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Program

Wednesday 22 May 2013

Université libre de Bruxelles, Forum A, Campus de la Plaine, 1050 Bruxelles – *Metro: Delta station*

17h-19h: **General STOLON Meeting**

19h-22h: Medicinal plants museum visit. Opening reception « Belgian Cheese and Beer »

Thursday 23 May 2013

Université catholique de Louvain, Auditoire central C, Avenue Mounier 51, 1200 Bruxelles - *Metro: Alma station*

9h-9h30: **Welcome**

9h30-9h40: **Congress opening**

- **Professor M.-P. Mingeot, President of Louvain Drug Research Institute (UCL)**
- **Professor J.-M. Kauffmann, Vice-Dean of Faculty of Pharmacy (ULB)**
- **Professor J. Quetin-Leclercq, Dean of Faculty of Pharmacy (UCL)**

Session 1: Chairs: M. FREDERICH (Liège) & E. CHOSSON (Rouen)

9h40-10h05: **M. Simmonds** (KEW Garden-UK)
Plant hunting – the relevance of botanical collections for the authentication of plants

10h05-10h40: **S. Krief** (Museum National d'Histoire Naturelle-France)
Health and use of natural substances by wild chimpanzees

10h40-11h: *Oral communication:* **A. Abedini** - Identification des substances antimicrobiennes de l'extrait hydro-méthanolique d'*Hyptis atrorubens* Poit. (Lamiaceae)

11h-11h20: Coffee break

11h20-11h30: Stolon 2012 Award

11h30-11h50: Presentation of the laureate of Stolon 2012 Award: **Philippe Ndjolo Okusa** (UMONS-ULB) - Etude phytochimique et de l'activité antimicrobienne directe et indirecte de *Cordia gilletii* De Wild (Boraginaceae)

Session 2: Chairs: Y.-F. POUCHUS (Nantes) & M.-A. LACAILLE-DUBOIS (Dijon)

11h50-12h25: **J. Bero** (UCL-Belgium)
From traditional medicine to new antiparasitic compounds

12h25-13h: **V. Butterweck** (University of Applied Sciences and Arts - Switzerland)
Pharmacokinetics of Natural Products: The missing puzzle piece in the efficacy of phytotherapeutics?

13h-13h20: *Oral communication:* **Y. Fromentin** – Extraction and intramolecular cyclisation of guttiferone A

13h20-14h30: Lunch and **poster session**

Session 3: Chairs: J. BOUSTIE (Rennes) & L. VOUTQUENNE-NAZABADIOKO (Reims)

14h30-15h05: **P. Duez** (ULB-UMons-Belgium)
First-line toxicological models for genotoxicity, teratogenicity and nephrotoxicity assessment of herbal products

15h05-15h25: *Oral communication:* **J. Hubert** – Centrifugal partition extraction as an efficient tool for natural extract fractionation and METABOLITE screening

15h25-15h45: *Oral communication:* **A. Dieu** – Antibacterial compounds from *Cladonia incrassata*

15h45-16h: Coffee break

Session 4: Chairs: T. HENNEBELLE (Lille) & F. CHAPELAND-LECLERC (Paris Descartes)

16h-16h35: **Qihe Xu** (King's College London - UK)
From anti-fibrotic herbs to international collaboration on good practices in Chinese herbal medicine research

16h35-17h30: **Poster session**

19h30: **Gala dinner**

Friday 24 May 2013

Université catholique de Louvain, Auditoire central C, Avenue Mounier 51, 1200 Bruxelles - *Metro: Alma station*

9h-9h30: **Welcome**

Session 5: Chairs: O. GROVEL (Nantes) & P. BOIRON (Lyon)

9h30-10h05: **G. Morlock** (Hohenheim University - Germany)
Chromatography combined with bioassays, MS and other hyphenations - the direct link to the compound indicating the effect

10h05-10h40: **Y. Vander Heyden** (VUB - Belgium)
Herbal fingerprints: development and data analysis

10h40-11h: Coffee break

11h-11h20: *Oral communication:* **E. Lautié** – Valorization of the nutrient-rich Yam bean (*Pachyrhyzus sp*.) for Central and West Africa

11h20-11h40: *Oral communication:* **S. Komaty** – Extraction of *Pseudevernia furfuracea* compounds by ionic liquids on microwave-assisted extraction

Session 6: Chairs: A. MAMBU (Limoges) & N. RUIZ (Nantes)

11h40-12h15: **R. Verpoorte** (Leiden University – The Netherlands)
Metabolomics: gateway to discoveries

12h15-12h20: **Congress closure: E. Seguin (AFERP) & Y.-F. Pouchus (STOLON)**

12h20-13h: Lunch

13h30-17h: **Visit of National Botanic Garden of Belgium (Meise)**

PLANT HUNTING – THE RELEVANCE OF BOTANICAL COLLECTIONS FOR THE AUTHENTICATION OF PLANTS

Monique S.J. Simmonds & Christine J. Leon

Royal Botanic Gardens, Kew, Richmond, Surrey, UK

Reliable assurance about the identity of medicinal plants is a worldwide need spanning research, regulatory and industrial sectors. The need for assurance is increasing as the trade in medicinal plants becomes global and there is lack of clarity in plant quality and the supply chain. This is in part due to changes in land use and in some parts of the world unregulated practices and the global trade of medicinal plant material as well as products derived from this material through the internet. There are growing concerns about material being adulterated as well as the increased use of substitutes that impact efficacy and safety. These concerns are fully justified given that not only are the bulk of medicinal species (60-80%) wild-harvested but changes in land use and widespread crop failures are placing increased pressure on the supply of quality plants from cultivated sources.

Despite a rapidly expanding pharmacognosy literature, international standards in good research practice using, for example, reliable reference materials and validated methods for quality control are still in their infancy. This presentation outlines recent efforts at the Royal Botanic Gardens, Kew to assist different organisations validate their plant material. This talk will be illustrated by the use that Kew is making of its botanical collections and the taxonomic expertise of its staff to develop authentication methods that are appropriate to different users. This includes the use of the existing collections at Kew but also the research and field work needed to establish a collection of validated material that is being used for the authentication of plants used in traditional Chinese medicine.

HEALTH AND USE OF NATURAL SUBSTANCES BY WILD CHIMPANZEES

Sabrina Krief

UMR 7206, Muséum national d'histoire naturelle, krief@mnhn.fr

More than 30 years ago, researchers observed an unusual behaviour suggesting a medicinal use of plants by wild chimpanzees at Gombe, Tanzania (Wrangham & Nishida, 1983). Research related to potential selfmedicative behaviour in apes extended to several sites and ape species in Africa. They showed that ingestion of whole rough leaves is a widespread behaviour in chimpanzees correlated with intestinal parasite infection with which they may alleviate symptoms caused by intestinal parasites such as cestods and nematods (Wrangham & Goodall, 1989; Wrangham, 1995). Later, consumption of bitter pith of *Vernonia amygdalina* by sick chimpanzees has been described (Huffman & Seifu, 1989). Reliefs of symptoms were observed and chemical compounds isolated from this species, confirming the therapeutic effect associated with this behaviour (Huffman et al., 1993; Ohigashi et al., 1994). In order to further investigate this topic of research, we conducted chimpanzee behavioral and health monitoring, botanical and phytochemical and as well as mosquito collections in the Kanyawara and Sebitoli area, in North-West of Kibale National Park (KNP), Western Uganda (0 13'-0 41'N and 30 19'- 106 30 32'E). In a collaborative project with colleagues from ICSN (CNRS, France), Uganda Wildlife Authority and Makerere University, we have investigated the bioactivities of plant parts of low nutritive value ingested by wild eastern chimpanzees (*Pan troglodytes schweinfurthii*) in KNP. New bioactive compounds have been isolated from leaves and barks (Krief et al., 2004, 2005, 2006). Among these, two novel compounds with antiplasmodial properties have been isolated from the *Trichilia rubescens* leaves (Krief et al., 2004) that wild chimpanzees from the Kanyawara community in KNP rarely consume. Occasionally, the Kanyawara chimpanzees also ingest soil, which has been shown to enhance the antiplasmodial activity of the *T. rubescens* leaves (Klein et al. 2008). We have recently confirmed that these chimpanzees are infected by at least four *Plasmodium* species including *P. reichenowi*, and two new species phylogenetically close to *P. falciparum* the parasite responsible for the deadliest infections in humans, and the *P. vivax* type (Krief et al., 2010). In addition, our results suggest a high prevalence in natural conditions. However, the parasite species, to some of which humans are susceptible, are not know to lead to malaria-related severe clinical symptoms in chimpanzees and our long-term health monitoring confirms these observations. Besides ingestion of compounds with antiplasmodial properties, we explore the hypothesis that other behavioural factors, such as avoidance of places where vectors are abundant might contribute to the apparent mild malaria morbidity in great apes. Analysis of the pattern of chimpanzee nest sites associated to a negative correlation between nest numbers and mosquito abundance and suggested that the selection of nest sites in locations with less diverse and numerous anophelines may limit the risk of infection (Krief et al., 2012). Ingestion of plant parts having pharmacological properties as well as avoidance of the night-biting anopheline mosquitoes are potential behavioural adaptations that would lead to a decrease in the clinical signs of malaria. Data records extending back to early 20th century indicate that the Kibale region has become moister which would likely lead to an increase in mosquito abundance. In addition, human population density increased by 300% between 1959 and 1990 (Naughton-Treves, 1998). It is possible that chimpanzees, which had been adapted over thousands of years to forest vegetation, vectors and parasites, might face novel threats to their health as changing climate and land conversion force then to live in fragmented forests and to use more frequently the forest edge.

FROM TRADITIONAL MEDICINE TO NEW ANTIPARASITIC COMPOUNDS

Joanne Bero

Université Catholique de Louvain, Louvain Drug Research Institute, Pharmacognosy Research Group, Avenue E. Mounier, 72, B-1200 Brussels, Belgium

Parasitic diseases are still responsible for many health problems. Among them, African trypanosomiasis or sleeping sickness caused by *Trypanosoma brucei*, malaria transmitted by *Plasmodium* species of which the most dangerous is *Plasmodium falciparum* and leishmaniasis. Plant biodiversity and knowledge of traditional healing allow, as it was the case for artemisinin, to open new ways in the field of therapeutic. In the work we will present, we analyzed the activity of several plants from Benin selected by ethnobotanical and bibliographical studies. These plants are used in traditional medicine as antimalarials. Crude extracts from powders of leaves, twigs, roots or aerial parts were prepared by maceration. These extracts were studied for their antiparasitic activities by *in vitro* tests on *Plasmodium falciparum*, *Trypanosoma brucei brucei* and *Leishmania mexicana mexicana*. In addition, cytotoxicities were analysed to determine the selectivity of crude extracts. The dichloromethane extracts of *Keetia leucantha* were selected for further investigations. So we tested the *in vivo* antimalarial activity of the dichloromethane extract of twigs which showed 40.7 % inhibition in mice infected by *Plasmodium berghei* at 100 mg/kg/day and the total aqueous extract which had a 30.8% inhibition at 200 mg / kg / day.

Known antiparasitic compounds were identified and quantified by LC-MS in the dichloromethane extract. As they could not account for the total activity observed, we isolated by bioguided fractionation several triterpenic esters, vanillin derivatives, a sterol and a coumarin. The structure determination of isolated compounds was performed by NMR studies and high resolution mass spectrometry. The isolated compounds were then studied *in vitro* for their antiplasmodial activities. Several of them showed a sub-micromolar antiplasmodial activity, including some triterpenic esters which are about 10 times more active than ursolic acid.

References:

Bero J., Ganfon H., Jonville M.C., Frédérich M., Gbaguidi F., DeMol P., Moudachirou M., Quetin-Leclercq J. In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J. Ethnopharmacol., 2009, 122, 439-444.

Bero J., Hannaert V., Chataigné G., Hérent M.F., Quetin-Leclercq J. In vitro antitrypanosomal and antileishmanial activity of plants used in Benin in traditional medicine and bio-guided fractionation of the most active extract. J. Ethnopharmacol., 2011, 137, 998-1002.

Bero J., Herent M.-F., Schmeida-Hirschmann G., Frédérich M., Quetin-Leclercq J. In vivo antimalarial activity of Keethia leucantha twigs extracts and in vitro antiplasmodial effects of their constituents. J. ethnopharmacol. 2013 submitted.

PHARMACOKINETICS OF NATURAL PRODUCTS: THE MISSING PUZZLE PIECE IN THE EFFICACY OF PHYTOTHERAPEUTICS?

Veronika Butterweck

Institute for Pharma Technology, School of Life Sciences, University of Applied Sciences Northwestern Switzerland

In recent years the number of studies investigating the pharmacodynamic effects of botanicals has increased exponentially, often reporting pharmacological effects of botanical extracts with insignificant bioactivities obtained in irrelevant *in vitro* bioassays. The data interpretation from these *in vitro* assays for their efficacy in animals and humans is based on the assumption that a sufficient concentration of active constituents can reach the target sites of action in the body. This interpretation can be misleading since the pharmacokinetic properties of a compound are completely ignored. Although important, there is still limited information available regarding herbal pharmacokinetics. This might be due to the following reasons: (i) the active constituents are not known; (ii) the study of herbal pharmacokinetics is extraordinarily complex because extracts are multicomponent mixtures which contain several chemical constituents. Therefore concentrations of single compounds in the final product are in the lower mg range per dose. (iii) The resulting plasma concentrations are often in the µg to pg per liter range. As a consequence analytical methods determining bioavailability and pharmacokinetics of natural compounds have to be sufficiently sensitive. Advanced techniques such as GC-MS/MS or HPLC-MS/MS can be used nowadays to accomplish these goals. A better understanding of the pharmacokinetics and bioavailability of natural compounds can help in designing rational dosage regimen; and it can further help to link data from pharmacological assays with clinical effects. In this presentation, pharmacokinetic studies will be discussed that have been conducted for some of the top-selling botanicals worldwide, including artichoke, echinacea, mangosteen and valerian.

FIRST-LINE TOXICOLOGICAL MODELS FOR GENOTOXICITY, TERATOGENICITY AND NEPHROTOXICITY ASSESSMENT OF HERBAL PRODUCTS

Pierre Duez

Laboratoire de Pharmacognosie, de Bromatologie et de Nutrition Humaine, Université Libre de Bruxelles, Belgium; Service de Chimie Thérapeutique et de Pharmacognosie, Université de Mons, Mons, Belgium

The increasing use of traditional herbal medicines around the world requires more scientific evidence for their putative harmlessness. To this end, a plethora of methods exist, more or less satisfying and more or less validated. In this post-genome era, new “omics” methods (transcriptomics, proteomics, metabolomics) are being proposed for toxicity assessment, with many potential advantages compared to conventional ones. The presentation will focus on (i) possible strategies to assess genotoxicity, teratogenicity and nephrotoxicity of medicinal plants and their extractives; (ii) recent progress in the use of “omics” technologies in this field; and (iii) advantages and limitations of promising methods.

Literature and safety reports show that structural alerts, *in silico* and classical *in vitro* and *in vivo* predictive methods are most often used. The current trend to develop “omics” technologies to assess genotoxicity, teratogenicity and nephrotoxicity is promising but most often relies on methods that are still not standardized and validated. Hence, it is critical that toxicologists in industry, regulatory agencies and academic institutions develop a consensus, based on rigorous methods, about the reliability and interpretation of endpoints. It will also be important to regulate the integration of conventional methods for toxicity assessments with such new “omics” technologies.

FROM ANTI-FIBROTIC HERBS TO INTERNATIONAL COLLABORATION ON GOOD PRACTICES IN CHINESE HERBAL MEDICINE RESEARCH

Qihe Xu, MD, PhD

Co-Director, Centre for Integrative Chinese Medicine
Department of Renal Medicine, King's College London (KCL), London, UK

At KCL, our research is focused on fibrosis, a common pathology characterised by excessive accumulation of extracellular matrix that often leads to chronic organ failure. It affects all major organs and is a leading cause of mortality. Currently, there is no specific anti-fibrotic drug and the condition represents an important therapeutic area of unmet need.

In the past six years, we employed novel assays to explore anti- and pro-fibrotic activities of herbs used in traditional Chinese medicine (TCM). **17 herbal formulae, 11 individual herbs and 5 herbal compounds** were found to be anti-fibrotic and 3 herbs were pro-fibrotic. These laid the foundation for the ***King's Centre for Integrative Chinese Medicine***, which specialises in mechanistic studies and further R&D of anti-and pro-fibrotic herbals, and ***Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era*** (GP-TCM), the 1st EU-funded Seventh Framework Programme (FP7) Coordination Action dedicated to informing the best practice and harmonising TCM research through interdisciplinary exchanges among TCM experts and scientists.

The GP-TCM project studied the **state of the art of TCM research**, especially focusing on those most relevant to the health of EU citizens. With its large pool of expertise across 24 countries including 15 EU member states, the consortium provided fora and collaboration platforms on quality control, extraction technology and component analysis, toxicology, pharmacology and regulatory issues of Chinese herbal medicine (CHM), and also on acupuncture studies, with a particular emphasis on the utilisation of a **functional genomics** approach, i.e. by addressing whole profiles of DNA, RNA, proteins, metabolites and biological activities through comprehensive bioinformatics analysis.

With its 3.5-year programme and 10 interactive work packages (WPs), GP-TCM made great efforts to identify the state of the art in the various aspects of TCM research, to develop guidelines and to agree on **priorities, challenges and opportunities**, as summarised in the open-access **GP-TCM *Journal of Ethnopharmacology* special issue** (http://www.sciencedirect.com/science/journal/03788741/140). Based on polls of opinions among consortium members and non-members, high-quality efficacy/effectiveness, safety and mechanistic studies were identified as grand priorities and that the TCM legacy in general and its management of chronic diseases in particular were regarded grand opportunities. Consortium members cast their votes of confidence in omics and systems biology approaches to TCM research and believed that quality and pharmacovigilance of TCM products are not only grand priorities, but also grand challenges. Non-members, however, gave priority to integrative medicine, concerned on the impact of regulation of TCM practitioners and emphasised intersectoral collaborations in funding TCM research.

To ensure sustainable EU-China collaboration in TCM research beyond the lifespan of GP-TCM (May 2009 - October 2012), the FP7 consortium led the establishment of a new not-for-profit organisation, known as **the GP-TCM Research Association** (www.gp-tcm.org). Launched in April 2012, the Association has officially succeeded the missions and legacies of the FP7 GP-TCM project since

November 2012. It will remain a devoted link between EU, China and other parts of the world, especially dedicated to dissemination, validation and further development of good practice guidelines through interregional, interdisciplinary and intersectoral collaborations in TCM research.

In summary, due to its personalised and function-oriented features and its holistic and pre-emptive approaches, TCM is highly complementary to the current model of Western medicine and thus represents an important field for future research. Through face-to-face meetings, outreach events, teleconferences, project website (http://project.gp-tcm.org/), newsletters (http://www.gp-tcm.org/news-list/), dissemination reports in public media and scientific magazines, as well as more than 100 deliverable reports (http://project.gp-tcm.org/about/deliverables/), the GP-TCM project widely disseminated good practice guidelines and met all the planned objectives and milestones.

Looking forward, prospects for the whole area largely depend on how experts in different disciplines and stakeholders in different regions and sectors collaborate and how much funding is invested into this field. To reach the goals of **better quality, safety and efficacy** in the future of TCM, the GP-TCM consortium proposes that the rules of **integrity** (good practice), **integration** (collaboration) and **innovation** must be followed.

CHROMATOGRAPHY COMBINED WITH BIOASSAYS, MS AND OTHER HYPHENATIONS - THE DIRECT LINK TO THE COMPOUND INDICATING THE EFFECT

Gertrud E. Morlock

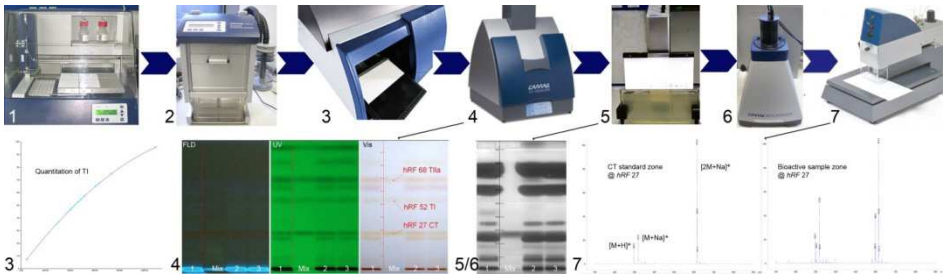
Chair of Food Science, Justus Liebig University Gießen, Heinrich-Buff-Ring 26, 35392 Gießen, Germany

Initially advocated by T. Hirschfeld, the term hypernation (super-hyphenation) was coined to place all of the required spectrometers into a single system so that all of the spectroscopic/-metric information is obtained in a single run [1]. Hypernation represents a logical, rapid and efficient strategy for obtaining the maximum possible information out of a single separation. Problems associated with column-based super-hyphenations are much less challenging in planar chromatography-based hyphenations: The open planar system is highly adaptive to different sensitivities, cost-effective by modular instrumentation compared to the status quo in analysis, generating less data due to targeted access to points-of-care on the plate, and directly accessible for the respective optimal detector solvent.

The eluent is evaporated after high-performance thin-layer chromatography (HPTLC) and not impacting the different detectors. The latter is extremely relevant for effect-directed detection with bioassays. HPTLC is highly compatible to the direct application of bioassays onto the chromatogram and can be the direct link to the compound indicating the effect, especially after elution of the zone of interest into a high-resolution mass spectrometer. Existing super-hyphenations are for example [2, 3]:

- HPTLC-UV/Vis/FLD-bioassay-HRMS
- HPTLC-UV/Vis/FLD-ATR FTIR
- HPTLC-UV/Vis/FLD-NMR

Examples are given in the field of natural product search and food analysis [4-7].



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HERBAL FINGERPRINTS: DEVELOPMENT AND DATA ANALYSIS

Y. Vander Heyden, G. Alaerts, M. Goodarzi, C. Tistaert, J. Viaene, B. Dejaegher

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Worldwide, herbs are used for preventive and therapeutic goals. Therefore, identification and quality control of these products of natural origin is required. Determination of some of the active compounds does not always allow assessing their total intrinsic quality. Since 1991 the World Health Organization accepts fingerprint chromatography as identification and quality evaluation technique for medicinal herbs. In fingerprint development, the goal is to create general conditions to maximize the peak capacity within an acceptable analysis time.

A fingerprint can be developed for a number of reasons: identification, classification or calibration purposes. Identification is to confirm that a sample is originating from the herb expected and to exclude that it is another, i.e. to attain a better quality control of the herbs. Classification can be performed to classify samples according to, for instance, their origin. This can be either a geographic origin or to distinguish between natural and synthetic compounds, e.g. vanillin from herbal, synthetic or microbiologic origin. Such evaluation is most often visualized by a principal component analysis, occasionally by a cluster analysis. However, it might also be by building a classification model or performing a similarity analysis. The latter techniques allow defining class borders or limit values. A multivariate calibration can be performed when the herb or its extract also can be characterized by an activity, e.g. an antioxidant or a cytotoxic activity. The activity then can be modelled as a function of the complete chromatogram. The most commonly used modelling techniques are stepwise multivariate regression, principal component regression and partial least squares. The goal of the modelling can be either to built models that are able to predict the activity for future samples based on the chromatogram (e.g. the antioxidant activity from green tea) or to identify the main compounds/peaks responsible for a given activity. In the presentation the different applications will be considered.

METABOLOMICS: GATEWAY TO DISCOVERIES

R. Verpoorte, N. Yuliana, H.K. Kim and Y.H. Choi

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Metabolomics has developed in a major tool in all types of research in the past decade. Functional genomics, plant resistance, quality control of food and botanicals, you name it. The basis of metabolomics is making unbiased observations with highly reproducible analytical tools, followed by a biostatistical analysis to find correlations between all the available data. That is a systems biology approach, which may lead to new discoveries.

This can be illustrated by the identification of the active compounds in medicinal plants. By measuring the metabolome of different extracts, accessions or fractions of a medicinal plant and combining these data with those of biological activity, signals related to the compounds related to activity can be found. That may include prodrugs and synergy in case of in-vivo experiments. To be able to measure all metabolites present in e.g. a plant, we developed comprehensive extraction as a way to rapidly identify the active compounds in a plant (Yuliana et al. 2011). NMR-based metabolomics of the fractions obtained with this method and combining these data with adenosine receptor binding activity data allowed the identification of flavonoids as the active compounds in *Orthosiphon stamineus* leaves.

A very different but quite exciting discovery made through the NMR-based metabolomics was the Natural Deep Eutectic Solvents (NADES). The data we collected in NMR-based metabolomics made us to ask the question “why are a few very simple molecules always present in considerable amounts in the spectra of any organism”? They must have a basic function in living cells. They include sugars, amino acids (e.g. proline, alanine, glutamine, asparagine), choline, and organic acids (e.g. malic, lactic, succinic acid). Sugars serve for storage and energy, the other compounds are in amounts that does not make sense to consider them only as metabolic intermediates. We found that mixtures of organic acids with bases form ionic liquids, e.g. malic acid and choline, whereas neutral solids may form deep eutectic solvents, e.g. sugars with choline or malic acid. NADES have a polarity like ethanol, and are excellent solvents for natural products, including DNA, proteins and polysaccharides, often with orders of magnitude higher solubility than in water. In our hypothesis many cellular and physiological functions are connected with the occurrence of NADES in nature (Choi et al. 2011).

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YH Choi, J van Spronsen, Y Dai, M Verberne, F Hollmann, IWCE Arends, G-J Witkamp, R Verpoorte (2011) Are Natural Deep Eutectic Solvents the missing link in understanding cellular metabolism and physiology? Plant Physiol. 156; 1701-1715.

IDENTIFICATION DES SUBSTANCES ANTIMICROBIENNES DE L'EXTRAIT HYDRO-METHANOLIQUE D'*HYPTIS ATRORUBENS* POIT. (LAMIACEAE)

Amin Abedini[1, 2], Vincent Roumy[1, 2], Séverine Mahieux[1, 3], Murielle Biabiany[4], Céline Rivière[1, 2], Sevser Sahpaz[1, 2], François Bailleul[1, 2], Christel Neut[1, 3], Thierry Hennebelle[1, 2]

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Les essais biologiques préliminaires de quatre extraits des feuilles et des tiges de *Hyptis atrorubens* Poit. (Lamiaceae) nous ont permis de sélectionner l'extrait hydro-méthanolique des tiges pour des études ultérieures. Cet extrait a été testé sur un panel de 46 micro-organismes *in vitro*. L'extrait hydro-méthanolique de tiges a été actif contre 29 micro-organismes (19 bactéries, 5 levures et 5 dermatophytes). La meilleure activité antibactérienne a été trouvée contre trois bactéries Gram positif (*Staphylococcus epidermidis* 10282, *Staphylococcus epidermidis* 5001 et *Enterococcus faecalis* C159-6), et une bactérie Gram négatif (*Stenotrophomonas maltophilia*). La bioautographie a permis l'isolement et l'identification de quatre composés antibactériens de cette plante : l'acide rosmarinique, le rosmarinate de méthyle, l'isoquercétol et l'hypéroside. Les valeurs de CMI et de CMB de ces composés et de leurs combinaisons ont été déterminées par la méthode de micro-dilution contre huit bactéries pathogènes. La meilleure activité inhibitrice et bactéricide a été trouvée pour le rosmarinate de méthyle (0,3 mg/ml). Notre étude a démontré pour la première fois l'activité antimicrobienne de composés identifiés chez *Hyptis atrorubens.*

EXTRACTION AND INTRAMOLECULAR CYCLISATION OF GUTTIFERONE A

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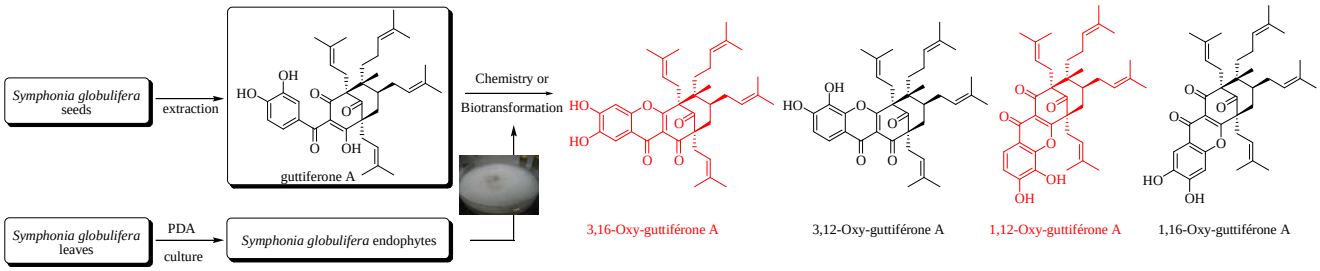

Guttiferone A is isolated from *Symphonia globulifera* seeds collected in French Guyana. This natural compound exhibits interesting biological activities such as trypanocidal, antiplasmodial and leishmanicidal properties. In order to generate potentially new active structures from Guttiferone A we have used advantageously either a chemical step or a biotransformation that produce new xanthones. Selective chemical oxidation lead to 1,12-Oxy-guttiférone A and 3,16-Oxy-guttiférone A. Biotransformation with some yeast or with *Symphonia globulifera* fungal endophytes selectively lead to 3,16-Oxy-guttiférone. The other two xanthones have been generated as a mixture, work in progress targeting the selective production of these two compounds.

CENTRIFUGAL PARTITION EXTRACTION AS AN EFFICIENT TOOL FOR NATURAL EXTRACT FRACTIONATION AND METABOLITE SCREENING

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Centrifugal Partition Extraction (CPE) is an innovative solid-support free separation technique derived from Centrifugal Partition Chromatography (CPC), with a column design characterized by less cells of larger volume. These particular features make possible to pump the mobile phase at higher flow rates while maintaining a good hydrodynamic behavior of the biphasic solvent system. Here are presented different applications of CPE, either for the highly productive purification of ionic molecules when combined to the strong ion-exchange displacement mode or for the rapid and selective fractionation of complex crude extracts when using a triphasic solvent system in a sequential elution mode. The respective results indicate that CPE offers promising perspectives in natural product research not only for preparative and pilot-scale purification purposes, but also for the determination of crude extract composition and screening of bioactive molecules.

ANTIBACTERIAL COMPOUNDS FROM *CLADONIA INCRASSATA*

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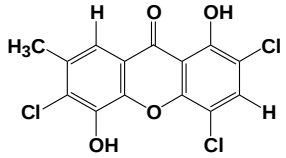
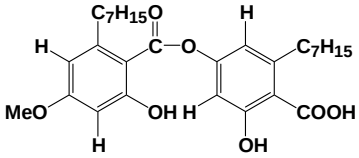
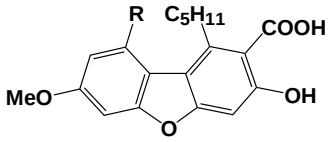
Cladonia incrassata is a composite lichen growing in Limousin, a suitable French region for the development of lichens. Acetone extract of *C. incrassata* was found in our previous study to exhibit a strong antimicrobial activity. Purification of this extract was initiated to track down active compounds. Bioassay-guided fractionation resulted in the isolation of a novel trichloroxanthone (**1**) and (-)-usnic acid. Three other known compounds (**2**, **3**, **4**) were also isolated for the first time from this lichen. A bioautographic protocol was used to evaluate the inhibition of growth of *Staphylococcus aureus* and the IC_{50} of (-)-usnic, didymic and condidymic acids were determined ($IC_{50} < 5\ \mu g/mL$).



1,5-dihydroxy-2,4,6 -trichloro-7-méthylxanthone (**1**)



Prasinic acid (**2**)



R = C_5H_{11} : condidymic acid (**3**)
R = C_3H_7 : didymic acid (**4**)

VALORIZATION OF THE NUTRIENT-RICH YAM BEAN (*PACHYRHIZUS SP.*) FOR CENTRAL AND WEST AFRICA

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This international and multidisciplinary project aimed at showing that this underutilized and highly nutritious legume root crop, showing a wide adaptation and high yield potential, can lead to greater food supply, more sustainable farming systems and new options for generating income in the rural and urban poor Central and West Africa. During this four-year initiative main results were obtained in: (1) improving the availability of yam bean germplasm for Central and West Africa especially high-yielding genotypes (2) developing the "gari" processing of storage roots (3) studying the chemical content of seeds, pods and leaves in order to provide a diversity of use of these by-products. While yam bean seeds are known to contain a toxic isoflavonoid, different strategies were developed in order to make them usable for consumption in a close future as pods are consumed in Asia. First different genotypes were screened using different kinds of analytical tools as microwave assisted extraction-solid phase extraction-ultra high performance liquid chromatography or near infrared spectroscopy to identify the less toxic ones. Then the feasibility of decreasing the amount of toxic compounds through food processing was studied and, *in vitro* and *in vivo* models were used to test the toxicity of different samples. The influence of the maturity of the seed containing pods was also studied.

EXTRACTION OF *PSEUDEVERNIA FURFURACEA* COMPOUNDS BY IONIC LIQUIDS BASED ON MICROWAVE-ASSISTED EXTRACTION

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Pseudevernia furfuracea (treemoss) is one of lichens used as raw materials in the perfume and cosmetic industries (1900 tons/year). The extracts used are classically prepared with organic solvents using heat-reflux extraction, a time- and solvent-consuming method. The objective of our work is to find innovative methods of extraction and purification which are efficient, cleaner and selective. Due to the interest of ionic liquids as alternative green solvents and of microwave-assisted extraction (MAE) as process with rapidness and high efficiency we decided to combine them for the extraction of *P. furfuracea.* This extraction was compared to the classical extraction with the Volatile Organic Solvents (VOCs). The efficiency of the extraction methods was evaluated using TLC coupled to a Camag® spectrophotodensitometer and HPLC analysis. The cytotoxicity of ILs and solvents used was evaluated on HaCaT (normal cell lines) and B_{16} (murine cancer cell lines).

[1] Joulain D., Tabbachi R. *Flavour and Fragrance Journal* 2009, 24, 105-116, 2009**.** [2] Lapkin A. A., Plucinski P. K., Cutler M., *Journal of Natural Products*; 2006: 69; 1653-1664 ; Lu Y., Ma W., Hu R., Dai X., Pan Y., *Journal of chromatography A,* 2008: 1208; 42-46 ; Bonny S., Paquin L., Carrié D., Tomasi S. , *Analytica Chimica Acta*, 2011, 707, 69– 75.

POSTER 1

ANTIQUORUM SENSING AND ANTIMICROBIAL ACTIVITY OF AROMATIC SPECIES FROM SOUTH AMERICA

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Quorum sensing (QS) is a bacterial communication mechanism that depends on microbial population density[1]. The interruption of QS is one example of an antipathogenic effect and can be used in the treatment of bacterial infections[2]. We investigated the anti-QS and antimicrobial properties of six essential oils from aromatic species from Argentina: *Salvia oficinallis*, *Minthostachys mollis*, *Satureja odora*, *Schinus molle*, *Lepechinia floribunda* and *Artemisia annua*. The anti-QS activity was determined using UV-visible spectrophotometry to measure the violacein production by *Chromobacterium violaceum*. The effective concentration of bioactivity at which the QS activity was reduced at 50% (MQSIC), was calculated. The antimicrobial activity was determined using *C. violaceum*, *Escherichia coli*, *Listeria innocua* and *Staphylococus aureus* as indicators. The minimum inhibitory concentration (MIC) and the minimal bactericide concentration (MBC) were determined by broth microdilutions method for each strain. Probit analysis was performed to analyze the violacein production data. All the oils have potential anti-QS activity at sub-lethal concentrations on *C. violaceum*. The *M. mollis* oil showed a greater anti-QS activity: diluted at 0.02% (v/v), violacein production decreased 90%. *S. molle* essential oil showed the lowest MQSIC (0.005%, v/v). *S. molle* and *M. mollis* oils exhibited the highest bacteriostatic (0.32% (v/v) for both oils) and bactericidal activity (0.64% (v/v) and 1.13% (v/v), respectively.

[1]Gram *el al.* International Journal of Food Microbiology (2002) 78:79-97. [2]Rasch *et al.* Applied and Environmental Microbiology (2005) 102: 826-837.

POSTER 2

ETHNOBOTANICAL AND BIOLOGICAL STUDY OF SOME HERBS USED AS ANTIDIABETIC IN SOUTHERN KATANGA AREA (DR CONGO)

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This study aims to inventory and evaluate biologically herbs traditionally used in the management of diabetes in the southern Katanga Province (DR Congo). Fifty-one persons, among which 49 traditional healers, were interviewed for their knowledge on diabetes identification and management, on plants and plant parts used, on methods of preparation and routes of administration. This enquiry yielded 95 plants species from 45 families. These were identified with the help of Professor Jean Lejoly (Université Libre de Bruxelles) by comparing harvested samples with reference vouchers of the Kipopo herbarium. The root bark is the organ most used, water decoction is the principal preparation mode and oral use is the predominant way of administration. This survey shows that Fabaceae (23.1 %), Euphorbiaceae (7.3 %), Apocynaceae and Loganiaceae (4.2 %, each) are the most represented families. From these 95 herbs, 31.5 % have already been reported for their use in diabetes management. Nine herbs were selected for biological study according to their popularity (most cited herbs) and the relative paucity of information on their potential antidiabetic activity. *In vivo* tests carried out with methanolic extracts (100 mg/kg) of the nine plants on a *Cavia porcellus* L. (guinea pig) Oral Glucose Tolerance Test (OGTT) model[1], indicate ($p < 0.05$) that all tested species are antihyperglycemic and most active species are *Albizia adianthifolia*; *Cassia occidentalis*, and *Vitex madiensis* with reductions of apex glucose blood concentration by 41.4 %, 43.5 % and 57.8 %, respectively. These species would be the source of future antidiabetic compounds.

[1]Matteuci, E. and Giampetro, O. Journal of Ethnopharmacology (2008) 115:163-172.

POSTER 3

PRELIMINARY ANTITUMOR AND HYPOXIA-SELECTIVE ANTIANGIOGENIC SCREENING OF SOME AFRICAN MEDICINAL PLANTS

Emilie Bony[1], Gabrielle Chataigné[1], Julia Bensemhoun[1], Romain Boidot[3], Thomas Genonceaux[1], Robert Kiss[2], Olivier Feron[3], Joëlle Quetin-Leclercq[1]. [1]Pharmacognosy Research Group, LDRI, UCL, Brussels, Belgium; [2]Laboratory of Toxicology, ULB, Brussels, Belgium; [3]Institute of Clinical and Experimental Research, UCL, Brussels, Belgium.

The relationship between angiogenesis, cancer growth and metastasis has been well-established. Angiogenesis involves the proliferation and migration of endothelial cells which plays a crucial role in solid tumor development. Moreover, hypoxia is recognized as a hallmark of most tumor types, triggers angiogenesis and accounts for an increased resistance to anticancer treatments. Therefore, hypoxia is a highly selective parameter allowing the identification of new effective compounds in decreasing tumor proliferation without side effects on healthy tissues. Based on these considerations, we realized a screening of 25 African plants for antiangiogenic and antitumor activity by measuring the cytotoxic activity of 68 plant extracts on different cell types. The antiangiogenic activity was assessed on endothelial cells (HUVEC) both in hypoxic and normoxic conditions allowing the determination of a normoxia/hypoxia (N/H) ratio, and antitumor activity was evaluated on 3 different cancer cell lines (LoVo, PC-3, and U373). Among the 68 plant extracts screened, 9 exhibited a high hypoxia-selectivity with a N/H ratio $\geq$ 2, the dichloromethane extract from *C. owariensis* being the most promising extract with a N/H ratio of 7.3 and high cytotoxicity on cancer cells. This study has demonstrated the successful use of hypoxia in the screening of plant extracts with antiangiogenic activity. Further fractionation of these crude extracts, which possess antitumor and hypoxia-selective antiangiogenic activity, is still required to identify new hypoxia-selective compounds.

POSTER 4

PHYTOCHEMICAL INVESTIGATION OF WALNUT LEAVES ESSENTIAL OIL; ANTIOXIDANT EVALUATION USING VARIOUS METHODS

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Leaves of Juglans regia L. have been widely used in folk medicine to cure many diseases including; venous insufficiency and haemorrhoidal symptomatology, and for their antidiarrheic, antihelmintic, depurative, and astringent properties. Most literature on the bioactivity of *J. regia* leaves has focused on their phenolics, but volatiles profiling was the subject of limited previous investigations, with little information on essential oil and its pharmacological effects. Thus in the present study, we contribute, for the first time, to elucidate the volatile constituents of fresh leaves of *J. regia* from Algiers region, using two different extraction methods: hydro-distillation assisted by microwave heating (MAHD) and classic hydro-distillation (HD) , and the evaluation of their antioxidant Activity. Extraction time of 1 h with MAHD provided yields 0.05% (w/w) greater than HD yields 0.03% (w/w) obtained after 3 h. Analysis by GC-MS and GC-FID have shown similar qualitative composition, but a large difference in terms of contents of the constituents was observed[1-2]. Antioxidant capacity of the oil was evaluated by three different methods: the scavenging effect on DPPH radical (2, 2-diphenyl-1-picrylhydrazyl), reducing power (the ability of essential oil to reduce Fe^{+3}) and β-Carotene/ linoleic acid bleaching assay (the ability of oil to inhibit the conjugated diene hydroperoxides arising from linoleic acid oxidation). The essential oil exhibited high antioxidant activity which was comparable to the reference standards at the same dose. These antioxidant properties are due to the chemical composition of essential oil that is rich in terpene and oxygenated terpene.[3]

[1]Boukhari *et al.* Helv. Chim. Acta (DOI: 10.1002/hlca.201200359), in press. [2]M. A. Farag JEOR (2008) 20: 323-327. [3]Rather *et al.* Phytomedicine (2012) 19:1185– 1190.

POSTER 5

RESEARCH OF ARGINASE INHIBITORS FROM NATURAL SOURCES

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Growing evidence suggests that arginase misregulation plays a key role in the physiopathology of essential hypertension. We previously showed[1] that arginase downregulates nitric oxide (NO) biosynthesis by substrate competition (L-arginine) and that endothelial dysfunction accompanying essential hypertension involved a NO production decrease. In mammalian cells, L-arginine is metabolized by two major pathways : nitric oxide synthase catalyzes its oxidation to L-citrulline and nitric oxide whereas arginase catalyzes its hydrolysis to L-ornithine and urea. These data suggest that arginase inhibitors have a great potential for the essential hypertension treatment by increasing NO availability.[2] Synthetic arginase inhibitors currently available are not usable for clinical treatment. Higher plants also utilize arginine and possess an arginase regulation system.[3] Consequently, plant material is an interesting source for arginase inhibitors research. First results concerning phytochemical investigations of sunflower seeds extracts (*Helianthus annuus*, Asteraceae), known as a source of arginase inhibitors[4], derived from chlorogenic acid, will be presented.

[1]Demougeot, C. *et al. Life Sciences* (2007) 80:1128-1134. [2]Demougeot, C. *et al. Journal of Hyperte*nsion (2008) 26:1110-1008. [3]Howe, G.A. *et al. The Journal of Biological Chemistry* (2004) 279:45998-46007. [4]Muszynska, G. and Reifer, I. *Acta Biochimica polonica* (1970) 17:247-252.

POSTER 6

ANTI-BIOFILM ACTIVITY OF RED FRUITS EXTRACTS: FOCUS ON ELLAGITANNINS ISOLATED FROM RASPBERRIES

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In the context of the prevention of dental caries or cavities (tooth decay) by natural foodstuff sources, the anti-biofilm activity of dry commercial extracts of four red fruits: cranberry (*Vaccinium macrocarpon*), raspberry (*Rubus idaeus*), blueberry (*Vaccinium myrtillus*) and acerola (*Malpighia punicifolia*), were assessed on yeasts of the *Candida* genus, which are present in the human oral cavity: *Candida albicans*, *C. glabrata* and *C. parapsilosis*. The anti-biofilm activity of these extracts was investigated using the XTT method, considering the anti-adhesion activity (biofilms aged of 2h). Raspberry extract would be efficient against the three species of *Candida* demonstrating an anti-adhesion activity from the concentration of 10 mg/mL (*C. albicans*) or from 20 mg/mL (*C. glabrata* and *C. parapsilosis*). Thus, raspberry was then selected for a phytochemical investigation. An extraction with a polarity gradient was performed on acetone extract from frozen mature and immature fruits to study the influence of the fruit maturation on the anti-adhesion activity. The two hexane extracts and the ethyl acetate extract obtained with immature raspberries showed an anti-adhesion activity on *C. albicans* at low concentrations (0.3; 0.3 and 0.07 mg/mL respectively).Their bioassay-guided fractionation by combined chromatographic methods (column chromatography: Sephadex ® LH 20 gel and preparative HPLC) permitted the obtention of ellagitannins mixture and then ellagitannins fractions of which main compounds were identified by ESI-SM. The anti-adhesion activity of these fractions was significant at very low concentrations (the order of 20 µg/mL) suggesting a real interest. In conclusion, this work demonstrated for the first time the fungal anti-adhesion potency of raspberry ellagitannins. They could prevent *Candida* growth as mono-species biofilms associated with dentures (oral candidiasis) and mixed biofilms (dental plaque).

POSTER 7

ANTIFUNGAL PLANT EXTRACTS AS NATURAL FOOD CONSERVATIVES

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Fungal contamination is a major issue for the food industry. To tackle this issue while simultaneously satisfying consumers increasing demand for synthetic conservative free products, plant extracts were investigated as natural antifungal solutions. 60 fungal contaminants isolated from contaminated cakes and brioches provided by our industrial partners were purified and identified. 6 representative contaminants were chosen as fungal targets. 19 plants selected for their potential antifungal proteins content were extracted and screened *in vitro* for antifungal activity. The water extract of *Amaranthus* sp. seeds (E1) and of *Brassica oleracea* (var. *capitata* f. *rubra*) seeds (E2) showed promising activity against respectively 3 and 4 out of 6 contaminants. *In vitro* evaluation of antifungal activity before and after proteins digestion with proteinase K as well as *in situ* antifungal activity testing of extracts containing products are undergoing.

POSTER 8

DECREASING γ-SECRETASE CLEAVAGE BY PLANT EXTRACTS SELECTED THROUGH ETHNOPHARMACOLOGY

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Background and Aim of the study: β-Amyloid peptide (Aβ) accumulation plays a key role in neurodegeneration in Alzheimer's dementia. In order to select candidates for the treatment of this disease, we investigated the activity on Aβ production of extracts obtained from 9 plant species traditionally used for dementia treatment in Benin or in Madagascar. *Methods and Results:* Several extracts were tested on CHO cells overexpressing the human neuronal Aβ precursor (APP695) to measure variations of APP processing (by Western-blotting) and, for the most active, of Aβ-amyloid production (by ECLIA). We observed, at non-toxic concentrations, that *Pterocarpus erinaceus* stem-bark aqueous extract displayed effects similar to those of DAPT (γ-secretase inhibitor) on APP processing: a significant increase in CTF/APP ratio joined to an important decrease of Aβ production. Moreover; both decreased γ-secretase-mediated APP cleavage in a membrane assay. *Conclusion:* This active extract is worth further studies to isolate the compounds responsible for the observed activity, to analyze its mode of action and determine its clinical potentials.

POSTER 9

MODULATION OF SKIN PIGMENTATION BY HERBAL EXTRACTS

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The main target of known melanogenesis inhibitors is tyrosinase (EC 1.14.18.1), the enzyme that catalyzes hydroxylation of L-tyrosine to DOPA and the oxidation of o-diphenols to o-quinones. Five medicinal plants were selected after an ethnobotanical survey among Rwandese traditional healers; twenty seven extracts of increasing polarity were prepared. After a cytotoxicity test of all extracts, their modulating effect was tested on malignant melanocytes pigmentation, on human and mushroom tyrosinase-catalyzed pigmentation (*in vitro* and by TLC-autography) and on human tyrosine hydroxylase activity (Pomerantz method). Some extracts inhibit melanogenesis (e.g. *Protea madiensis* Oliv. (Proteaceae) ethyl acetate extract; possible hypopigmentation use) whereas others activate melanogenesis (e.g. *Sesamum angolense* Welw. (Pedaliaceae) ethyl acetate extract; hyperpigmentation use); some present dual effects depending on concentration, indicating the simultaneous presence of inhibitors and activators. The bio-guided fractionation of *Protea madiensis* ethyl acetate extract yielded for the first time in this plant the compounds 2-tridecanone and oleic acid which inhibit human tyrosinase. The ethyl acetate extract of *Sesamum angolense* yielded a pentacyclic triterpenoid, probably an ursolic acid derivative, which stimulates mushroom tyrosinase activity. Full characterization of this compound structure and activity is under investigation. Promising identified hypopigmentant and hyperpigmentant molecules were identified which could be used by the cosmetics industry and warrant further clinical studies. The present study allowed to confirm the informations received from traditional healers.

POSTER 10

ETHNOPHARMACOLOGICAL SURVEY OF PLANTS USED AGAINST SICKLE CELL DISEASE IN LUBUMBASHI AREA (DR CONGO)

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An ethnobotanical survey was carried out in the area of Lubumbashi /DR Congo by interviewing thirty-five traditional practitioners about their knowledge on plant recipes used against drepanocytosis. Forty-seven plants used in folk medicine in the management of Sickle Cell Disease (SCD) and/or related illness as swellings, bone aches, anaemia, infectious and parasitic diseases have been inventoried regarding their different modes of preparation and posologies. For each cited herb, samples were collected and identified by comparing with herbarium collections at the Institut National d'Etudes Agronomiques (INERA) with the help of a botanist, Prof. Jean Lejoly. Voucher specimens were deposited for each plant at the Faculty of Sciences/University of Lubumbashi. Three families, Euphorbiaceae, Moraceae and Fabaceae, were represented in majority (20 species). Twenty-one species were quoted by more than one traditional practitioner. The modes of preparation and administration are mostly and respectively aqueous decoctions (52 %) and per os (60 %) while the most used part of the plants are the roots (40 %). According to this survey, Ficus capensis Thumb., Hymenocardia acida Tul. and Ziziphus mucronata Willd. are the most important and useful plants to treat the anaemia and sickle cell disease. In fact at least five traditional practitioners quoted each of them. These plants are under investigation for pharmacological and phytochemical studies against falciformation.

POSTER 11

AUREALIS™ IMPROVES SKIN MOISTURIZING AND EXHIBITS ANTI-WRINKLE PROPERTIES: FROM IN VITRO STUDY TO CLINICAL EVIDENCE

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Without adequate moisture, skin can become dull and lifeless. Moisturizing is therefore essential to achieving healthy, radiant skin. Treating dryness from the inside out by restoring the effective glycosynthesis allows the skin to regain proper hydration over time and may prevent the first signs of aging[1]. In the extracellular matrix, glycoaminoglycans (GAG) are the main compounds implicated in the hydration process. Hyaluronic Acid (HA), the most important of these compounds, regulates skin moisture levels thanks to its excellent water-retention properties[2]. The flowers of *Citrus aurantium* var *amara*, widely distributed and cultivated all over the world, are traditionally used for its soothing properties. However, no scientific evidence is available to ensure this activity on human skin. Aurealis™, a developed extract of flowers of bitter orange, was assessed on its ability to enhance the production of hyaluronic acid (HA) on an *in vitro* model of normal human epidermal keratinocytes (NHEK). After 72h stimulation at 0.2 mg/mL, the release of HA was increased by 44%. These first results were confirmed on normal human skin explants. Aurealis™, tested at 0.1% during 10 days, showed respectively 33% and 183% increase of HA and GAG in the extracellular matrix of the epidermis. A double-blind *vs.* placebo *in vivo* study was then conducted, under dermatologist control during 3 months, on 2 groups of 20 volunteers. The measurement of wrinkle depth by 3D profilometry revealed a 6.6% and 9.1% reduction at days 56 and 84, respectively. The Aurealis™ group demonstrated a 10.8% overall decrease in wrinkle depth *vs.* placebo. These results confirm the effective use of Aurealis™ as moisturizing and anti-wrinkle agent.

[1]Chuttani A, Gilchrest BA (1995) Skin. In: Mazurek S, ed. *Handbook of Physiology*; Section 11: Aging. New York: Oxford University Press. pp 309–324. [2]Papakonstantinou E, Roth M and Karakiulakis G (2012) *Dermato-Endocrinology* 4:3, pp 253–258.

POSTER 12

EFFECTS OF AERIAL PARTS EXTRACTS OF *JUSTICIA SUBSESSILIS* OLIV. ON METHICILLIN - RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

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In traditional Burundian medicine, the aerial parts of *Justicia subsessilis* Oliv. are used in the treatment of diverse bacterial infections. In this context, we studied the effect of this plant against MRSA. Successive extracts of aerial parts powder with solvents of increasing polarity were prepared by percolation. After a preliminary phytochemical screening, the antibacterial activity of these extracts were evaluated on two MRSA strains using broth microdilution and agar diffusion methods. A modulating effect on antibiotic resistance was studied for the extracts without direct antibacterial activity. The methanolic extract showed antibacterial effect on both MRSA strains with a MIC of 250 µg/ml; whereas the dichloromethane and aqueous extracts were active at 500 µg/ml and the hexane and ethyl acetate extracts were inactive. Antibiograms effected on Mueller Hinton agar incorporating this latter extract however led to restoration or enhancement of some antibiotics activity. Diameters of inhibition considerably increased in the presence of oxacillin (from 11.7 to 25 mm), amoxicillin (from 13.2 to 26.6 mm), ofloxacin (from 9.5 to 25.2 mm) and ampicillin (from 10.8 to 24.3 mm). The methanolic extract showed antibacterial effect against MRSA strains that may support the use of this plant in the treatment of bacterial infections in traditional Burundian medicine.

POSTER 13

METHOD OF EVALUATION OF ANTIOXYDANT AND SCAVENING RADICALS PROPERTIES OF ACTIVE SUBSTANCES IN CARAPA PROCERA DC-A CAMEROONIAN PLANT

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Many diseases are reslut of a discoder between production of Oxygen Reactive Secies (ORS) and ability of body to neutralize and correct damages due to oxidative effects. These diseases are known as « Oxidative Stress ». On the other band, some others diseases are inducced by the presence of chemicals elements witch one many single, non-paired electrons on the external layer and able of free existence and called « free radicals ». Many plants of Cameroon flora are used by traditional doctors to relieve or treat patients. Some of the plants contain active principles whose activity is linked to inhibition of ORS. Researches of compounds presenting antioxydant and scavenging activity were made on methanolic and chloroformic back extracts of Carapa procera DC (Meliaceae). The plant was harvested in Abon bang (East region of Cameroon) and is used as antipyretic by BAKA's pygmy people. Research of scavening radical and antioxydants compunds by TLC shoxn on chrmatograms yellow stains on white background revealing that the degradation of betacarotene were inhibited, thus bringing out the antioxydant activity. Elsewhere, chromatograms expressed by DPPH (2,2 – diphényles – I – pierylhydrazy) showed white stains on violet background revealing scavenging activity. Phytochemical studies of Carapa procera lead to characterized groups of actives substances including scavealing phenols, flavonoids, and tannins.

POSTER 14

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 $\leq$ 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.

POSTER 15

MESURE DU POUVOIR ANTI-INFLAMMATOIRE DES EXTRAITS ETHANOLIQUES DE *FRAXINUS ANGUSTIFOLIA* ET *PISTACIA LENTISCUS*

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Fraxinus angustifolia et *Pistacia lentiscus,* sont des plantes utilisées dans la médecine traditionnelle pour le traitement de diverse pathologies notamment les inflammations. Notre travail s'est intéressé à l'étude de l'activité anti-inflammatoire de l'extrait éthanolique d'écorces de *Fraxinus angustifolia* et de feuilles de *Pistacia lentiscus.* L'activité anti-inflammatoire de l'extrait éthanolique de ces deux plantes sur une inflammation aiguë chez les souris induit par la carragénine a montré une diminution significative de l'œdème aussi bien pour le Diclofenac (molécule de référence) que pour l'extrait éthanolique dès la 2ème heure et atteint son maximum à la 3ème heure, de 37.73% pour les feuilles de *Pistacia lentiscus,* alors que l'extrait éthanolique d'écorces de *Fraxinus angustifolia* a montré un pourcentage d'inhibition important de 50, 96% à une dose de 200 mg/kg.

POSTER 16

YEAST KILLER TOXIN AGAINST TWO CANDIDA SPECIES IN MIXED BIOFILM

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A yeast killer toxin produced by the yeast *Pichia anomala* (*Pa*KT) was characterized by the wide spectrum of its antimicrobial activity. The susceptibility of *Pneumocystis carinii*, *Candida albicans* to *Pa*KT has been demonstrated by in vitro attachment tests and in vivo infectivity assays. The aim of this study was to evaluate the antimicrobial activity of *Pa*KT on different *Candida* biofilms, mimicking the colonization of medical devices by microorganisms. This study compared the development of the mixed *C. albicans* and *C. krusei* biofilm with or without *Pa*KT. Several parameters such as temperature (25, 30, 37°C) and time course (2, 24, 48, 72, 96 hours), were investigated to obtain *C. albicans* or *C. krusei* biofilms[1]. In terms of the mixed biofilms, *C. albicans* was the most prevalent in the biofilm at early stages (2h and 24h incubation). But at 48h and 72h, *C. krusei* widely colonized the stainless steel surface (10-fold more). And the number of *C. krusei* was higher from 24h incubation on the mixed biofilm compared to the biofilm constituted by *C. krusei* only. The mixed *C. albicans* and *C. krusei* biofilms favoured *C. krusei* development. The addition of *Pa*KT in the mixed biofilm, induced a significant reduction in both *C. albicans* and *C. krusei* development. From 24h incubation at 25°C, only 28% of *C. krusei* and 47% of *C. albicans* are present in the mixed biofilm with *Pa*KT to compare with the mixed biofilm without *P. anomala*. It is interesting to note that the effect of the killer toxin was more important on *C. krusei*. At 48h, only 4% of *C. krusei* cells were alive compared to 17% of *C. albicans* in mixed biofilm with *P. anomala*. This effect of the killer toxin was quantity-dependent. These results strongly support the potential interest for the development of new strategies for prevention and control opportunistic microorganisms colonizing medical prosthetic implants based on the yeast killer phenomenon.

[1]Séguy N, Mazars E. 2009. *J. Mycol. Med*., **19**: 204-205.

POSTER 17

STUDY OF THE ANTIFUNGAL ACTIVITY OF DIFFERENT EXTRACTS OF *CENTAUREA DILUTA AIT. SUBSP. ALGERIENSIS* (COSS. & DURIEU)

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The genus *Centaurea* (Asteraceae) comprises more than 500 species, most of them growing around the Mediterranea and in Western Asia. *Centaurea* species have long been used for their biological properties, mainly as anti-inflammatory, antipyretic, cytotoxic, antibacterial, antiproliferative and antifungal. Phytochemical researches revealed that flavonoids and sesquiterpene lactones are mainly responsible for major pharmacological properties. Aerial parts of *Centaurea diluta Ait. subsp. algeriensis* (Coss. & Durieu), an endemic species to Algeria and Maroc[5], were collected from the Aïn Maabed near Djelfa in the central part of Northern Algeria in June 2012. Extract of aerial parts of *C. diluta Ait.* (Asteraceae) were obtained after two treatments. First air-dried material (1500 g) was powdered and macerated at room temperature with MeOH/H_2O (77:23, v/v; 15 L) for 48 h, three times, and concentrated. The obtained residue was extracted by solvents with increasing polarities to yield six extracts: Petroleum ether (0.1 g), $CHCl_3$ (4.0 g), EtOAc (3.6 g), *n*-butanol (19.2 g); MeOH (1.2 g) and aqueous (95.2g). All of these fractions were tested against phytopathogens, including *Fusarium oxysporum*, *Cladosporium cucumerinum*, *Botrytis cinereae*, *Colletotrichum*, *Rhodotorula* [P1], *Pseudomonas syringae* [P2], *Pseudomonas syringae*. First results show only the petroleum ether extract was active against *Fusarium oxysporum* (MIC: 62.5 µg/ml). This extract will be further fractionated to elucidate the active compound(s).

POSTER 18

ETUDE PHYTOCHIMIQUE DES RACINES DE *GLYPHAEA BREVIS* (MALVACEAE)

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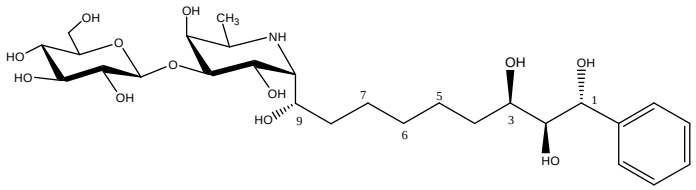
Glyphaea brevis Spreng. (Monach.), a Malvaceae, is widely distributed in Africa and South America. It is valued as vegetable and various therapeutic uses such as treatment of respiratory diseases, diarrhea, and dysentery are reported. Recent work also highlighted its anticonvulsant properties. The phytochemical investigation of the methanol aqueous 80% extract of the roots of this species led to isolation and characterization of 10 new aryl alkyl *C*-glycosides and a new cinnamide glucoside derivative. The structure elucidation of these compounds was established by analysis of spectroscopic data (HRESIMS, and 1D and 2D NMR).



POSTER 19

ANTITRYPANOSOMAL COMPOUNDS FROM THE ESSENTIAL OIL AND EXTRACTS OF *KEETIA LEUCANTHA* LEAVES WITH INHIBITOR ACTIVITY ON *TRYPANOSOMA BRUCEI* GAPDH

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Keetia leucantha is a West African tree used in traditional medicine to treat several diseases among which parasitic infections. The dichloromethane extract of leaves was previously shown to possess growth inhibitory activities on *Plasmodium falciparum*, *Trypanosoma brucei brucei* and *Leishmania mexicana mexicana* with low or no cytotoxicity (>100 µg/ml on human normal fibroblasts) (1, 2). In continuation of our investigations on antitrypanosomal compounds, we analyzed the major triterpenic acids in this dichloromethane extract by LC–MS and also the leaves essential oil obtained by hydrodistillation by GC-FID and –MS. Forty-one compounds were identified in the oil whose percentages were calculated using the normalization method. The essential oil, seven of its constituents and the three triterpenic acids were evaluated for their antitrypanosomal activity on *Trypanosoma brucei brucei* bloodstream forms (*Tbb* BSF) and procyclic forms (*Tbb* PF) to identify an activity on the glycolytic process of trypanosomes. The oil showed an IC_{50} of 20.9 µg/ml on *Tbb* BSF and no activity was observed on *Tbb* PF. The best antitrypanosomal activity was observed for ursolic acid with IC_{50} of 2.5 and 6.5 µg/ml respectively on *Tbb* BSF and *Tbb* PF. The inhibitory activity on a glycolytic enzyme of *T. brucei*, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was also evaluated for betulinic, olenaolic and ursolic acids, phytol, α- and β-ionone. The three triterpenic acids and β-ionone showed inhibitory activities on GAPDH with oleanolic acid being the most active with an inhibition of 72.63% at 20 µg/ml. These results may in part explain antitrypanosomal activities (3).

[1]Bero, J., Ganfon, H., Jonville, M.C., Frederich, M., Gbaguidi, F., Demol, P., Moudachirou, M., Quetin-Leclercq, J., 2009. Journal of Ethnopharmacology 122, 439–444. [2]Bero, J., Hannaert, V., Chataigne, G., Herent, M.-F., Quetin-Leclercq, J., 2011. Journal of Ethnopharmacology 137, 998–1002. [3]Bero, J., Beaufay C., Hannaert, V., Herent, M.-F., Michels P.A., Quetin-Leclercq, J., 2011. Phytomedicine 20, 270– 274.

POSTER 20

ANTICANCER EFFECT OF ROOTS AND SEEDS EXTRACT OF ALGERIAN *PEGANUM HARMALA* L.

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Peganum harmala L. (Zygophyllaceae) is distributed in a large area of the world and can grow in semi-arid and pre-desert regions. This plant is rich in alkaloids, including β-carbolines derivatives (harmine, harmaline, harmalol, harman, tetrahydroharmine and harmol) and quinazoline derivatives (vasicine and vasicinone). Because of its wild spectrum of physiological activity, Peganum harmala found its way in traditional medicine. Its alkaloids have been found to present anticancer, antibacterial and antiviral activities. However, the most important effect of harmala alkaloids is certainly the inhibition of monoamine oxidases that leads to hallucinogen effects. Indeed, evidence indicates that harmine and harmaline are hallucinogenic in humans. In this study, we evaluated the cytotoxic effects of roots and seeds extracts on a human lung adenocarcinoma epithelial cell line (A549), a human glioblastoma cell line (U373), a human glioma cell line (Hs683), a human melanoma cell line (SK-MEL-28), a mouse melanoma cell line (B16F10) and a human breast cancer cell line (MCF7) (0.01 – 100 µg/ml). The cell viability was determined using MTT test. Both extracts showed potent antiproliferative activity against all cells in a dose-dependent manner, IC50 ranging from 1 to 13 µg/ml. In vitro quantitative videomicroscopy analyses focused on anti-proliferative effects on A 549 cell lines and indicated that the extract exhibit a cytostatic effect.

POSTER 21

REVEALING THE ANTI-TUMORAL EFFECT OF ALGERIAN *GLAUCIUM FLAVUM* ROOT AGAINST HUMAN CANCER CELLS

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Glaucium flavum (*G. flavum*) is a plant from the Papaveraceae family native to Algeria where it is used in local traditional medicine to treat warts. *G. flavum* root crude alkaloid extract inhibited breast cancer cell proliferation and induced G2/M phase cycle arrest and apoptosis without affecting normal cells, which is a highly awaited feature of potential anti-cancer agents. *G. flavum* significantly reduced growth and vascularization of human glioma tumors on chicken chorioallantoic membrane (CAM) *in vivo*. The chromatographic profile of the dichloromethane extract of *G. flavum* root showed the presence of different constituents including the isoquinoline alkaloid protopine, as the major compound. We report for the first time that *G. flavum* extract may represent a new promising agent for cancer chemotherapy.

POSTER 22

ANTI-AGES ALGERIAN VEGETAL EXTRACTS. PHYTOCHEMICAL STUDY OF *DAUCUS AUREUS* DESF. (APIACEAE)

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In a large project on Algerian plants, we are interested in their phytochemistry and biological properties (PHC franco-algérien Tassili, 2012-2016). Particularly, in diabetes, a chronic hyperglycaemia occurs which is responsible for complications of diabetes through advanced glycation end-products (AGEs) formation.[1] SONAS lab recently developed an automated HTS assay, suitable for compounds and extracts, to evaluate their anti-AGEs potential.[2] This assay was used to select potent anti-AGEs extract from a selection of plant species traditionally used for the treatment of diabetes in Algeria.[3] Among them, EtOAc and BuOH extracts from *Daucus aureus* (Apiaceae) were selected and their phytochemistry studied. Moreover, MeOH extract led to the isolation of flavonoid O-glucosides, which structures were elucidated by spectroscopic methods, including UV, MS, 1D and 2D NMR.

[1]WHO, Diabetes. Available at: http://www.who.int/topics/diabetes_mellitus/en/. Accessed 20 June 2012. [2 a)] Derbré *et al.* Anal. Bioanal. Chem. (2010**)**, 398, 1747; [b)] Séro *et al.* Food Chem. Submitted. [3]See Amel Achouri poster.

POSTER 23

PRELIMINARY STUDY OF PHENOLIC COMPOUNDS IN *MISCANTHUS X GIGANTEUS* AND THEIR EFFECTS ON BATERIAL GROWTH

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Miscanthus x giganteus is a perennial grass hybrid well known for its ability to tolerate stressful environment such as low or high temperature, organic and metal pollution. This study focused on the chemical composition of phenolic compounds in roots of giant *Miscanthus* grown in contaminated and non-contaminated soils and their effect on the growth of bacteria under different conditions. The phytochemical investigation of chloroform and methanol root extracts was conducted using reverse phase high performance liquid chromatography (RP-HPLC) and liquid chromatography coupled with mass spectrometry (LC-MS). The antimicrobial activities of these extracts against 6 strains including *Stenotrophomonas rhizophila, Pseudomonas aeruginosa,* and 4 strains of *S. maltophilia* were tested using microplate assay. The results showed no qualitative difference in the extract compositions between plants grown in contaminated or non-contaminated conditions. The presence of chlorogenic acid was confirmed and structures of 4 other derivatives were proposed based on UV and MS/MS spectra. We observed effects of these extracts on the growth of bacteria and these influences may change when experiments are taken at different temperatures. For most strains, the presence of methanol extract may promote the growth rate of bacterial strains and circumvent the negative impact of an antibiotic (nalidixic acid) or a metal (zinc), while chloroform extracts showed inhibitory impact on the growth of strains.

POSTER 24

UNUSUAL AMINO ACIDS AND MONOFLUOROACETATE FROM *DICHAPETALUM MICHELSONII* (UMUTAMBASHA), A TOXIC PLANT FROM RWANDA

Virginie Esters[1], Charles Karangwa[2], Monique Tits[1], Pierre Francotte[3], Bernard Pirotte[3], Anne-Catherine Servais[4], Marianne Fillet[4], Jacques Crommen[4], Elmar Robbrecht[5], Arlette Minet[6], Thierry Grisar[6], Luc Angenot[1], Michel Frederich[1] [1]Laboratory of Pharmacognosy, Centre Interfacultaire de Recherche du Médicament (CIRM), University of Liège, Belgium; [2]Laboratory of Pharmacology and Toxicology, Department of Pharmacy, National University of Rwanda, Rwanda; [3]Laboratory of Medicinal Chemistry and [4]Laboratory of Analytical Pharmaceutical Chemistry, CIRM, University of Liège, Belgium [5]Department of Vascular Plants, National Botanic Garden of Belgium, Belgium; [6]Center for Cellular and Molecular Neurobiology (CNCM), University of Liège, Belgium.

In the course of our investigations on Umutambasha in order to identify its convulsant principles, small quantities of monofluoroacetate were detected (capillary electrophoresis with an indirect UV detection, ^{19}F-NMR spectroscopy) in stem bark, leaves, and fruits of this plant newly identified as *Dichapetalum michelsonii* Hauman. Conclusive evidence for a monofluoroacetate presence came from its isolation from the freeze-dried extract of stem bark. Three free unusual amino acids, named, *N*-methyl-α-alanine, *N*-methyl-β-alanine, and 2,7-diaminooctan-1,8-dioic acid, described for the first time in a plant, and known trigonelline were also isolated from the stem bark of *D. michelsonii* by preparative HPLC performed with HILIC support. Structure elucidations were mainly achieved by spectroscopic methods (^{1}H-NMR, 2D-NMR, MS) and by comparison with authentic references. These unusual amino acids were detected by a fast, reliable TLC analysis in all our batches of Umutambasha, suggesting that they could be used for identification purposes in case of human or livestock intoxications. Finally, electro-encephalographic recordings and behavioural observations performed in mice suggested that the convulsive patterns produced by Umutambasha are the consequence of monofluoroacetate presence in *D. michelsonii.*

POSTER 25

NEW PRENYLATED CHALCONE FROM *FISSISTIGMA LATIFOLIUM* AS A MODULATOR OF BCL-XL/BAK AND MCL-1/BID INTERACTIONS

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It is now well established that the over-expression of anti-apoptotic proteins such as Bcl-xL and Mcl-1 plays a role in cancer development and can be correlated with resistance to cancer therapeutics. These proteins are thus considered to be challenging targets for the development of novel anti-cancer treatment, but a strict inhibition of Bcl-xL may result in apoptosis "escape" through Mcl-1 pathway.[1] The search for dual inhibitors is therefore essential to restore the apoptotic process. Thus, similarly to the methodology used for Bcl-xL/Bak interaction[2], an affinity displacement assay using fluorescent polarization, based on the binding of a fluorescein-labeled peptide (BH3-domain of Bid) to Mcl-1, has been developed. A biological screening on the modulation of Bcl-xL/Bak interaction was initially conducted on 9 000 plant extracts, leading to the isolation of new active compounds such as meiogynin A[2] or kingianin G[3]. The most active extracts were also screened on Mcl-1/Bid interaction. The bioguided fractionation of the EtOAc bark extract from *Fissistigma latifolium* led to the isolation of one new prenylated chalcone with potent binding affinity for Bcl-xL and Mcl-1, and four new substituted monoterpenoids. The isolated compounds possess a carbon skeleton that we suggest to be biologically formed *via* an enzymatic catalysis of a Diels-Alder reaction. In this communication, we report the development of the bioassay on Mcl-1/Bid interaction and the phytochemical analysis of *Fissistigma latifolium*.

[1]Tse *et al*. Cancer Res. (2008**)** 68:3421-3428. [2]Litaudon *et al.* J. Nat. Prod. (2009), 72: 480–483. [3]Leverrier *et al.* Phytochemistry (2011) 72:1443-1452.

POSTER 26

ANTI-PLASMODIAL ACTIVITY OF *DICOMA TOMENTOSA* (ASTERACEAE): UROSPERMAL A-15-O-ACETATE IDENTIFIED AS THE MAIN ACTIVE COMPOUND

Olivia Jansen[1], M. Tits[1], L. Angenot[1], JP Nicolas[2], JB Nikiema[3], M. Frédérich[1]. [1]Laboratoire de Pharmacognosie, Centre Interfacultaire de Recherche du Médicament (CIRM), Université de Liège, Belgium ; [2]Association Jardins du Monde, 15, rue St Michel, 29190 Brasparts, France ; [3]Unité de Formation et de Recherche en Sciences de la Santé, Université de Ouagadougou, Burkina Faso.

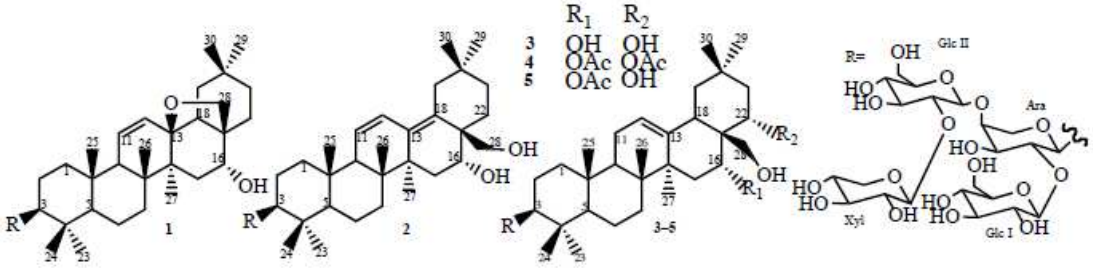
Dicoma tomentosa (Asteraceae) is traditionally used in Burkina Faso to treat malaria. The aim of the present study was to investigate the anti-plasmodial potential of this plant and to isolate the active compounds. All 8 crude extracts obtained from the whole plant showed *in vitro* antiplasmodial activity against two *Plasmodium falciparum* strains (3D7 and W2) and strong activity (IC50<5µg/ml) was observed for 4 extracts. This confirms the traditional use and the promising anti-malarial potential of the plant. However, most of the active extracts also exhibited cytotoxicity, but no haemolytic activity. An *in vitro* bioguided fractionation allowed to isolate and identify urospermal A-15-O-acetate, a sesquiterpene lactone (melampolide) as the major anti-plasmodial compound of the plant (IC50 < 1 µg/ml against both 3D7 and W2 strains). This product was also found to be the main cytotoxic compound (SI = 3.3). While this melampolide has already been described in the plant, this study is the first report on the biological properties of this compound. Its high activity merits further study to elucidate its mechanism of action against *P. falciparum*. The active extracts of *D. tomentosa*, as well as urospermal A 15-O-acetate, displayed only a moderate selectivity, and further investigations are needed to assess the safety of the use of the plant by the local population.

POSTER 27

POLYCARPAEA CORYMBOSA AS SOURCE OF CYTOTOXIC TRITERPENOIDS

Mahenina Jaovita Manase[1], Anne-Claire Mitaine-Offer[1], Tomofumi Miyamoto[2], Chiaki Tanaka[2], Stéphanine Delemasure[3], Patrick Dutartre[3], and Marie-Aleth Lacaille-Dubois[1]. [1]EA 4267 (FDE/UFC), Laboratoire de Pharmacognosie, Université de Bourgogne, BP 87900, 21079 Dijon Cedex, France, [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812–8582, Japan, [3]Cohiro, UFR de Médecine de Dijon, BP 87900, 21079 Dijon Cedex, France.

Four new triterpenoid saponins **1**–**4**, were isolated from *Polycarpaea corymbosa* Lamk. var. *eriantha* Hochst, along with the known apoanagallosaponin IV (**5**). The isolation was achieved by successive solid/liquid column chromatography (VLC, FLASH, MPLC) and the structures were elucidated by spectroscopic data analysis (1D and 2D NMR, HR-ESIMS). Compounds **1**, **3**–**5** were evaluated for cytotoxity against SW480, DU145 and EMT6 tumor cell lines, and **1** was the most active (IC50 4.61-22.61 μM), in comparison with etoposide. Compound **2** tested against SW480 and a cardiomyoblast cell line (H9c2) was inactive (IC50 >50 μM).

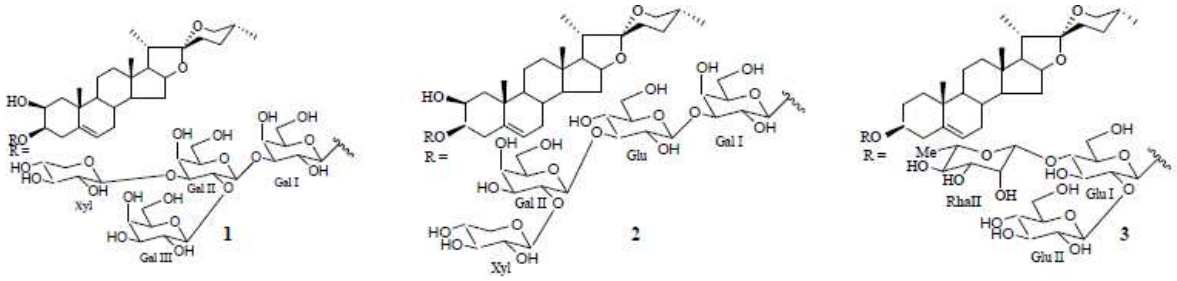


POSTER 28

CYTOTOXIC STEROIDAL SAPONINS FROM *ALLIUM FLAVUM* L.

Abdelmalek Rezgui[1], Anne-Claire Mitaine-Offer[1], Thomas Paululat[2], Stéphanie Delemasure[3], Patrick Dutartre[3] and Marie-Aleth Lacaille-Dubois[1]. [1]Laboratoire de Pharmacognosie, EA 4267, FDE / UFC, UFR Pharmacie, Université de Bourgogne, 7, Bd Jeanne d'Arc, BP 87900, 21079 Dijon, France. [2]Universität Siegen, OC-II, Naturwissenschaftlich-Technische Fakultät, Adolf- Reichwein-Str. 2, D-57076 Siegen, Germany. [3]Cohiro, UFR Médecine,7, Bd. Jeanne d'Arc, BP87900, 21079 Dijon, France.

Three new steroidal saponins **1-3** were isolated from *Allium flavum* L. (Amaryllidaceae) using successive solid /liquid chromatographic techniques (VLC, MPLC, and Flash). Their structures were elucidated on the basis of extensive 1D and 2D NMR experiments and mass spectrometry. The three spirostane-type saponins were chosen for cytotoxicity bioassay against a human cancer cell line (colorectal SW480) and showed moderate cytotoxic activity compared to an anticancer control, doxorubicin.



POSTER 29

BIOACTIVE STEROIDAL SAPONINS FROM ASPARAGACEAE SPECIES

Jacqueline Eskander[1], Catherine Lavaud[2], and Ola K. Sakka[3]. [1]Pharmacognosy Department, Helwan University, POB 11795, Ain-Helwan, Cairo, Egypt. [2]Institut de Chimie Moléculaire de Reims (ICMR), UMR-CNRS 7312, BP 1039, 51687 Reims cedex 2, France. [3]Department of Chemistry, American University of Cairo, POB 74, New Cairo 11835, Egypt.

Asparagaceae previously classified to the family Liliaceae, is divised in seven subfamilies among them Agavoideae and Nolinoideae which include many species growing in dry area, and ornemental plants. These families are known to be a rich source for steroidal saponins and sapogenins used as raw material for hormones synthesis, in addition to other various biological activities. In a frame of phytochemical studies on plants growing in Egypt of medicinal value, we studied three species of Asparagaceae: *Agave macroacantha*[1], *Yucca de-smetiana*[2], and *Beaucarnea recurvata*[3]. Twenty-one steroidal saponins were isolated among them eight are new compounds. Their structures were assigned using a combination of 2D NMR techniques and confirmed by ESI-MS^n. The saponins of *Y. de-smetiana* were evaluated for their antitumoral activity against four cell lines. A molecular modeling approach was performed to establish direct influence of conformational criteria on cytotoxicity activity.

[1]J. Eskander, C. Lavaud, D. Harakat, *Fitoterapia*, **2010**, 81, 371-374. [2]J. Eskander, O.K. Sakka, D. Harakat, C. Lavaud, *Med Chem Res*, **2013**, DOI 10.1007/s00044-013-0497-4. [3]J. Eskander, C. Lavaud, D. Harakat, *Phytochemistry*, **2011**, 946-951.

POSTER 30

MULTIDISCIPLINARY APPROACH OF INSECTS METABOLITES : THINKING OUTSIDE BOXES

L.-M. Dao[1]; A. Maciuk[1]; S. Cojean[1], F. Roblot[1], J. Orivel[2], P. Coulerie[3], A. Magro[4], C. Ducamp[4], F. Ramon-Portugal[4], C. Duplais[2],, E. Poupon[1], B. Figadère[1]. [1]UMR 8076 CNRS BioCIS, Faculté de Pharmacie, Université Paris-Sud. [2]UMR CNRS-INRA Ecofog, UAG, Kourou/Cayenne, Guyane. [3]IAC, Nouméa, Nouvelle-Calédonie. [4]ENFA, Toulouse.

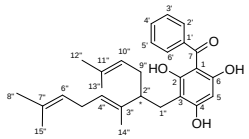
When compared to flora, insect world is commonly referred as a vast, underexplored source of biodiversity, either from the taxonomy, ecology, chemistry or biology point of view. Chemistry can federate several scientific fields, as it encompasses intra or interspecies communication, metabolomic specificities, and beyond, biological and potentially therapeutic activities. Our group of laboratories federates several expertises and expectations, based on the knowledge chemistry can bring up. Chemistry and antiplasmodial activities of compounds are described here for ants, bugs and ladybugs, showing that the exploration of insect chemical space can be fascinating for biologists as well as pharmacognosists.

POSTER 31

ANTIMICROBIAL ACTIVITY OF PRENYLATED BENZOYLPHLOROGLUCINOL DERIVATIVES FROM THE LEAVES OF *GARCINIA GOUDOTIANA*

Sania Mahamodo[1], Céline Rivière[2,3], Christel Neut[2,4], Amin Abedini[2,3], Vincent Roumy[2,3], Thierry Hennebelle[2,3], Sevser Sahpaz[2,3], Dorothée Razafimahefa[1], Bakonirina Razanamahefa[1], François Bailleul[2,3] and Bakolinirina Andriamihaja[1]. [1]Laboratoire de Chimie Appliquée aux Substances Naturelles, Faculté de Sciences, Université d'Antananarivo, BP 566, Antananarivo 101, Madagascar, [2]Univ Lille Nord de France, F-59000, Lille, France, [3]UDSL, Laboratoire de pharmacognosie, EA 4481 (GRIIOT), F-59000, Lille, France, [4]UDSL, INSERM U995, UFR Pharmacie, F-59000 Lille, France.

Within the framework of a research project entitled Biodiversity Conservation and Drug Discovery in Madagascar of the International Cooperative Biodiversity Groups (ICBG-Madagascar) aimed at exploring flora of Madagascar, one endemic Clusiaceae, *Garcinia goudotiana* (Planch. & Triana) P. Sweeney & Z.S. Rogers, locally known as Kimbaletaka, has been selected for its antimicrobial activity. The crude extract as well as methylene chloride and ethyl acetate partitions of this species demonstrated antimicrobial activity against several strains, in particular bacillus and cocci Gram+ (*Staphylococcus*, *Enterococcus*, *Corynebacterium*) with a CMI ranging from 19.5 to 78 µg/mL. The bioactivity-directed fractionation of the methylene chloride partition led to the isolation of two new prenylated benzoylphloroglucinol derivatives (**1**-**2**). Other compounds are still in identification.



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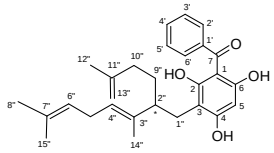

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POSTER 32

PLANTLET EXTRACT OF RHEALBA® OATS: EVIDENCE OF PROTEIN-FREE CONTENT FOR APPLICATION ON ATOPIC SKIN

Anne Mandeau, Valérie Teysseyre, Marie-Françoise Aries, Véronique Crebassa-Trigueros, Jean-François Boé, Bernard Fabre Centre de R&D Pierre Fabre, Toulouse, France.

Owing to their high content of flavonoids and saponins, plantlets of *Avena sativa* L. (Poaceae) are likely to possess anti-inflammatory and immunoregulatory properties of value in the treatment of atopic dermatitis (AD). Atopic patients and especially children are prone to sensitisation toward aero-allergens or dietary proteins (such as ovalbumin, peanut, cereal proteins, etc.) With a view to its potential use in atopic subjects at risk of developing sensitisation to dietary proteins, we prepared a plantlet extract without proteins and isolated 2 flavonoids, isoorientin-2''-*O*-arabinoside and isovitexin-2''-*O*-□arabinoside, and two saponins, avenacosides A and B. The absence of protein in this extract was evidenced by electrophoresis and Western immunoblotting. Furthermore, Western immunoblotting demonstrated the absence of cross-reaction between grain and plantlet proteins. We evaluated the anti-inflammatory activity of the plantlet extract and its compounds *in vitro* in a model of keratinocyte inflammation*:* 6-keto prostaglandin F1α production was inhibited by the plantlet extract and isoorientin-2''-*O*-arabinoside. Intracellular interleukins production in activated T lymphocytes was also inhibited, demonstrating their immunoregulatory activity in vitro. These biochemical and pharmacological properties and its proven protein-free composition make oat plantlet extract an ingredient of potential interest in topical products intended for atopic subjects.

POSTER 33

VASORELAXANT PROANTHOCYANIDINS FROM *HYMENOCARDIA ACIDA* TUL.

F. Nsuadi-Manga[1,4], C. El Khattabi[1], J. Fontaine[1], G. Berkenboom[1], P. Duez[2], C. Noyon[3], P. Van Antwerpen[3], J. Lami-Nzunzu[4], and S. Pochet[1]. [1]Laboratory of Physiology and Pharmacology, [2]Laboratory of Pharmacognosy, Bromatology and Human Nutrition and [3]Laboratory of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Université Libre de Bruxelles, Bd du Triomphe, 1050 Brussels, Belgium, [4]Laboratory of Bio-Organic Research, Faculty of Pharmaceutical Sciences, Université de Kinshasa, BP 212 Kin XI, D. R. Congo.

Decoctions of *Hymenocardia acida* root bark (Euphorbiaceae) are traditionally used in D. R. Congo for the treatment of many diseases including hypertension. In a previous study, we showed that the methanolic extract from this part of plant has an endothelium-dependent vasorelaxant effect on isolated rat thoracic aorta. This study was conducted to determine the nature of the compounds responsible for the vasorelaxant activity. *Hymenocardia acida* root bark (HaRB) methanolic extract was fractionated on a column of polyamide to yield 3 fractions (F1, F2 and F3). The vasorelaxant effect of the different fractions was studied on endothelium-intact aortic rings pre-contracted with phenylephrine (PE, 1 µM). As the compounds responsible for the vasorelaxant activity appeared to be high-molecular weight proanthocyanidins, we carried out the analysis of the thiolysis products of the most active fraction by liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS). Fraction F3 was the most potent fraction and elicited a concentration-dependent relaxation (96.6 ± 1.8 % at 3 µg/mL, EC_{50} = 1.0 µg/mL, n = 8). The thin-layer chromatography and the HPLC profiles indicated that this fraction contained presumably polymeric compounds. The LC/ESI-MS analysis of the thiolysis products showed that the fraction F3 is a mixture of procyanidins and prodelphinidins, with a predominance of procyanidins. The mean degree of polymerization was found to be 15. The vasorelaxant activity of *Hymenocardia acida* root bark appears to be partly due to polymeric procyanidins and prodelphinidins.

POSTER 34

BURKINABINS, ANTISICKLING VANILLIC ACID DERIVATIVES FROM *ZANTHOXYLUM ZANTHOXYLOIDES*, A PLANT USED IN TRADITIONAL MEDICINE IN BURKINA FASO

B. Ouattara, O. Jansen, P. Fondu, I.P.Guissou, L. Angenot, M. Tits, M. Frédérich - Université de Liège (ULg). Laboratoire de pharmacognosie, Centre Interfacultaire de Recherche sur le Médicament (CIRM), Belgique.

Sickle cell anemia is a public health problem in many countries particularly in Africa. It is a genetic disease responsible of many clinical complications in affected patients. The root bark of *Zanthoxylum zanthoxyloides Lam* (Rutaceae) is widely used in folk medicine for its antisickling properties in Burkina Faso and other West African countries[1]. With the aim to confirm this empirical practice, we tested different extracts of this plant with a deoxygenating device[2] and we studied the phytochemical composition of the extract.[3]. The samples were particularly rich in vanillic acid derivatives; three new divanilloylquinic acids showed the best antisickling properties: 3-4-O- divanilloylquinic acid or burkinabin A, 3-5-O-divanilloylquinic acid or burkinabin B and 4-5-O-divanilloylquinic acid or burkinabin C.[3] This plant could have an interesting place in the treatment of sickle cell disease and burkinabins could be used for the standardization of the future herbal medicine.

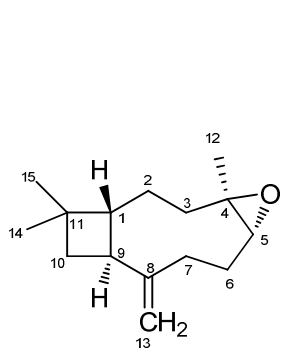
[1]Bossopki, I., 2003. Etude des activités biologiques de *Fagara zanthoxyloides Lam*. (Rutaceae). Pharmacy thesis. Faculté de Médecine, de Pharmacie et d'Odontostomatologie, University of Bamako, Mali [2]Fall *et al.* British journal of Haematology (1998) 103:957-959. [3]Ouattara *et al.* Phytomedicine (2009) 16:125-129.

POSTER 35

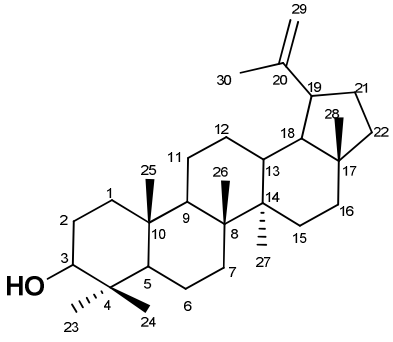
PHYTOCHEMICAL STUDY OF *CROTON MAYUMBENSIS* J. LÉONARD (EUPHORBIACEAE)

J.P Ondo[1-2], J.B Bongui[2], A. Elomri[1], P. Vérité[3], J. Lebibi[2], E. Seguin[1]. [1]Université de Rouen, UMR CNRS 6014, COBRA, UFR Médecine-Pharmacie, 22 Bd Gambetta, 76000 Rouen, France, [2]Laboratoire de substances naturelles et de synthèse organométallique, Université des Sciences et Techniques de Masuku, BP 901 Franceville, Gabon, [3]Equipe ABTE/ Toxemac, "Toxicologie de l'environnement : milieux aériens et cancers", Laboratoire de Chimie Analytique, Université de Rouen, UFR Médecine-Pharmacie, 76183 Rouen Cedex, France.

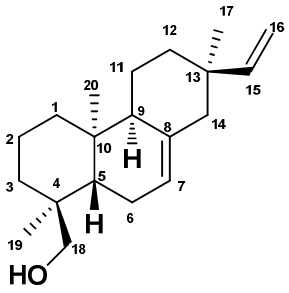
Croton mayumbensis is traditionally used to treat malaria symptoms in Haut-Ogooué, Gabon. This tree which can reach 30 m high grows in the rainforest. The aim of this study was to determine the phytochemical composition and to evaluate the *in vitro* antiplasmodial activity and the cytotoxicity of extracts of this plant. Extracts from stem barks of *C. mayumbensis* were obtained by lixiviation at room temperature using dichloromethane (CH_2Cl_2). Various methods of separation and purification led to the isolation from this extract of caryophyllene oxide, lupeol and isopimarol. In addition, crude extract showed low antiplasmodial activity (IC_{50} = 19.1 µg/ml) against W2 strain of *P. falciparum* and weak cytotoxicity against myelomonocytic THP1 cells (LD_{50}= 181.6 µg/ml).



Caryophyllene oxyde



Lupeol



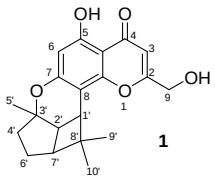
Isopimarol

POSTER 36

A NEW MEROTERPENOID ISOLATED FROM ROOTS OF *PTAEROXYLON OBLIQUUM* RADLK

Daniel Agostinho, Leslie Boudesocque, Isabelle Thery-Kone, Alain Gueiffier, Cécile Enguehard-Gueiffier, Hassan Allouchi. UMR INRA 1282 Infectiologie et Santé Publique, Equipe Recherche et Innovation en Chimie Médicinale, Université de Tours François Rabelais, UFR des Sciences Pharmaceutiques Philippe Maupas, 31 avenue Monge, 37200 Tours, France.

The roots of *Ptaeroxylon obliquum* Radlk. (*Rutaceae*) afforded a new meroterpenoid, ptaerobliquol **1**, together with chromones, ptaeroxylinol acetate **2** and peucenin **4**, and coumarins, scopoletin **5** and prenyletin **3**. Ptaerobliquol was isolated using centrifugal partition chromatography followed by silica gel column and identified by extensive NMR and single crystal X-ray analyses. A biosynthetic pathway is proposed for this new compound.



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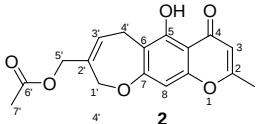

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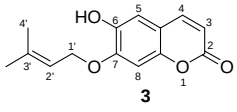

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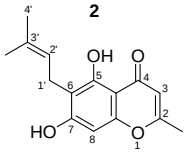

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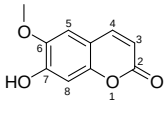

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POSTER 37

FORMATION OF ALKALOID ARTIFACTS MAY CONTRIBUTE TO EXPLAIN *MONDIA WHITEI* (HOOK.F.) SKEELS BIOACTIVITY

Valérian Bunel, Marie Hamel, Pierre Duez, Caroline Stévigny. Laboratory of Pharmacognosy, Bromatology and Human Nutrition, Faculty of Pharmacy, Université Libre de Bruxelles (ULB), Brussels, Belgium.

Mondia whitei (Hook.f.) Skeels (Apocynaceae) is used across sub-Saharan Africa to treat a vast range of diseases and health conditions. The root is commonly chewed by men for its aphrodisiac and tonic properties, but it is also used for the treatment of asthenia and depression; these indications are suggestive for an effect at the central nervous system's level[1-2]. Consistent with the botanical family of *M. whitei*, previous phytochemical analyses have revealed the presence of alkaloids whose structures still remain unknown[3-4]. Along the isolation and characterization of *M. whitei* alkaloids, these were found to be resulting from the formation of an artifact issuing from the known aromatic compound[4] 2-hydroxy-4-methoxybenzaldehyde. The classical extraction process of alkaloids, based on the acid/base equilibrium of this class of compounds, requires the use of alkalizing substances, such as ammonia. This base reacts with the aldehydic carbonyl of 2H4MBZA through a nucleophilic substitution reaction, yielding a new nitrogen compound. We next investigated 2H4MBZA's reactivity towards the neurotransmitters: epinephrine, norepinephrine, dopamine, serotonin and γ-aminobutyric acid (GABA). Except for epinephrine, these biogenic amines were able to react with 2H4MBZA, forming derived products whose putative structures were confirmed via mass spectrometry analysis. We suggest that the formation of such derivatives may explain the bioactivity of *M. whitei* in modulating the affinity of the neurotransmitters towards their receptors.

[1]Pedersen *et al.* J Ethnopharmacol (2008) 119:542-8. [2]Martey and He, Journal of Pharmacology and Toxicology (2010) 5:460-468. [3]Kerharo, J. *La Pharmacopée Sénégalaise Traditionnelle : plantes médicinales et toxiques*, ed. E.V. Frères. 1974, Paris. [4]Oketch-Rabah, H.A. J Diet Suppl (2012) 9:272-84.

POSTER 38

FIVE NEW NORLUPANES FROM THE HEARTWOOD OF *DIPTEROCARPUS COSTATUS* C.F. GAERTN (DIPTEROCARPACEAE)

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The *n*-hexane extract of *Dipterocarpus costatus* C.F. Gaertn (Dipterocarpaceae) heartwood exhibited significant both cytotoxic and antimalarial activities. Chromatographic fractionation of this extract led to the isolation of five new norlupanes, together with 4 known common terpenoids (*i.e.* β-sitosterol, caryophyllene oxide, clovane-2,9-diol and β-elemene), as well as 2 dammarane triterpenes from dipterocarpol group (*i.e.* dipterocarpol and dammarenediol II). Their structures were determined on the basis of spectroscopic methods, including 2D NMR experiments, and GC-EI-MS analysis with comparison to reference data, particularly for the identification of sesquiterpenes. In vitro antiplasmodial (against the chloroquin resistant *Plasmodium falciparum* FcB1 strain, evaluated by inhibition of the uptake of [^{3}H]-hypoxanthin) and cytotoxic (against PC3, MDA-MB-231, HT-29 and HCT116 human cancer cell lines and rat myoblast-derived L-6 cells) activities of the isolated compounds are also reported. Among the tested compounds, one norlupane, bearing an endoperoxide function, displayed potent antimalarial activity, with an IC_{50} of 5.13±3.03 µM, while it was poorly cytotoxic against both mammalian and rodent cells.

POSTER 39

HALOGENATED DERIVATIVES OF PHEOPHORBIDE A, A PHOTOACTIVABLE COMPOUND ISOLATED FROM YOUNG CARPINUS BETULUS LEAVES

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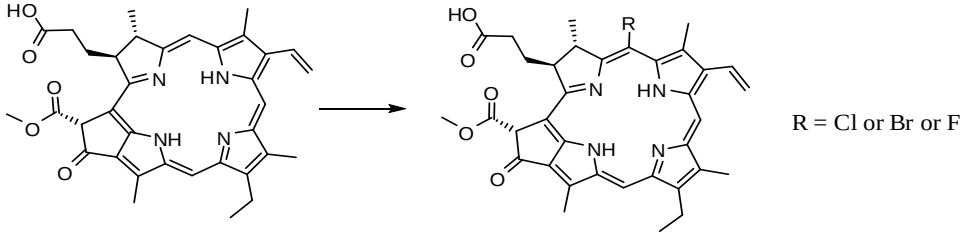
As part of our ongoing research for anticancer compounds from the Walloon Region forest[1], EtOAc extract from *Carpinus betulus* leaves was phytochemically studied, leading to the bioguided isolation of pheophorbide a. Evaluation of the growth inhibitory activities of pheophorbide a using MTT colorimetric 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.



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POSTER 40

CHEMICAL ENGINEERING ON *MAMMEA NEUROPHYLLA* EXTRACT TO IMPROVE ANTI-AGES POTENTIAL

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Natural products (NPs) and their derivatives (NDs) represent more than 50% of approved therapeutic agents over the last 30 years, due to their structural diversity and ability to interact with biological targets.[1] Although NPs have been marginalized by Big Pharma, approaches involving generation of new leads and scaffolds from NPs could be now favoured.[2] For example, chemically engineered extracts (CEEs) consist of a chemical diversification from simple and accessible raw materials such as some natural extracts.[3] In this context, first experiments were undertaken and results will be presented. First, we proposed and validated a general protocol from chemical reactions on a selected extract to purification and characterization of active and original compounds. Then, we valorised *Mammea neurophylla* methanolic bark extract selected for its inhibition of Advanced glycation endproducts (AGEs) but devoid of new compounds (flavanols, procyanidins and condensed tannins). The well-described acid-catalyzed cleavage of condensed tannins in the presence of various nucleophiles was used.[4] Original and active NDs were obtained.

[1]. D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2012**, 75, 311. [2]. G. T. Carter, *Nat. Prod. Rep.* **2011**, 28, 1783. [3]. I. Ayelen Ramallo *et al. Acc. Chem. Res.* **2011**, 44, 241. [4]. L. Ferchichi *et al. Phytochemistry* **2012**, 78, 98.

POSTER 41

CHEMODIVERSITY OF ENDEMIC SPECIES *WIKSTROEMIA* (THYMELAEACEAE) FROM FRENCH POLYNESIA

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Many species of *Wikstroemia* genus (Thymelaeaceae family), used as traditional medicinal plants in Asia and Pacific areas, are well known to possess various interesting therapeutic properties[1,2]. Three endemic species of *Wikstroemia* are found in French Polynesia: *W. coriacea*, *W. raiateensis* and *W. johnplewsii* and we report herein a phytochemical investigation to establish their chemical composition and pattern[3]. Sixteen main constituents of stem bark or stem were isolated and identified by spectroscopic techniques. Comparison of the chemical profile of these species put in evidence the chemodiversity of these three endemic species[4].

References: [1]Borris, R. P. *et al*. *J Ethnopharmacol*., (1988) 24, 41-91.[2]Pétard, P. (1986) Plantes utiles de Polynésie et Raau Tahiti, Haere Po No Tahiti ed., Tahiti. [3]Ingert, N *et al*. Molecules (2013) 18, 2988-2996. [4]Guezennec, J., Moretti, C., and Simon, J. C. (2006) Substances naturelles en Polynésie française, IRD ed.

POSTER 42

A STUDY OF THE STRUCTURE-ACTIVITY RELATIONSHIP OF KERMADECINE A

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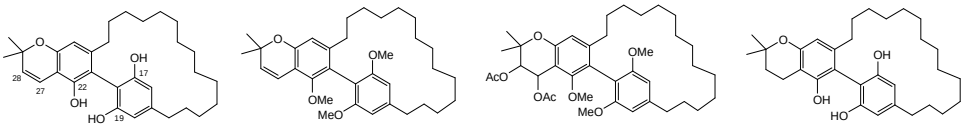
The kermadecine A is a natural molecule has been isolated for the first time from the bark of a New Caledonian plant, *Kermadecia elliptica*, Proteaceae. It showed a significant activity against cancer cells lines KB and L1210, with IC_{50} values within the micromolar range[1]. In order to carry out a structure-activity relationship study of kermadecine A (1) ,thirteen molecules were prepared, to explore the contribution of phenolic groups 17, 19 and 22 to its cytotoxic activity on one hand, and to determine the influence of the functionalization of the pyran ring's double bond 27.28 on this activity on the other hand. The IC50 of these compounds were determined on three different cancer cells lines: U937, HL60 and KB. The results showed the important role of the phenolic groups in the biological activity to the degree that methylated derivative on the three phenols (2) completely lost its activity. However, it is important to note that the functionalization of the double bond 27.28 (3) restored the cytotoxic activity. Furthermore, kermadecine A in which the double bond 27.28 is reduced (4) led to a more active compound than the molecule itself.



1 2 3 4

[1]Jolly C., Thoison O., Martin M.-T., Dumontet V., Gilbert A., Pfeiffer B., Léonce S., Sévenet T., Guéritte F., Litaudon M., Cytotoxic turrianes of *Kermadecia elliptica* from the New Caledonian rainforest, *Phytochemistry*, 2008, 69, 533-540.

POSTER 43

ANTIANGIOGENIC CYCLOARTANES FROM *GARDENIA OUDIEPE* (RUBIACEAE) AND THEIR SEMISYNTHETIC ANALOGS

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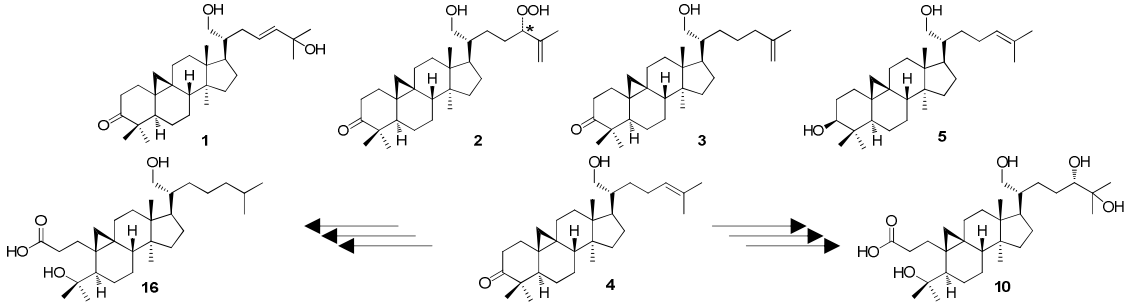
Three new cycloartanes (**1**-**3**), along with two known cycloartanes (**4**-**5**) were isolated from *Gardenia oudiepe*, an endemic Rubiaceae from New Caledonia. According to their anti-angiogenic activity determined by chemiluminescent screening assay for detection of VEGF's receptor-1 ligands, **4** was found to be the most potent and has been chosen for semisynthesis of twelve derivatives in order to improve the activity and to establish the structure-activity relationships. The IC_{50} value of **4**, **5** and **16** were determined as 1.3, 4.0 and 4.4 μM respectively.



POSTER 44

PHYTOCHEMICAL STUDY OF THE ALPHITONIA XEROCARPA BARKS (RHAMNACEAE)

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This work consists of phytochemical study of the bark of Alphitonia xerocarpa (Rhamnaceae), a New-Caledonia endemic plant. From the chloroform extract, the ceanothic acid (major constituent), and two new lupan derivatives (1-3) were isolated. The purification of the hydro-methanolic extract led to the isolation and structural elucidation by 1D and 2D NMR of 19 compounds : lyoniresinol, maesopsin, rutoside, uridine, three glycosylated phenols, eleven dammarane derivatives including ten new saponins, and finaly a new glucoside of ceanothic acid. Screening tests were performed to evaluate the cytotoxic and antibacterial properties of isolated compounds only two of them (2-3) showed high biological activity. The MIC was determined against S. aureus and E. faecalis, and the IC50 value was calculated by a WST-1 test using KB cell line.

POSTER 45

CONTRIBUTION TO THE CHEMOTAXONOMIC STUDY OF SPECIES OF THE GENUS *DESMODIUM* OF CAMEROON

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Constantly looking for new drug substances, the pharmaceutical industry has always turned to natural substances of plant origin very often. Of these substances, hepatoprotective plants are attracting increasing attention. *Desmodium adscendens* a species of the family Fabaceae has long been used in traditional medicine to treat liver ailments. The chemotaxonomy which is one of the methods used in the exploration of medicinal plants, says that if a species of has an interesting potential therapeutic substances through it synthesizes, a species of the same genus or family is more likely to be also promissing in this field. In this study, we will focus on four species of the genus *Desmodium* belonging to the family Fabaceae (legume). *Desmodium* species used for this study are of diverse origin and crop after flowering. The different species were collected in sa 'a (*Desmodium adsendens*). In Bangangté (*Desmodium incanum*) and Dschang (*Desmodium uncinatum* and *D. intortum*). The species were all identified in the National Herbarium of Cameroon.Studies show that chemical screening: alkaloids, tannins, flavonoids, anthocyanins, leucoanthocyanes, mucilages, sugar reducers, saponins, sterols and triterpenes are preent in all species of Desmodium with concentrations more likely higher in *D. intortum* and *D. uncinatum*. Chemical screening studies showed that alkaloids, tannins flavonoids, anthocyanins, leucoanthocyanes, mucilages, sugar reducers, saponins, sterols and triterpenes are present in all species of *Desmodium* with concentrations more likely higher in *D. incinatum* and *D. intortum* reflecting the similary between the different species used. The active principales identified in Desmodium adsendens including soyasaponines I and III, vitexin and isovitexin were identified among other *Desmodium* ; other compounds such as kaempferol, apigenin, quercetin and rutin were also identified in some *Desmodium* among which some for the first time in *D. incanum*. Further studies on ethnobotanical and pharmacological study of these species associated with the isolation of chemical compounds identified should be undertaken.

POSTER 46

CHEMICAL CONSTITUENTS OF THE LICHEN *DERMATOCARPON LURIDUM* (WITH.) J.R. LAUNDON

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Lichens are fungal and algal/cyanobacterial symbioses resulting in the production of specific metabolites. We precisely performed a phytochemical study on a foliose lichen *Dermatocarpon luridum*, found on rock near river[1]. Extraction by three successive solvents was conducted on dried crush material, namely aqueous, chloroform and acetone extracts. The aqueous extract was purified by a cation exchange resin. Then HPTLC-UV and HPLC-DAD-MS^n techniques were applied to detect and purify mycosporines-like amino acids[2], which are water soluble and low molecular weight compounds, having a strong UV absorption maximum between 310 nm and 365 nm, and a high molar extinction coefficient (ε = 28 000 to 50 000 $M^{-1}.cm^{-1}$). These compounds are known to play an important role in the protection of the organisms against UV radiation. As the first step of investigation, mycosporine glutamicol (fig.1a) was recognised in *Dermatocarpon luridum* by HPLC-DAD-MS^n. Preparative methods are in progress to isolate mycosporines-like amino acids in this extract. Three compounds were isolated from acetone extract, including a phytosphingosine-type ceramide, (2*S*,3*S*,4*R*)-2-[(2'*R*)-2'-hydroxytetracosanoylamino]-1,3,4-octadecanetriol, with two polyols, volemitol and mannitol[3,4]. This is the first report of a phytosphingosine-type ceramide from lichen. The structures of these compounds were elucidated by spectroscopic methods.

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POSTER 47

PURIFICATION OF NON-IONIC PEPTIDES BY CO-CURRENT CENTRIFUGAL PARTITION CHROMATOGRAPHY

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Hydrophobic non-ionisable peptides are not soluble in most common solvents and are thus difficult to purify by preparative reversed-phase HPLC, normally used for industrial production. The challenge exits to develop alternative purification chromatographic processes using suitable solvents and providing good yields, high purity and sufficient productivity. Centrifugal Partition Chromatography (CPC), combined with the co-current elution mode – was successfully applied to the purification of different bioactive peptides (modified cyclosporine A, protected exenatide), or a protected synthetic intermediate of bivaluridin. This original solution was developed after the study of the effect of the peptide on the hydrodynamic behavior of the two phases, and the visualization of the flow patterns using the Visual-CPC device. Critical impurities were efficiently eliminated and these peptides were recovered in high yield and high productivity.

POSTER 48

SELECTION OF CLUSIACEAE AND CALOPHYLLACEAE EXTRACTS BASED ON DEREPLICATION AND ANTI-INFLAMMATORY PROPERTIES

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Inflammation is associated with many pathogenic disorders including endothelial dysfunction. Calophyllaceae and Clusiaceae which are rich in polyphenolic compounds such as coumarins, xanthones, benzophenones and biflavonoids[1] are well-known for their anti-inflammatory properties[2]. Bark, leaves and occasionally fruits of thirteen plants belonging to the genus *Calophyllum*, *Mesua* (Calophyllaceae), *Garcinia* (Clusiaceae) and native from Malaysia, were extracted using DCM and MeOH as the solvents. Extracts of interest were selected according to two distinct criteria. Firstly, a dereplication analysis was conducted though HPLC-PDA-MS^n. Secondly the VCAM-1 surface-expression of (TNF-α)-stimulated endothelial cells from human umbilical veins (HUVECs) was evaluated. It appeared that several extracts particularly rich in xanthones and phenylcoumarins significantly decreased inflammatory marker expression. In this context, a new phenylcoumarin was identified as the major component of the bioactive fruits DCM extract from a *Mesua*.

[1] V. Cechinel Filho *et al. Chem. Biodivers.* **2009**, 6, 313-327. [2] J. Gonzalez-Gallego *et al. Br. J. Nutr.* **2010**, 104, S15-S27.

POSTER 49

ELICITATION OF GALANTHAMINE AND LYCORINE BIOSYNTHETIC PATHWAY WITH DEUTERIUM-LABELLED-4'-*O*-METHYLNORBELLADINE ADDED TO *LEUCOJUM AESTIVUM* BULBLETS

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The most studied Amaryllidaceae alkaloid is galanthamine, an acetylcholinesterase inhibitor, marketed as hydrobromide salt, for the treatment of Alzheimer's disease[1]. A second important alkaloid is lycorine possessing antiviral, antimalarial, anti-inflammatory and anti-tumor activities. *In vitro* cultures can be used as an alternative way to obtain these valuable metabolites. The aim of this work consists on enhancing the biosynthetic pathway of these alkaloids. The deuterium-labelled common precursor of galanthamine and lycorine, 4'-*O*-methyl-d_3-norbelladine, was first synthesized with a yield of 33%[2]. This labelled compound was then incorporated into the liquid medium of *Leucojum aestivum* bulblets. It highly stimulated the synthesis of both native galanthamine (4.76% DW, V/S 1% DW in the control sample) and native lycorine (2.62% DW, V/S 0.01% DW in the control sample) after its biotransformation by bulblets justified by the presence of both deuterated galanthamine and lycorine quantified by LC-MS and GC-MS.

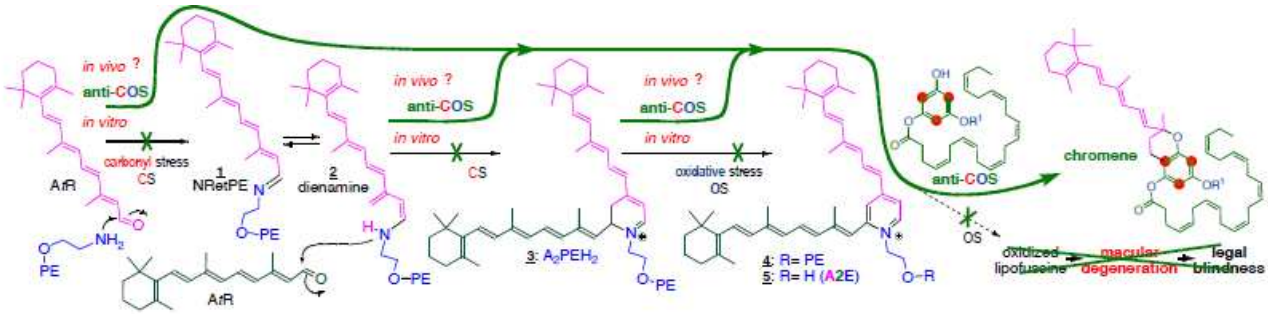
[1] Ptak *et al.* J. Nat. Prod. (2009) 72, 142-147. [2] El Tahchy *et al.* J. Nat. Prod. (2011) 74, 2356-2361.

POSTER 50

LIPO-PHLOROGLUCINOLS SYNTHESES AS ANTI-CARBONYL AND –OXIDATIVE STRESSORS (ANTI-COS) IN NEURODEGENERATIVE DISEASES (STARGARDT)

Claire Vigor[1], Céline Crauste[1], Philippe Brabet[2], David Cia[3], Thierry Durand[1] and Joseph Vercauteren[1]. [1]Institute of Biomolecules Max Mousseron (IBMM), UMR CNRS-UMI & II 5247, Department of Bioactive Lipids Synthesis, Faculty of Pharmacy, 15 av. C. Flahault 34093 Montpellier; [2]Inserm U1051, Institute for Neurosciences of Montpellier (INM); [3]UMR Inserm 1107, Laboratory for Neurosensory Biophysics, Faculty of Medecine and Pharmacy, Clermont-Ferrand.

All-*trans*-retinal (A*t*R) formed in the normal visual cycle is carried out from photoreceptor cells by an ABCA4 protein. *Abca4* gene mutations lead to the dysfunction of this flipase causing an accumulation of A*t*R inside the photoreceptor disks to form the Schiff base *N*-retinylidene-phosphatidyl-ethanolamine (NretPE) **1**, through a carbonyl stress (CS) mechanism on phosphatidyl-ethnolamine (PE). Rearranged dienamine of NretPE **2** adds further onto a second molecule of A*t*R in excess (CS) towards the A2PEH2 **3**, which stabilizes into the pyridinium A2PE **4**, through an oxidative stress (OS). Upon the daily phagocytosis of distal part of outer segments of photoreceptors by retinal pigment epithelium (RPE), **4** is hydrolyzed into lipofuscin A2E **5**, considered as the main causing agent for macular degeneration. We are synthesizing a series of anti-COS phloroglucinols (naturally anti-OS) from natural or synthetic sources. Structural modifications include *O*-alkylations (R1= alkyl groups), to increase nucleophily, thus anti-CS properties. To improve their bioavailability and induce a vectorization process to retinal tissue, more lipophilic polyphenols are designed: due to the high content of membrane of photoreceptors in polyunsaturated fatty acid (PUFA), phloroglucinols are linked to PUFAs such as docosahexaenoic acid (DHA, C22:6 n-3), to obtain newly synthesized "lipophenols" that revealed to be more active *in vitro*.



POSTER 51

SUBSTITUTED FLAVONES, A NEW ANTIMALARIAL CHEMOTYPE

Flore Nardella[1], Valérie Collot[2], Silvia Stiebing[2], Martine Schmitt[1], Marcel Kaiser[3,] Bernard Weniger[1], Ermanno Candolfi[4], Catherine Vonthron-Sénécheau[1]. [1]UMR CNRS 7200 Laboratoire d'Innovation Thérapeutique, Faculté de Pharmacie, Université de Strasbourg, 64701 Illkirch, France, [2]Centre de Recherche et d'Etude dur le Médicament de Normandie, EA 4258 FR CNRS 3038 INC3M-SF 4206 ICORE, Boulevard Becquerel, Université de Caen Basse-Normandie, 14032 CAEN Cedex, [3]Swiss Tropical and Public Health Institute, University of Basel, 4002 Basel, Switzerland, [4]Institut de Parasitologie et de Pathologie Tropicale de Strasbourg, Faculté de Médecine, 3 rue Koeberle, 67000 STRASBOURG.

Recent reports of increased tolerance to artemisinin derivatives - the most recently adopted class of antimalarials - have prompted a need for new treatments. However, many of the therapies currently in development use known antimalarial pharmacophores chemically modified to overcome the liabilities of their predecessors [1]. Although these compounds may become important in the treatment of malaria, it would be preferable to discover chemotypes with novel mechanisms of actions. To identify novel antimalarial leads, we have screened our plant extracts library using *Plasmodium* whole-cell proliferation assays with cultured intraerythrocytic parasites. The isolation of an active biflavonoid from *Campnosperma panamense* (Anacardiaceae) [2, 3] led us to the development of simplified synthetic analogs. Structure-activity relationship study is still in progress, but several active compounds have already been synthesized. Indeed, in preliminary studies conducted in a *P. berghei* malaria mouse model, MR70 enabled to reverse the parasitemia of 50% of the infected mice with four daily intraperitoneal doses of 100 mg/Kg. MR 70 stands out as a starting point for a medicinal chemistry lead optimization effort. It belongs to no class of molecules currently in development or used in therapeutic [4]. This work has been funded by the French *Fondation pour la Recherche Médicale.*

[1] Olliaro P., Wells T.N. ***Clin. Pharmacol. Ther.*** **(2009)** 85, 584; [2] Weniger B, Vonthron-Sénécheau C, Arango GJ, Kaiser M, Brun R and Anton R. ***Fitoterapia*** **(2004)**, 75, 764; [3] Weniger B, Vonthron-Sénécheau C, Kaiser M, Brun R, Anton R. ***Phytomedicine*** **(2006)**, 13, 176; [4] http://**www.mmv.org**/research-development/rd-portfolio, le 14/03/2013.

POSTER 52

PHYTOCHEMICAL INVESTIGATION OF A LICHEN FROM BRITTANY, *STEREOCAULON EVOLUTUM* GRAEWE

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Previous studies[1] confirmed that the lichen genus *Stereocaulon* could be an interesting source of novel secondary metabolites and one of these fruticose lichens, *S. evolutum*, was harvested in Brittany (France) on schisteous rocks. Phytochemical studies of the three extracts obtained by solvents of increasing polarity (*n*-hexane, acetone and THF) afforded seven compounds whose structures were elucidated by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, ESI-MS). The compounds belong to four different classes including a very common depside atranorin (5.6 g) [1]; monoaromatic phenols methylbetaorcinol carboxylate (159 mg) and atranol (9.9 mg) which could result from atranorin degradation[2]; depsidones lobaric acid (95.7 mg) and stictic acid (19.5 mg); diphenyl ethers sakisacaulon A (8 mg) and lobarin (6 mg), not so frequent in lichens[1,3,4]. Other compounds are currently under identification on *S. evolutum* which is for the first time phytochemically investigated.

[1]Ismed *et al*., Fitoterapia (2012) 83, 1693-1698. [2]Ismed F, Rennes1-UEB PhD, 2012. [3]Huneck S *et al.*, Progress in the chemistry of organic natural products. NewYork: Springer; (2001) 218-21. [4]Morita *et al*., Bioorganic & Medicinal Chemistry Letters (2009) 19:3679-3681.

POSTER 53

LC-HRMS/MS INVESTIGATION OF A NEW MARINE-DERIVED *PENICILLIUM* SPECIES FOR THE RAPID DETECTION OF ORIGINAL BIOACTIVE COMPOUNDS

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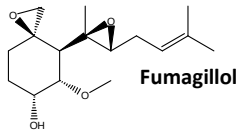
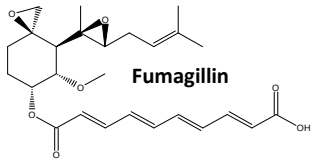
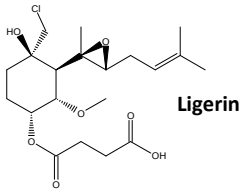
In a previous study, bioguided fractionation of an extract of *Penicillium ligerum*, a new species of a marine-derived fungus, led to the isolation of ligerin. This chlorinated sesquiterpene derivative of fumagillin exhibited an antiproliferative activity with a high selectivity against osteosarcoma cell lines and demonstrated *in vivo* antitumor activity. In this work, natural structural analogs of ligerin were searched by investigations of the metabolic fingerprints of four different *P. ligerum* strains. For that purpose, we developed a LC-HRMS/MS-based strategy: a fragmentation modelization was established by thoroughly studying the fragmentation patterns of ligerin and its analogs fumagillin and fumagillol. The automatized search for diagnostic ions in the metabolic profiles together with the use of the HRMS model allowed the detection and the structural identification of several new compounds related to ligerin. This study shows the interest of LC-HRMS/MS analyses for rapid investigations of the chemical diversity of marine-derived fungi in order to consider the targeted purification of original bioactive secondary metabolites.







POSTER 54

HOST-PLANT/FRANKIA RELATIONSHIPS IN ACTINORHIZAL SYMBIOSES : INFLUENCE OF THE CAPABILITY TO SPORULATE *IN PLANTA* ON THE METABOLIC PROFIL THE PLANT

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The association between *Alnus spp* and the actinobacteria *Frankia* takes place among actinorhizal symbioses. During this interaction, *Frankia* induces in its host-plant, the formation of a new organ in the roots, the nodule, in which trophic exchange take place between the two partners. The bacteria give the host reduced nitrogen, thanks to its capability to fix nitrogen from atmosphere, and benefits in return of organic compounds issued from photosynthesis of the plant. Actinobacteria are known for their good resistance to drastic environmental conditions, often linked to their capability of sporulation. All the *Frankia* strains are able to sporulate in pure culture. But, only certain strains, called Sp+, conserve this ability under symbiotic conditions, in nodule's cells (*in-planta sporulation*)[1,2]. Genetic differences are already observed between Sp+ and Sp- strains. Our aim is to detect eventual signal molecules associated to the presence or the absence of sporanges from the symbiote in the nodule. For this study, from 48 biological samples, we did an exploring analysis of metabolites synthesized in the nodules or the roots from *Alnus Glutinosa*, from Sp+ or Sp- sites (N+ : nodule extract from Sp+ tree, N- : nodule extract from Sp- tree, R+ : root extract from Sp+ tree, R- : root extract from Sp- tree). Polar extracts (MeOH/Eau) were analysed by UHPLC/UV-DAD/ESI-QTOF in order to compare the content in secondary metabolites. Sugars were also analysed after derivatisation by GC/MS-QQQ. Preliminary results of PCA show that some secondary metabolites are discriminant between N+ and N-. This difference is less visible for root extracts. This could explain specific modification during the symbiose.

[1]Vanden Bosch *et al*. (1985) Amer. J. Bot. 72:99-108. [2]Van Dijk C (1978) 81:601-615.

POSTER 55

TISSUE SPECIFIC ACCUMULATION OF RESVERATROL OLIGOMERS IN GRAPE GROWING WASTE PRODUCTS

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Wine growing strongly depends on pesticides to prevent grape diseases and yield losses. The French action plan Ecophyto 2018 aims to reduce nationwide use of pesticides by 50% within ten years. The use of green pesticides considered environmentally friendly is one of the ways toward an era of truly sustainable agriculture. Our project aims to develop green inputs to fight downy mildew, the major grape diseases in the Loire Valley. Grape canes a non-recycled waste of wine industry (1-5 tons per hectare per year) may have considerable potential as a source of bioactive compounds, especially resveratrol oligomers that are stilbene polyphenols. Resveratrol and wine polyphenols are known to exert *health-promoting* effects including antioxidant, anticancer, anti-inflammatory and cardioprotective properties. Additionally, resveratrol is a major phytoalexin produced by plants in response to various stresses and promotes disease resistance. We initiate the present work with the purification of resveratrol mono- and oligomers (dimers, trimers and tetramers) by CPC to develop quantitative analyses of grape cane stilbenes by UPLC-MS. We characterized the distribution of stilbenes in different tissues of grape canes by fluorescence microscopy and UPLC-MS analyses and after tissue dissections. Resveratrol oligomers were mainly accumulated in vascular tissues. The composition and distribution of stilbenes in grape canes tissues was altered by the degree of fungal infection in vineyard plots. First *in vitro* assays highlighted an antifungal activity of the polyphenol-rich grape cane extract against downy and powdery mildew. These results allow us to consider grape byproducts as a promising source of bioactives substances for development of biopesticides.

POSTER 56

BIODEREPLICATION OF CROTON SPP EXTRACTS TO POINT OUT MOLECULES RESPONSIBLE OF THE ANTIPLASMODIAL ACTIVITY

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Bioguided fractionation is the most used approach to help discover active compounds in complex extracts. Nevertheless, this strategy implies repetitive biological assays and tedious fractionation steps. We developed a method based on mass spectrometry to directly detect in the crude extract compounds binding to heme[1], as this characteristic is the most common mechanism of antiplasmodial drugs, including quinolones and artemisinin. Here we present an improved protocol as well as its application to a series of natural extract (extracts by different solvents of different parts of several *Croton* spp.). This biodereplication approach allows focusing the isolation efforts to the most promising compounds.

[1]Muñoz-Durango K, Maciuk A, Harfouche A, Torijano-Gutiérrez S, Jullian J-C, Quintin J, Spelman K, Mouray E, Grellier P, Figadère B; *Anal Chem*, 2012 (84) 3324−3329.

POSTER 57

PHYTOCHEMICAL ANALYSIS OF TREE ROOT EXUDATES *IN SITU* AND THEIR POSSIBLE IMPLICATION IN TREE N-ACQUISITION STRATEGY

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Eperua falcata (Aublet), a late-successional species abundant in French Guiana, has developed an original strategy concerning N-acquisition by largely preferring nitrate, rather than ammonium[1].Given this preference for nitrate, we hypothesized that root exudates would promote nitrate availability by a) enhancing nitrate production by stimulating ammonium oxidation or b) minimizing nitrate losses by inhibiting dentrification. Root exudates were collected *in situ* in monospecific planted plots. The phytochemical analysis of these exudates and of several of their corresponding root extracts was achieved using UHPLC/DAD/ESI-QTOF. Our results show that (i) the distinct exudation patterns observed are related to distinct root morphologies, and this was associated with a shift in the root flavonoid content, (ii) a root extract representative of the diverse compounds detected in roots showed a significant and selective metabolic inhibition of isolated denitrifiers *in vitro*, and (iii) in soil plots the abundance of nirK-type denitrifiers was negatively affected in rhizosphere soil compared to bulk. Altogether this led us to formulate hypothesis concerning the ecological role of the identified compounds in relation to N-acquisition strategy of this species.

[1]Schimann *et al*. Soil Biol. Biochem. (2008) 40:487-494.

POSTER 58

PRELIMINARY STUDIES OF NUTRACEUTICALS: THE WAY FORWARD TO PREVENT ADVERSE HEALTH EFFECTS OF A CASSAVA-DOMINATED DIET

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Oxidative damage is a biological hallmark of cassava poisoning. We asked whether local food crops may be used as nutraceuticals to initiate prevention against the toxic effects of cassava. We performed a case-control study of 20 subjects severely affected by konzo vs. 20 age- and gender- matched controls from rural Bandundu, the world most konzo-affected region. Levels of oxidant markers F2-isoprostanes were assessed using state-of-the art proteomic methodologies (LC-MS). We also analyzed methanolic extracts of green vegetables widely grown in Bandundu using the 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging assay by Thin Layer Chromatography and ELISA. Crops of interest included vegetables, mushrooms and spices that were compared to vitamin C, vitamin E or quercetin as references. We showed a clear correlation between levels of F-2 isoprostanes isomers (reaching up to 50-times normal reference values) and konzo-associated disability (Spearman $r = 0.7$, $P < 0.01$). Inhibitor concentrations (IC_{50} ± standard deviation) for samples were found to be 33.99± 1.93(*Pteridium aquilinum*) 35.72 ± 4.19 (D*ioscorea praehensilis*), 74.27±8.52 (*Mannihot utilisima*), 92.8 ± 35.83 (*Psophocarpus scandens*) 105.58 ±2.83 (*Raphia sese*), 122.37±2.83 *(Megaphrynium macroschtachyum*), 145.22 ± 35, 8 (*Auricularia sp*) vs. 0.72 ± 1.37 (vitamin C), 34.65 ± 0.45 (α- tocopherol), or 1.99 ± 0.15 (quercetin). *Pteridium aquilinum* and *Dioscorea praehensilis* showed antioxidant activities comparable to those of vitamin E. Select food crops may be used as nutraceuticals in campaigns to mitigate the health effects of cassava neurotoxicity when acceptance of modern intervention trials is not guaranteed**.**

POSTER 59

CLASSIFICATION OF ALGERIAN *MENTHA* SPECIES BY NMR-BASED METABOLOMICS

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^{1}H-NMR-based metabolomics have been applied to identify potential NMR-markers capable of distinguishing three *Mentha* species e.g. *Mentha pulegium* L., *Mentha rotundifolia* auct. and *Mentha spicata* L. These were collected in various locations in Algeria with the aim to establish a quality control protocol based on NMR profiles. Samples were dried in the shade, powdered and extracted with ethanol-water (1:1). Extracts were evaporated to dryness and dissolved in deuterated methanol-water (1:1). Presaturation NMR spectra were measured on Bruker BioSpin GmbH to suppress the solvent signals. For Principal component analysis (PCA-X), the ^{1}H NMR spectra of all plant extracts were binned every 0.04 ppm and normalized to the total area of the peaks. The PCA scores, plotted on a 2-dimensional space defined by the PC1 and PC2 axes, clearly discriminated either the species or metabolites content among the same species according to the cultivation region. All six *Mentha pulegium* were clearly separated along PC1 and PC2 according to harvesting locations; by contrast, the harvesting locations were divided into two groups along PC1 for both *M. rotundifolia* and *M. spicata*. NMR data analysis also indicated that variabilities arise from increases and/or decreases in aliphatic and aromatic protons intensities, suggesting varying metabolites concentrations according to location. A strong separation of *Mentha pulegium* from *Mentha rotundifolia* and *Mentha spicata* using OPLS-DA demonstrated a clear metabolic discrimination between species. This metabolomic study indicates several diagnostic NMR signals which could be used as bio-markers for the *Mentha* identification and quality insurance.

POSTER 60

EXTRACTION OPTIMIZATION AND METABOLITE PROFILING OF NINE LICHENS BY LC-ESI-MS/MS

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Lichens are symbiotic organisms harboring bacterial communities on the surface and inside of their thalli [1-3]. In order to develop chemical ecology study on this mini-ecosystem, the extraction process needs to be optimized to obtain the complete metabolite profiles. The aims of this study are (1) the optimization of lichen extraction using Taguchi experimental design and HPLC analysis and (2) the realization of qualitative metabolite profiling of various marine (*Lichina confinis*, *L. pygmaea*), maritime (*Roccella fuciformis*, *R. phycopsis*) and inland (*Collema auriforme*, *C. cristatum*, *C. fuscovirens*, *Leptogium lichenoides*, *Synalissa ramulosa*) lichens by LC/MS. Various parameters of the extraction method: grinding system, kind of solvents, solid/liquid ratio and stirring were shown to influence the efficiency of extraction. Differences and similarities of the metabolite profiling of these nine species extracted with the optimized method were underscored using LC-MS. Some compounds were identified by fragmentation pathway and/or after isolation by NMR analysis.

[1] Cardinale, M. et *al.*,, *Environmental microbiology reports* **2011**, 4, 23-28; [2] Bates, S.T., et *al*., *Applied and Environmental Microbiology* **2011**, 1309-1314; Bjelland, T. et *al*., *Environmental Microbiology Reports* **2011**, 3, 434-442.

POSTER 61

CHARACTERIZATION OF FLAVONOIDS GLYCOSIDES IN TRAVELLER'S TREE LEAVES (*RAVENALA MADAGASCARIENSIS* S.) BY HPLC-DAD-ESI-MS/MS

Catherine Mervoyer, Benedicte Portet, Jocelyn Giboulot, Christian Lubrano. Centre de Recherche Yves Rocher, 101 Quai Roosevelt, 92444 Issy les Moulineaux Cedex. France.

Native from Madagascar, *Ravenala madagascariensis* S., is a famous plant which the common name, "Traveller's tree" is explained by its large boat-shaped bases of leaves that could store rainwater easily used by thirsty travellers. The available scientific reports on phytochemical composition are very limited[1,2]. Considered as a potential source of active ingredients, we characterized for the first time, flavonoids from the leaves of *Ravenala madagascariensis* S. by high performance liquid chromatography 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, 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. In the next step, quantification was performed by HPLC-DAD and the total amount of flavonol glycosides could reach 6% (w/w) of dry extract and repartition of flavonoids was variable depending on the date of harvest. The phytochemical profile obtained could be a powerful tool to establish analytical specifications in order to assess the quality control of traveller's tree extracts used as raw materials for cosmetic applications.

. Rabarisoa *et al*., Canadian journal of plant science (1981) 61, 691-695. **2.** Williams *et al*., Biochemical Systematics and Ecology (1977) 5, 221-229.

POSTER 62

LOOKING FOR PHENOXAZONIC COMPOUNDS FROM TWO SPECIES OF THE GENUS *PYCNOPORUS* AND *TRAMETES VERSICOLOR* AND COMPARATIVE CHROMATOGRAPHIC ANALYSIS BY LCMS-ESI-HRMS ORBITRAP

Welti, S., Wang, Q., Duhal, N., Moreau, P.-A. & Courtecuisse R.

Pycnoporus cinnabarinus (Jacq.: Fr.) P. Karst is well known for its phenoxazonic compounds such as cinnabarin, cinnabarinic acid, pycnoporin and tramesanguin. These constituants, biosynthesized from 3-hydroxyanthranilic acid, form red pigments which are responsible for the intense red orange color of these fungi. Moreover, *P. cinnabarinus* showed antitumour activity particularly with regard to the cinnabarinic acid and the cinnabarin (Dias & Urban, 2009). The genus *Pycnoporus* P. Karst., very closely related to the genus *Trametes*, gathers four main species: *P. sanguineus*, *P. coccineus*, *P. puniceus* and *P. cinnabarinus*. Within *Pycnoporus cinnabarinus*, Gripenberg (1963) distinguished 3 different types of phenoxazinone-producing strains either producing only cinnabarin, or producing cinnabarin and cinnabarinic acid, or producing tramesanguin and cinnabarin. Does it depend of the species or not? The aim of our study is to carry out chromatographic analysis coupled with extractive mass spectra of two ethyl acetate extracts of *P. cinnabarinus*, *P. sanguineus* but also those of *Trametes. versicolor* (L. : Fr.) Lloyd. *P. sanguineus* showed cinnabarinic acid, cinnabarin and tramesanguin as preponderant compounds while *P. cinnabarinus* only showed cinnabarin and tramesanguin. Traces of cinnabarin were found in *Trametes versicolor*, corroborating the close relationship between the two genera. In conclusion, although further studies should be achieved with a larger panel of species from genera *Trametes, Pycnoporus* and *Leiotrametes* Welti & Courtec., this study brings the first data as to the taxonomic value of the phenoxazonic compounds in the *Pycnoporus* and *Trametes* group. Further experiments will probably allow to correlate the phylogenetic results based on molecular data with this chemotaxonomic approach.

POSTER 63

DEGRADATION OF ROTENONE IN YAM BEAN SEEDS (*PACHYRHIZUS SP.*) THROUGH FOOD PROCESSING

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The purpose of this research is to screen different processes for *Pachyrhizus* seeds able to decrease or even eliminate rotenone, a toxic isoflavonoid. These seeds are very interesting because of their high contents in proteins (28.3%) and lipids (26.3%)[1]. Different kinds of processes (drying, roasting, boiling, frying, alcohol extraction) and traditional Beninese culinary recipes were tested and rotenone was quantified by a validated method associating microwave extraction, solid phase extraction (SPE) and HPLC-UV[2,3]. The influence of removing the tegument was also investigated. In the best conditions, an important degradation of rotenone was obtained with up to 80% removal. The most effective methods were drying and roasting the seeds and macerating their flour in local alcohol. This rotenone degradation/elimination induced a decrease of samples toxicity which was confirmed by cytotoxic assays.

[1] Grüneberg *et al*. *Journal of the American Oil Chemists Society* (1999) 76 (11): 1309-1312. [2]Lautié E *et al*. (2012) *Journal of Separation Science*, 36, 758–763. [3] Lautié E *et al*. (2011) *Food Chemistry*, 131 (4):1531-1538.

POSTER 64

BIOACTIVE PHENOLIC COMPOUNDS FROM WILD SALAD SPECIES USING SPECTROPHOTOMETRY AND HPTLC CHROMATOGRAPHY

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The great interest or antioxidant phenolic derivatives used in food supplements and foodstuffs for health benefits requires fast chemical analyses and efficient control methods of raw natural resources. The phenolic profile of plant materials from various species is, indeed, complex and variable. Three wild salads (*Cichorium intybus, Lactuca serriola, Taraxacum officinale*) were investigated for their phenolic compound content regarding their medicinal properties[1-3] using optimized parameters of spectrophotometry and cellulose HPTLC chromatography. Total caffeic acid derivatives and flavonoids amounts were evaluated using spectrophotometry. The use of high performance plates and of spray-application obtained with an automated sample applicator ATS3 gave us sharper bands and a higher sensitivity. Major phenolic compounds contents of each plant, *i.e*., chicoric acid, chlorogenic acid, luteolin-7-O glucoside were compared. We might now use anti-oxidative tests directly on the plate to confirm and ensure the real activity and compare its level in our samples.

[1]Fons *et al.* Phytochemistry (1998) 49: 697-702. [2]Schütz *et al.* Journal of Ethnopharmacology (2006) 107: 313-323. [3]Rammal *et al.* Phytothérapie (2008) 6:184-186.

POSTER 65

NON-ANTHOCYANIN POLYPHENOLS QUANTIFICATION IN *EUTERPE OLERACEA* FRUITS BY A UHPLC−LTQ-ORBITRAP MS METHOD

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High antioxidant and anti-inflammatory activities have been observed from non-anthocyanin polyphenols of *E. oleracea* fruits[1-2]. The aim of this work was to quantify major non-anthocyanin polyphenols by an accurate UHPLC−LTQ-Orbitrap MS method. Fruits were harvested in Pará state (Brazil), processed to pulp and lyophilised. 0.5g of dry pulp powder was defatted by sonication with petroleum ether. The residue was then extracted five times with 5mL MeOH each time for 30 min (optimized conditions giving recovery rates > 90%). The extract was evaporated to dryness with a RapidVap® evaporator at 35°C. Solubilization of the dried extract was realised using 40% MeOH. For the UHPLC quantification, a HSS C18 column (1.8µm) was used with a gradient elution of MeOH and H_2O both with 0.1% HCOOH and the ionisation source (ESI) was operated in NI mode. 26 compounds were identified, among them 7 identified for the first time in this fruit. Total error and accuracy profiles were used as validation criteria. Calibration in the matrix was found to be more accurate than calibration without matrix. Trueness, repeatability, intermediate precision, selectivity, response function, linearity and LOD/LOQ for 12 non-anthocyanin phenolic compounds were evaluated and the quantification method validated.

[1]Kang *et al*., Food Chem. (2010) 122:610–617. [2]Kang *et al*., Food Chem. (2011) 128:152–157.

POSTER 66

MODULATION OF DNA DAMAGE REPAIR AND TRANSLESION SYNTHESIS: A POSSIBLE MECHANISM FOR NATURAL PRODUCTS CHEMOPREVENTION AND INDIRECT GENOTOXICITY?

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Balance between DNA damage and repair appears vital to the cellular machinery, to health and longevity. Given the complexity of DNA lesion tolerance and repair mechanisms and the high number of proteins and cofactors involved, we hypothesize that natural products from food and medicinal plants may interfere in these processes, notably by activating or inhibiting repair.Original methods were developed to probe the modulation of major mechanisms by natural products: *(i)* fidelity of translesion synthesis (TLS), a DNA damage tolerance mechanism that relies on specialized DNA polymerases (pols) able to insert a nucleotide opposite a lesion on the template strand (capillary electrophoresis); *(ii)* kinetics of rejoining strand breaks arising from damage and excision repair (comet assay); *(iii)* capacity of double-strand breaks repair by non-homologous end-joining (NHEJ), a preponderant mechanism in eukaryotes (on-chips microelectrophoresis); *(iv)* capacity of base excision repair, the major repair pathway responsible for removal of small DNA lesions (oligonucleotide repair chips). All methods were validated and could be applied to the study of modulation by natural products, including common flavonoids and dietary plants extracts, in FHs 74 Int cells. None of tested flavonoids inhibits repair; quercetin increases non-specific endonuclease activity, apigenin and epicatechin increase the excision of damages; sakuranetin increases non-specific enzymatic activities and decreases or increases specific activities. Raw plant extracts variously modulate activities. Although some of these protocols represent a simplification of the complexity of the *in vivo* organization of DNA into chromatin, data obtained so far show that herbals are likely to interfere in TLS and repair capacity.

POSTER 67

SIMULTANEOUS QUANTIFICATION OF TWO TOXIC POLYPHENOLS APPLIED TO THE SELECTION OF GENOTYPES OF YAM BEAN (*PACHYRHIZUS SP.*) SEEDS

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The purpose of this research was to develop and validate a rapid quantification method able to screen many samples of yam bean seeds to determine the content of two toxic polyphenols, namely pachyrrhizine and rotenone. The analytical procedure described is based on the use of an internal standard (dihydrorotenone) and is divided in three steps: microwave assisted extraction, purification by solid phase extraction and assay by UHPLC. Each step was included in the validation protocol and the accuracy profiles methodology was used to fully validate the method. Finally the validated dosing intervals range from 0.25 mg to 5 mg pachyrrhizine and from 0.58 mg/g to 4 mg/g rotenone per gram of seeds. Around 130 samples from different accessions, locations of growth and harvest dates were screened. Pachyrrhizine concentrations ranged from 3.29 mg/g to lower than 0.25 mg/g while rotenone concentrations ranged from 3.53 mg/g to lower than 0.58 mg/g. This screening along with PCA and DA analyses allowed the selection of the more interesting genotypes in terms of low concentrations of these two toxic polyphenols.

POSTER 68

MOLECULARLY IMPRINTED POLYMERS: TOWARDS RECEPTOR MIMICS FOR DRUG DISCOVERY

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Molecularly Imprinted Polymers (MIPs) are based on selective recognition properties of synthetic cross-linked materials possessing cavities complementary to a template molecule in size, shape and position of the functional groups. In recent years, MIPs received an increasing attention in the field of natural products for their application in the drug discovery process. In this work, new synthesis strategies were developed to obtain imprinted cavities able to mimic the flexibility and mobility exhibited by receptor/enzyme binding pockets. For the study of working conditions, Quercetin (Qu) was chosen as a representative template considering it is one of the most active compounds in the flavonoids family, commonly found in medicinal and food herbal products. Bulk polymerization (BP), precipitation polymerization (PP) and suspension polymerization (SP) were investigated to obtain optimal chromatographic materials. The Qu MIPs (Fig. 1) were evaluated as sorbents for SPE and HPLC in order to confirm the presence of imprinted cavities and to evaluate their selectivity, respectively. By SPE, successful imprinting of the MIPs is confirmed when the retention appears distinctly superior on MIPs than on NIPs (Corresponding control polymers or non-imprinted polymers). The best SPE results were obtained for MIPs prepared by the SP method. Micrographs obtained by SEM reveal the large distribution of sizes and the irregular shape of particles prepared by BP. On the contrary, spherical beads with a well-controlled size distribution were obtained by SP indicating better perspectives for their use in chromatography. The MIPs were packed into HPLC columns in order to study their selectivity towards a set of analogues and non-analogues of quercetin. Thanks to the improved chromatographic efficiency afforded by SP, the developed MIPs are highly promising for the screening of lead compounds from complex systems such as natural products.

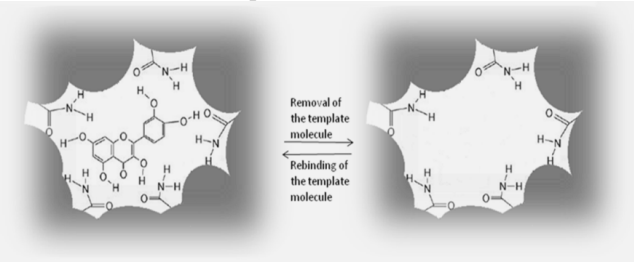

Figure 1: Schematic representation of a quercetin imprinted polymer

POSTER 69

USE OF MEDICINAL PLANTS AROUND THE MOUNTAIN FOREST OF KAHUZI-BIEGA NATIONAL PARK, DR CONGO

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The Kahuzi-Biega National Park, located in the east part of DR Congo, was created in 1970 to protect the lowland gorillas and their habitats which cover 600 km^2 of lowland and highland forests. The high density of population and the high rate of poverty lead to over-use of natural resources. The traditional medicine also constitutes a pressure on the forest, when parts of vegetable species, especially woody species, are taken without standards[1]. Given the wide diversity of ethnics groups around the Park, the study has evaluated *(i)* if the ethnic membership of the healers does influence their knowledge and use of medicinal plants[2]; and *(ii)* the impacts on the vegetation of the Kahuzi-Biega forest. Ethnobotanical survey was carried out in 26 villages with 49 professional healers among the Shi, Havu, and the "Batwa" communities. 218 plants were identified among them 46% are collected from the protected area. The statistics tests have shown a significant difference in the use of species among the communities. But a similarity was observed between the Shi and Batwa groups. Some species are most requested, which supposes they could be vulnerable but also could contain interesting phytochemical compounds which warrant further studies.

[1]Mehdioui R. & Kahouadji A. Bulletin de l'Institut Scientifique, Rabat, section Sciences de la Vie, (2007) 29:11-20. [2]Rasoanaivo P. Colloques international « pratiques soignantes, éthique et sociétés », Unesco de Lyon, France. (2005).

POSTER 70

H E D R I N E: A NEW TOOL TO DETECT HERB-DRUG INTERACTION

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Epidemiological studies continue to show that many European people use Complementary and Alternative Medicine (CAM) in pursuit of health and well-being. "Complementary medicine" refers to use CAM together with conventional medicine, such as using phytotherapy products with conventional evidence-based medicines. Be aware that some dietary supplements, natural products may interact with medications; HEDRINE (for Herb Drug Interaction Database) have been created. This website lists clinical studies or cases reports of interactions (or absence of interaction). HEDRINE also indicates potential interactions by a pharmacodynamic or pharmacokinetic mechanism. All records are in French. Each interaction is accompanied by a summary of different observations reported, references with a link to the editor website of the scientific publication. The interpretation is also facilitated by a color code classifying the intensity of each interaction.

A pilot version of the database is accessible at the following address: http://hedrine.ujf-grenoble.fr.

POSTER 71

CHEMOMETRICS FOR DEVEVELOPING ANALYTICAL SEPARATION TECHNIQUES IN PHARMACOGNOSY

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The success of chemometrics over the last decades is well established in the development of analytical separation techniques. In pharmacognosy the complexity of the samples needs to be handled with different analytical separation techniques eg LC-MS, LC-LC, GC-MS. Chemometrics provides the tools to optimize these techniques in an elegant way. The objectives of this paper are to provide concrete applications. Focus will be on alkaloids (ajmaline, papaverine and noscapine) and other pharmaceutical natural products such as oligomeric procyadinins. It also may used on peaks with different concentrations (typically main compound and its impurities). The importance of targeting robustness of the separation at an early stage of the development of separation methods will also be emphasized. This is a key to avoid a complete new development of analytical procedures at the validation stage.

POSTER 72

SELECTION OF ALGERIAN MEDICINAL PLANTS ACCORDING TO A SCREENING OF THEIR ANTI-AGES PROPERTIES

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In a large project on Algerian plants, we are interested in their phytochemistry and biological properties (PHC franco-algérien Tassili, 2012-2016). Particularly, in diabetes, a chronic hyperglycaemia occurs which is responsible for complications of diabetes through advanced glycation end-products (AGEs) formation.[1] SONAS lab recently developed an automated HTS assay, suitable for extracts, to evaluate their anti-AGEs potential.[2] This assay was used to screen different parts of 20 Algerian plants belonging to various families. Pressurized liquid extraction (PLE) led to 82 DCM and MeOH extracts. Seven were selected for their anti-AGEs properties and subjected to dereplication using LC-DAD-MS^n. Some of them were selected for a bioguided fractionation.

[1]. WHO, Diabetes. Available at: http://www.who.int/topics/diabetes_mellitus/en/. Accessed 20 June 2012. [2]. a) S. Derbré *et al. Anal. Bioanal. Chem.* **2010**, 398, 1747; b) L. Séro *et al. Food Chem.* Submitted.

POSTER 73

SYNERGY OF *MUCUNA PRURIENS* FOR PARKINSON'S DISEASE : MOLECULES AND MECHANISMS

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Mucuna pruriens (Fabaceae) seed powder is used in Indian Ayurvedic medicine against Parkinson's disease. It contains up to 5% of L-DOPA[1]. Yet the efficacy of the traditional remedy (5-15g powder daily) has been shown to be better than equivalent doses of L-DOPA : clinically, a shorter T_{max} and a higher C_{max}[2], and *in vivo* less dyskineasias[3]. Our goal is to identify molecules having a biological activity susceptible to explain the synergy effect. Our approach is a systematic assessment of minor compounds by bioguided fractionation or biodereplication to identify and characterize the activity of other compounds besides L-DOPA. Down this project, methodological developments are implemented to provide original tools, including MS online monitoring, to efficiently detect the activity of different compounds on different targets directly within the extract. Targets include DOPA-decaboxylase, monoamine oxidase, catecholamine O-methyl transferase, BBB transport, dopaminergic and cholinergic receptors, P-GP modulation, neuroprotection.

[1]Siddhuraju et *al*. Food Chemistry 2001. [2]Katzenschlager R et *al*. J Neurol Neurosurg Psychiatry 2004 [3]Lieu CA et *al*. Parkinsonism Relat Disord 2010.

POSTER 74

IDENTIFICATION AND BIOACTIVITIES OF ALKALOIDS ISOLATED FROM *ISOLONA COOPERI*

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Isolona cooperi Hutch. & Dalz. (Annonaceae) is a tropical rainforest tree from West Africa traditionally used as tonic and against trypanosomiasis. Only the essential oil has been chemically investigated so far[1]. Stem bark and root extract have been fractionated and yielded several compounds, among them five alkaloids whose structures have been elucidated using MS and NMR. Biological assays on several parasites have been performed. Chemical and biological data are presented and discussed in perspective with the traditional use of this plant.

[1] Boti JB, Koukoua G, N'Guessan TY, Muselli A, Bernardini AF, Casanova J. *Phytochem. Anal.*, 2005, 16, 357-363.

POSTER 75

IN VITRO ANTIBACTERIAL EFFECT OF LAURIC ACID ON *PAENIBACILLUS LARVAE*, CAUSAL AGENT OF AMERICAN FOULBROOD

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American foulbrood (AFB) is a serious disease that affects the larvae of honeybee *Apis mellifera.* It is caused by *Paenibacillus larvae*, a resistant spore forming bacterium, which remains viable through long periods of time and survives environmental adversities[1]. This plague is usually controlled with antibiotics which could leave toxic residues in honey and by products. Laboratory and field trials were conducted to evaluate the effectiveness of lauric acid, an innocuous saturated fatty acid, in the prevention and control of AFB in honeybee colonies. The antimicrobial activity of lauric acid against ten *P. larvae* strains was determined by the broth microdilution method. *In vitro* assays showed the lowest concentration of lauric acid that cause the inhibition of the bacterial strains, The minimal inhibitory concentration (MIC) and the minimal bactericide concentration (MBC) mean values were 20,33 µg mL^{-1} (range: 13,54 - 27,08 mg mL^{-1}). Lauric acid toxicity to adult honeybees was tested by two different methods and registered after 24h, 48h and 72h. The LD_{50} (Lethal Dose 50) mean values obtained were 216,6 mg/bee (24h); 166,9 mg/bee (48h); 137,6 mg/bee (72h) by the oral administration method; and 1.352 mg/plate (24h), 1.255 mg/plate (48h) and 1.214 mg/plate (72h) by the complete exposure method. The LD_{50} indicated that complete exposure method would be the safer one to administer the fatty acid on beehives. The results obtained for lauric acid in the present study contribute to the screening of alternative natural compounds to control AFB in the apiaries. This way, toxicological risks and other undesirable effects, such as resistances due to the indiscriminate use of antibiotics, would be avoided.

[1]Genersch E et al. (2006) International Journal Systematic Evolution of Microbioly, 56:501-511.

POSTER 76

ANTIPARASITIC HYBRIDS OF *CINCHONA* ALKALOIDS AND BILE ACIDS

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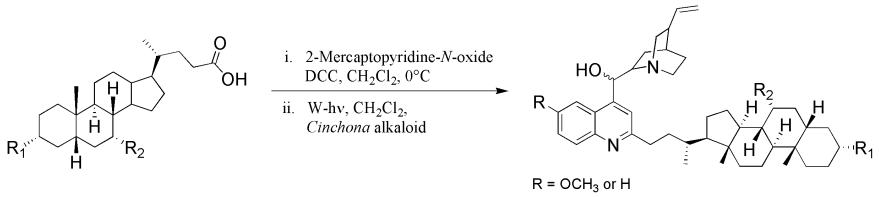
A series of 16 hybrids of *Cinchona* alkaloids and bile acids was prepared by means of a Barton-Zard decarboxylation reaction. Quinine, quinidine, cinchonine and cinchonidine were functionalized at position C-2 of their quinoline nucleus by radical attack of a *nor*-cholane substituent. The compounds were evaluated *in vitro* for their antitrypanosomal, antileishmanial and antiplasmodial activities, along with their cytotoxicity against normal human fibroblast cells. Seven compounds showed promising trypanocidal activity with IC_{50} in the same range as the commercial drug suramine. Moreover all the 16 hybrids showed antiplasmodial activity ($IC_{50} \leq 6$ µg/ml), particularly those containing a *nor*-chenodeoxycholane moiety with IC_{50} comparable to those of the natural alkaloids, and good selectivity indexes.



POSTER 77

PHYTOCHEMICAL STUDY, DIRECT AND INDIRECT ANTIMICROBIAL ACTIVITIES OF *CORDIA GILLETII* DE WILD (BORAGINACEAE)

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Infectious diseases remain a serious public health problem both in developing countries, where they are the main cause of the high mortality rates recorded, and in industrialized countries where there is an alarming incidence of antibiotic resistance. There is thus an increasing need for new compounds that can act by a direct antimicrobial effect or by an indirect effect, inhibiting resistance mechanisms of microorganisms. Medicinal plants, particularly those traditionally used against infectious diseases in developing countries, are a probable source for these types of compounds. In this context, Cordia gilletii De Wild (Boraginaceae), a medicinal plant from which root barks and leaves are traditionally used against infectious diseases in Democratic Republic of Congo, was investigated for biological activities and phytochemical composition. Root bark extracts showed interesting biological activities: (i) antimicrobial properties, acting directly (bactericid and bacteriostatic effects against gram positive and gram negative bacteria, respectively) or indirectly (enhancement or restoration of antibiotic activity on resistant strains); (ii) inhibitory effect on the expression of two Pseudomonas aeruginosa QS genes, lasB and rhlA; (iii) antiplasmodial effect against a chloroquine sensitive strain of Plasmodium falciparum; (iv) antioxidant effect determined by the free radical DPPH quenching. Leaves extracts showed only antiplasmodial activity. Root barks extracts with the highest direct (methanol extract) and indirect (n-hexane extract) antimicrobial properties were fractionated to isolate and to identify the active compounds. To bio-guide the fractionation, the culture medium for the detection of active compounds on chromatographic plates (TLC-bioautography) was optimized. The compound ferulaldehyde, isolated from the methanol extract, showed antimicrobial, antioxidant and antiplasmodial properties. From the n-hexane extract two compounds were isolated, lupeol and friedelin. Lupeol showed indirect antimicrobial effect by decreasing the MIC of some antibiotics against MRSA; whereas friedelin was inactive. Although these three compounds have already been described in other plant species, this is the first report of their occurence in Cordia gilletii; the indirect antimicrobial effect of lupeol is described for the first time in this work. As it belongs to the family of Boraginaceae, a family well known as one of the most important sources of pyrrolizidine alkaloids (PAs), Cordia gilletii is susceptible to contain these toxic compounds that were consequently researched. A GC-MS analysis did not reveal the presence of PAs (detection limit, 2 ppm) in root barks and leaves extracts of C. gilletii, suggesting a lack of PA-related toxicity of this plant. This reassuring finding needs to be confirmed with samples harvested at different locations. This work reveals that C. gilletii may act against pathogenic microorganisms by: (i) a direct antimicrobial effect (partly due to férulaldéhyde); (ii) the enhancement or restoration of antibiotic activity against resistant strains (effect of lupeol); and (iii) an inhibitory effect on the expression of quorum sensing regulator genes, decreasing the virulence of microorganisms. These actions could help to fight infections caused by resistant strains.

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