role in the development of dental caries. Although fluoride and other preventive efforts have led to a dramatic decline in dental caries, the ability to control the actual infection has been limited. **The aim** of this work was to investigate the effect of a Grape Seed Extract (GSE) on the growth and adherence of *S. mutans*. **Methods:** Cytotoxicity assay of GSE was used to determine the non-toxic concentrations to the Hep-2 cell line. The free radical scavenging activity of GSE was measured by using the DPPH assay. Antimicrobial activity of GSE was examined on *Streptococcus mutans* to determine the MIC (microtiter plate method). The potentiality of GSE on the adherence (biofilm) was tested on growing bacterial cells on the bottom of cell culture plate. In order to check the effect of the treatment on DNA fragmentation, *S. mutans* was allowed to infect the Hep2cell line in the presence and absence of treatment. **Results:** The non-toxic dose of GSE and its major constituents (gallic acid, catechin and epicatechin) was 15%, 7%, 5% and 15% respectively. All treatments have the ability to inhibit the growth and formation of *S. mutans* biofilm with priority to epicatechin (80.98% & 66.25% respectively). The epicatechin could completely inhibit the DNA fragmentation induced by *S. mutans*. **Conclusion:** GSE, especially epicatechin showed an interesting action on *Streptococcus mutans* and could be used for the lowering of this potentially cariogenic species in oral cavity.

PL8 Antimicrobial activity and simultaneous determination of some phenolic compounds of *Inula helenium* (L.) ssp. *turcoracemosa*

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I. helenium is a perennial herb up to 1 – 2 m with thick aromatic rhizomes and is represented by 4 subspecies in Turkey. Its roots have been used traditionally against a variety of ailments including asthma, cough, bronchitis, tuberculosis, indigestion, chronic enterogastritis and helminthic diseases (1 – 3). In this study, antimicrobial activity of the methanol extracts of flowers, leaves and radix of *I. helenium ssp. turcoracemosa* was determined against *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. albicans* and *C. tropicalis* by agar dilution method. All extracts exhibited antibacterial and anticandidal activity with different MIC values ranging from 50 to 800 µg/ml. Radix extract was found more active against gram positive bacteria and yeasts, especially against *C. tropicalis* (MIC: 50 µg/ml). Also, chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempferol were investigated qualitatively and quantitatively in the plant parts by RP-HPLC. While all the investigated compounds were determined in the flower extract, only chlorogenic and caffeic acids were determined in the radix extract. Consequently, phenolic compounds could be partially responsible for the antimicrobial potential of the plant as well as sesquiterpene lactones known as the main constituents of the plant. References: 1. Zhao, Y-M. et al. (2006) *Chem. Biodivers.*, 3:371 – 384, 2. Davis, P.H. (1982) Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh, 3. Huo, Y. et al. (2010) *J. Pharm. Biomed. Anal.*, 51:942 – 946.

PL9 Protective effect against neuronal cell and antibacterial effect of mulberry fruit extracts

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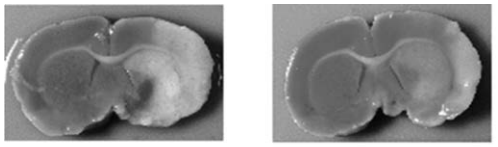
We carried out functional evaluation on the protective effect on cerebral cell and antibacterial activities with mulberry fruits extracts. As a result, 1% HCl-MeOH extract showed 37% cytoprotective effect on hydrogen peroxide, also Cyanidin-3-glucoside (C3G) identified mulberry fruits and cyanidin showed 52%, 76%, respectively, protective effects on oxygen-glucose deprivation (OGD). In the antibacterial activity of mulberry fruit extracts, MeOH-Cheongil extract showed the highest inhibitory activity. And *Salmonella typhimurium* was shown inhibitory rate more than 70% in all treatment groups. Also *Klebsiella pneumoniae* was shown inhibitory activity in all treatment groups, too.



Control Mulberry fruit extract

PL10 Comparison analysis of biochemical compound in Glycyrrhiza glabr roots from two localities of Iran (Bojnurd) and Afghanistan (Herat)

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Liquorice (*Glycyrrhiza glabra* Family *Leguminoceae*) is a very popular medicinal plant in the world. It, also known as licorice and sweetwood, is native to the Mediterranean and certain areas of Asia. Licorice rhizomes are used in herbal medicines for health effects and it contains more than 100 various useful compounds including phenolics and triterpene saponins (glycyrrizin). In this study, the content of some biochemical compounds (sugar, phenol and protein) important in pharmacy, food industry and economics were compared in *G. glabra* roots gathering from two localities of Iran (Bojnurd) and Afghanistan (Herat). Data showed that higher content of sugar (39.74 mg.gDW) was in Herat against Bojnurd (23.61). Bojnurd locality showed higher content of total phenolic compounds (167 mg.gDW) than Herat (73.43). Protein content was higher in roots gathered from Herat (20.32 mg.gDW) than Bojnurd. It seems that there is a correlated between the content of secondary metabolite production and climate condition. Therefore, environmental conditions are important factors in production of secondary metabolites in liquorice plants.

PL11 Herbal tourism – A new approach to special-interest eco-travels: Lessons and initiative from Serbia

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In contrast to mass tourism where the sun, sand and sea constitute the only tourism resource, nature-based tourism activities respond to people's desire to participate in tours with different aims such as relaxation, discovery, learning and escaping to nature, and getting away from the routine of everyday life. As one of nature-friendly tourism forms, herbal tourism, could be defined as provision of sustainable and responsible collecting of curative, aromatic, edible and seasoning plants to general public and organised groups, supported by interpretative and educational services and facilities, based on ethnopharmacological tradition. Thus, the purpose of this research is to introduce a new form of tourism, focused on presentation, education and sustainable use of autochthon

plants – preliminarily named herbal tourism. Also, this study will give insight into properties of herbal species and their distribution in Serbia, which could further develop herbal tourism sites and routes. Proposed herbal tourism destinations could further support scientific research of plants and enhance rural areas, with numerous environmental and financial problems that are evident in developing countries, such as Serbia.

PL12 Phenolic profiling of Rumex L. species by means of the LC-MS/MS

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Rumex L. genus, traditionally known as the sorrels and the docks, is a genus that comprises about 200 species. Although native to Europe and Asia, nowadays it is introduced almost everywhere. They have use in traditional medicine as antiseptic and antidiuretic cures, whereas the roots of some *Rumex* species are used for its laxative, depurative and tonic properties. Sorrel is also often used in French cooking. Previous chemical studies have detected various constituents among which are flavonoids, anthraquinones and tannins. The objective of this work was to determine differences in overall phenolics composition of selected Rumex species: *R. patientia* L., *R. acetosa* L., *R. acetosella* L., *R. crispus* L, *Rumex obtusifolius* L. and *Rumex balcanicus* Rech. LC-MS/MS technique was used for quantitative analysis of 80% ethanolic extracts of aerial parts, roots, leaves, flowers and stems of selected *Rumex* species. Fourtyfive reference compounds from different phenolic classes were selected: benzoic acid derivatives, cinnamic acid derivatives, lignans, coumarins and flavonoids (flavonoid aglycons, flavonoid-*O*-glycosides and flavonoid-*C*-glycosides). Principal component analysis (PCA), a multivariate analysis technique, was employed for data analysis in order to evaluate a „phenolic profile"of investigated species. High content of hyperoside and isoquercitrine differentiates *R. acetosa* from other *Rumex* species. Abundant content of apigenin and cinaroside, as well as lower content od gallic acid, distinguish *R. acetosella* and *R. acetosa* samples from other investigated ones. High amount of rutin is determined in *R. patientia* species samples. This work showed significant quantitative and qualitative differences in "phanolic profile" of investigated species.

PL13 Comparison analysis of drying methods on extract, sugar, protein and phenol contents of licorice (Glycyrrhza glabra)

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Licorice (*Glycyrrhza glabra*) belongs to Fabaceae family and is one of the most important medicinal plants for pharmacology and ulcers and gastric cancer. Some extraction methods affect the quality and quantity of secondary metabolites. Thus, an experiment was carried out to study the effect of different drying methods on some biochemical compounds such as proteins, sugars and phenol content of *G. glabra*. Samples were divided to the same weight (30 g) and dried in different temperature conditions: air condition (37 °C) and two oven temperatures (35 and 65 °C). Then, samples were extracted with 80% ethanol solvent in Soxhlet apparatus during 2 hours. The results showed that the highest extraction was observed in oven dried tissue at 35 °C (11.20%). The highest amount of total sugar, protein and phenol were obtained in air dried tissue samples (61.39 $mg.g^{-1}$ dry weight), oven with 35 °C (22.31 $mg.g^{-1}$ dry weight) and oven with 65 °C (89.67 $mg.g^{-1}$ dry weight), respectively. The lowest amount of total sugar, protein and phenol were achieved in oven with35 °C (25.39 $mg.g^{-1}$ dry weight), oven with 65 °C (9.37 $mg.g^{-1}$ dry weight) and air dried (59.42 $mg.g^{-1}$ dry weight), respectively.

PL14 Phenolic profile and antioxidant activity of buckwheat (Fagopyrum esculentum) herb and root extracts

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Buckwheat (*Fagopyrum esculentum* Moench.) is a plant from Polygonaceae family, commonly used as a pseudocereal since it is nutritionally similar to grains. Due to high rutin content, it is traditionally used as a remedy for cardiovascular diseases. While numerous phenolic compounds were identified in this plant, only several have been quantified so far. The objective of this work was to obtain detailed phenolic profile of 80% ethanolic extracts of rhizoma, stems, leaves, flowers, and complete aboveground parts of buckwheat cultivated in Serbia. Total of 45 phenolics, including phenolic acids, flavonoids, coumarins and lignans, were quantified by LC-MS-MS technique. In addition, antioxidant activity was evaluated for herb and rhizoma extracts, regarding their ability to reduce Fe^{3+}-TPTZ complex, scavenge DPPH, O_2^-, OH and NO radicals, and inhibit lipid peroxidation. Dominant phenols in all investigated extracts were quercetin and its glycosides – rutin, isoquercitrin, quercitrin and hyperoside, accounting for 16 – 18% of herb, flowers and leaves dry extract, thus making buckwheat a species extraordinarily rich in flavonols. Phenolic acids were less abundant, their total content not exceeding 4%, with quinic, 5-*O*-caffeoylquinic and (to a lesser extent) protocatechuic acid as the most significant. Both herb and rhizoma extracts exhibited high reduction capacity and radical-scavenging potential, in some cases higher than that of commercial antioxidants. In general, herb exhibited higher activity, which is in line with found higher phenolics content. Obtained results confirm *F. esculentum* to be a rich source of bioactive natural products and a valuable potential ingredient of functional foods.

PL15 Quality evaluation of herbal drug "Saussureae radix"

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The Saussureae radix has been used for treatment of abdominal pain, vomiting, diarrhea, chronic inflammation, and antibacterial effect. The quality of these herbs has been affected by many factors such as collection time, place, temperature, cultivation environment and manufacturing process. Costunolide, dihydrocostunolide, costuslactone, dihydrocostuslactone, α-costol, saussurea lactone, and costuslactone are main active components in Saussureae radix according to the literature. We used costunolide and dihydrocostunolide as marker compounds for quality evaluation. 38 samples of Saussureae were collected from those habitats in Korea and China. The developed HPLC-DAD method was applied to investigate for quality control of Saussureae radix sample. The content of costunolide varied from 0.674% to1.812% and for dihydrocostunolide, the content varied from 1.310% to 3.035%. The all samples showed similar pattern.

PL16 Monitoring contents of atractylenolide I and atractylenolide III in *Atractylodes Japonica*

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The quality of herbal medicines is dependent on many factors including harvest seasons, plant origins, drying processes and other factors. The monitoring of herbal medicines was required processing for quality control. *Atractylodes japonica* belongs to the Asteraceae family. This herb has been cultivated in east Asia such as Korea, Japan and China. It showed analgesic, antibacterial, antidepressant, antiinflammantory and antitumer activity. Atractylenolide I and Atractylenolide III were major compounds of *Atractylodes japonica*. An established HPLC-DAD method was used to monitor contents of atractylenolide I and III in 30 *Atractylodes japonica* sample obtained from Korea and China. The results shows that atractylenolide I content falls in the range 0.02 - 0.19% and atractylenolide III content in the range 0.04 - 0.31%. In this study, we identified

differentiate the quality of *Atractylodes japonica* sample from different species and collected loactions.

PL17 Neuroprotective effect of compound isolated from *Cynanchum paniculatum*

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Cynanchum paniculatum, belongs to Asclepiadaceae has been used to treat various disease, such as invigorate blood, alleviate edema and to relieve pain and toxicity for a long time. 4,5-Dimethoxypyrocatechol was isolated from the 80% methanol extract of *Cynanchum paniculatum*. 4,5-Dimethoxypyrocatechol had neuroprotective effect on the glutamate-induced cellular oxidative death in HT22 cells. Furthermore, we found that reactive oxygen species (ROS) accumulation and Ca^{2+} concentration by oxidative stress were reduced by 4,5-dimethoxypyrocatechol in HT22 cells.

PL18 Marbank – A biobank of arctic marine organisms

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Marbank is a marine biobank located in Tromso, Norway. Marbank has a national responsibility for collecting, preserving and cataloguing marine organisms from Norwegian waters for research, commercial and exploitation purposes. The mission of Marbank is to provide an easy accessible repository of marine resources from Arctic and sub-Arctic areas for R&D institutions and industry that search for bioactive compounds in marine organisms. In addition to the collection in Tromso, Marbank coordinates a network of marine collections in Norway. The overall aim of the network is to better coordinate marine collections in order to make resources and data more accessible to both Norwegian and international users. Marbank is established in close cooperation with the Norwegian Ministry of Fisheries and Coastal Affairs.

PL19 Micropropagation and identification of O-palmitoyl-3-O-β-D-glucoside of β-sitosterol in the medicinal species *Lopezia Racemosa*

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Lopezia racemosa Cav is a traditional medicinal plant with anti-inflammatory and anti-cancer properties. Among the secondary compounds of interest is O-palmitoyl-3-O-β-D-glucoside of β-sitosterol (LR1) that seems to participate in the medicinal effects of this species. The aim of this work was to obtain an efficient micropropagation system of *L. racemosa* to compare the active metabolites contents between *in vitro* and field-grown plants. Cultures were initiated from nodal segments of in vitro germinated seedling inoculated onto Murashige and Skoog (MS) medium supplemented with BA individually or in combination with NAA. Optimum culture conditions for shoot organogenesis were determined. Multiple shoots were generated via indirect organogenesis on medium with either BA 0.5 mg/L or combined with NAA 2.5 mg/L. The elongated shoots were successfully acclimated to greenhouse conditions. Thin layer chromatography (TLC) and proton nuclear magnetic resonance spectroscopy (1 H-NMR) analysis showed the presence of LR1 in callus, regenerated shoots and the control plants. Therefore, the protocol also provides an effective means for the in vitro conservation of *L. racemosa* that produce pharmaceutically interesting anti-inflammatory and anti-cancer metabolites.

PL20 Profiling of cereal-derived alkylresorcinols with antigungal activity against *Fusarium oxysporum*

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Alkylresorcinols are non-isoprenoid phenolic lipids present in several commercial plants of Poaceae family. *In vitro* studies on alkylresorcinols have shown to possess antimutagenic, antimicrobial, cardioprotective activities among others. As part of our research on antifungal agents, a chemical characterization focused on alkylresorcinol profiling and quantification by HPLC-ESI-MS and Fast Blue RR-based colorimetric method, respectively, were performed on alkylresorcinol-enriched extracts (AEE) from wheat, corn, oats, barley and wheat bran flours. Several alkylresorcinol were identified in each product, whose common structural feature was the alkenyl side chain. Wheat bran was found to have the highest alkylresorcinol content followed by barley, wheat, oats, and maize flours. In addition, *in vitro* antifungal assay was performed on each AEE against *Fusarium oxysporum*. All AEE exhibited dose dependent antifungal activity at different levels. All data were correlated by principal component analysis (PCA). A composition-activity relationship was observed.

PL21 Chemical and biological profiling on rhizobacteria from *Physalis peruviana* with antagonistic activity agaisnt *Fusarium oxysporum*

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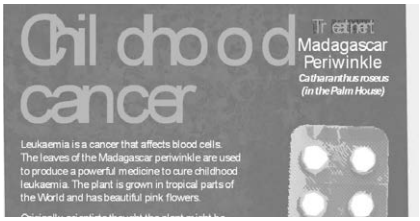
School of Sciences, Universidad Militar Nueva Granada, Cajicá, Colombia, AA 49300

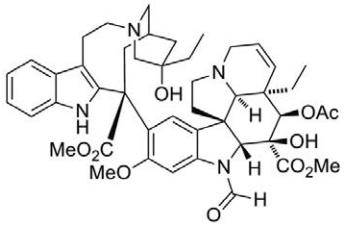
From *Physalis peruviana* rhizosphere were isolated 174 bacterial strains, which were tested for their *in vitro* antagonism against *Fusarium oxysporum* and their capability for promoting germination on *P. peruviana* seeds. From this set of rhizobacteria, 11 strains were found to have antagonistic activity and only 6 possessing effects on germination. In order to chemically characterize these strains, indole and alkylresorcinol quantification by colorimetric methods, as well as medium polarity metabolic profile from these antagonistic rhizobacteria by high resolution liquid chromatography coupled to mass spectrometry (HPLC-MS), were performed for bacterial cultures. All chemical and biological data were analyzed by multivariate statistics using principal component analysis (PCA). A close relationship was found between indole content and germination. In addition, antagonistic activity was correlated at different levels. Indole derivatives were found to be the most common compounds into the LC-MS profile in bacterial cultures.

PL22 The medicinal plant collection at the University of Oxford Botanic Garden

Foster A
University of Oxford Botanic Garden, Rose Lane, Oxford, OX1 4AZ, U.K

The Botanic Garden was founded in 1621 as a physic garden to grow plants for medicinal purposes. In the subsequent 390 years the focus on medicinal plants diminished somewhat. In 2010, a new and unique, modern medicinal plant collection was established and is now being used for teaching of undergraduates, postgraduates, visiting students and academics. It is also being used in schools education programmes (from 5 years to 18 years old) and to educate local doctors (General Practitioners) about plant derived medicine (both herbal and conventional). The aims and objectives of the new collection and its impact on the education of both our general visitors and specialist groups will be presented.





Snapshot from a family trail leaflet, Vincristine, *Catharanthus roseus*

PL23 Antioxidant activity and polyphenolic composition of water knotweed (*Polygonum amphibium* L.) ethanolic extracts

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As a part of our ongoing phytochemical investigation of plants from *Polygonum* genus (Polygonaceae), we investigated the chemical composition and antioxidant acitivity of *P. amphibium* collected on Vlasina lake in Serbia. Plants from this genus are used in traditional medicine, diet, and as ornamental plants. Polyphenolic profile of 80% ethanolic extracts of stems, leaves, flowers, and entire herb was determined by quantitative LC-MS/MS analysis of 45 phenolics, and by spectrophotometric measuring of the total phenolic and flavonoid content. The antioxidant activity was evaluated by measuring FRAP ability of the extracts and their scavenging capacity towards DPPH, OH, NO and O_2^- radicals. Dominant phenols in all investigated extracts were found to be quercetin and its glycosides – hyperoside and isoquercitrin, accounting for up to 4% of leaves dry extract. Among flavan-3-ols, catechin, epicatechin and gallic acid ester – epigallocatecin gallate – were present mostly in stems (~0.9%). Gallic acid was the most abundant phenolic acid (up to 0.5% of leaves and herb dry extract). High DPPH and O_2^- scavenging ability (IC_{50} 6.8 – 7.4 μg/mL and 5.5 – 8.5 μg/mL, respectively), and moderate ability of scavenging OH (IC_{50} 135 – 188 μg/mL) and NO (IC_{50} 82.6 – 102 μg/mL) radicals, were observed in herb extracts, as well as high FRAP ability (461 – 466 mg eqv. ascorbic acid/1 g dw), and total phenolic content (300 – 307 mg eqv. gallic acid/1 g dw). Flavonoids content was 38.5 – 39.7 mg eqv. quercetin/1 g dw.

PL24 Potent NF-κB inhibitors from endophytic fungus of a taxus plant

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NF-κB inhibitors isolated from natural sources that induce apoptosis are promising new anticancer agents. A potent NF-κB inhibitor (NFW9C-17) was isolated from an unidentified endophytic fungus of a *Taxus* plant from Pakistan named NFW9. The NF-κB inhibitory concentration (IC_{50}) of NFW9C-17 was 0.20 μg/mL (0.47 μM) when using HeLa cells. Rocaglamide (IC_{50}= 0.075 μM) was used as a positive control. The NF-κB inhibitory effect of NFW9C-17 is in agreement with previous literature reports. It is being further evaluated to uncover the mechanism through which it exhibits the attenuating effect on NF-κB. Three cancer cell lines, HT-29 colon cancer cells, HeLa cervical cells, and MDA-MB-231 hormone independent breast cancer cells, are being used to evaluate the effects of NFW9C-17. The observed effects have shown to be concentration dependent and the activity is comparable to the positive control, daunomycin, a potent chemotherapeutic agent. Our findings suggest that NFW9C-17 might be involved in the induction of apoptosis of treated cells. Evaluation of the biological activity of NFW9C-25, a new natural product analog from this species with NF-κB activity (IC_{50}= 0.7 μg/mL; IC_{50}= 2.06 μM), is ongoing to determine the structure activity relationship of both natural compounds.

PL25 Quantitative analysis of epicatechin metabolites in human serum using UHPLC-MS-MS

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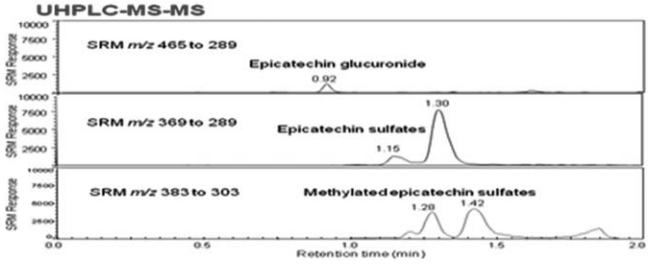
Flavanols such as epicatechin occurring in the seeds of Theobroma cacao (cocoa beans) are associated with beneficial health effects. When cocoa seed-based products are ingested, epicatechin is absorbed and converted into epicatechin glucuronide, epicatechin sulfates, and epicatechin methylsulfates, which can be found in human serum. A UHPLC-MS-MS method was developed for rapid quantitative analysis of epicatechin glucuronide, epicatechin sulfates, and epicatechin methylsulfates in serum. The concentrations of the epicatechin metabolites in blood serum were measured using a Shimadzu Nexera UHPLC with an LCMS-8030 triple quadrupole mass spectrometer with collision-induced dissociation and selected reaction monitoring.



PL26 Sarsasapogenin stimulates melanogenesis in melan-a cells

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Sarsasapogenin (SAR) is a steroidal sapogenin that is used as starting material for the industrial synthesis of steroids. It has various pharmacological benefits, such as antitumor and antidepressant activities. Since its effect on melanin biosynthesis has not been reported, we used murine melanocyte melan-a cells to investigate whether SAR influences melanogenesis. In this study, SAR significantly increased the melanin content of the melan-a cells from 1 to 10 μM. Based on an enzymatic activity assay using melan-a cell lysate, SAR had no effect on tyrosinase and DOPAchrome tautomerase activities. It also did not affect the protein expression of tyrosinase-related protein 1 and DOPAchrome tautomerase. However, protein levels of tyrosinase and microphthalmia-associated transcription factor (MITF) were strongly stimulated by treatment with SAR. Therefore, our reports suggest that SAR treatment may induce melanogenesis through the stimulation of tyrosinase and MITF expression in melan-a cells. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2010 – 0025362).

PL27 Natural products from plants and their synthetic analogs against pests

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As USDA and DoD joint efforts to search for effective, environmentally friendly vector borne disease transmission controlling agents, natural products isolated from plant extracts belonging to various families were evaluated for activity against mosquitoes. The compounds were tested against *Ae. egypti* for activity as larvicides and repellents. The most popular mosquito repellent has recently been found to have neurotoxic effects and thus new compounds with varying modes of action are needed for public health and military applications. These active compounds were isolated from plants in Asteraceae, and Rutaceae plant families. A moderately active amide isolated from a member of the Rutaceae family has been modified to obtain highly potent repellents with three times longer duration of protection (> 6 days) than DEET when tested on cloth patch assay against *Ae. egypti* female mosquitoes. Isolation, synthesis of analogs and biological activities of these compounds will be discussed.

PL28 Ethnobotanical and phytochemical studies of medicinal plants of minority groups in southern china

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Medicinal plants used traditionally by the Zhuang in Jingxi of SW Guangxi, the Dong in Liping of SE Guizhou, and the De-ang in SW Yunnan were investigated and documented, based on the ethnobotanical approaches. Some important species in folk societies were selected for further studies through phytochemical method. We have documented 329 medicinal plant species from the Dragon-boat Festival market in Jingxi County of Guangxi. To evaluate the medicinal plants, some samples were screened phytochemically, including *Selaginella moellendorfii*, *Pilea cavaleriei* subsp. *crenata* and *Corydalis saxicola*.. Traditional medicinal plant species with 157 species have been investigated from the Dong communities in Liping, southeast Guizhou. Totally 31 compounds were isolated, in which 11 are new ones, with one novel structure, from three species. Ninty-two species of medicinal plants used traditionally by the De-ang ethnic group in southwest Yunnan were documented. In total 44 compounds were isolated from *Piper boehmeriaefolium* and *Remusatia vivipara*, in which 12 are new ones. Conclusively, it is a shortcut to use ethnobotanical approaches to study medicinal plants from the ethnic societies, which harbor rich medicinal plant resources. Ethnobotany can be a tool for new drug discovery.

PL29 Characterization of brain bioactive polyphenols as disease modifying agents in Alzheimer's disease

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Alzheimer's disease (AD) is a devastating disorder that strikes 1 in 10 Americans over the age of 65 and almost half of Americans over 85 years old. The odds of developing AD double every five years after age 65. There is mounting evidence that dietary polyphenols may beneficially influence AD. The Center of Excellence for Research on Complementary and Alternative Medicine (CERC) in Alzheimer's disease defines our vision for an integrated multidisciplinary program of preclinical research projects all linked by the unifying scientific theme of understanding the potential protective roles of grape-derived polyphenols in AD. In 2008, a marketing report suggested that more than eight percent of nutraceutical consumers indicated that they had purchased food products aiming to prevent an undesirable condition, and fifty percent of consumers reported purchasing foods to manage or treat conditions. Much of this consumer demand for therapeutic food products is for foods containing polyphenols. However, because polyphenolic compositions and bioactivities vary considerably due to plant-growth environments, there are problems with the preparation of grape-derived polyphenolics (and other dietary polyphenolics). For the same reason, there are also issues that complicate the harvest, storage and processing/preparation of certain dietary sources of polyphenols. These limitations prompted us to assemble groups of interdisciplinary scientists with expertise in Alzheimer's disease and nutritional-botanical sciences to design a series of studies with the ultimate goal of isolating and identifying bioactive polyphenolic compounds from dietary grape sources that are capable of providing beneficial Alzheimer's disease-modifying activities. The studies conducted in our Center employ mechanistic-based approaches to validate sensitive and reliable translational tools to detect and assess relevant signatures of brain bioavailable polyphenols as disease modifying agents in Alzheimer's disease by measuring efficacy as well as other outcomes mechanistically. The anticipated goals of our Center have thus far been met by successfully identifying select dietary grape polyphenol compounds capable of attenuating AD-type cognitive dementia in models of AD-type β-amyloid pathogenesis. We recently succeeded in the isolation and structural identification of brain bioavailable, bioactive polyphenol metabolites from dietary grape products. Our studies have also provided for the first time the much need information for an ongoing translational Phase II clinical study exploring the feasibility of developing a select polyphenol preparation for the treatment of AD.

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