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STOLON
Sciences végétales et fongiques
Facultés de Pharmacie

AFERP
Association Francophone pour l'Enseignement et la Recherche en Pharmacognosie

2ème Symposium international AFERP-STOLON

- *Biodiversité et substances naturelles* -

Lyon - 15 au 17 juillet 2015

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2ème Symposium International AFERP-STOLON

« Biodiversité et Substances Naturelles »

LYON, du 15 au 17 Juillet 2015

Université Claude Bernard Lyon 1

Institut des Sciences Pharmaceutiques et Biologiques (ISPB)

Campus de Rockefeller, Amphi C Bâtiment Cier et Médiathèque
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Partenaires du Symposium AFERP-STOLON 2015

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2ème Symposium International AFERP-STOLON

« Biodiversité et Substances naturelles »

LYON, du 15 au 17 Juillet 2015

PROGRAMME

Mercredi 15 Juillet 2015	
15h30-18h00	**Accueil des participants**
16h00-17h50	**AG STOLON (salle 105, 1er étage, bâtiment CIER)**
Chairman : *Marie-Geneviève Dijoux-Franca - Pr Lyon* ***Gestion micro*** ***:*** *Vincent Walker*	
18h05-18h15	**Ouverture du Congrès**
18h15-19h00	**J.L. Wolfender** (Université de Genève) « *Metabolomics and innovative biological tests, new perspectives in the chemistry of natural products* »
19h00-22h30 A partir de 20h ...	Cocktail d'accueil « *Du côté du Jardin Botanique …* » Visite possible des collections d'anatomie et d'histoire naturelle Médicale du Musée Testut Latarjet sur le site de la Faculté de Médecine et Pharmacie *Ce musée rassemble des pièces d'anatomie et des collections d'histoire naturelle médicale issues de la Société de Médecine de Lyon et obtenues auprès d'illustres médecins/scientifiques lyonnais (Petit, Testut, Latarjet, Ollier, Lacassagne, …) tout ceci dans un cadre restituant l'atmosphère des cabinets de curiosités d'antan (collection d'anatomie générale, d'anatomie comparée, d'anthropologie criminelle, d'égyptologie …).*  *Musée Testut Latarjet*

Jeudi 16 Juillet 2015	
Axe 1. Biodiversité, Ecologie et Développement durable	
***Chairman** : Marie-Noëlle Vaultier - MCU Nancy (STOLON) Marion Millot - MCU Limoge(AFERP)* ***Gestion micro :** Camile Rozier*	
8h30-9h00	**B. David** (Laboratoires Pierre Fabre) *«New regulations for accessing your biodiversity samples - from theory to practice... »*
9h00-9h30	**A. Favel** (Université Aix Marseille) *« CIRM-CF a Biological Resource Center dedicated to the preservation of filamentous fungi of interest to agro-industries and their utilization»*
9h30-10h30 ***Communications orales***	9h30-9h50 **K. Cottet** (Université Paris-Descartes) « *Comparative metabolomics studies between African and Amazonian* Symphonia globulifera *by LC-tandem MS and NMR* » 9h50-10h10 **G. Chiapusio** (Université de Bourgogne Franche Comté) « *The relevance to study natural plant phenolic productions under environmental changes: the case of* Sphagnum *peatlands* » 10h10-10h30 **L. Bornancin** (Université Perpignan Via Domitia) « *The role of cyanobacterial lipopeptides in structuring a tropical marine ecosystem* »
10h30-11h00	Pause-café – Posters
***Chairman** : Gwenaël Ruprich-Robert - MCU Paris V (STOLON) Marion Girardot - MCU de Poitiers (AFERP)* ***Gestion micro :** Clément Labois et Carole Le Fur*	
11h-11h30	**P.A. Moreau** (Université de Lille 2) *«True morels: from morphology to DNA, advances in taxonomy»*
11h30-12h00	**S. Prado** (MNHN Paris) « *Chemical ecology of endophytic fungi*»
12h00-12h10 ***Flash Posters***	12h00-12h02 **M. Vansteelandt** (Université de Toulouse) « *Inside the medicinal plants: the fungal endophytes, a hidden community as a source of bioactive compounds* » 12h02-12h04 **A. Poinso** (Université de Toulouse) « *SOD inhibitors research from endophytic extracts* » 12h04-12h06 **A. André** (ICSN, Gif-sur-Yvette) « *Viability assay of fungal endophytes extracts on normal Human dermal fibroblasts for potential cosmetic application* » 12h06-12h08 **A. Gadea** (Université de Rennes) « *Lichen chemistry and grazing by the sub-Antarctic snail* Notodiscus hookeri » 12h08-12h10 **F. Vautrin** (Université de Lyon) « *Profiling of primary metabolites of* Poaceae *root exudates in soil* »
12h10-13h40	Déjeuner et Session Posters

Axe 2. Méthodologies et approches pour l'étude des substances naturelles actives	
***Chairman** : Marina Kritsanida, MCU Paris Descartes (AFERP) Gilles Comte Pr Lyon* ***Gestion micro :** Jihane Hamzaoui*	
13h45-14h15	**F. Chemat** (Université d'Avignon et des Pays de Vaucluse) *« Green extraction of Natural Products as tools for obtaining Food, Pharmaceutical and Cosmetic ingredients »*
14h15-14h45	**F. Bourgaud** (Université de Lorraine) *«Biosynthesis of furanocoumarins in higher plants: fundamental aspects and practical applications in the production of non-toxic* Citrus*»*
14h45-15h45 ***Communications orales***	14h45-15h05 **D. Parrot** (Université de Lyon) « *Phenylpropanoids pathway: Bioinformatics and modeling approaches to increase the production of metabolites of interest* » 15h05-15h25 **L. F. Nothias-Scaglia** (ICSN, Gif-sur-Yvette) « *Molecular networking an innovative tool for natural products research: Application to the phytochemical studies of* Euphorbia *species* » 15h25-15h45 **P. Le Pogam** (Université de Rennes) « *LDI-MSI : Distribution of metabolites within* Ophioparma ventosa »
15h45-15h51 ***Flash Posters***	15h45-15h47 **T. Péresse** (ICSN, Gif-sur-Yvette) « *Detection and isolation of prenylated stilbenes from various* Macaranga *species using molecular networking* » 15h47-15h49 **A. Quero** (Université d'Amiens) *« Phytochemical analysis of* Camelina sativa *during seed development »* 15h49-15h51 **B. Thiombiano** (Université Amiens) *« Mass spectrometry characterization of the esterified flaxseed molecules: from a lignan complex to a phenylpropanoid polymer »*
15h51-16h30	Pause-café – Posters
***Chairman** : Florence Souard - MCU Grenoble (AFERP) Marie-Geneviève Dijoux-Franca - Pr Lyon* ***Gestion micro :** Florian Vautrin*	
16h30-17h00	**E. Gontier** (Université de Picardie Jules Verne) *« Metabolites and plant secondary metabolism; examples of approaches for the study of biosynthesis and valorization of plant chemodiversity »*
17h00-17h30	**J. Hubert** (Université de Reims Champagne Ardennes) « *Chemical profiling of natural extracts: A* ^{13}*C NMR-based dereplication strategy*»
17h30-18h10 ***Communications orales***	17h30-17h50 **J-B. Gallé** (Université de Strasbourg) « *Isolation of vismiones from* Psorospermum *species and synthetic approach toward Leishmanicidal molecules* » 17h50-18h10 **M. Tsoukalas** (Université de Strasbourg) *« New pregnane glycosides from* Cynanchum *spp. (*Apocynaceae*) with antidiabetic potential »*

18h10-18h20 ***Flash Posters***	18h10-18h12 **I. Zebiri** (Université de Reims Champagne Ardennes) *« Phytochemical study of* Dendrobangia boliviana *»* 18h12-18h14 **P. Bernard-Savary** (Club de CCM, Chromacim) *« Accelerated discovery and profiling of physiologically active components in complex samples by HPTLC-EDA-HRMS »* 18h14-18h16 **P. Waffo Teguo** (Université de Bordeaux) *« Centrifugal partition chromatography applied to grapevine and wine chemistry »* 18h16-18h18 **M-A Tribalat** (Université de Nice Sophia Antipolis) *« Exploration of the biosynthetic pathway leading to saraines, original alkaloids produced by the sponge* Haliclona sarai *»*
18h20-19h00	**Session posters**
19h45	Diner de Gala *« Au fil de l'eau … »*

Vendredi 17 Juillet 2015	
Axe 3. Médecines traditionnelles / MAC	
***Chairman** : Serge Michalet - MCU Lyon (AFERP) Didier Blaha - MCU Lyon (STOLON)* ***Gestion micro :** Rosa Padilla*	
8h30-9h10 ***Communications orales***	8h30-8h50 **M. Sauvain** (IRD Lima, Pérou) *« In vitro Anti-*Helicobacter pylori *activity of plants used for gastrointestinal disorders in traditional medicine from coast, Andes and central forest of Peru »* 8h50-9h10 **V. Julllian** (Université de Toulouse) *« From Malaria to cancer:* Quassia amara *and Simalikalactone E (SkE) »*
9h10-9h20 ***Flash Posters***	9h10-9h12 **C. Shalukoma** (Université libre de Bruxelles) « *Typology of healers in traditional medicine around the Kahuzi-Biega national Park,a World Heritage Site in danger, DR Congo »* 9h12-9h14 **J. Ngezahayo** (Université libre de Bruxelles) « *Antibacterial activity of pentacyclic tripenoids acids from* Platostoma rotundifolium *aerial parts »* 9h14-9h16 **O. Danton** (Université de Clermont-Auvergne) « *Extraction of natural antalgics from plants used in traditional medicine in Mali : Study of* Pericopsis laxiflora *»* 9h16-9h18 **D. Guedon** (Laboratoires Arkopharma) « *Genotoxicity testing of herbal medicinal products containing quercetin: avoiding pitfalls in Ames tests »* 9h18-9h20 **S. Michel** (Université Paris-Descartes) *« Association des amis du musée François Tillequin* »
9h20-9h40 ***Communication orale***	9h20-9h40 **A. Harfouche** (Université de Paris Sud) « *Synergy of* Mucuna pruriens *for Parkinson's disease: Molecules and mechanisms »*
9h40-10h10	**A. Maciuk** (Université Paris-Sud) - **G. Cousyn** (DGCCRF) *«Dietary supplements: the spirit of the laws»*

10h10-10h40	Pause-café – Posters
Gestion micros : *Hoang Nam Pham et Carole Le Fur*	
10h40-12h10	**Table ronde : *« L'arrêté plantes en questions »*** Animée par **P. Champy** (Université Paris-Sud) et **S. Boutefnouchet** (Université Paris-Descartes) Participants : **V. Siranyan**, Université Lyon1, MCU Droit Santé Publique, membre de du conseil central D de l'Ordre des Pharmaciens. **B. Montreuil**, Pharmacien, Bron, Président du Syndicat des Pharmaciens du Rhône. **B. Dal-Gobbo**, Médecin généraliste, Bourg en Bresse. **D. Barret**, Laboratoires Iphym, Lyon. **G. Cousyn**, DGCCRF.
12H10-12h30	Remise de prix Clôture du congrès (E. Seguin AFERP et Y.F. Pouchus STOLON)
12h30-13h45	Déjeuner
14h30-17h30	Visite du Musée des Confluences http://www.museedesconfluences.fr/

Samedi 18 Juillet 2015

Journée Post-Congrès proposée par Chromacim/Club CCM

8h15-19h00	Cette journée comportera une sortie sur le terrain et des visites : ***« Découverte de la Chartreuse : Lichens et plantes alpines, Monastère de la Grande Chartreuse, Cave et distillerie de Voiron, Chocolaterie Bonnat »*** Vous trouverez le programme détaillé de la journée ainsi que l'application d'inscription sur le lien suivant (inscription obligatoire, 50 euros, tout compris : transport, repas, visite). ⇨ cliquez ici : **Journée du 18 Juillet 2015, Sortie sur le Terrain : Découverte de la Chartreuse !** http://clubdeccm.inviteo.fr/index.php?onglet=6 Le nombre de places sera au maximum de 48 personnes. N'hésitez pas à vous inscrire dès maintenant !

Résumés
Communications orales
Flash Posters

O1 – Conférence plénière invitée

METABOLOMICS AND INNOVATIVE BIOLOGICAL ASSAYS, NEW PERSPECTIVES IN THE CHEMISTRY OF NATURAL PRODUCTS

Jean-Luc Wolfender

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

The comprehensive analysis of a plant metabolome is very complex [1]. The large chemical space occupied by natural products (NPs) is directly linked to a high variability of their intrinsic physicochemical properties that render their separation, detection and characterization challenging. In order to identify all these metabolites, crude plant extract profiling is essential. This is a task that requires methods providing high chromatographic resolution for detailed profiling or high throughput for rapid quantification or fingerprinting analysis. Furthermore these methods should give on-line spectroscopic information for the identification of each individual metabolite for dereplication purposes. In this respect hyphenated techniques such as LC-MS and LC-NMR have played a key role over the last three decades [2]. In phytochemical analysis, the recent introduction of Ultra High Pressure Liquid Chromatography (UHPLC) systems using sub-2 µm packing columns have allowed a remarkable decrease in analysis time and increase in peak capacity, sensitivity and reproducibility compared to conventional HPLC. In complement to this powerful chromatographic method, the introduction of benchtop high resolution MS instruments provide sensitive detection and accurate MS and MS/MS information for dereplication. For *de-novo* identification of NPs on-line LC-NMR, introduced in the early 90's, has evolved towards sensitive at-line microflow NMR approaches combining pre-concentration of the LC peaks of interest prior to NMR measurement (LC-SPE-NMR, capillary NMR: CapNMR). With such methods structure determination of targeted compounds at the low microgram level is possible and complement the search in MS and chemotaxonomic data bases for dereplication. The potential and limitations as well as some new trends in the development of UHPLC-MS and micro NMR will be discussed. In particular examples related to metabolomics and *de novo* biomarker identification will be presented. The impact of these technologies in NP research studies and perspective of use of related state-of-the-art methods in terms of evolution or revolution in the field will be discussed.

1. Wolfender JL, Rudaz S, Choi YH, Kim HK. Plant metabolomics: from holistic data to relevant biomarkers. *Curr. Med. Chem.* 2013 **20:** 1056-90.
2. Wolfender J-L, Marti G, Thomas A, Bertrand S. Current approaches and challenges for the metabolite profiling of complex natural extracts. *J Chromatogr A* 2015 **1382:** 136-164.

Axe 1. Biodiversité, écologie et développement durable

O2 – Conférence invitée

NEW REGULATIONS FOR ACESSING YOUR BIODIVERSITY SAMPLES - FROM THEORY TO PRACTICE…

Bruno David

Institut de Recherche Pierre Fabre, 3 avenue Hubert Curien, BP 13562, F-31035 Toulouse, France.

Legal access to biodiversity is now a strategic point for every natural product researcher in either academic or industrial sectors. The need to share the fair and equitable benefits arising from the utilization of genetic/biological resources established by the Rio Convention (1992) was reaffirmed in Nagoya (2010)[1]. On 12 October 2014, the Nagoya Protocol entered into force internationally, therefore national laws[2], the European regulation EU N°511/2014[3] are to be clearly understood and followed.

The presentation will describe the new practical processes for legally accessing to our biodiversity samples from national[2], European[3] or international collects. Prior Informed Consent (PIC), Mutually Agreed Term (MAT) contracts, administrative formalities and obligations will be detailed. The main issues of the Nagoya protocol implementation will be discussed: definition of scope, utilization of commodities, establishment of clear, fair, transparent and efficient rules of access in every country, dispute settlement and multilateral benefit sharing mechanism …

These laws could play a pivotal role in unleashing the power of biodiversity in a sustainable way and a spirit of fair, shared and equitable development. However, the new access laws should not be overly complex and burdensome for both academic and industrial researchers in order to avoid paradoxical effects. In fact, without effective, practicable and secure access, no valorisation and no benefit sharing will be possible toward the source countries by the pharmaceutical and cosmetics industries or university researchers. Sustainable and compliant access should be facilitated in the interest of all stakeholders: source countries, local populations, academic researchers, industries, patients and customers of biodiversity derived products.

[1] http://www.cbd.int/abs/doc/protocol/nagoya-protocol-en.pdf
[3] http://ec.europa.eu/environment/nature/biodiversity/international/abs/index_en.htm
[2] http://www.assemblee-nationale.fr/14/ta/ta0494.asp

O3 – Conférence invitée

CIRM-CF A BIOLOGICAL RESOURCE CENTER DEDICATED TO THE PRESERVATION OF FILAMENTOUS FUNGI OF INTEREST TO AGRO-INDUSTRIES AND THEIR UTILIZATION

Anne Favel

UMR1163 BBF Biodiversité et Biotechnologie Fongiques, Faculté des sciences de Luminy - ESIL 163 avenue de Luminy CP 925 13288 Marseille cedex 09

O4

COMPARATIVE METABOLOMICS STUDIES BETWEEN AFRICAN AND AMAZONIAN SYMPHONIA GLOBULIFERA BY LC-TANDEM MS AND NMR

Kévin Cottet,[a] Eirini Kouloura,[b] Maria Halabalaki,[b] Marina Kristanida,[a] Jean-Duplex Wansi, [c] Guillaume Odonne,[d] Christophe Duplais,[e] Emmanuel Micros,[f] Sylvie Michel, [a] Leandros A. Skaltsounis,[b] Lallemand Marie-Christine[a]*

[a]*Laboratoire de Pharmacognosie, UMR CNRS 8638 COMETE, Université Paris Descartes Sorbonne Paris Cité, 4 Avenue de l'Observatoire 75006 Paris France.* [b]*Division of Pharmacognosy and Natural Product Chemistry, Departement of Pharmacy, Univeristy of Athens,* Panepistimiopolis, Zografou 15771, Anthens Greece

Introduction: *Symphonia* globulifera is a widespread tropical species belonging to the clusiaceae family and containing secondary metabolites exhibiting interesting biological activities [1]. This species shows morphological variations which seem to be dependent of his ecological distribution [2], moreover data from literature concerning phytochemical compositions of *S.g.* from different regions mismatch [1].

Objectives and methodology: We decided to investigate metabolome compositions of four *S.g.* plants parts from different areas (Cameroun and French Guiana) using a metabolomics based study. Next to a quasi exhaustive extraction (CH_2Cl_2/MeOH 1:1, under sonication), leaves, bark, latex and twigs extracts were divided into a polar and an apolar phase prior to be analyzed with an UPLC-MS/MS system (Thermo Orbitrap) and NMR (Bruker 600 MHz).

Results: Both statistical analyses reveal these differences mainly come from apolar compounds as shown in Fig 1. Fifty-four known compounds responsible of the metabolome variation have been putatively identified using fragmentation patterns. Biological activities of these compounds reveal a large proportion of them (31%) have an anti-microbial or anti-parasitic activity, giving clues about a metabolome adaptation of a same species to different microbial pressure.

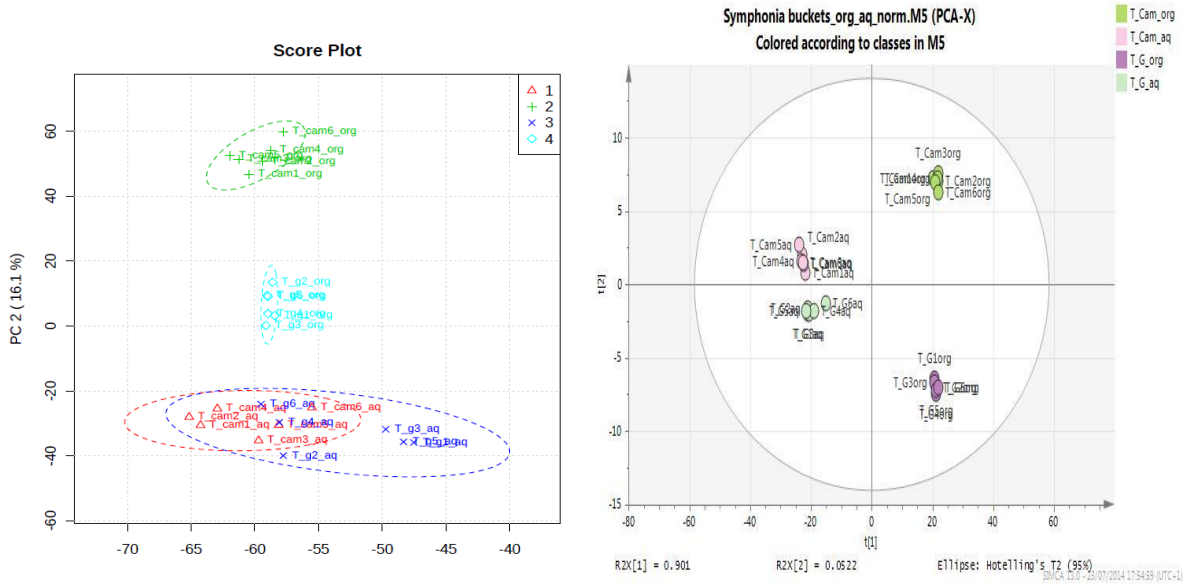

Fig. 1: PCA analysis along PC1 and PC2 from twigs LC-MS data in negative mode (a) (pink: polar Cameroon, grey: polar French Guiana, green: aplolar Cameroon, purple : apolar French Guiana) and from NMR data (b) (red : polar Cameroon, orange: polar French Guiana, green : apolar Cameroon, blue : apolar French Guiana)

[1] : Y. Fromentin *et al*, Planta Medica, 2015, 81(2), 95-107.
[2] : Dick CW, Heuertz M.. Evolution 2008; 62: 2760–2774

O5

THE RELEVANCE TO STUDY NATURAL PLANT PHENOLIC PRODUCTIONS UNDER ENVIRONMENTAL CHANGES: THE CASE OF *SPHAGNUM* PEATLANDS.

G Chiapusio, VJ Jassey, ML Toussaint, P Binet

University of Bourgogne Franche Comté, ChronoEnvironment UMR CNRS 6249,USC INRA, BP 71 427, FR-25 211 Montbéliard cedex. France.

The accumulation of phenolics in vascular plant tissues is considered as a common adaptive response of plant to adverse environmental conditions [1]. Nevertheless, a clearer understanding of secondary plant metabolic response to environmental changes is still needed to facilitate our knowledge in plant defense and plant-plant or plant-microbial communities' interactions. In particularly, we hypothesize that environmental changes strongly influence aboveground-belowground interactions by modifying chemical interactions that alter ecosystem attributes and functions.

Since 2008, our original research focuses on *Sphagnum* allelochemical interactions in a french peatland where an experimental moderate climate forcing (1 up to 3°C) was created by Open Top Chambers over vegetation. Objectives are to understand 1) the production, degradation and allelopathic effect of living *Sphagnum* polyphenols and 2) how warming affects *Sphagnum* allelochemical interactions and the peatland functionning.

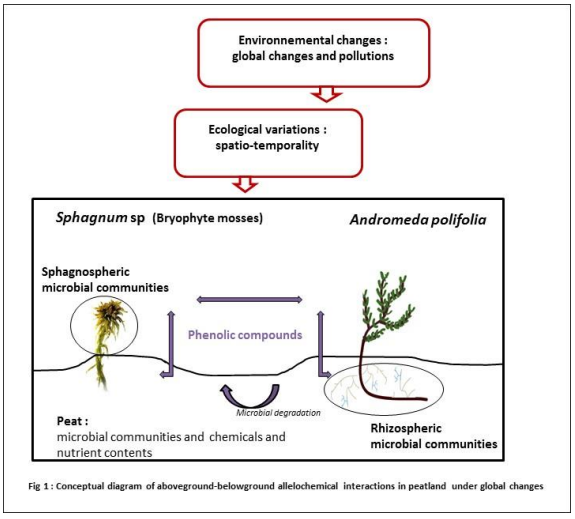

Fig 1 : Conceptual diagram of aboveground-belowground allelochemical interactions in peatland under global changes

Results show that water-soluble phenolics are produced by living *Sphagnum fallax* (around 1 mg g^{-1} DW) and that *Sphagnum*-peroxidases constitute their main oxidative system (10^{-3} U/g DW). Phenolics play a crucial role in the micro distribution of associated *Sphagnum* micro-organisms (specially the top predators) but also on surrounding *Andromeda* mycorrhiza symbioses (Fig 1). Under climate change, *Sphagnum* decreased its phenolic concentrations (by around -1.5 times) [2] and so greatly impact phenolic-microbial interactions [3].

Such experimental warming interact to shape plant secondary metabolism and consequently allelochemical interactions found in peatland but such research still remains an important challenge for scientists [4].

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O6

THE ROLE OF CYANOBACTERIAL LIPOPEPTIDES IN STRUCTURING A TROPICAL MARINE ECOSYSTEM

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In the lagoon of Moorea, French Polynesia, we have identified a relatively simple tropical marine ecosystem consisting of two primary producers (two filamentous cyanobacteria, *Lyngbya majuscula* and *Anabaena cf. torulosa*), two herbivorous molluscs (the opisthobranchia *Stylocheilus striatus* and *Diniatys dentifer*), an omnivorous nudibranch (*Gymnodoris ceylonica*) and a carnivorous crab (*Thalamita coerulipes*). *L. majuscula* and *A. torulosa*, that can proliferate across a wide sandy area and even on corals, are prolific producers of secondary metabolites, mainly cyclic lipopeptides[1], which may either be toxic or act as feeding deterrents to potential consumers. The sea hare *S. striatus* is able to sequester and modify some secondary metabolites produced by *L. majuscula*[2,3]. However, the fate of the metabolites in other species, and more broadly the role of lipopeptides in inter-kingdom signaling, is unknown. In this model ecosystem, our ultimate goal is to highlight the cascading effects of chemical mediators in multi-trophic relations; expression by primary producers, mechanisms of tolerance and sequestration of "toxins" acquired from dietary sources, and chemical recognition mechanisms in intra-specific or inter-specific relationships. The first aim of our research was to complete the metabolic profile of both cyanobacteria. Focusing our attention on *A. torulosa* we isolated new acyclic peptides, derived from the known cyclic laxaphycins[4], and characterized them using extensive NMR experiments (1D and 2D NMR: COSY, TOCSY, HSQC, HMBC, NOESY) and mass spectrometry (HR mass spectrometry and fragmentation by MS^n). It is the first time that acyclic analogs of laxaphycins have been described although numerous samples have been studied during the two last decades. The vast majority of secondary metabolites have now been characterized for both cyanobacteria and will be discussed. The two metabolic profiles and the first results concerning the sequestration of cyanobacterial metabolites along the trophic chain will be presented.

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O7 – Conférence invitée

TRUE MORELS: FROM MORPHOLOGY TO DNA, ADVANCES IN TAXONOMY

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True morels (genus *Morchella*, *Ascomycota*) perfectly illustrate the difficulties which taxonomists have to face for four centuries in classifying and naming biodiversity. Species delimitation, in such groups in which morphology is hardly interpretable, collides with our own limit of interpreting the few available characters, and requires an addition of ecological, biogeographical, and now molecular data.
Are morels two, three, or rather twenty, thirty or more species? "Integrative" taxonomy, which associates these various pieces of answers in order to propose a robust species concept, also rises up new questions, especially on evolutionary history, geographical patterns, and capacities of dispersion of morels.

O8 – Conférence invitée

FUNGAL ENDOPHYTES CHEMICAL ECOLOGY: NATURE AND ROLE OF THE FUNGAL SECONDARY METABOLITES

Soizic Prado.

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Most of the plants in natural and anthropogenic ecosystems are colonized by unapparent and symptomless microorganisms (bacteria and fungi) called endophytes. Indeed, these are invading the living tissues (leaves, stems and roots) of the host plant without causing any symptom of disease. In addition, endophytic fungi have been shown to establish mutualistic associations with their host plants and conferring fitness benefits such as tolerance to biotic and abiotic stresses. This mutualistic relationship between endophytes and host-plant is regulated by the production of various metabolic factors which nature, origin and mechanisms of action remain to be investigated. Moreover, involvement and regulation of such metabolites in the functioning of the microbiome are still poorly understood despite their impressive and original chemical diversity often associated with relevant biological activities.

In this context, we studied and identified the cultivable fungal diversity of *Cephalotaxus harringtonia*, an Asian conifer tree of medical importance. We then focused on the isolated endophyte *Paraconiothyrium variabile* which the genome was recently sequenced. The chemical mediators of this fungal endophyte will be presented as well as their potential roles in the course of interactions with i) phytopathogens, ii) bacteria associated with the microbiome and iii) the host plant itself.

FP1

INSIDE THE MEDICINAL PLANTS: THE FUNGAL ENDOPHYTES, A HIDDEN COMMUNITY AS A SOURCE OF BIOACTIVE COMPOUNDS

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In the field of natural products, plants have proven their interest as a source of lead compounds in drug discovery. Nevertheless, in the context of biodiversity preservation, researchers recently focused their work on renewable sources, like micromycetes, which present the ability to modulate their metabolism depending on the substrate. Fungal endophytes are micro-organisms that grow into the internal tissues of the host-plant, *i.e.* a unique environment peculiar to each plant in a specific geographic location [1], and thus represent an especially exciting and relatively untapped source of new bioactive metabolites. The relationship between the host and its endophytes is described as symbiotic, but may change over time, from mutualism to parasitism [2]. Moreover, each endophyte also displays interactions with the entire microbiome of the plant. The project led in our research group is aiming at a better understanding of the communities of medicinal plants-associated endophytes, especially their diversity and their interactions, as well as the search for new antiparasitic compounds. Medicinal plants from South America and North Africa were investigated for their culturable fungal endophytes diversity: 409 ascomycetous strains were isolated from 21 plants freshly collected. ITS rDNA was sequenced for 346 isolates: 71% of the isolates belong to the Sordariomycetes class, and 29% are Dothideomycetes. Secondary metabolome of all the strains is currently under investigation concurrently with a primary bioassay screening on *Leishmania infantum*. Our major goals are to correlate the potential bioactivity to the UHPLC-ESI-QTOF MS profiles to highlight bioactive markers, and to correlate these chemical profiles to the DNA sequences to discriminate fungal strains using both molecular dereplication techniques and chemical diversity analysis.

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FP2

SOD INHIBITORS RESEARCH FROM ENDOPHYTICS FUNGI EXTRACTS

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SOD inhibitors are proven to be very interesting leads to cancer's treatment and cisplatine's resistance reversion (1). Natural substances are a huge "pot of gold" for new molecules research. So we are trying to find these SOD inhibitors from natural substances, and especially in fungal endophytes, because of the lack of knowledge concerning these fungi. The endophytes have been cultured on Malt-Agar medium from plants harvested in Amazonian forest, and extracted by EtOAc.

We developed an enzymatic test, reproducible, fast and well adapted in fast-screening research of SOD inhibitors. This method is based on pyrogallol colorimetric properties (2). The preliminary results show an anti-SOD activity in several endophytes fungi extracts. The test also allows us to determinate the anti-oxidant properties of the extracts.

However, in order to confirm the results, it should be supplied by LC-MS analysis, method that we have already started setting up. Thanks to these data, we will easily be able to perform the bio-guided extraction of the interesting metabolites and then the determination of their structure by NMR.

SOD: SuperOxide Dismutase

References: (1): Papa, Luena, Giovanni Manfredi, et Doris Germain. « SOD1, an Unexpected Novel Target for Cancer Therapy ». Genes & Cancer 5, no 1-2 (avril 2014): 15-21.

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FP3

VIABILITY ASSAY OF FUNGAL ENDOPHYTES EXTRACTS ON NORMAL HUMAN DERMAL FIBROBLASTS FOR POTENTIAL COSMETIC APPLICATION

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Objective: The 'Substances Naturelles et Biodiversité' team of the ICSN has a collection of leaf endophytic fungi from French Guiana. These fungi represent an innovative source of natural products, all of them have been characterized by sequencing (ITS), and their extracts have been tested in various bioassays including cytotoxicity on MRC5 (non-cancerous Human lung fibroblasts) and MDA-MB-435 (metastatic melanoma) cells [1]. In order to study the cosmetic potential of the extract collection, cytotoxicity on Normal Human Dermal Fibroblasts (NHDF) have to be tested and compared to the other cytotoxicity data available.
The cell viability of NHDF was assessed for eighty non-cytotoxic microorganisms extracts (exhibiting less than 25 % of cell proliferation inhibition after 72 hours of incubation on MRC5 and MDA-MB-435). The assay is based on the reduction of resazurin to resorufin by metabolically active cells (Cell Titer-Blue assay, Promega). The fluorescent signal of the product resorufin is measured in a multiplate reader (Wallac 1420 Victor^2 multilabel counter, PerkinElmer).
Results: Among eighty non-cytotoxic extracts at the concentration of 10 µg/mL on MRC5 and MDA-MB-435 cells, sixty-one appeared to be cytotoxic on NHDF after only a 24 hours period of incubation (less than 25% of cell proliferation inhibition).
Conclusion: In order to study the potential of leaf endophytic fungi as cosmetic active ingredients, classic cell lines used for viability assays (MRC5 and MDA-MB-435) are not representative of the sensibility of dermal cells. The use of NHDF to assess the non-cytotoxicity of these extracts is the first step for the biological study of the collection.

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FP4

LICHEN CHEMISTRY AND GRAZING BY THE SUB-ANTARCTIC SNAIL *NOTODISCUS HOOKERI*.

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The gastropod *Notodiscus hookeri* is the only molluscan species native from Sub-Antarctic islands and widespread on Possession Island. Two ecophenotypes exist in Crozet Archipelago. The mineral type which is characterized by a mineralized shell, is located along the coast line, and could be associated to the presence of calcium in the clay minerals of the soils. The organic type, characterized by an organic shell is distributed at high altitudes in the fell-field, without clay and exchangeable calcium [1]. *N. hookeri* feeds exclusively on lichens. Lichens are symbiotic organisms widely distributed in this Sub-Antarctic region, as crustaceous, foliaceous and fruticose thalli and their photobionts could either be cyanobacteria or green algae. Previous experiments highlighted that *N.hookeri* eats preferentially some lichens rather than others. Secondary metabolites could have an influence in snail's feeding choices. We intend to explore the diet of *N. hookeri* according to its ecophenotype and the distribution of secondary metabolites in lichens.
LC-UV-MS profiling of *Pseudocyphellaria crocata*, *Usnea antarctica*, and *Argopsis cymosa* was performed. In *P. crocata*, three main structural groups were found as major metabolites: depsides (tenuiorin and its derivatives), depsidones (stictic acid related compounds) and pulvinic derivatives. The dibenzofurane derivative usnic acid was identified as the major metabolite of *U. antarctica,* while depside atranorine and depsidone lobaric acid are mainly found in *A. cymosa*. Then, these compounds were quantified by HPLC-UV to study their effects (palatability or toxicity) on *N. hookeri* after validation of biological model of feeding choices.

Reference: [1].Charrier, M. *et al. PLoS ONE* **8,** (2013).

Mots clés :
Métabolisme du cholestérol, profilage enzymatique et métabolomique ciblé, cancer, statines.

FP5

PROFILING OF PRIMARY METABOLITES OF *POACEAE* ROOT EXUDATES IN SOIL

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The rhizosphere, the region of soil surrounding a plant root, is the site of highest microbial biomass and activity. It is here that many plant/bacteria interactions take place controlled mainly by root exudates (1, 2). A large body of knowledge suggests that root exudates may act as messengers that communicate and initiate biological and physiological interactions between plant roots and soil organisms (3). Root exudation clearly represents a significant carbon cost to the plant. Indeed, 5 to 20% of carbon fixed by plant being released to the rhizosphere soil (1). Plant-microorganism interactions have been studied extensively, but major questions remain unanswered because of an inability to characterise root exudates in the rhizosphere soil. To date, root exudates characterisation was carried out only using hydroponics cultures (3) and capture of root exudates *in situ (4)*, which is not a realistic model because of the lack of telluric bacterial communities and complex soil matrix. Therefore, the understanding of mechanisms that rule plant-microbes communication needs the integration of various disciplinary fields such as biology, ecology, genetics and metabolomics.

In this study, we proposed to analyse the metabolic profile of root exudates released into the Root Adhering Soil (RAS) by five *Poaceae.* Root exudates are divided into two classes: primary and secondary metabolites. Only the primary metabolites profiles (sugar, amino acids and organic acids) are presented in this study because of their high carbon content. Plants were grown in triplicates in greenhouse for 10 weeks and the RAS from each plant was immediately lyophilised and conserved at -80°C. Root material was carefully washed, immediately lyophilised and conserved at -80°C. Both RAS and root system were ground to obtain fine powder. Primary metabolites were then extracted from RAS and roots and analysed using GC-MS after a derivatization step.

Our results demonstrated (i) that we are able to characterize root exudates extracted from the root adhering soil and the root system, (ii) the presence of diversified carbon sources from each class of molecules with significant differences between the root and RAS, (iii) significant differences on quantity and composition of these classes of molecules according to plant species as tested by Pricipal Componant Analysis (PCA). For example, *Dactylis glomerata* exudes more in quantity and diversity of sugar, amino acids and organic acids, than *Festuca paniculata* which exudes only sugar.

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Axe 2. Méthodologies et approches pour l'étude des substances naturelles actives

O9 – Conférence invitée

Green extraction of Natural Products as Tools for Obtaining Food, Pharmaceutical and Cosmetic Ingredients

Pr. Farid CHEMAT

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This presentation will introduce a new and innovative area in the frontiers of chemistry, biology and processing: green extraction with special emphasis on medicinal and aromatic plants. Green extraction is a part of the sustainable development concept; its history, concept, principles and fundamentals will be described. We will pay special attention to the strategies and the tools available to make biorefinery greener. The representation will present the innovative research in this area these past five years in term of innovative techniques (microwave, ultrasound, pulse electric field...) and alternative solvents (ionic liquids, sub and supercritical fluid, agrosolvents, water...) applied to this new area green extraction of natural products with special examples applied to biorefinery concept.

A general definition of green chemistry is the invention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances. In relation of green extraction of natural products, this definition can be modified as follows: "*Green Extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/product*". The listing of the "six principles of Green Extraction of Natural Products" should be viewed for industry and scientists as a direction to establish an innovative and green label, charter and standard, and as a reflection to innovate not only in process but in all aspects of solid-liquid extraction. The principles have been identified and described not as rules but more as innovative examples to follow discovered by scientist and successfully applied by industry.

Eco-Extraction du Végétal : procédés innovants et solvants alternatifs. DUNOD, Paris, 2011. F. Chemat, J. Strube
Green Extraction of Natural Products. Theory and practice, Wiley-VCH, Weinheim, 2015.

O10 – Conférence invitée

BIOSYNTHESIS OF FURANOCOUMARINS IN HIGHER PLANTS: FUNDAMENTAL ASPECTS AND PRACTICAL APPLICATIONS IN THE PRODUCTION OF NON-TOXIC CITRUS

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Furanocoumarins are a subclass of polyphenolics which constitute important plant defense compounds. They are mainly found in 4 higher plant families: Apiaceae, Moraceae, Fabaceae and Rutaceae. These molecules have been documented as one of the most explicit example of chemical warfare between plants and insects: plants have elaborated new cocktails of furanocoumarin during the course of evolution while, in parallel, some insects have acquired the capacity to detoxify such compounds.

Furanocoumarin biogenesis in plants has remained elusive until recently at the molecular level. However, the first genes and enzymes involved in furanocoumarin synthesis have started to be described. As regards the linear furanocoumarin bergapten, starting from the precursor molecule *p*-coumaroyl CoA we now have 5 different enzymatic steps (one oxoglutarate-dependent dioxygenase, one prenyltransferase, one cytochrome P450 and one methyltransferase) out of a total of 6 that have been unraveled so far, the last missing step being marmesin synthase, provisionally assigned as another cytochrome P450. Biochemical studies have revealed that the pathway is tightly regulated by feedback loops down-regulating key-enzyme activities when furanocoumarins reach a high intracellular level. This down-regulation process is operating on several enzymes of the pathway and matches well with the necessity of the plant cells to protect themselves against the toxicity of the furanocoumarin they elaborate.

In addition to their ecological functions in plants, furanocoumarins are important toxicants in human diet, mainly found in some citrus species. This presentation will emphasize on the distribution of 27 coumarins and furanocoumarins found in 61 Citrus varieties from the 4 ancestral Citrus taxons (pummelos, mandarins, citrons and papedas). Based on this survey, we propose new breeding strategies to develop Citrus devoid of the toxic furanocoumarins.

O11

PHENYLPROPANOIDS PATHWAY: BIOINFORMATICS AND MODELING APPROACHES TO INCREASE THE PRODUCTION OF METABOLITES OF INTEREST

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Phenolic compounds are secondary metabolites produced by plants, whose physiological role is not fully elucidated. However, they are known in plant-environment relations (attractive or repulsive compounds), pigmentation of flowers and fruits and also for antioxidant properties that arouse much interest in the prevention and treatment of diseases. In the part of the BacHBerry project in which we are involved (BACterial Hosts for production of Bioactive phenolics from bERRY fruits to products - Project No. FP7-613793), we focus on metabolic engineering to propose sustainable alternative pathways to produce polyphenols and to determine which genes should be transferred into bacteria (*Lactoccocus lactis* and *Corynebacterium glutamicum*) for high-value phenolics production in scalable fermentation bioprocesses. In this context, we develop algorithms to reconstruct metabolic pathways from genome-scale networks, experimental data (-omics data) and databases taking into account regulatory pathways, stoichiometric data, cellular compartmentalization, etc. We propose an intuitive visualization tool and three exploratory algorithms (currently in development) to efficiently highlight heterologous pathways.

O12

MOLECULAR NETWORKING, AN INNOVATIVE TOOL FOR NATURAL PRODUCTS RESEARCH: APPLICATION TO THE PHYTOCHEMICAL STUDIES OF *EUPHORBIA* SPECIES

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Analysis of natural extracts by liquid chromagraphy coupled to mass spectrometry in tandem mode (LC-MS/MS) is considered to be one of the most versatile and holistic method to date. Typically, few hundreds to thousands of MS/MS spectra can be obtained from a single analysis in data-depend mode. Interpretation of these large amounts of datasets is time-consuming, often tricky and thus can be misleading. A recent innovative bioinformatic tool, named molecular networking, had been designed to assist interpretation of LC-MS/MS data [1,2]. In this presentation, we will expose the successful implementation of molecular networking in our on-going work on Corsican *Euphorbia* plant extracts [3]. LC-MS/MS analyzes were performed on QTOF apparatus. GnPS internet plateform was used to generate molecular networks [4]. Data were visualized with Cytoscape software [5]. Results indicated that molecular networking can efficiently be used to (i) map chemical diversity, (ii) detect unknown analogues of reference standards and (iii) identify potentially novel compounds. In order to isolate compounds highlighted thanks to molecular networking, an original structure-guided-based purification workflow has been designed. Based on these results, we believe that molecular networking is going to become an essential tool for natural products research community.

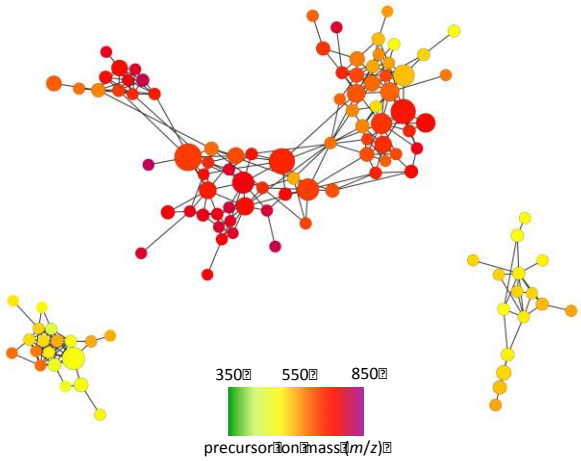

Fig. 1 **Representation of molecular networks**

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O13

LDI-MSI : DISTRIBUTION OF METABOLITES WITHIN *OPHIOPARMA VENTOSA*

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Ophioparma ventosa is a crustose mountain-dwelling lichen that contains a set of constant chemical constituents (haemoventosin, usnic, divaricatic and thamnolic acids) accompanied by variable additional compounds (e.g atranorin, various depsidones...)[1]. Our phytochemical investigation of Austrian samples of *O. ventosa* revealed the occurrence of a rare depside, miriquidic acid. Given *O. ventosa'*s aggressiveness and its trend to overrun neighboring lichens, the origin of such an array of variable compounds raises questions: does it correspond to several chemotypes or is it acquired through assimilation from overgrown lichens (the miriquidic acid-producing *Miriquidica ventosa* growing nearby our sample)? Thus, we checked whether the distribution pattern of miriquidic acid within *O. ventosa*'s thallus was compatible with such scenario.

Allocation of lichen substances in the lichen thalli is a challenge to discuss their role. The discovery of such distribution pattern most often relies on extraction of targeted tissues for chemical analyses, sometimes guided by specific UV features of the analytes[3]. Many lichen substances can also be stained by exogenously applied chemicals. Both approaches appear limited since sharp details of distribution are lost when analyzing bulk tissues while imaging techniques based on histochemistry does not distinguish between individual compounds and need a chemical treatment of tissue which may lead to distortions of localization[4]. Based on LDI-MS adequacy for dereplicative purposes in lichenology, we herein describe the first mass spectrometric imaging technique undertaken on lichen. LDI-MSI revealed the distribution pattern of all *O. ventosa*'s metabolites with an unprecedented spatial resolution in lichenology, reflecting their ecological significance. Thus, LDI-MSI appears to be of outstanding interest to advance the understanding of lichen biology.

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DETECTION AND ISOLATION OF PRENYLATED STILBENES FROM VARIOUS *MACARANGA* SPECIES USING MOLECULAR NETWORKING

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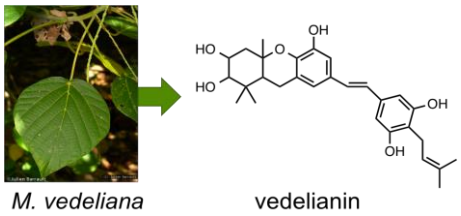
Phytochemical studies of plants of the genus *Macaranga* led to the discovery of a new familly of prenylated stilbenes with an original hexahydroxanthene moiety. Vedelianin (cf: **Fig 1**), isolated from the leaves of *Macaranga vedeliana*, endemic to New Caledonia, was the first member representative of this chemical series. **[1]** Studying these molecules is of great interest since they display promising antiproliferative activities at low concentration (nM) for specific tumor-derived cell lines, such as glioblastoma. Our project, aims to discover new active analogues and bio-precursors of these molecules in 20 species of *Macaranga* collected in various countries (Madagascar, Vietnam, New Caledonia). With the objective to study the most promising species, we have developed an LC/HRMS^2-based methodology, in which the data are sorted and grouped in the form of molecular networks and visualized with the software Cytoscape. The main features of the methodology will be presented and exploitation of generated molecular networks will be illustrated by concrete examples.



M. vedeliana vedelianin

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PHYTOCHEMICAL ANALYSIS OF *CAMELINA SATIVA* DURING SEED DEVELOPMENT

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Camelina sativa is considered as an alternative oilseed crop with interesting agronomic potential. This Brassicaceae is grown mainly for its oil which has an unusual fatty acid profiling particularly rich in polyunsaturated fatty acids [1]. The phytochemical composition of *Camelina sativa* seed is also distinguished by other compound of pharmaceutical interest (flavonols, glucosinolates) [2]. However few information is available on the accumulation dynamics of these compounds during seed development. Here, we show the accumulation kinetics (15, 25 and 45 days after flowering (DAF)) of various biochemical families involved in the seed quality. The metabolomic analyses were conducted on GC-FID, GC-MS and LC-MS to obtain the most integrative view of the seeds phytochemical composition. The coupling of these different analytical methods has enabled to monitor the content of 9 fatty acids, 3 glucosinolates, 6 flavonols, 12 amino acids, 7 non structural carbohydrates, 4 tricarboxylic acids and 10 others compounds.

The ACP produced from all the data collected enables clear discrimination between the different populations (15, 25 and 35 DAF) with the PC1 and PC2 explaining almost 85% of the variability between samples. The separation is especially important between 15 DAF and the other two development stages (25 and 35 DAF) and is associated with a high accumulation of fatty acids, glucosinolates, flavonols and sinapine. This discrimination is also associated with a high depletion in all primary metabolites except sucrose, galactinol and raffinose.

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FP8

MASS SPECTROMETRY CHARACTERIZATION OF THE ESTERIFIED FLAXSEED MOLECULES: FROM A LIGNAN COMPLEX TO A PHENYLPROPANOID POLYMER.

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One of the particularity of flaxseed is in the fact that most of the phenylpropanoid pathway is directed towards the biosynthesis of lignans, molecules that are now recognized for their phytoestrogen, antioxidant but also antifungal and antibacterial properties (Pauletti et al 2000). However despite their exceptionally high level accumulation in the form of a polymer formed by the association of lignan, hydroxycinamic acids and flavonoids with ester linkages via the 3-hydroxy 3-methyl glutaric acid (Struijs et al 2008, 2009) at a reduced level in the integument, the potential biological role of lignans in flaxseed remains unknown to this day.

Following some results obtained on germinating flax seed showing a release of lignans polymer in the exudates, questions about the initial structure of the molecule present in the seed arose.

To answer this, a characterization of existing molecules in mature flax seed integument was envisaged. Fractionation by semi-preparative HPLC on flaxseed integument extracts followed by GCMS and LCMS analysis before and after hydrolysis demonstrated that in addition to the already describded molecules, other lignans, oligolignols, flavonoids and organic acids are present in the lignans polymer. we also noticed a change in the proportions of the different metabolites depending on the polarity of the polymer which reflects the existence of different types macromolecules.

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O14 – Conférence invitée

METABOLITES AND PLANT SECONDARY METABOLISM; EXAMPLES OF APPROACHES FOR THE STUDY OF BIOSYNTHESIS AND VALORIZATION OF PLANT CHEMODIVERSITY

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Plant secondary metabolites are generally described as natural compounds involved in adaptation to environmental stresses [1]. Because some (most) of them have biological properties, their use as natural drugs has been largely developed in traditional medicine. Modern medicine is also researching new leads for the treatment of various diseases such as cancer, Alzheimer, VIH... Understanding which metabolites are accumulated in plants, how they are synthesized, what is controlling fluxes in the pathways are challenges of interest for pharmaceuticals, cosmetics, nutraceuticals but also for crop protection [2]. The most recent approaches, including omics, open new routes for academic researches involved in the study and valorization of plant chemodiversity [2]. As such, we developed a metabolomic platform to study lignan biosynthesis in *Linum* species [3]. Using MNR [4] and MS [5-8] as main analytical tools, we demonstrated that such phenylpropanoids are accumulated as a macromolecular complex in the teguments of *L. usitatissimum* seeds. These bioactive compounds most probably play a role of chemical barrier during the seed germination. The time course study of endo- and exo-metabolome during germination is thus a first step to describe the impact of lignans in the control of microbial diversity in the spermosphere [9]. Metabolomics approach, including *i)* chemical analysis and, *ii)* targeted/untargeted data analysis, then contributes to the understanding of the role of such lignans in seedling. Also, more complex lignans such as arytetraline (ATLs) harboring anticancer activities may be produced by other *Linum* species (*Linum perenne* and *L. album)*[10]. The use of correlation network analysis allowed the study of glycosylation/deglucosylation processes in the pathway of ATLs in hairy root cultures [10]. As for tropane alkaloids in *Datura innoxia* roots [11-12], correlation network analysis can be used to describe biosynthetic pathways [13] and to determine regulation points that can lead to the identification of bottlenecks in fluxes [10,13]. New biotechnological strategies can thus be chosen to improve plant secondary metabolite production but also plant selection [3-8, 14].

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O15 – Conférence invitée

CHEMICAL PROFILING OF NATURAL EXTRACTS: A 13C NMR-BASED DEREPLICATION STRATEGY

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Natural extracts from plants or microorganisms still represent invaluable sources of biologically active metabolites for the development of new drugs or cosmetics. The major challenge in the search for such metabolites arises from the extreme complexity of natural extracts or culture media that contain a wide diversity of molecules with distinct physical and chemical properties. At present, even if modern analytical and purification techniques are routinely available in most laboratories, a considerable work is still necessary to isolate and elucidate individual metabolite structures from crude natural extracts.

The aim of the present work was to develop a dereplication strategy for the identification of natural metabolites directly within mixtures [1]. Exploiting the polarity range of metabolites, the principle was to rapidly fractionate a multigram quantity of a crude extract by centrifugal partition extraction (CPE) [2]. The simplified and chemically diverse mixtures obtained are subsequently analyzed by 13C NMR. After automatic collection and alignment of 13C signals across spectra, Hierarchical Clustering Analysis (HCA) is performed for the pattern recognition of elution profiles. As a result, strong correlations between 13C signals of a single structure across the fraction series can be directly visualized as chemical shift clusters. Each cluster is finally assigned to a molecular structure by means of an in-house database containing structures and predicted ^{13}C NMR chemical shifts of natural metabolites. Through different real-life examples [3, 4], it is demonstrated here that the combination of a multi-gram-scale fractionation method with ^{13}C NMR and HCA pattern recognition of ^{13}C signals enables the rapid identification of the major known metabolites within crude natural extracts while avoiding tedious purification procedures.

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O16

ISOLATION OF VISMIONES FROM *PSOROSPERMUM* SPECIES AND SYNTHETIC APPROACH TOWARD LEISHMANICIDAL MOLECULES

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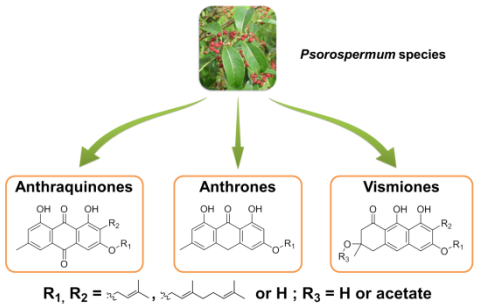

The screening of plants used in Cameroonian traditional medicine against parasitic diseases revealed significant leishmacidal activity for species belonging to the *Psorospermum* genus and led to the isolation of several prenylated anthranoids (bianthrones, anthraquinones, anthrones and a dihydroanthracenone of the vismione family). The activity of these compounds is inversely correlated with their oxidation state. The acetylvismione D showed the best activity against *Leishmania donovani* amastigotes with IC_{50} of 90 nM.

Further fractionations based on reversed-phase and centrifugal partition chromatography allowed us to isolate 9 vismiones from the CH_2Cl_2 bark extract of *P. glaberrimum*. All these compounds exhibited strong leishmanicidal activity (30 nM<IC_{50}< 827 nM).

This work revealed that the previously isolated anthranoids were mostly artefactual and derived from corresponding vismiones during the purification process. The development of a gram-scale isolation protocol of these highly unstable compounds when traditional ill-suited purification processes are used, will provide enough starting material for further hemisynthesis steps and allow the investigation of their mechanism of action. Synthetic approaches are also developed in this way.

O17

NEW PREGNANE GLYCOSIDES FROM *CYNANCHUM* SPP. (APOCYNACEAE) WITH ANTIDIABETIC POTENTIAL

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In the framework of our search for antidiabetic compounds capable of stimulating the secretion of the hypoglycemic hormone GLP-1 (glucagon-like-peptide 1), we selected some plants based on ethno-pharmacological and chemotaxomical criteria, orientated by the reported antidiabetic effects of *Hoodia gordonii* (Apocynaceae)[1]. This approach led to the selection of some Malagasy leafless *Cynanchum* species [2]. Both sourcing availability and GLP-1 secretagogue *in vitro* activity conducted to the focusing on two species of this *Cynanchum* taxon for further phytochemical investigation.

Bio-guided fractionation of the ethanolic extract of *Cynanchum marnierianum* led to the purification of two compounds, which were identified by combination of HR-ESI-TOF-MS and 1D and 2D NMR analyses as new pregnane glycosides, named marnieranosides A and B. Based on these findings, we then focused on the isolation of pregnanes present in the ethanolic extract of *Cynanchum menarandrense*. This work led to the purification and identification of 5 new pregnane glucosides along with 3 already known pregnanes, two of which formerly reported in the closely related *Caralluma* genus that is well characterized for its hypoglycemic activity [2]. Even though few compounds showed a weak stimulation of GLP-1 secretion in the STC-1 cell-based bioassay, the isolation of pregnanes previously isolated from plants with antidiabetic properties or showing structural similarities with the bioactive *Hoodia* pregnane P57, validated our approach combining both ethnopharmacological and chemotaxomical data for the discovery of secondary metabolites with antidiabetic potential.

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FP9

PHYTOCHEMICAL STUDY OF *DENDROBANGIA BOLIVIANA*

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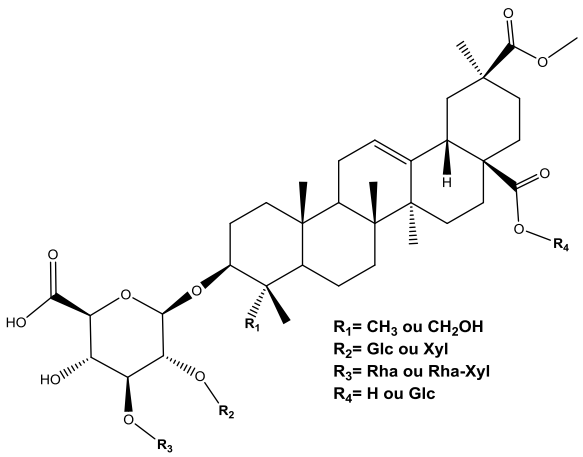
The phytochemical study of a plant of the family Icacinaceae, collected in the Amazon rainforest of Peru, was performed for the first time in order to search new bioactive molecules. This study highlighted twelve new structures of saponins with two known saponins which are Talinumùoside I and 3-O-β-D-glucuronopyranosylserjanic acid. The new molecules are glycosides of serjanic acid and phytolaccinic acid. The purification of these compounds from the methanolic extract was carried out using various chromatographic methods (VLC; flash chromatography; HPLC). The elucidation of the structures was performed by analysis of 1D and 2D NMR spectra (1H, ^{13}C, DEPT, COSY, HSQC, HMBC) and mass spectrometry. These compounds are evaluated for their hemolytic, cytotoxic (on fibroblasts) and antileishmanial activities (*Leishmani infantum* promastigotes).



FP10

ACCELERATED DISCOVERY AND PROFILING OF PHYSIOLOGICALLYACTIVE COMPONENTS IN COMPLEX SAMPLES BY HPTLC-EDA-HRMS

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On the one hand, the separation of thousands of compounds in a complex extract is thrilling, but may be still unsatisfactorilydue to coelutions. Hence, the question arises where to stop in high-sophisticated separation science? Which technical effort is economically justifiablein routine? On the other hand, the separation itsself does not imply an effect-directed answer to questions such as"Which compounds of the thousands of compounds are effective?". In contrast to high-sophisticated, comprehensive separation science, astreamlined methodology is presented in the following that is able toanswerthese effect-directed questions without the need for a perfect separation, however, with clear limitations [1, 2].

The first part of the streamlined methodology is an effect-directedscreening of up to 22 raw extracts in parallel. Thus, this part (HPTLC-UV/Vis/FLD-(bio)assay) can be described as a non-targeted, effect-directed detection of single or also coeluting effective compounds of the sample. The second part is a highly targeted characterization of the effective compoundsdiscovered via the hyphenation to structure elucidating techniques (HPTLC-HRMSor NMR or ATR FTIR).For a direct link, the hyphenation HPTLC-UV/Vis/FLD-(bio)assay-HPLC-HRMS or NMR or ATR FTIRis studied. Here, the bioactive zone of interest is selectively eluted via a short orthogonal column into the HRMS or into a microvial for NMR or ATR FTIRrecordings. Considerable information can be obtained when the same sample is applied multifold on the plate.Thus, information on effective compoundsin a complex sample and their sum formulaecan be obtained from a single chromatographic run.Depending on the bioassay or enzymatic assay selected, for example, antibiotics, œstrogens, acetylcholinesterase inhibitors, xanthine oxidase inhibitors or tyrosinase inhibitors are discovered in complex samples. HPTLC-UV/Vis/FLD-(bio)assay examples are given, taking 3 to 20 min per sample for the discovery of the bioactive components.

Every technique has its limitation. For volatile or oxidation-prone compounds, thisstreamlined methodology is limited in the information content. Nevertheless, it may serve as a surveyon effect-directed components in complex samples.Benefits may result from the side-by-side sample comparison, the matrix-tolerance, the avoidance of carry overand of discrimination,the always fresh adsorbent, the comparatively low-tech workflowas well as the multifold evaluation of the separated sample saved on the plate.

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FP11

CENTRIFUGAL PARTITION CHROMATOGRAPHY APPLIED TO GRAPEVINE AND WINE CHEMISTRY

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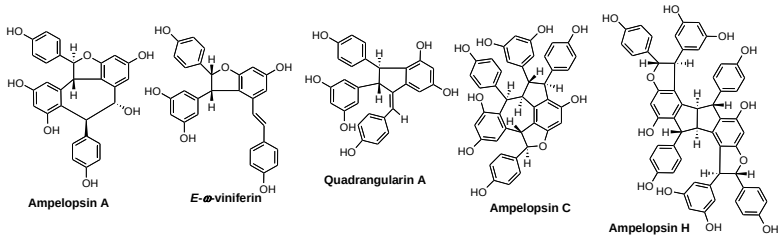
Grapevine is a rich source of health promoting compounds, including flavonoids, phenolic acids, and stilbenoids. Some of these have undergone extensive biological testing, particularly in regards to disease prevention and anti-aging activities.[1] However, there are many additional stilbenoids in wine that have yet to be identified and biologically tested. Their isolation and identification has been hindered by their minor concentrations and the complexity of the grapevine and wine matrix. In order overcome these obstacles, we have developed multiple chromatographic strategies, based on centrifugal partition chromatography (CPC), to afford stilbenoid rich fractions, and even pure compounds for compound identification. CPC is a very versatile separation technique used in the fractionation and purification steps of natural products.[2]. We developed new hyphenated techniques, like CPC-SPE-RMN. Leading us to identify six stilbenoids previously unidentified in wine. This includes four stilbenoid dimers, one tetramer, and the first instance of a stilbenoid trimer in wine (see Figure below).



Examples of Some Compounds Newly Identified in Wine.

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FP12

EXPLORATION OF THE BIOSYNTHETIC PATHWAY LEADING TO SARAINES, ORIGINAL ALKALOIDS PRODUCED BY THE SPONGE *HALICLONA SARAI*

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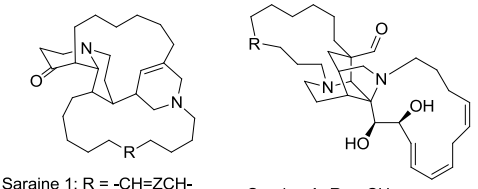
Metabolic pathways of alkaloids have been widely studied for terrestrial plants, but remains poorly understood in the marine environment. Sponges were shown to be highly active producers of original alkaloids. In particular, *Haliclona sarai* has been described to produce two families of saraines [1, 2] (Fig 1), related to petrosins and xestospongins from a biosynthetic point of view [3, 4] However, their intriguing metabolic pathways still remain unknown as no experimental data have been obtained to date. Different hypotheses can be proposed for the biosynthesis of the piperidine cores. Our chemical investigation led for the first time to the isolation of some haliclamines that led us to investigate nicotinic acid and acetate as precursors [5]. Feeding experiments were realized during one month on sponges kept in aquaria with different precursors labeled with ^{14}C: acetate (leading to carbon chains), tryptophan (precursor of nicotinic acid in animals), aspartic acid (precursor of nicotinic acid in plants) and ornithine (as possible negative control). After extraction and several steps of purification leading to the compounds of interest, the incorporation of ^{14}C was measured by radio-TLC detector. First results show that acetate and tryptophan are incorporated in haliclamines and saraines, in agreement with the hypotheses involving nicotinic acid and acetate as precursors of this biosynthetic pathway.



Saraine 1: R = -CH=ZCH-
Saraine 2: R = -CH_2-
Saraine 3: R = -CH_2CH=ZCH-

Saraine A: R = -CH_2-
Saraine B: R = -CH=ZCH-
Saraine C: R = -CH=ZCHCH_2-

Fig.1 Structure of saraines isolated from H.sarai

The team of IAEA-REL at Monaco is deeply acknowledged for assisting in the feeding experiments

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Axe 3. Médecines traditionnelles / MAC

O18

IN VITRO ANTI-*HELICOBACTER PYLORI* ACTIVITY OF PLANTS USED FOR GASTROINTESTINAL DISORDERS IN TRADITIONAL MEDICINE FROM COAST, ANDES AND CENTRAL FOREST OF PERU.

Alice Gadea [a], Denis Castillo Pareja [a, b], Valérie Jullian [a, d], Juan-Pablo Cerapio Arroyo [a, b], Geneviève Bourdy [c, d], Mercedes Gonzales de la Cruz [e], Mohamed Haddad [a, d], Pedro Vásquez-Ocmín [a, b], Fabio Espichán Jáuregui [a, b], Michel Sauvain [a, d]

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Helicobacter pylori is a Gram (-) bacteria responsible of stomach disorders like gastritis, ulcers and stomach cancer. In Peru, stomach cancer is the second one in prevalence and the first one in mortality. Because of the high percentage of recurrence and reinfection, it's necessary to find news antibacterial molecules, which are able to regulate the bacteria infection. Several natural substances are efficient antibacterial agents and some reputed gastroprotective remedies from worldwide traditional pharmacopeia seem to exhibit inhibitory activity on *H. pylori*. Therefore, selected Peruvian medicinal plants were evaluated *in vitro* against *H. pylori*. Ethnopharmacological surveys were carried out during four month (northern coast, central Andes and high Amazon) in medicinal plants markets and also in the wild, with the help of informants. All plants were selected for their use in case of gastrointestinal disorders, acidity, and ulcers. Altogether 112 species were collected and the anti-*H. pylori in vitro* was evaluated on a Peruvian patient clinical strain, using broth microdilution method. Three parameters were retained as biological activity marker: inhibitory concentration (IC_{50}), Minimal Inhibitory Concentration (MIC) and Minimal Bactericide Concentration (MBC).

The results showed that 23 plants extracts were active on *H. pylori* (MIC ≤ 1000 µg/mL). Among the active plants, 18 presented a bactericidal activity (MBC ≤ 1000 µg/mL). A bioguided fractionation of the active species (bacteriostatic and bactericidal activity) could be considered, using IC_{50} as evaluation criteria.

O19

FROM MALARIA TO CANCER: *QUASSIA AMARA* AND SIMALIKALACTONE E (SkE)

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In our constant effort to find new therapeutic solutions against malaria from traditional pharmacopeias, our team has confirmed at the end of an extensive ten years research program, the medicinal potential of a broadly used and cultivated plant all over Amazonia: *Quassia amara* L. (Simaroubaceae). A bioguided fractionation of this plant lead to the isolation of the Simalikalactone E (SkE). SkE has been first isolated from the traditional decoction made with *Q. amara* leaves. In order to investigate further its promising biological activities, we have then perfected the extraction protocol to achieve a reproductible yield of 40 mg/kg of plant.

In our recognized laboratory models, this product displayed very interesting antimalarial and anticancer activity *in vitro* and *in vivo*. SkE inhibited murine malaria growth of *Plasmodium vinckei petteri* by 50% at 1 mg/kg of body weight/day, by oral route [1]. In a model of chronic myeloid leukemia (CML) cells implanted in athymic mice, a significant reduction of tumor size was obtained after treatment with SkE at 1 mg/kg of body weight/day, by ip route [2]. In these experiments, SkE showed pharmacological properties equivalent and sometimes more effective than reference compounds used in classical therapy (such as chloroquine for malaria or Imatinib for cancer). Investigation of its mechanism of action on CML cells showed that SkE acts on the ERK pathway, inhibiting ERK1/1, MEK ½ et B-Raf phosphorylation [2]. We developed a quantification procedure of SkE in blood samples and analyzed mice blood after oral and ip administration of SkE. We found out that SkE is barely detectable 4h after administration. We suggested that SkE could be rapidly metabolized, and therefore, the *in vivo* antimalarial and anticancer properties may be due to its metabolites [3].

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FP13

TYPOLOGY OF HEALERS IN TRADITIONAL MEDICINE AROUND THE KAHUZI-BIEGA NATIONAL PARK,A WORLD HERITAGE SITE IN DANGER, DR CONGO.

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Several ethnobotanical surveys have demonstrated links between the folk medicinal practices with ethnic and geographic identity of healers, while many others concluded the opposite. Given this contrast, the present study aimed to establish a categorization that could organize healers, treated pathologies and profiles of used medicinal plants based on their ethnic origin and area of practice. A secondary goal was to evaluate whether certain medicinal species could be in danger, especially those considered essential both for healers and for gorillas of the park. A total of 88 healers, recognized as 'specialists' in their communities, were involved in the study. Multivariate analyses showed that the ethnic belonging and geographical location did not explain practices and knowledge of healers. However, using the IndVal method, differences were observed in their degree of specialization. Non-specialized healers (70 %) could be distinguished from healers specialized in the care of bone traumatisms and those specialized in reproductive organs (30 %). The Mantel correlation has shown a positive association ($r = 0.134$, $p < 0.05$) between the 'healers-plants' and 'healers-diseases' matrices. Forest species were the most collected (83 %), of which 47 % are also consumed by lowland gorillas. This indicates that healers who treat similar diseases often use similar herbs.

FP14

ANTIBACTERIAL ACTIVITY OF PENTACYCLIC TRIPENOIDS ACIDS FROM *PLATOSTOMA ROTUNDIFOLIUM* AERIAL PARTS.

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Platostoma rotundifolium is widely used by Burundian traditional healers against microbial infections, notably skin disorders. Preliminary tests have shown antibacterial activity of the extracts of aerial parts against both Gram positive and negative bacteria. The most active ethyl acetate extract was submitted to bioguided fractionation to isolate the major antibacterial compounds. At least three active compounds were visible on the TLC-bioautogramm (Fig. 1). The bioguided

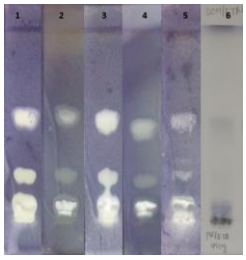
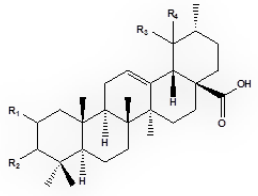


Fig. **1** : TLC-bioautography of the ethyl acetate extract of *Platostoma rotundifolium* aerial parts (40 µg). Mobile phase: dichloromethane/ethyl acetate (80:20). Bacterial strains: MRSA C100459 (1); MSSA ATCC6538 (2); MRSA ATCC 33591 (3); MRSA C98506 (4); *E. coli* ATCC 6538 (5). Bacterial growth visualization: methyl tetrazolium (lanes 1-5); white spots indicate inhibition zones of bacterial growth. Chemical detection: vanillin-sulfuric acid reagent (lane 6).



Compounds	R_1	R_2	R_3	R_4	**MIC (nM)**
Ursolic acid	H	βOH	βCH_3	H	17.5-68
Corosolic acid	αOH	βOH	βCH_3	H	17-68
Tormentic acid	αOH	βOH	βCH_3	αOH	64-524

fractionation process led to the isolation of these three active compounds. They were elucidated to be ursolic acid (**a**), corosolic acid (**b**) and tormentic acid (**c**) based on their spectral analysis and comparison to standard's spectra. Ursolic and corosolic acids showed significant antibacterial activities against *S. aureus* (methicillin-susceptible and -resistant strains) and *E. coli*, which may support the use of *P. rotundifolium* in traditional Burundian medicine. Such hydroxylated pentacyclic triterpenoid acids could point to new antimicrobial strategies that may help overcoming the antimicrobial resistances actually observed throughout the world.

FP15

EXTRACTION OF NATURAL ANALGESIC COMPOUNDS FROM PLANTS USED IN TRADITIONAL MEDICINE IN MALI : STUDY OF *PERICOPSIS LAXIFLORA*

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Nowdays, the urge to find new analgesics with less side effects is increasing and plants have always been a source of inspiration for new drugs. In Mali, plants are often used as part of traditional medicine. Traditional healers are taught by their parents how to treat people with plants from surroundings. Therefore, an ethnopharmacological study was conducted on plants used to treat pain in south of Mali (Districts of Bamako, Siby, Dioïla and Sikasso). 113 traditional healers were interviewed and 120 plants were recorded as used against stomach ache, headache, muscle ache, rheumatism, diabetic neuropathic pain, traumatic pain, etc… Among them, two plants were most often cited: *Cassia sieberiana,* mentioned 21 times (roots) and Pericopsis laxiflora, mentioned 11 times (leaves). Aqueous and methanolic extracts of both plants were screened with an in vivo acid acetic-writhing test showing a moderate anti-nociceptive activity at 300 mg/kg. Both methanolic and aqueous extracts of *Pericopsis laxiflora* displayed significant activities (respectively 43% and 34% of inhibition of abdominal cramps) while the methanolic extract of roots of *Cassia sieberiana* induced only 23% of inhibition. Further partitioning with solvents of increasing polarity and column chromatography were conducted. Results led us to purify fractions to isolate active molecules.

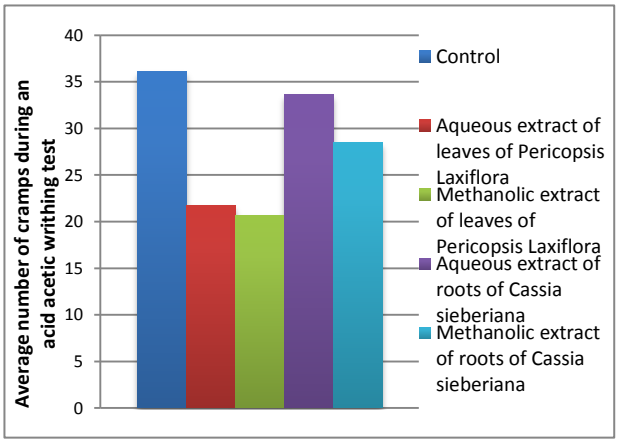

Fig. 1: Anti-nociceptive activity of four crude extracts

FP16

GENOTOXICITY TESTING OF HERBAL MEDICINAL PRODUCTS CONTAINING QUERCETIN: AVOIDING PITFALLS IN AMES TESTS

K.A. Ancolio Morcq[1], F. Finot[2]; C. Miette[1], O. Cariou[2], P. Abbe[1], M. Canari-Delorme[1], O. Legendre[1]; G. Brunet[2], E. Boccardo[1], N. Prigent[2], I. Mouche[2], M. Ballantyne[3], J. Clements[3], D. Guedon[1].

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Recently, new common quality, safety and efficacy standards for licensing herbal medicinal products (HMPs) have been established including assessment of genotoxic potential. Legal provisions and recommendations dealing with the evaluation of genotoxicity risks of HMPs are briefly detailed with testing strategy based on their characteristic features. Amongst them is the potential presence of genotoxicants with well-established safety profile which may be responsible for positivity in Ames tests. If the *in vitro* positivity observed can be clearly attributed to these specific constituents known to be devoid of *in vivo* genotoxicity, no further *in vivo* genotoxicity testing is required. The most ubiquitous of these in food and medicinal plants is quercetin. False positives may also be observed with the growth of “pseudorevertant” colonies due to a feeding effect of the herbal preparation containing amino acids, especially histidine. The “treat and wash” method is employed to avoid this possible bias. DMSO is the usual (extracting) solvent. Meanwhile, other solvents must be considered when testing herbal teas (water) or powdered herbal substances which imply different extraction solvents (water, ethanol 50%, ethanol, DMSO…) to cover the entire spectrum of constituents. It is well-known that DMSO enables easy dissolution of quercetin and affords an appropriate stability of the corresponding solutions. In order to properly consider the possible interference of quercetin with the assessment of genotoxicity potential of herbal medicines under non-standard conditions, the influence of different parameters (solvent, pH, concentration, exposure-time) on quercetin solubility, stability and mutagenicity in Ames test using *Salmonella typhimurium* TA 98 strain was investigated. Based on the current OECD 471 guideline, the influence of different Ames test conditions was also studied including presence or absence of S9, use of pre-incubation or plate-incorporation method. Possible modification of quercetin response when a “treat and wash” method is used has also been explored. A better understanding of the methodology to be applied to determine possible involvement of quercetin for genotoxicity activity has been reached. It is here verified that the “treat and wash” method does not cancel out the mutagenic response of quercetin.

FP17

ASSOCIATION DES AMIS DU MUSEE FRANÇOIS TILLEQUIN

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Récemment créée, la Société des Amis du Musée François Tillequin a pour objectif d'aider à valoriser les collections du Musée de Matière Médicale de la faculté de Pharmacie de l'Université Paris Descartes.

Le « Musée François Tillequin, collection de Matière médicale » représente un ensemble unique de premier plan par le nombre d'échantillons rassemblés environ 25 000 échantillons de drogues végétales et animales et d'objets liés à leur production, leur transport, leur traitement et leur emploi. Il a un double intérêt à la fois patrimonial et scientifique dont le profane et le chercheur peuvent tirer partie. Cette collection illustre l'histoire de la Pharmacie à travers les siècles puisqu'elle comprend des échantillons des XVIII, XIX et XXème siècles. Elle constitue également une source de travail pour les scientifiques : historiens des sciences, spécialistes de substances naturelles, ethnobotanistes…

O20

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

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Mucuna pruriens has been used since a long time in Ayurvedic medicine for the treatment of Parkinson disease. *M. pruriens seed* powder *contains high* amount of *L-DOPA (up to 5%),* an *amino acid used as a symptomatic treatment of PD. Several in vitro* and *in vivo studies have evaluated the antiparkinsonian efficacy of M. pruriens seed extract comparatively* to *pure L-DOPA*. *M. pruriens seed extract* was *shown* to be *two* to *three times more effective than synthetic L-DOPA* with or without dopa decarboxylase inhibitors, improving behavior in rat model of Parkinson disease. *Our approach consists in identifying the molecules* having a biological activity *and their mechanisms* susceptible to explain the synergy effect. *We developed several enzymatic assays based on mass spectrometry as detector to monitor the activity of different compounds on different targets, including inhibition of monoamine oxidase, dopadecarboxylase and/or catecholamine O-methyltransferase. Furthermore, our purpose in this study was the evaluation of the agonist activity of the isolated compounds on dopaminergic and cholinergic receptors. The structural analysis identified the structure of several new molecules* isolated from the hydroethanolic seed extract of *Mucuna pruriens.* We also investigated the potential antiparkinsonian activity of these new compounds.

O21 – Conférence invitée

DIETARY SUPPLEMENTS: THE SPIRIT OF THE LAWS

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[2]DGCCRF

Dietary supplements have been going mainstream for a decade now, and this growth will definitely maintain itself in the future, implying a significant and coveted market. Regulations of these products have also significantly evolved to accompany this trend, introducing new texts and setting new rules. Several characteristics of this sector can explain current tension between the actors, institutions and communities involved: 1) the pace of this evolution and the way it has taken has been largely influenced by European institutions, forcing EU member states to adopt an unified approach of this products; 2) Due to the efforts of the industrial sector to market products with health benefits, dietary supplements are at the frontier between food and medicinal products; 3) besides vitamins and minerals, which have a longstanding scientific background for their use and effects, herbal and other natural substances are widely used in dietary supplements.

These aspects make the status, scope and limits of dietary supplements highly debated especially in France. Legitimacy to regulate and even sell these products overlap between EU and state members institutions, and between the food and pharmaceutical sectors, leading to divergent opinions and finally hindering a clear visibility of the future of dietary supplements.

We propose to depict the current context with examples of ingredients that have attracted attention and crystallized misunderstandings. The spirit of the laws, especially the division of powers, will be explained through these examples to help understanding the situation and make an educated guess on the upcoming regulatory context of dietary supplements.

Table ronde

« L'ARRETE PLANTES EN QUESTIONS »

Animée par **A. Maciuk** (Université Paris-Sud) et **S. Boutefnouchet** (Université Paris-Descartes)

Participants : **V. Siranyan**, Université Lyon1, MCU Droit Santé Publique, membre de du conseil central D de l'Ordre des Pharmaciens. **B. Montreuil**, Pharmacien, Bron, Président du Syndicat des Pharmaciens du Rhône. **B. Dal-Gobbo**, Médecin généraliste, Bourg en Bresse. **D. Barret**, Laboratoires Iphym, Lyon. **G. Cousyn**, DGCCRF.

Résumés
Posters

P18

PHYTOCHEMICAL ANALYSIS OF *ATROPA BELLA-DONNA* FRUITS USED BY THE INVASIVE FRUIT FLY *DROSOPHILA SUZUKII*.

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Drosophila suzukii (Diptera: Drosophilidae), an invasive fruit fly native to Asia, was recorded for the first time in North America and in the south of Europe in 2008. Unlike most other frugivorous *Drosophila* species using over-ripe or rotting fruits, *D. suzukii* is able to infest fresh fruits before their maturity by using a serrated ovipositor [1]. This fly causes damages on fleshy fruits of cultivated plants (cherry, strawberry, raspberry...) and is thus considered as an invasive pest in agricultural production system. Since 2008, *D. suzukii* has moved to the north of France and, nowadays forms important populations in Picardy region. Cultivated plants infested by *D. suzukii* are well documented in invaded areas but recent work in our laboratory shows that this Diptera is also a very polyphagous species, able to infest numerous wild fruits, some of them being considered to be highly toxic [2].
As *D. suzukii* represents an excellent biological model for understanding the conditions and factors leading to a successful biological invasion [3], we investigated the phytochemical content of the fruits of one of these toxic plant species collected *in natura*, *Atropa bella-donna*, at three maturation stages using TLC and LC-MS approaches. At the same time, the fruits were studied to determine their potential suitability for *D. suzukii* in laboratory conditions. Infestation experiments were also conducted using *D. melanogaster*, a widely distributed and non-invasive fly. First phytochemical results showed significant differences in qualitative and quantitative metabolic profiles along the fruit maturation process. These differences will be discussed in the context of their contribution to the success of *D. suzukii* invasion.

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P19

BACTERIAL COMMUNITIES ASSOCIATED WITH POLLEN FROM WIND-POLLINATED TREES ANALYZED BY PCR-TTGE (TEMPORAL TEMPERATURE GEL ELECTROPHORESIS)

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Université de Montpellier, UFR Sciences Pharmaceutiques - [1]Laboratoire de Botanique, Phytochimie et Mycologie ; UMR 5175 CEFE ; Substances Naturelles et Médiations Chimiques - [2]Laboratoire de Bactériologie ; UMR 5569 HydroSciences ; Pathogènes Hydriques Santé Environnements - [3]UMR 95 QualiSud ; Contaminants de la Chaine Alimentaire - BP 14491, 34093 Montpellier cedex 5, France.

Pollen from wind-pollinated plants is the predominant cause of pollinosis which corresponds mainly to allergic rhinitis and conjunctivitis. Bacterial communities associated to leaves and roots surface were analyzed for many years with both culture-dependent and independent methods. However bacterial communities associated to pollen were rarely investigated. Mixed microflora consisting of Gram-positive and Gram-negative mesophilic bacteria, thermophilic actinomycetes and fungi have been highlighted on allergenic pollen grains [1]. In the domain of plant protection, *Pseudomonas syringae* pv *actinidiae* was also detected on the kiwi pollen grains [2] but only with culture dependent approaches. The aim of this study is to perform a preliminary analysis of the bacterial communities associated with pollen by applying PCR-TTGE methods. A PCR anchored on the bacterial 16S rRNA gene followed by a TTGE analysis were performed on DNA extracts from different pollen samples. Pollen samples were collected from various anemophilous plants (*Pinus*, *Cupressus,…*) in Montpellier between February and May 2013. The results showed that PCR-TTGE is a suitable tool since bacterial profiles were obtained for each pollen samples. Particular care should be taken for DNA extraction. Further analyses should be undertaken to confirm these preliminary results.

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P20

BIOLOGICAL CONTROL OF *AGROBACTERIUM VITIS* USING ESSENTIAL OILS OF *ORIGANUM COMPACTUM* AND *THYMUS CILIATUS*

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Agrobacterium vitis is a host-specific pathogenic bacterium that causes grape crown gall disease affecting vine growth and production worldwide. This disease is difficult to control because of the absence of effective control methods. Many strategies are used for the management of crown gall, including biological control [1]. The potential use of the antimicrobials properties of essential oils (EO) for the biological control of grapevine crown gall was investigated. The main objective of this research work is to evaluate the antibacterial activity of essential oils (EO) of thyme (*Thymus ciliatus*) and oregano (*Origanum compactum*) on the growth of *A. vitis*. The extraction of EO was performed using hydrodistillation-type procedure. The chemical compositions of the two EO were established by GC-MS analyses, and their antibacterial activity was tested *in vitro* against *A. vitis* (strain S4). Our results show that these EO have important antibacterial activities against *A. vitis*. The Minimum Inhibitory Concentration (MIC) was determined by Bioscreen test and was shown to be 0.15 mg.mL-1 and 0.3 mg.mL-1 for oregano and thyme, respectively. Oregano and thyme exhibit an antibacterial effect with an inhibition percentage in the order of 67.5% and 41.7%, respectively. We also tested the antibacterial activity of oregano EO *in planta* (Tomato) to control *A. vitis*.

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P21

ENDOPHYTIC FUNGAL DIVERSITY IN MEDICINAL PLANTS OF WESTERN AND CENTRAL PARTS OF UKRAINE

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Plants of the *Herniaria* genus (Caryophyllaceae) are widely distributed in Europe, Asia, and North Africa and both plants are widely used in Ukrainian folk medicine for the treatment of kidney stones, gout, hernias, and respiratory disorders. *Herniaria* genus is a source of original bioactive saponins[1]. However little is known about the phytochemistry of other secondary metabolites particularly those biosynthesized by the endomicrobiome of this genus. Endophytes are microorganisms that exist within the tissues of living plants. Fungal endophytes have been demonstrated to be a rich and reliable source of biologically active and/or chemically novel compounds[2]. Since there is no currently available information on endophytic fungi of HG and HP, a project was initiated to geneticaly and biochemicaly characterize fungal endophytes of HP and HG in Ukraine. The samples were collected in 2014 during the flowering season in the western (HG) and the central (HP) parts of Ukraine in the months of June and July. We have successfully isolated 54 strains from the stems, flowers of HG and 39 strains from the stems, flowers, leaves, roots of HP. Based on BLASTn results after ITS PCR amplification and sequencing (Eurofins MWG Operon) it was determined that all isolates belong to the *Dothideomycetes* class. Phylogenetic analyses revealed that endophyte fungal microbiome was dominated by *Alternaria* genus in HG and HP (98 % and 77 % respectively). At least 1 HG and 7 HP strains were identified as *Cladosporium* and 2 HP strains– as unclassified *Didymellaceae*. Our future goal is to investigate and compare the metabolome of the plants and their endophytes with a particular attention to secondary metabolites, especially the triterpenoids.

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P22

CONTRIBUTION TO THE PHYTOCHEMICAL STUDY OF *DIGITALIS CARIENSIS*

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The genus Digitalis (Plantaginaceae) is represented by 9 species in the flora of Turkey, of which D. cariensis Boiss. ex Jaub. & Spach is endemic for Turkey and East Meditarranean element [1]. The plant is known as "Mugla foxglove" and distributed in southern Anatolia [2]. Previous phytochemical studies on D. cariensis resulted in the isolation of pregnane and furostanol glycosides along with phenyl ethanoid glycosides [3]. This presentation described the isolation and structure elucidation of additional steroid glycosides from the aerial parts of this plant. A methanolic extract of the aerial parts was submitted to successive solid/liquid preparative chromatographic methods, i.e. vacuum liquid chromatography (VLC) and medium-pressure liquid chromatography (MPLC) over silica gel and RP 18 yielding four saponins. Their structures were elucidated by using by 1D and 2D NMR spectroscopic techniques (^{1}H-^{1}H COSY, NOESY, HSQC, HMBC), and FABMS as a new spirostane-type glycoside, (25R)-5α-spirostan-2α,3β,23β-triol 3-O-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, a new cardenolide glycoside, 3β,14β,16β-trihydroxy-card 20(22)-enolide 3-O-β-D glucopyranosyl-(1→4)-β-D-digitoxopyranoside together with two known steroid saponins described for the first time in D. cariensis, (25R)-5α-spirostan-2α,3β-diol 3-O-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and (25R)-5α-furostane-2α,3β,22α,26-tetrol
3-O-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl 26-O-β-D-glucopyranoside [4].

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P23

PHYTOCHEMICAL INVESTIGATION OF THE LICHEN *NEPHROMA LAEVIGATUM* AND ITS ENDOLICHENIC FUNGI

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Lichens are symbiotic association between a fungus (mycobiont) and cyanobacteria and/or algae (photobiont). During our ongoing work on exploring the chemical biodiversity of lichen from the French Limousin region, a screening has been performed on numerous lichen acetonic extracts. *Nephroma laevigatum* has shown interesting activities against HT-29 cancer cell lines and towards the inhibition of fungal biofilms. Thus, it has been selected for further investigations. However, lichen resources are limited and endolichenic fungi are a wide reservoir for access to bioactive compounds. Recent studies show that endolichenic fungi or bacteria isolated from lichens produced unique and novel secondary metabolites. *Nephroma laevigatum* is a fungal and cyanobacterial (genus *Nostoc*) symbiosis. This lichen is known to produce chlorinated anthraquinones [1] and mycosporines. Phytochemical study of *Nephroma laevigatum* as well as endolichenic fungi cultivation and identification by barcoding (PCR amplification of the ITS with ITS4 and ITS5 primers [2], sequencing and comparison to gene libraries) are underway. The first results reveal that: (a) fractionation of *Nephroma laevigatum* dichloromethane extract led to in the isolation of one triglyceride (Trilinolein) for the first time from this lichen and chlorinated anthraquinones [3] (b) the three isolated fungi belong to the genus *Nemania*. Structures were elucidated by spectroscopic methods (IR, UV, 1D and 2D-NMR, HRMS) and other compounds are currently under identification. Biological evaluation of pure compounds will be realized and the comparison of the chemical profiles of endolichenic fungi and those of the lichen will be carried out.

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PHYTOCHEMICAL ANALYSIS OF LEAVES OF *DOLICHANDRONE SPATHACEA* (BIGNONIACEAE)

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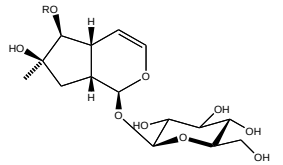
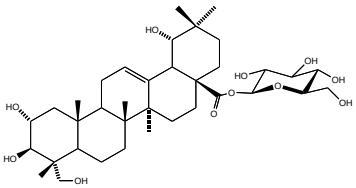
Dolichandrone spathacea (L.f.) K. Schum. named “mangrove trumpet tree” is a common smooth tree growing wild in riverbanks, swamps and mangroves of the Asia-Pacific area. The leaves of this traditional medicinal plant are used in different countries to treat oral thrush (as mouthwash), flatulence and bronchitis (juice of leaves) [1,2,3]. A single report in literature yielded the detection of flavonoids, triterpenes and tannins [4].

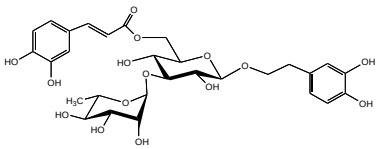
Chemical investigation of the methanolic extract of leaves of a sample collected in Vietnam, furnished 16 iridoids, 3 saponins, 3 phenylethanoid glycosides, 4 flavonoid glycosides, 3 monoterpenic acids, 5 phenolic acids and 1 megastigman glucoside. Among these compounds, five are new.

With regard to the traditional use of the leaves against various infections and according to literature results demonstrating an antimicrobial activity of the methanolic extract against MRSA [4], we looked for the antimicrobial potency of the extracts and isolated compounds from this plant. Our results are presented on a second poster (A. Abedini *et al.* “Antimicrobial evaluation of *Dolichandrone spathacea* and *Paypayrola guianensis*”).



R = phenylpropanoid ester ou monoterpenic ester





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P25

CALOPHYLLUM CALABA L.: AN EXAMPLE OF SUSTAINABLE VALORIZATION OF THE BIODIVERSITY IN OVERSEAS

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In the current context of green chemistry, tropical plant biomass comes as a promising raw material. The biodiversity of Overseas is exceptional and the eco-development opportunities abound, especially in the oilseeds field. Seed oils are potential sources of health products and innovative cosmetics.

Calophyllum species, widely used in traditional medicine, are currently studied for their therapeutic potential [1], [2].

Fig. 1 : Fruits of Galba

In Guadeloupe, the most widespread Calophyllum species is *C. calaba*. The seed oil from this plant is marketed by Phytobôkaz company as “Galba oil” and is produced respecting the environment. Phytobôkaz aims at conserving biodiversity through the valorization of natural products from plants. This strategy is part of a whole agro-ecological process to develop sustainable models of plantation, as is the case with *C. calaba*.

Seeds from *C. calaba* provide good yields of an oil with interesting biological properties. A phytochemical study of the unsaponifiable matter of this oil was performed. It showed the presence of six major compounds with pyranochromanone structure, which were isolated and characterized by various analytical and spectroscopic methods (LC/MS, MS/MS, NMR). The chemical features of this Guadeloupean species will be presented and compared to those of other species of Calophyllum.

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P26

CHARACTERIZATION OF ALKALOIDS AND CAROTENOIDS, A DEFENSE COCKTAIL ON COCCINELLIDAE EGGS' SURFACE

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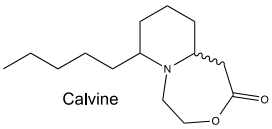

Ladybirds (Coccinellidae: Coleoptera) are known to produce sophisticated secondary metabolites, including alkaloids, used mainly for defense. These molecules are present in different life stages, from eggs to adults. Our attention was drawn to the *Calvia quatuordecimguttata* (L.) species eggs, that are covered with red drops. Behavioral studies show that this secretion, produced during egg laying, very effectively repels ladybird predators. The minute amounts of secretion available are a challenge for the chemist. After collection with SPME fibers under a stereo microscope, secretions were analyzed by LC-UV-ESI-QToF. They contain calvine analogs and carotenoids. Carotenoids are believed to be a reliable signal of the toxic or distasteful presence of the calvine alkaloid. This example shows how ecologists and chemists benefit from joint efforts to explore chemical ecology mysteries.

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WHICH METABOLITES FROM HEMP ROOT DO INDUCE SEED GERMINATION OF THE PARASITIC WEED *PHELIPANCHE RAMOSA?*

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Phelipanche ramosa (Orobanchaceae) is a non-chlorophyllous parasitic plant with a wide host range, including hemp. It is a growing concern for hemp culture as no solution is proposed so far to reduce its impact and spread. Latent orobanche seeds within the soil germinate only when exposed to host root exudates. Various germination-inducing phytochemicals have been identified in other host plants (sunflower, rapeseed, weeds…) but not in hemp roots[1]. One characteristic of this interaction is the very high sensitivity of parasite seeds to host plant compounds, present in the rhizosphere at concentration down to the nano- or picomolar level, rendering their isolation challenging. Our goal is to identify such compounds in hemp root exudate. The adopted strategy consists in growing hemp hydroponically and extracting chemicals from root exsudate (or culture medium) by SPE. Root exudate concentrates are subjected to bioguided fractionation[2]. We developed a systematic method to track compounds bearing activity, based on automatic recognition of *m/z* peaks and correlating the bioactivity to different fractograms obtained with different chromatographic methods. One compound ($C_{17}H_{26}O_4$) has been isolated. The very low quantity led us to perform 1D and 2D NMR on a 950 MHz instrument.

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P28

STUDIES ON NATURAL BIO-INSECTICIDES FROM CAMBODIAN PLANT BIODIVERSITY TO CONTROL MALARIA AND DENGUE VECTORS

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The main objective of the present work is to enhance Southeast Asian plant biodiversity, particularly from Cambodia, for the evaluation of insecticide or insect repellent properties of plant extracts to be used in vector control programs, respectful of the environment. Ethnobotanical surveys conducted in medicinal plant shops in different markets and in the National Center of Traditional Medicine of Phnom Penh enabled the selection of plants used in traditional medicine for their insecticidal/ repellent activity. This study was completed by literature search obtained from Cambodian books published by the National Center of Traditional Medicine. All in all, 73 plants were mentioned for their insecticidal/ repellent activity and five plants were selected for the evaluation of insecticidal properties. After collecting the plants, extracts were prepared following a standardized methodology. A study of the behavioral responses of *Aedes aegypti* and *Anopheles minimus* at three concentrations of plant extracts were performed using an excito-repellency test system[1,2]. Results showed that *Strophanthus scandens* leaves hexanic extract is the only one to exert a repellency on the two mosquitoes species (*An. minimus* at the concentration of 2.5% and *Ae. aegypti* at 5%). The obtained results confirm the traditional use of *S. scandens*, and show that Cambodian plants extracts could be a promising environmental friendly alternative to synthetic insecticides for which resistances have recently been observed.

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P29

SCREENING OF MEDITERRANEAN MUSHROOMS TO IDENTIFY NATURAL LDHA INHIBITORS

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Human lactic acid dehydrogenase (LDH) is a tetrameric enzyme composed of two subunits, LDHA and LDHB. Although LDHB is ubiquitously expressed, LDHA expression is predominantly found in skeletal muscle and other highly glycolytic tissues. LDH catalyzes the reversible transformation of pyruvate into lactate in anaerobic conditions, coupled with NADH oxidation into NAD+. LDH thus regulates the final step of anaerobic glycolysis. It is now a well-known fact that most malignancies rely on a high rate of glycolysis to produce the energy necessary for tumor growth and progression. Numerous studies have demonstrated that natural substances can be considered as strong therapeutic resources in the fight against cancer. We sought to identify natural LDH inhibitors within fungi. 160 fungal extracts were obtained by ultrasonic sequential extractions, using 4 different solvents, of 40 Mediterranean edible mushrooms. We then screened the 160 fungal extracts for their potential LDH-inhibitory properties. Through this method, we have identified 7 fungal extracts that harbor LDH inhibitory properties. Because these active extracts originate from different extraction solvents, we suspect that the LDH inhibitory properties of these fungal extracts are exerted by different types of molecules. Further bioguided purification will carried out to identify bioactive compounds. Our findings thus point to the interest of investigating the potential application of these mushroom-derived substances in therapeutic strategies.

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P30

BIOGUIDED FRACTIONATION AND ISOLATION OF CYTOTOXIC COMPOUNDS FROM AN ACTINOBACTERIUM ISOLATED FROM AN AUSTRIAN LICHEN

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Lichens, due to their symbiotic feature between fungi and algae (Chlorophyta or Cyanobacteria), are well-known as a rich source of original compounds.[1] However, recent studies revealed the presence of a microbiota, consisting of bacteria, associated to these organisms.[2] These microorganisms may represent an underexplored reservoir of novel species of potential interest in the discovery of novel lead compounds.

Actinobacteria are known for their ability to produce compounds of clinical and pharmaceutical importance.[3] *Nocardia ignorata* was selected between several strains isolated from the Austrian lichen *Collema auriforme*[4], based on its interesting cytotoxicity evaluated on two different cell lines (HaCaT, B16). In order to identify bioactive compounds, the extract was purified by bioguided fractionation. After several days of growing, the culture media was collected and extracted with XAD-7 resin. The extract was then purified using flash chromatography and HPLC/DAD and the potential cytotoxicity of the fractions and/or pure compounds was measured on HaCaT and B16 cells.

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3 Bérdy,. J J. Antibiot. 58. 1–26. (2005)
4 Parrot, D. et al. Sci. Rep. in revision

P31

Effect of Heavy Metals on Invasive Knotweed (*Fallopia* spp.) and the Combined Effect of Metal Pollution and Plant Colonization on MultiDrug Resistance (MDR) Phenotypes in Soil Bacteria

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The presence of heavy metals in the environment, which is largely due to anthropic activities, represents a stress for all living organisms, impacting ecosystem structure and function. At the microbial scale, the cellular toxicity of metals could alter microbial communities by enhancing MDR bacterial phenotypes through mechanisms of resistance co-selection [1]. Some plant species are able to grow on heavy metal polluted soil; this is the case of the invasive complex *Fallopia* which is able to accumulate metals, leading this plant to be dominant in high polluted urban environment [2]. On the other hand plants are known to be major drivers of soil microbial communities structure and functioning through rhizodeposition principally [3], and *Fallopia* sp. has been shown to exert significant effects on soil bacterial communities through its secondary metabolites content [4].

In this context we wanted to evaluate the effect of metal pollution on *Fallopia* secondary metabolism, and the combined effect of metal pollution and plant colonization on bacterial MDR resistance phenotypes. In this aim, we undertook a mesocosm experiment where rhizome fragments of *Fallopia* were grown in greenhouse in soil pot artificially polluted or not with heavy metals (Pb, Zn, Cd, Cr). Our results show that (i) heavy metals delay plant growth but did not affect plant height after 3 month nor aerial part dry weights, only belowground part dry weights were lowered; (ii) each plant genotype could be discriminated on PCA plots at each collecting time and for each plant part, but the effect of metals was only detectable for both plant parts at 1 month and not at 3 ; (iii) the antibiotic resistance profiles of soil bacteria were affected by plant or metals but the combined effect of plant and metals was not explained by addition effects, but rather by interaction effects which indirectly confirms the importance of metal-induced change on plant metabolism.

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P32

THE ROLE OF *AGROBACTERIUM FABRUM* SPECIFIC GENES IN BACTERIA-PLANT INTERACTION: PLANT METABOLOMIC APPROACH AND BACTERIAL FITNESS STUDY

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Agrobacterium sp. is a genus of bacterial species living in the soil and rhizosphere of plants with which they establish commensal interactions or favorable PGPR type. When they harbor a Ti plasmid (for *Tumor inducing*), they also can be phytopathogenic bacteria causing Crown gall disease, an economically important phytobacteriosis affecting many plants of agronomic and horticultural interest. To fight against this disease, it is important to better understand the ecology of *Agrobacterium* and the adaptations of the different species that allow them to coexist and survive in their natural habitats. A comparative genomic study showed that *Agrobacterium* species differ in genomic regions that are specific to them and that would confer adaptations to particular ecological niches.The annotations of the specific genomic regions of one of these species, *A. fabrum*, indicate some functions linked to plant compounds (sugar, phenolic compounds...), suggesting the involvement of these genes in bacteria-plant interactions [1]. Furthermore, the functional study of one of these genomic regions has allowed to characterize a degradation pathway of hydroxycinnamic acids [2]. We hypothesize that the specific gene regions of our model *A. fabrum* are involved in commensal interactions established with a plant partner. Firstly, the aim of this study is to determine by a metabolomic approach if some of these specific genomic regions are able to modify the phenolic compounds content of roots colonized by bacteria and which plant metabolites are modified. Secondly we studied the involvement of these genomic regions in the fitness of bacteria.

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P33

STUDY OF PLANTS AND RESISTANT BACTERIA ADAPTED IN METALLIFEROUS AREAS BY METABOLOMIC AND MICROBIOLOGICAL APPROACHS

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Anthropization largely contributes to the emergence and transmission of resistance genes in the environment, and highly contaminated soils such as metalliferous sites could be seen as hot spots for the expression of multi drug resistance (MDR) phenotypes (1). Some plants and microorganisms are able to withstand and accumulate metals in their different parts. It is reported that these plants have shown the ability to defend against herbivore and pathogens (2). We hypothesise that this defensive effect of plants is due to their change of metabolism in the adaption processes with high metal level. These compounds may play an essential role on the expression or the dispersion of MDR phenotypes, and thus influence bacterial growth. In this study, we have compared using UHPLC-DAD/ESI-QTOF the metabolites profiling of *Pteris vittata*, a well-known hyperaccumulator plant found in Dai Tu mining iron ore in Thai Nguyen province (Vietnam) and used as phytoremediation and control plants which were collected in non-polluted area. Extracts of three different parts (root, stem and leaf) were analyzed using standard protocols for extraction, purification and characterization of natural compounds. Statistical analysis (i.e. Principal Component Analysis and ANOVA followed by Tukey's HSD test) were performed to identify specifically the compounds over-expressed in polluted areas. In parallel, resistant bacteria from soil samples collected in this mining area were enumerated on generalist media (1/10 tryptic soil agar) supplemented with various combinations of antibiotics and metals in order to evaluate the prevalence of MDR phenotypes. Taxonomic diversity of resistant bacteria was further investigated by 16S rDNA sequencing. The results showed out discriminant compounds in the metabolic profiles of *Pteris vittata* when they are grown on diversely contaminated soils. Increased and decreased compounds were preliminary identified. Beside, over two hundreds isolates with MDR phenotypes which include a large range of species belonging to genera such as *Pseudomonas, Stenotrophomonas,...* were quantified and identified showing a rich diversity of resistant bacterial populations in the rhizosphere of plant from metal-polluted soils. These findings suggested further investigations on the impact of discriminant plant metabolites on resistant bacteria, in order to determine how metals and plant metabolites modulate resistance to antibiotics.

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FAST IDENTIFICATION OF RADICAL SCAVENGING COMPOUNDS FROM *SECURIGERA VARIA* BY BIOACTIVITY-GUIDED FRACTIONATION AND ^{13}C NMR-BASED DEREPLICATION

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Securigera varia (Fabaceae) is a common Eurasian herbaceous perennial plant that enables erosion control, roadside planting and soil rehabilitation. The crude MeOH extract of *S. varia* aerial parts was shown to exhibit a significant radical scavenging activity. The aim of this study was to identify the compounds responsible for this activity. For this purpose a bioactivity-guided fractionation procedure involving centrifugal partition extraction (CPE) combined to the stable DPPH radical scavenging assay was developed. The major active compounds present as simplified mixtures in the CPE-generated fractions were rapidly identified by using a recently developed dereplication method based on ^{13}C NMR, pattern recognition of NMR signals by hierarchical clustering analysis and a natural metabolite database. Semi-preparative HPLC was then used as an orthogonal technique to isolate minor and unknown active compounds. As a result, one new and twelve known flavonoid glycosides together with three nitropropanoylglucopyranoses were identified. The new compound was confirmed to be apigenin 7-*O*-□-D-glucopyranosyl-(1□2)-□-D-glucuronopyranoside by 2-D NMR analyses and mass spectrometry.

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IDENTIFICATION AND PURIFICATION OF STILBENES FROM SOME CYPERACEAE AND STUDY OF THEIR NEUROPROTECTIVE EFFECTS.

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Abstract: Stilbenes are phenolic compounds of plant secondary metabolism, whose distribution within the plant kingdom is limited to species that have acquired during the evolution the ability to synthesize these molecules. Their impacts and their biological activities such as neuroprotective, anticarcinogenic, antioxidant effects have already touched several topics. Among the stilbene, resveratrol, base molecule stilbene, is a particularly active molecule. It is considered a neuroprotective compound. Feng *et al* (2009) have shown that resveratrol could directly bind to $A\beta_{42}$, interfere in their aggregation, change the conformation of the oligomers and thus reduce cytotoxicity of these oligomers **[1]**. Resveratrol and its derivatives could be a parade of Alzheimer's disease by limiting its effects. It is in this context that the purpose of our work arose. First, we have isolated and identified these molecules in some species of the sedge family. Phytochemical studies were performed using a set of analytical and preparative strategies by means of analytical and preparative HPLC and CPC (Centrifugal Partition Chromatography) for obtaining pure molecules and LC-Mass and NMR for identification of compound isolated. Twelve compounds, mostly isolated and purified from the Cyperaceae. Secondly, we studied the effect of these stilbene on neuronal cytotoxicity induced by β-amyloid peptide with PC12 cells. We used as a study model of neuronal cells (PC12 cell line) brought into contact with the peptides $\beta A_{25\text{-}35}$. Initially, we studied the effect of these stilbene on the viability of PC12 cells in the absence of βA to determine if these compounds alone could induce toxicity on cells. Then we looked for their potential to inhibit neuronal death induced by the peptide. Among the stilbenes isolated two compounds showed a strong anti-amyloid activity.

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P36

RAPID AND GREEN EXTRACTION ASSISTED BY MICROWAVE AND ULTRASOUND OF CEPHARANTHINE, A BISBENZYLISOQUINOLINE ALKALOID FROM *STEPHANIA ROTUNDA*

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The tuber of *Stephania rotunda* is used to treat malaria in traditional medicine in many Asian countries. Cepharanthine is a bioactive bisbenzylisoquinoline alkaloid of the plant and exhibits interesting in vitro antiplasmodial activity. Two innovative and ecologic technologies were proposed to extract cepharanthine from *S. rotunda* tuber: ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). Various parameters of these processes were studied to optimize the extraction of cepharanthine from the tuber. Quantification of cepharanthine was performed by a validated HPLC method. MAE was the most effective method, in a closed vessel system, with the solvent ethanol-water (50:50, v/v), ratio solid-liquid 1/20, power 100 W, temperature 80°C and extraction time 15 min. UAE, with solvent ethanol-water (50:50, v/v), solid-liquid ratio 1/30 (w/v) and extraction time 10 min, was more ecologic because it didn't need any heating and was shorter than MAE. Statistical analyses showed that these two methods exhibited the same efficiency for the extraction of cepharanthine and both methods were more efficient than percolation. MAE and UAE allowed the optimal extraction of cepharanthine from *S. rotunda* tuber when the solvent used was ethanol-water in comparison with dichloromethane. Both eco-extractions, using ethanol-water mixture, were found to be promising alternative methods for the extraction of cepharanthine from *S. rotunda*.

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PHYTOCHEMICAL ANALYSIS AND ANTIDIABETIC ACTIVITY OF *PLUMERIA ALBA* LINN. (APOCYNACEAE) ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Plumeria alba (Apocynaceae) is a plant used in Togolese traditional medicine to treat diabetes. The present study was designed to investigate the effect of total hydro-alcoholic extract and active fraction of *P. alba* root on diabetes. Phytochemical screening, TLC and HPLC/DAD analysis revealed the presence of polysaccharides and phenolic compounds such as coumaric derivates. The effect of total hydro alcohol extract at 250 mg/kg and supernatant fraction obtained after polysaccharides precipitation in cold alcohol were measured on a model of diabetic rats (fructose-enriched fat diet and streptozotocin). Analysis of lipid profile indicate that fructose-enriched fat diet increased blood cholesterol, triglycerides and HDL (High density lipoprotein) levels in diabetic untreated rats compared to normal control rats. The administration of total extract (250 mg/kg/day) and supernatant fraction of *P. alba* (100 mg/kg/day) during 14 days significantly reduced lipid parameters (total cholesterol, $p < 0.001$; triglycerides, $p < 0.01$; HDL : $p < 0.05$).These results suggest that total extract and supernatant fraction of the *P. alba* exhibit significant antidiabetic and hypolipidemic properties in streptozotocin induced diabetes animals. Supernatant fraction is one of some purifications without toxic solvent and is similar to traditional use condition of *P. Alba.*

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DIFFERENT PHYTOCHEMICAL COMPOSITIONS OF PROPOLIS SAMPLES COLLECTED IN IVOIRY COAST, AFRICA

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Propolis, or bee glue, is a natural resinous hive product collected by honeybees from buds and exudates of various trees and plants. Mixed with beewax and salivary enzymes, it is used to fill in cracks and holes in the hive as well as a chemical weapon against intruders. It is well known that the chemical composition of propolis depends on the flora at the site of collection. Therefore propolis are generally classified as "poplar-type" in temperate zones and "green Brazilian", "*Clusia", "Macaranga"* as well as Mediterranean-type in tropical zones [1]. The aim of this work was i) to study the phytochemical composition of EtOH extracts from six batches collected in different regions of Ivory Coast, using GC/MS and HPLC/UV/MS^n profilings followed, when necessary, by 1D and 2D NMR analysis, and ii) to evaluate their antioxidant and anti-AGEs activities using respectively DPPH and BSA assays. One of the six propolis samples, originating from Katiola, exhibited an unusual chemical composition (flavonoids and phenolic acids derivatives) associated to a poplar-type propolis [2]. The EtOH extract showed a high antioxidant activity of 1066±15 µmol TE/g (control: rosemary EtOH extract at 591±21 µmol TE/g) and an excellent anti-AGEs activity with an IC_{50} of 20 µg/mL (control: *Styphnolobium japonicum* EtOH extract at 90 µg/mL). The other propolis extracts exhibited, as expected for tropical type samples, triterpenoids as major constituents accompanied with minor polyphenols such as prenylated flavanones, chlorogenic acid, or biflorin. Therefore the composition of the propolis collected in Katiola appears as quite unusual and we are now working on the analysis of its botanical origin.

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P39

OPTIMISATION OF A COLORIMETRIC ASSAY FOR HIGH THROUGHPUT SCREENING OF NATURAL ARGINASE INHIBITORS

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Background: Arginase is an enzyme that converts L-arginine to L-ornithine and urea. Its activity could be involved in various diseases such as hypertension and leishmaniasis [1]. It is therefore a potential target for new treatments. Natural products could be a source of arginase inhibitors but current methodologies in this area have to be improved [2] in particular due to the lack of reproducible and high throughput screening assays. **Aim**: We optimized and validated a colorimetric method for the screening of arginase inhibitors. **Method**: We improved the method of Corraliza *et al.* [3], which is based on the reaction of urea with alpha-isonitropropiophenone, by using a low amount of purified liver bovine arginase instead of cell lysates and by miniaturizing the assay. We validated our assay by determining kinetic properties of arginase with and without the reference arginase inhibitor S-(2-boronoethyl)-L-cysteine (BEC). We then screened ten pure natural compounds and determined their inhibitory properties. Finally we tested plant extracts for their activity on arginase. **Main results**: We accurately measured arginase activity by using only 0.25 unit of enzyme per well of a microplate. We confirmed its Michaelis-Menten kinetics and found Vm and Km values of 8.15 nmol urea.min^{-1} and 50.5 mM respectively. As expected BEC was a potent arginase inhibitor, with a half maximal inhibitory concentration (IC50) of 3.3 µM. We also determined the IC50 values of ten pure natural compounds and found that the most potent was chlorogenic acid (IC50 = 10.6 µM). Interestingly our most active *helianthus* extract so far could also inhibit significantly arginase (IC50 = 12 mg/L), which may be correlated with its high amount of chlorogenic acid (~13%). **Conclusion**: Our simple, cost-effective and highly reproducible method can be used for routine screening of new arginase inhibitors in natural sources.

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P40

CARNOSOL PURIFICATION FROM *ROSMARINUS OFFICINALIS* BY CENTRIFUGAL PARTITION CHROMATOGRAPHY, FROM LABORATORY TO INDUSTRY

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Rosemary (*Rosmarinus Officinalis*) is an aromatic herbal plant belonging to the Lamiaceae family and known for its medicinal and taste properties. Recent studies have shown its pharmacologic activities for cancer chemoprevention and therapy due to phenolic compounds presence like carnosol, carnosic acid and rosmarinic acid. Carnosol was more specifically evaluated for anti-cancer properties in prostate, breast, skin, leukemia and colon cancer showing promising results. Its purification is required at lab-scale for toxicology studies and at industrial scale for production as an active ingredient. Centrifugal Partition Chromatography (CPC) is a preparative separation technique working with a biphasic liquid system. One phase is used as the mobile phase when the other is the stationary phase hold in place by centrifugal fields. Manufacturers have recently designed small columns for method development, saving solvent and time. In this context, we will present the CPC method development and carnosol purification from a Rosemary leaves extract on a lab-scale instrument, highlighting the advantages of the CPC technique on natural products purification. Then transfer towards large scale production can be achieved through a new methodology based on the experimental observation of apparatus and solvent system behaviors, resulting in the production of pure carnosol from Rosemary leaves extract on an industrial scale apparatus.

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DEVELOPMENT OF A PHARMACOPHORIC DECONVOLUTION METHOD : THE PROOF OF CONCEPT

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Traditionally in natural-product research, concentrated extract samples are screened in bioassays. Such extracts are complicated mixtures and sometimes the biological activity is mainly due to one or two compounds. Isolating each compound from a crude extract is too onerous and time-consuming.[1] The traditional approach of bioassay-guided isolation of natural products allows targeting the bioactive compounds. Bioactive compounds are traditionally identified and characterized following the fractionation and purification of the bioactive extract, guided by bioactivity assays. However, bioguided fractionation has a lot of disadvantages, such as isolation of trivial molecules. In addition, this method is still time consuming and tedious. That is why we have devised a new faster method based on differential analysis 2D-NMR and the use of the hyphenated method HPLC-SPE-NMR.

The proof of concept was made with the identification of eleganolone as the bioactive compound of *Bifurcaria bifurcata* extract. The same results were obtained with both methods but the pharmacophoric deconvolution method developed here is less time- and quantity of raw material- consuming.

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P42

CHEMICALLY ENGINEERED EXTRACTS OF *HYPERICUM PERFORATUM* (ST JOHN'S WORT) AS SOURCES OF BIOACTIVE COMPOUNDS TO PREVENT ENDOTHELIAL DYSFUNCTION

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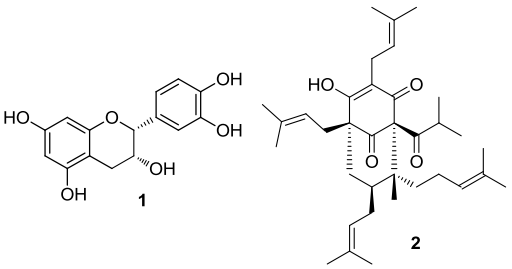
Graft rejection remains a serious concern in transplantation therapy. As endothelial dysfunction plays a prominent role in transplant rejection, finding new ways to prevent this process is a huge challenge. Recently, Rouger *et al.* evidenced the significant antiinflammatory and immunomodulatory properties of polyphenolic compounds from tropical *Calophyllaceae* and *Clusiaceae* plants on endothelial cells [1]. Plants from the *Hypericaceae* family biosynthesize similar natural products and some species (*e.g. Hypericum perforatum*) are cultivated and available in large amounts. To preserve biodiversity and valorize medicinal plants growing in the Loire Valley, the project HYPROTEC attempts to 1/ improve access to a library of original and bioactive polyphenols by chemical modifications of *H. perforatum* extracts ; 2/ evaluate their potential to prevent endothelial dysfunction [2].

The present work describes the biological activities of two libraries of natural products derivatives purified from *H. perforatum* chemically engineered extracts (CEEs). The first one was obtained from the methanolic root extract using the well-described acid catalyzed cleavage of tannins in presence of various nucleophiles to produce semisynthetic analogues of epicatechin **1**. The second library was achieved from the cyclohexanic flowering tops extract subjected to either oxidation or acylation to provide originals hyperforin derivatives **2**.



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ANTI-INFLAMMATORY ACTIVITY OF A *CARICA PAPAYA* LEAF EXTRACT

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Obtained from a *Carica papaya* (pawpaw) aqueous leaf extract, Gencix® is a fluid powder dentifrice used to inhibit bacterial growth as well as to protect gums. *Carica papaya* extracts were previously reported to exhibit anti-inflammatory activities which could be associated with flavonoids, saponins, tannins, glycosides or polyphenols generally found in the leaves[1,2]. Therefore, after succesive extraction of Gencix® using DCM, EtOAc and H_2O, respectively, the anti-inflammatory activity of each extract was evaluated. The release of Tumor Necrosis Factor-alpha (TNF-α, a pro-inflammatory cytokine) by human monocytic cells derived from an acute monocytic leukemia (THP-1) was monitored after a *Porphyromonas gingivalis* LPS activation. Both DCM (inhibition: 60.8% at 500 µg/mL) and EtOAc (inhibition: 54.9% at 500 µg/mL) extracts were active in comparison with dexamethasone used as the reference compound (inhibition: 52.3% at 1 µg/mL) when a lower activity was detected for the aqueous extract (inhibition: 30.1% at 500 µg/mL). Since the DCM extract also appeared as cytotoxic, a bioguided fractionation was firstly undertaken on the EtOAc extract. Results of a preliminar dereplication study using LC-DAD-MS^n will be presented together with attempts to identify anti-inflammatory compounds through this bioguided fractionation.

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BIOACTIVE MOLECULES OF *ANVILLEA RADIATA*

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Anvillea garcinii subsp. radiata (Cosson & Durieu) is a wild plant that grows predominantly in the steppes of north Africa (Morocco and Algeria) and found in areas of the Middle East. It is widely used in traditional medicine for the treatment of dysentery, gastric-intestinal disorders, chest cold and has been reported to have hypoglycemic activity as well as antifungal activity.

Germacranolides previously isolated using conventional procedures (maceration or soxhlet followed by silica gel column chromatography) from the aerial parts of *Anvillea garcinii* or *A. radiata* possess anti-tumor activities [1,2]. However this plant contain also less regarded flavonoids.

In order to be able to valorize these two molecule families we develop an Accelerated Solvent Extraction (ASE) method in two steps to selectively extract germacranolides then flavonoids.

Major germacranolides were isolated using Centrifugal Partition Chromatography (CPC) with a heptane/ethyl acetate/methanol/water (1:5:1:5 v/v/v/v) system in elution gradient. 9β-hydroxyparthenolide and 9α-hydroxyparthenolide were identified by mass spectrometry (MS) and ^{1}H NMR and ^{13}C NMR and represent respectively 3 and 2 % of the plant material. Minor flavonoids were characterized by HPLC-MS chlorogenic acid derivatives, glycoside and aglycone flavonols were identified.

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BACOPA MONNIERI EXTRACT INCREASES RAT CORONARY FLOW AND PROTECTS AGAINST MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

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Bacopa monniera (Brahmi) is a traditional Ayurvedic medicinal herb in India and Pakistan widely used for its nootropic effect. Brahmi is also used for cardiotonic effect but this latter property has been poorly validated by pharmacological studies. For this purpose, the present study investigated the effects of *B. monnieri* on heart function in physiological and pathological conditions, in rats.

Experiments were conducted in isolated hearts from male Wistar rats using the Langendorff preparation and coronary flow, left ventricular developed pressure (LVDP), heart rate (HR) were measured. In a first experiment, hearts were perfused with Krebs-Henseleit buffer (KHB, control) or with an ethanolic extract of *B. monnieri* (30 and 100 μg/ml) for 30 min. In a second experiment, isolated hearts were pretreated with KHB or with the extract for 10 min. Then, ischemia (30 min) followed by reperfusion (30 min) with KHB was applied.

In normally perfused hearts, as compared to control, *B. monnieri* significantly increased coronary flow by 63±13% (30μg/ml) and 216±21% (100μg/ml). There were also small increases in LVDP, but no change in HR in response to both concentrations of *B. monnieri*.

In hearts subjected to ischemia/reperfusion, *B monnieri* increased LVDP was 84±10% (30μg/ml), 82±10% (100μg/ml) and 52±6% (control) as well as functional recovery, a global index of cardiac contractile function.

In conclusion, our study revealed that *B. monnieri* induces significant effects on cardiac function in physiological and pathological conditions. These data suggest that *B. monnieri* might be a novel cardioprotectant strategy. Our prospects would be to identify compounds involved in these cardiac effects.

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COUMARINS FROM AERIAL PARTS OF *CACHRYS LIBANOTIS* L., APIACEAE

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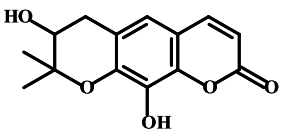
Cachrys is a genus belonging to the family of Apiaceae. This family consists of more than 400 genera widespread in more than 3000 species [1, 2]. It is represented by 55 genera in Algeria [3]. *C. libanotis* L. species is present in the Mediterranean area. *Cachrys libanotis* were collected in El Kala near the coast (Wilaya El Taref) in the east part of Algeria, during the flowering period.

Phytochemical studies of the aerial parts led to isolation of two new coumarins, 8-hydroxy decursinol **(1),** meranzin hydrate-3-acetyl-3'-*O*-*β*-glucopyranoside **(2)** and together with eight known compounds : osthole, meranzin hydrate, meranzin hydrate- 3'-*O*-*β*-glucopyranoside, 3'-methoxy dihydroseseletin, 8-methoxy marmesin, nodakenin, 8-hydroxy nodakenin, hesperidin. Their structures were determined by 1 and 2-D NMR techniques.



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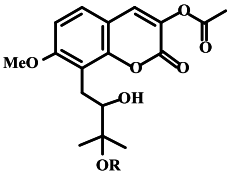

R = O-β-glucose 2

References :

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[3] P.Quezel, and S.Santa (1963).Nouvelle flore de l'Algérie et régions désertiques et méridionales, Tome II. Edition CNRS, Paris

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P47

A NEW PHENYLPROPANOID DERIVATIVE ISOLATED FROM *CARTHAMUS TINCTORIUS* L.

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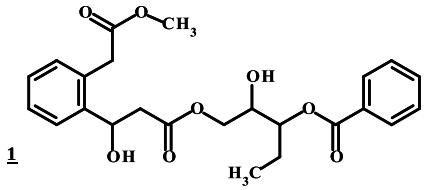
Compositae is the larget family in dicots all over the world and there are about 200 genus including 2000 species disputed in China, one of them is Genus *Carthamus*. Genus Carthamus has 18-20 species in the world in total and two species in China, one is *Carthamus tinctorius* L. and the other is *C. lanatus* L. [1] The dry flower of *C. tinctorius* L. is a pretty widely-used traditional medicine in China, which is also called red flower or safflower. It is cultivated all over China. The safflower does hold an effective impact on eliminating the stasis and invigorating the blood circulation. The pharmacological studies as well indicate that safflower possesses several medicinal functions such as activating uterus, lowering hypertention and hyperlipemia [2]. Meanwhile it is endowed with properties as anti-inflammatory and analgesic, anti-aging and improving cardiovascular functions, etc. A large variety of compounds are isolated and identified from *C. tinctorius* L., for instance, flavonoids, alkaloids, lignanoids, polyacetylenes, spermidines, organic acids, etc. In the course of a continuing search for active compounds from *C. tinctorius* L., a new phenylpropanoid derivative, named 2-hydroxy-1-(3-hydroxy-3-(2-(2-methoxy-2-oxoethyl) phenyl) propanoyloxy) pentan-3-yl benzoate (**1**), along with *β*-daucosterol and stigmasterol were obtained from the aerial part of *C. tinctorius* L..



1

References: [1] Science Press, Flora of China (1983). Beijing: *Science Press*. 78(1):186.

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P48

LENTINULA EDODES: CHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF SPOROPHORES AND SHIITAKE-CONTAINING DIETARY SUPPLEMENTS

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Lentinula edodes (Shiitake) is an edible medicinal mushroom appreciated for its flavor and its taste. It bears many biological activities and is commercialized in numerous dietary supplements. [1]
In the present study, we compared the chemical profile and the antioxidant activity of this mushroom and those of mushroom-containing dietary supplements. Both matrices were consecutively extracted with four solvents of increasing polarity as previously described [2]. The chemical profile of the different extracts was analyzed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The total phenolic content was estimated by Folin-Ciocalteu reagent. Antioxidant activity was evaluated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent using TLC and spectrophotometry [3].
Our results suggested significant differences of the phenolic content from shiitake sporophores and shiitake-containing dietary supplements. We also found an antioxidant activity in both mushroom and dietary supplement as described in literature [4].

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P49

SEPARATION AND CHARACTERIZATION OF APPLE BIOACTIVE MOLECULES BY HYPHENATION OF HPLC AND HPTLC WITH MASS SPECTROMETRY

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Apple is one of the most consumed fruit in the world and well known for its benefice on health. Apple is a natural resource of active molecules, such as flavonoids and triterpenes that possess numerous biological properties: antioxidant, anti-inflammatory or anticancer. Thus the study and the characterization of apple bioactive molecules becomes of a great interest.
Each compound family presents many isomeric and isobaric molecules that have to be separated using chromatographic methods to achieve their identification. The separation can be optimized by HPTLC or HPLC and compound characterization should be performed with hyphenation to mass spectrometry.
In this work, the apple flavonoids are characterized as well by HPLC/DAD/MS² than by HPTLC/Maldi. In apple pomace extracts, the HPLC/DAD/MS² analysis allows to characterize and understand ionization and fragmentation of flavonoid family. Moreover in apple juice enriched, different glycosylated derivatives of quercetin are identified thanks to their HPTLC separation. Hyphenation HPTLC/Maldi allows identifying quercetin derivatives and detecting minor compounds coeluted. That are distinguished by their aglycone (Kaempferol *m/z* 285; Phloretine *m/z* 273).
The apple pomace triterpene family is analyzed by HPLC/ELSD/MS onto two different chromatographic systems. 2 groups of molecules were separated on silica-C18 column: one corresponding to triterpenic acids and the other to triterpene derivatives containing a coumaryl group whereas on porous graphitized carbon (PGC) it was the position isomers of triterpenic acids that could be separated and identified.

P50

METABOLOMICS ANALYSIS OF *GALIUM ODORATUM* (L.) SCOP.: IMPACT OF THE PLANT POPULATION ORIGIN AND GROWTH CONDITIONS.

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Galium odoratum is a plant used in traditional medicine and to prepare beverages. This work aimed at studying the impact of plant origin and growth conditions on the metabolite content of the plant. **Material and methods**- Aerial biomass of *Galium odoratum* was collected from five natural populations (*in situ* conditions) and from controlled environment (*ex situ* conditions). **Results**- Quantitative analysis of selected phytochemicals including phenylpropranoids and iridoids showed clear differences between the plants from nature and those of controlled growth conditions as well as internal variation within the group. The metabolomic approach emphasized the decrease of the secondary metabolites pool paralleled by an increase of the carbohydrates in *ex situ* conditions. **Conclusion**- Metabolomics approaches using ^{1}H-NMR and HPLC is worth to consider for studying the impact of climate factors on the regulation of the phytochemical profile in relation to the origin of the plant material.

P51

METABOLITES ANNOTATION RANKING USING MARKOV CHAIN ALGORITHM

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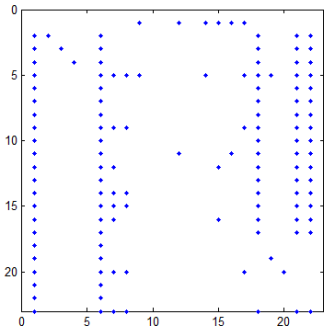

Identification of small molecules, often called metabolites remains a difficult task. It is one of the largest challenges in chemistry today and has even been considered as the main bottleneck in metabolomics for the last decade. High resolution mass spectrometry (HRMS) has become a powerful tool for metabolites annotation and dereplication but despite its high accuracy (> 1 ppm) it is still tricky to attribute a single empirical formula to one *m*/*z* value. In the last decade, several strategies have been proposed in order to get rid of less probable empirical formula, using heuristic filtering [1] or Bayesian statistics [2]. Here we propose an alternative method based on molecular pathways and the metabolome concistency concept [3] in order to further filter and rank compounds output according to metadata such as species or metabolic pathway. The implementation involves the Markov chain algorithm proposed by Brin and Page for the Google PageRank [4].

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P52

ANTIOXIDANT PROPERTIES AND CHOLINESTERASES INHIBITORY EVALUATION OF CANNABISIN F ISOLATED FROM HEMPSEED CAKES (*CANNABIS SATIVA* L.)

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Industrial hemp (*Cannabis sativa* L.) is cultivated in many countries for textile and nutritional interest. Hempseed constitutes an excellent source of healthy unsaturated fatty acids and therefore is used for the production of oil. Byproducts of this industry are not currently considered as having high economic added value. Nevertheless, hempseed and its residues are a source of amide derivatives of caffeic acid [1], among which some possess predominant radical scavenging activity [2]. Thus hempseed cakes constitute a real interesting crude material that should be recovered for the extraction of biologically active compounds. We performed a phytochemical study of hempseed cakes leading to the isolation of *trans*-caffeoyl derivatives and a series of cannabisin type compounds. Since one of the current drug discovery approaches for Alzheimer disease (AD) treatment focus on compounds which can restore the brain acetylcholine (ACh) levels and reduce the oxidative stress [3], cannabisin F was evaluated as a potential inhibitor of acetyl and butyryl cholinesterases (EeAChE and eqBuChE), and as an antioxidant (ORAC FL test). The results obtained showed a low activity of such compound against both cholinesterases (respectively 45,12 ± 0,23 % and 8,05 ± 0,19 % at 5µM). However, its ability to reduce the amount of peroxyl radicals potency displayed a strong antioxidant activity (10.25 µmol of trolox equivalents) corresponding to about three times that of ferulic acid used as reference compound. This preliminary but promising result encourages us to carry on with the evaluation of the products from *Cannabis sativa* L., in order to detect new leads for AD.

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P53

SEVEN LICHEN EXTRACTS: PROMISING SOURCES OF INHIBITORS OF *CANDIDA* BIOFILMS

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In the context of prevention or treatment of infections involving fungal biofilms (in particular associated with *Candida* yeasts) with new natural substances, 7 lichen extracts were found to display significant anti-adherent activity against *Candida albicans* yeasts ($p<0.05$) for concentrations lower than 15 µg/mL. The aim of this study was to characterize their chemical composition and anti-biofilm activity.

Their anti-biofilm activity was studied against *C. albicans* biofilms aged of 24h (mature biofilms) on polystyrene substrates, using XTT method. Besides CFU counts and trypan blue staining were realized. HPLC analyses of these extracts were performed as a first chemical profile investigation and optionally completed by a bioguided fractionation and purification carried out by chromatographic columns and preparative HPLC. The identification of isolated compounds was performed by NMR and MS.

This study showed that the activity was conserved against a mature biofilm: all extracts demonstrated anti-biofilm activity for concentrations lower than 10 µg/mL, especially *Evernia prunastri* and *Ramalina fastigiata*, two tree lichens, whose activity was observed as soon as 24h and persisted at 48h of biofilm treatment. CFU tests confirmed the anti-biofilm activity and demonstrated a decrease of the amount of yeast cells constituting the biofilm. Besides, the yeasts of the biofilm were alive after treatment, suggesting a non lethal effect. This work suggested a dispersant or detaching action of these extracts not targeting the fungal cell membrane. HPLC profiles of the two most active extracts reveal similarities in the chemical content and especially the presence of one common depside: evernic acid. Its implication in the anti-biofilm activity is highlighted by preliminary bioguided fractionation of these extracts. A thorough biological investigation will be conducted on this compound.

Thus, it was demonstrated the preventive and curative potential of lichen metabolites against *C. albicans* biofilms.

Acknowledgements: CNRS

P54

EXPLORING THE METABOLOME OF A MARINE-SOURCED *PENICILLIUM UBIQUETUM* STRAIN TOWARD THE ISOLATION OF CYTOTOXIC COMPOUNDS

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During a biological screening realised on marine-sourced *Penicillium* sp., extracts of a *P. ubiquetum* strain isolated from mussel sampled in the Loire estuary showed cytotoxicity against KB cell line but no activity on other assays regardless of the culture medium [1]. This species has been recently described, and very little is known about its metabolites [2]. We engaged a work in order to describe the metabolome of this species and to isolate the cytotoxic compounds. For this purpose, cultures were performed following the OSMAC approach [3] using 7 different media and two osmotic conditions: distilled water (DW) and seawater (SW). Results showed that fungal growth was enhanced by seawater whereas salinity was unfavourable to the production of metabolites. When tested for bioactivity, CYA extracts were found the most active with an IC_{50} of 7 µg/mL for CYA-SW. All extracts were analysed by LC-UV/DAD-HRMS and the metabolic profiles obtained were dereplicated using an in-house R-package consisting in automated data processing and database search. None of the terrein and okaramines described for this species in literature was found in any extracts. The mycotoxin citrinin was the major metabolite for all extracts except one and its quantification showed that sea water is a limiting factor for its production, except for YES and MES media. 14 other substances were identified and were new for this species: their variations and consistency of production were analysed. Isolation and structure determination of the compounds responsible for the cytotoxicity of the CYA-SW extract are currently ongoing: some of them will be presented.

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P55

NATURAL DEEP EUTECTIC SOLVENTS (NaDES): REACHING THE UNATTAINABLE

Alexis Lavaud[1], Mickaël Laguerre[1], Simona Birtić [1], Gaetan Pichon[1], Anne-Sylvie Tixier-Fabiano[2], Marc Roller[1], Farid Chemat[2], Antoine C. Bily[1]

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During the past decade, a new generation of solvents with a great potential for many industrial applications, emerged. They were first coined as *deep eutectic solvents* by Abbott et al. [1] in 2003 from the Greek term "eutektos" meaning "well melting". These solvents are combinations of compounds with lower melting points than their individual components, thus enabling the mixtures to be used as ambient temperature solvents. While deep eutectic solvents gained more and more interest, suspicion began to grow that life had long discovered them. Accordingly, it has recently been postulated that naturally occurring sugars, amino acids, quaternary ammoniums, and organic acids may form, when combined in the proper proportions, a liquid phase in plant cell called natural deep eutectic solvents (NaDES). Such liquids are supposed to maintain plant integrity and resistance to harsh environmental stresses, including drought, desiccation and extreme temperatures [2]. In the present work, a variety of NaDES were prepared by a simple, economical and environmentally-friendly process [3]. Various plant materials including olive leaves were subjected to NaDES-assisted extraction. A considerable increase of important bioactive compound levels was reached indicating the great potential of NaDES for the cosmetic sector.

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P56

LDI-MS AS A VERSATILE AND ULTRA-FAST DEREPLICATION APPROACH FOR LICHENS

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Lichens are self-sustaining symbiotic systems (fungus and green alga and/or cyanobacteria) producing a wide range of unique compounds which exert various and significant biological activities[1]. Successful harnessing of this unique chemodiversity relies on early and refined dereplication techniques to reduce time and costs in further downstream analyses. Yet, current dereplication approaches remain time-consuming and costly, stimulating efforts to develop mass spectrometry methods without neither hyphenation nor tedious sample preparation[2,3]. As such, MALDI-TOF-MS is an attractive tool since it performs fast and cheap and possibly automated analyses. However, its most challenging application in the realm of natural products are matrix interferences that obscure signals below *m/z* 700-1000 Da[4].

Many lichen compounds display close structural similarity to known MALDI matrices and usnic acid was consequently reported as a working MALDI matrix[5]. Independently from such matrical properties, it was presumed that other phenolics might likewise exhibit auto-ionization upon laser light exposure.

The present study enlightens the auto-ionization properties of all main classes of lichen metabolites. The versatility of negative-ion mode LDI-MS is further assessed on various lichen crude extracts by comparison (i) to LC-ESI-MS, used as a benchmark and (ii) to preliminary identification of metabolites isolated from different samples. Altogether, such results emphasize the tremendous interest of LDI-MS as a blitz-screening compatible dereplication approach in lichenology.

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P57

CHEMICAL STUDIES OF *BACILLUS WEIHENSTEPHANENSIS,* A LICHEN-ASSOCIATED BACTERIUM

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While lichens are usually described as a mutualistic symbiosis between fungi and algae, recent studies highlighted the diversity of associated bacterial assemblages. [1] These microorganisms are promising sources of structurally diverse and potent bioactive compounds. As part of our search for novel sources of bioactive natural products, *Bacillus weihenstephanensis* isolated from the lichen *Peltigera hymenina* (Brittany, France) had been selected for it promising production of cytotoxic and/or antibacterial compounds. In fact, *Bacillus* species are known for their production of versatile and bioactive secondary metabolites. [2]

To improve the production yields and the diversity of therapeutic interest metabolites, we focused our studies on the bacterial growth conditions. First, we studied the influence of nutrients on the production of metabolites using various nutritional element carriers. In addition, influence of incubation periods was determined by comparing the chemical profile of the extracts obtained from cultures stopped at different time. At least, we have started to use small-molecules for metabolism elicitation. Our studies combined (*i*) LC-MS analyses and data processing methods like GNPS [3] for visualizing sample profile and diversity, (*ii*) antibacterial and cytotoxic tests as bio-guided assays, and (*iii*) semi-preparative HPLC, NMR data and exact mass analyses for the elucidation of chemical composition of bacterial extracts. This approach allowed the evaluation of the diversity and redundancy of the compounds in various extracts depending on culture conditions. It will lead to the production of potential therapeutic agents.

References:
[1] a) Grube, M., *et al*., *Fungal Biol*, **2009**, 23, 72-85; b) Grube, M., *et al*., *ISME J*, **2009**, 3, 1105-1115 ; c) Bates, S T., *et al*., *Appl. Environ. Microbiol*, **2011**, 77, 1309-1314 [2] Chaabouni I., *et al*., *Bacillus thuringiensis* Biotechnology, Springer, **2012**, 18, 347-366 [3] GNPS: Global Natural Products Social Molecular Networking

P58

OCCURRENCE OF CYTOCHALASIN E, AN N-CONTAINING FUNGAL METABOLITES IN THE LICHEN *PLEUROSTICTA ACETABULUM*.

M. Millot[a], S. Delebassée[a], E. Pinault[b] and L. Mambu[a]

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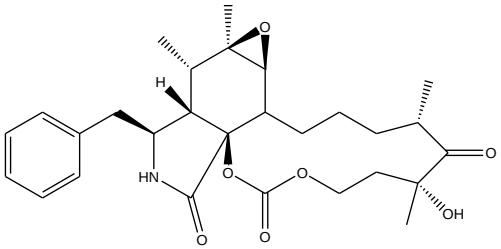
Two samples of the lichen *Pleurosticta acetabulum,* were collected in the suburb of Paris (sample 1) and in Limoges (sample 2). Investigation of anti-proliferative activity of acetonic extracts on HT-29 cancer cell lines has shown a significant difference between the two samples (IC_{50} = 6 µg/mL and IC_{50} = 35 µg/mL respectively). The aim of this work is to find out the compound responsible for the biological activity and to understand the differences observed between the two samples.



Anti-proliferative activity of extracts and fractions has been measured by MTT. Fractionnation and purification have been done by different chromatographic methods. Structures of isolated compounds have been determined by spectroscopic methods (IR, UV, NMR and MS). Chemical profil of 7 samples from different localizations in France has been realized by TLC and HPLC. LC-MS quantification has been realized in order to quantify the active molecules in the extracts.

Bioguided fractionation of the two samples led to the identification of an N-containing compound which has been identified as cytochalasin E. Preliminary results of LC-MS quantification, confirm the presence of this metabolite in all samples and show some quantitative variability between samples for various geographic areas.

The anti-proliferative activity and pro-apoptotic affect of extracts and fraction has been correlated with the presence of cytochalasin E. The plausible biosynthetic origine of this compound will be discussed.

P59

AN OPTIMIZED EXTRACTION PROCEDURE FOR THE PRODUCTION OF HIGH ADDED VALUE EXTRACTS FROM LICORICE ROOTS USING GREEN TECHNOLOGIES

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Licorice (*Glycyrrhiza glabra* L.) is one of the most widely used plants due to its sweet taste and beneficial properties [1]. According to current studies, licorice extracts possess a wide range of biological activities such as antioxidant, anti-inflammatory, antidiabetic, hypocholesterolemic, antimicrobial and anticancer activities [2]. The aim of the present study is the development of a green procedure for the treatment of licorice to produce high added value extracts with high phenolic content and significant antioxidant and tyrosinase inhibitory activity. The extraction process was performed in two consecutive steps. Initially, the raw material was extracted with supercritical CO_2 (SFE) to recover the non-polar antioxidant compounds belonging to the class of flavonoids, while the residue was treated with different ratios of water/ethanol and varying conditions using the accelerated solvent extraction (ASE) technique. In order to identify the optimal conditions in SFE and ASE methods, a Response Surface methodology was applied [3] and the yield (%), free radical scavenging activity (against DPPH and ABTS), total phenolic content (mg Gallic acid/g extract), total flavonoid content (mg Quercetin/g extract) and tyrosinase inhibition (% inhibition) were defined as the responses for both processes. In conclusion, a green extraction procedure based on SFE and ASE techniques was established and led to extracts with antioxidant and skin whitening properties, which could be used for the development of novel cosmeceuticals.

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P60

MANGIFERIN DISTRIBUTION AMONG *COFFEA* SPECIES

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Mangiferin, a xanthone with potent antioxidant and pharmalogical properties [1], was isolated from the leaves of a wild coffee species, *Coffea pseudozanguebariae* Bridson [2]. To determine their relevance as biological diversity markers, mangiferin and hydroxycinnamic acid esters (HCE), other antioxidant phenolic compounds [3] were studied in leaves of 14 *Coffea* species from Africa and 9 from Madagascar**,** using LC-MS, HPLC, histochemical and statistical methods.

Mangiferin accumulation in leaves of the 23 species analysed appeared as species characteristic but seemed independent of their geographic origin and their phylogenetic relationships. Using an original HPLC method, we demonstrated mangiferin accumulation in seven African *Coffea* taxa. Interestingly, none of the 9 Madagascan species studied accumulated mangiferin. As a potent antioxidant, it is probably involved in plant defense. Its external localisation in fruit pericarp confirms the hypothesis of a protecting role against UV [4]. However, its presence in abaxial leaf epidermis and absence from the adaxial one indicated that mangiferin may be involved in other defense mechanisms such as response to biotic stress [5]. These Coffee leaves and fleshy part of their fruits with high content of mangiferin and HCE have great potential with added health benefits as beverages and masticatory products already traditionally used in Africa and Asia [6].

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P61

ANTI-MICROBIAL AND ANTI-PROLIFERATIVE ACTIVITIES OF ASCOMYCOTA AND BASIDIOMYCOTA EXTRACTS

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Minimum global macrofungal species diversity is expected around 49.500 worldwide [1]. Little of macrofungi species have been recently studied for bioactive metabolites [2]. These include polysaccharides, proteins, lipids together with a large diversity of secondary metabolites, *i.e*., polyphenols, terpenoids, alkaloids. Fungal metabolites represented potential therapeutic agents against many diseases as Alzheimer, diabetes, malaria, infectious diseases and cancer [3-6].

We selected macrofungal species (*Basidiomycota*, *Ascomycota*) growing in the Mediterranean area to be screened for their biological properties of great interest. Macrofungi have been investigated for their chemical composition using various solvent polarities with extraction yield up than 30%. HPLC and TLC profiling have been performed. In parallel some of the crude extracts have been evaluated for their anti-microbial activity by disc diffusion method and for anti-proliferative activities in HCT116 colon adenocarcinoma cell line. Bioguided purifications of the bioactive extracts are in progress to identify promising therapeutic compounds.

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P62

HPTLC-ESI-MS AND HPTLC-FLUORESCENCE METHODS FOR IDENTIFICATION AND QUANTIFICATION OF DARUTOSIDE IN *SIGESBECKIA ORIENTALIS* EXTRACTS

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Sigesbeckia orientalis L. (Asteraceae) is a small shrub native to India and widely distributed in tropical and temperate parts in the world. In some countries, this herb is an important traditional medicine to treat skin disorders. In Malagasy Pharmacopeia, the leaves are used externally as protective covering for wounds and burns to stimulate wound healing. We confirmed the real interest of this plant for cosmetic application by the development of an extract of leaves of *Sigesbeckia orientalis* really efficient for sensitive skins. Our phytochemical investigations showed that diterpenoids are one of the main groups of secondary metabolites. Thus, we developed a convenient and reliable analysis method to assess the quality control of the leaves and darutoside was selected as marker. RP-18 HPLC with UV detection at 210 nm was often used to identify terpenoids. Nevertheless, the methodology is limited in its ability to separate all components of interest in hydroethanolic extracts of *Sigesbeckia* due to the presence of a resin-gum (mix of oligosaccharides and terpenoids). The aim of our study was to develop a quantitative analysis of darutoside in leaves extracts by HPTLC-fluorescence. The method was performed on a TLC scanner apparatus on Si60 HPTLC plates. The mobile phase was chloroform/methanol/water in the ratio (65/25/4, v/v/v). For revelation, the plates were sprayed with primuline reagent then scanned in fluorescence mode in a TLC scanner at 366. Darutoside was previously identified by ESI-MS with TLC interface. We applied our method for quantification of darutoside on our leaves samples collected in Madagascar. We observed that the amount of darutoside is variable depending on the date of harvest. April to June is the best period to harvest the leaves because darutoside reaches up to 1.5 % of dry extract. This present study described for the first time the quantification of darutoside in *Sigesbeckia orientalis* leaves extracts by HPTLC-fluorescence method.

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P63

VARIABILITY OF *CALOPHYLLUM TETRAPTERUM* PHYTOCONSTITUENTS IN RELATION WITH THEIR ANTI-INFLAMMATORY PROPERTIES

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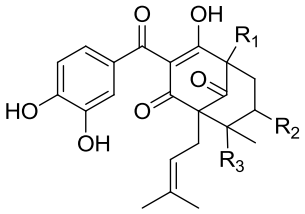
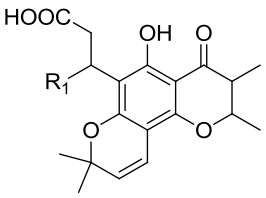
Several pantropical *Clusiaceae* and *Calophyllaceae* species are traditionally used to treat inflammatory diseases [1] since they biosynthesize polyprenylated polyphenols with broad anti-inflammatory properties [2-4]. In our search for original natural products able to prevent inflammation, 56 DCM and MeOH extracts originating from 10 Malaysian *Clusiaceae* or *Calophyllaceae* species were evaluated. Each of them was submitted to a HPLC-PDA-MS^n dereplication analysis together with a bioassay monitoring the expression of the Vascular Cell Adhesion Molecule 1 (VCAM-1) on human endothelial cells. A marked difference was observed between two DCM leave extracts obtained from distinct batches of *Calophyllum tetrapterum*. Indeed, a first extract rich in polyprenylated benzophenones showed 29% inhibition of VCAM-1 at 0.5 µg/mL, whereas the second extract, mainly containing chromanone acids, didn't exhibit any activity at the same concentration. This chemovariation, which can be attributed to the collection site and the harvesting period, will be discussed.



Polyprenylated benzophenone



Chromanone acid

References: [1] M. S. de Melo *et al.* Evid. Based Complement. Alternat. Med. (2014) 2014: 960258. [2] X.-H. Su *et al.* Chem. Biodivers. (2008) 5: 2579-2608. [3] N. Anantachoke *et al.* Pharm. Biol. (2012) 50: 78-91. [4] U. M. Acuña *et al.* Curr. Top. Med. Chem. (2009) 9: 1560-1580.

P64

MODIFICATION OF MAIZE XYLEM SAP METABOLITES IN RESPONSE TO SEED INOCULATION BY THE PGPR *AZOSPIRILLUM LIPOFERUM* CRT1

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Plant growth-promoting rhizobacteria (PGPR) colonize the plant rhizosphere and enhance root and shoot growth while stimulating local, and systemic, resistance to biotic, and abiotic, stresses [1]. Because of the potential agronomical benefits of PGPRs on crop plants, large research efforts have been devoted these past decades to isolate PGPR strains, sequence their genomes, describe the multitude of effects of these strains on the physiology of their host and highlight potential phytostimulatory genes in bacterial genomes. Despite these efforts, the mechanisms of action of PGPRs on their host physiology are poorly understood. In this study, we focus on the ability of a commercial PGPR strain, *Azospirillum lipoferum* CRT1, to enhance maize leaf growth. In order to see whether PGPR stimulated roots send a growth-stimulatory chemical message to the leaves, we have conducted a metabolomic analysis of maize xylem (ascending sap). Two cultivars of maize, FuturiXX (RAGT) and Seiddi (Caussade), were selected because previous studies have shown that only the second one displays growth enhancement after seed inoculation by *A. lipoferum* CRT1 [2].
Maize seeds were pre-germinated with the PGPR strain in petri dishes. Plants were grown indoors in non-fertilized natural grass soil maintained at field capacity and analysed 11 days post-sowing. In order to assess growth enhancement of the two maize cultivars by the PGPR strain, we measured the photosynthetic quantum efficiency (Ft/Fm') with a fluorimeter on the most recent mature leaf (second leaf). Xylem sap was collected with a Scholander pressure chamber, derivatized with a trimethyl silylation agent (MSTFA) and analysed by GC-MS (adapted from [3]). More than one hundred standards were analysed in parallel to identify xylem sap compounds. Our data reveal that the xylem sap extracts contained only few cellular or phloem contaminants. Xylem metabolic fingerprints differed between maize cultivars and upon inoculation. The photosynthesis (growth)-enhancing incompatible interaction generated the largest xylem metabolite profile change. In conclusion, the analysis of plant xylem metabolites proved to be very informative to explore the mechanisms of plant-microbe interactions. It is a novel plant metabolomics tool that we coin as xylenome.

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P65

CHARACTERIZATION OF ANTIOXIDANTS FROM *DETARIUM MICROCARPUM* GUILL. ET PERR. USING HPLC-DAD COUPLED WITH PRE-COLUMN DPPH ASSAY

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The present study aimed at identifying antioxidant constituents from the leaves of *Detarium microcarpum* (Fabaceae), an African tree species which is widely used in traditional medicine and as a food. A pre-column DPPH-HPLC assay was performed in order to speed up the process by allowing direct detection of the bioactive compounds. Indeed, this method has been reported as a rapid and effective screening of radical scavenging compounds in complex mixtures, such as plant extracts [1]. Its principle relies on the ability of antioxidant constituents to be oxidized upon reaction with DPPH radical. On this basis, peak areas of bioactive compounds are reduced in the HPLC chromatogram after spiking the extract with DPPH. At a wavelength of 280 nm, ten major peaks were observed on the chromatogram of a methanolic extract of *D. microcarpum* leaves. Comparison with the extract spiked with DPPH clearly showed that peak areas of five constituents were significantly reduced after radical pretreatment. These assumed antioxidant compounds were thus isolated. Four flavonol glycosides (quercetin 3,7-*O*-dirhamnoside, myricitrin, isoquercitrin and quercitrin) as well as gallic acid were identified. These five phenolics were subsequently evaluated for their individual DPPH radical scavenging effects and have all exhibited good or very good activities, ascertaining the efficiency of the screening method to detect antioxidant constituents from complex biological matrices.

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P66

SCREENING OF FIFTEEN PLANTS FROM THE NATIONAL PARK OF KAHUZI-BIEGA (RDC) USED IN TRADITIONAL MEDICINE.

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According to the WHO, the actual antibiotic resistance problematic constitutes a real threat for the public health. There is therefore an urgent need for new active compounds resulting in an increasing interest for plants which are an important source of secondary metabolites. The fifteen analysed plants were selected on the basis of an ethnobotanical survey that classified them as medicinal plants. Interestingly, these plants also appear to be eaten by lowland gorillas (*Gorilla berengei graueri*), eventually for automedication. The plants were harvested in the National Park of Kahuzi-Biega in DR Congo and for each plant a voucher specimen has been deposited in the National Herbarium of Belgium. The minimum inhibitory concentration (MIC) of the plant extracts (methanol and aqueous) were determined by microdilution methods against *S. aureus* (methicillin-susceptible strains) and *E. coli*. The initial tests showed that the methanol and aqueous extract of *Pleiocarpa pycnantha* Stapf. are active (from 250 µg/ml to 32 µg/ml). Whereas the others extracts showed no effect. After a phytochemical screening it appears that we have essentially flavonoids and triterpenes in most of the methanol extracts. No alkaloids have been found.
Some tests are still running about testing the effect of the association of the plants with antibiotics. Only the plants possessing the highest probability of exhibiting active antibacterial activity according to the ethnobotanical survey have been tested.

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ANTIMICROBIAL EVALUATION OF *DOLICHANDRONE PATHACEA* AND *PAYPAYROLA GUIANENSIS*

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Nowadays the research on natural products with antimicrobial properties has a great importance in the medical field and food-cosmetic industries. This study investigates on the bioactive compounds isolated from plants used in traditional medicine for the treatment of various infectious diseases. We evaluate the antimicrobial activity of several medicinal plant extracts from Algeria, Guyana, Vietnam, Peru and Senegal against 22 microorganisms including Gram positive/negative bacteria and yeasts. Development of microbiological evaluation methods such as bioautography, measurement of MIC (minimum inhibitory concentration) on microplates, and screening protocol using a multiple inoculator (Steers), was one of our goals. In this study, we present the results obtained on two traditional medicinal plants: *Dolichandrone spathacea* (Bignoniaceae) used in Vietnam to treat flatulence, bronchitis and oral thrush, and *Paypayrola guianensis* (Violaceae) an endemic plant of Guyana used as a febrifuge. We have investigated the antimicrobial activity of five extracts of these two plants (*D. spathacea and P. guianensis*) in order to identify of bioactive compounds. The results reveal for the first time the presence of several new compounds as natural and antimicrobial substances in every plant (the lowest MIC=41.6 µg/ml). In the literature antimicrobial studies often concern a limited number of strains from one or two species. In our study, we chose to determine the MIC against a large spectrum of microorganisms, which may enable us to discover extracts with a large-scale activity. The Synergy (activity for mixtures of compounds) and *in vivo* studies may be interesting in the future for a valorization approach of these compounds.

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IN VITRO AND *IN VIVO* EVALUATION OF THE SAFETY OF SOME BENINESE PLANTS USED IN TRADITIONAL MEDICINE

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About 80% of population in developing countries use traditional remedies in their usual health care [1] and plants used in traditional medicine are an interesting alternative to expensive and hardly available modern medicines, mainly in rural areas. Moreover, they are a promising source of new drugs structurally innovative. Therefore it is important to investigate their biological properties and we focused on 5 beninese plants: *Byrsocarpus coccineus* Schumach. & Thonn (Connaraceae), *Carpolobia lutea* G.Don (Polygalaceae), *Holarrhena floribunda* T.Durand & Schinz (Apocynaceae), *Keetia leucantha* (K. Krause) Bridson and *Keetia venosa* (Oliv.) Bridson (Rubiaceae). In order to validate their safety, we evaluated toxicity of dichloromethane extracts and also aqueous decoctions being the major formulation traditionally used, *in vitro* on two cellular strains, WI38 and J774, and in *vivo* on female NMRI mice according to the highest tolerated dose model [2]. All lipophilic extracts and aqueous decoctions showed a low cytotoxicity on both strains (IC_{50} >35 and 100µg/ml respectively) and no toxic signs with a total cumulative dose of 800 mg/kg (i.p. and p.o. respectively). These results provide evidence of their safety in acute model and the different extracts will be investigated *in vitro/ in vivo* to validate their traditional uses, especially antiparasitic ones for most of them.

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[2] DNDi, Drugs for Neglected Disease initiative; Drug screening for Kinetoplastids diseases, April 2009

P69

ISOLATION AND IDENTIFICATION OF POTENTIAL ANTIMALARIAL COMPOUNDS FROM ENDEMIC PLANTS OF REUNION ISLAND

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Malaria is known as the most important parasitic disease around the world with 584 000 malaria deaths worldwide in 2013 [1]. Due to the problem of increased parasite resistance, natural products from endemic plants of Reunion Island, hot spot of promising biodiversity, could represent an important source of new antimalarial drugs. The aim of this thesis research focuses on the evaluation of potential antiplasmodial activity of medicinal plants from Reunion Island. A global screening of plants extracts from Reunion Island was performed on *Plasmodium falciparum* 3D7 chloroquine-sensitive strain revealed by colorimetric method as described in previous reports [2]. *Monimia rotundifolia* was then selected due to its promising *in vitro* activity against *Plasmodium*. Bioguided fractionation was realized using Prep HPLC techniques and led to the isolation of aporphine-type alkaloids from *Monimia rotundifolia* leaves dichloromethane extract. Further investigations are in process to confirm the antiplasmodial activities of these alkaloids and to determine their structures.

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P70

ANTIOXIDATIVE AND WOUND HEALING PROPERTIES OF *CRATAEVA ADANSONII* DC

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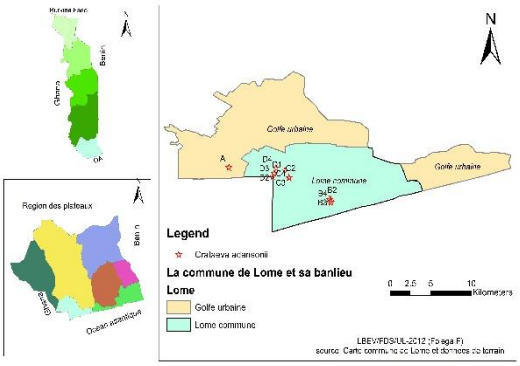
Leaves of *Crataeva adansonii* DC, a small bush found in Togo, are widely used in traditional medicine to cure infectious abscess. Traditional healers harvest only young leaves and sprouts early in the morning in order to prepare their drugs. To validate this ancestral practice, we performed a phytochemical screening of various *C. adansonii* leaves samples collected in different places, at different time and at different ages.

Using antioxidant activity as selection criteria, optimal extracts were obtained with sprout leaves, collected at 5:00 am in Djidjolé. Wound healing potential was then investigated for several extracts *in vitro* on keratinocytes stimulated by *Staphylococcus aureus* proteins. Anti-inflammatory activity was highlighted with flavonoids-rich extracts, especially against TNF□. Those results validate the traditional practices and the potential of *C. adansonii* as wound healing drug.



P71

RESEARCH OF ANTIMICROBIAL AND RESISTANCE MODIFYING AGENTS FROM BENINESE PLANTS USED IN TRADITIONAL MEDICINE

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In a world of increasing resistance to current antibiotics, search of novel therapeutic options is urgently needed. The aim of this work was to screen plant crude extracts for direct or indirect (inhibition of resistance) antimicrobial activity.

Four crude extracts from 12 plants traditionally used in Benin for the treatment of infections were obtained by successive extraction with hexane, dichloromethane, ethyl acetate, and methanol. All extracts were tested for their direct (MIC) and their indirect (in combination with common antibiotics) antimicrobial activities, using as test organisms *Staphylococcus aureus* MRSA ATCC33591 (resistant to β-lactams by production of β-lactamase and of an additional PBP [PBP2a] with low affinity for most β-lactams) and SA1 (resistant to quinolones by overexpression of the efflux pump NorA). Direct antimicrobial activity was tested by determination of Minimal Inhibitory Concentrations (MIC). Indirect activity was screened by looking for interaction between antibiotics and extracts using checkerboard titration and calculation of Fractional Inhibitory Concentration Index (FICI). Combined antibiotics were ampicillin (resistance due to β-lactamases and PBP2a), oxacillin (resistance due to PBP2a only), norfloxacin (substrate for efflux by NorA) and moxifloxacin (poor substrate of NorA). Synergy was defined by a FICI ≤ 0.5 and additivity, by a FICI ≤ 1.

The dichloromethane extract of *Vitellaria paradoxa* leaves, the methanol extracts of *Vitellaria paradoxa*, *Cola gigantea* stem barks, and *Topinanthus bangwensis* aerial parts showed activity on MRSA ATCC33591 (MIC 500 mg/L) and the methanol extract of *Cola gigantea* stem barks and the dichloromethane extract of *Spirospermum penduliflorum* aerial parts, on SA1 (MIC 250-500 mg/L).

The methanol extracts of *Vitellaria paradoxa* and *Cola gigantea* leaves and stem barks were additive or synergistic with oxacillin and ampicillin against MRSA ATCC33591 (FICI: 0.28 – 1), suggesting a possible inhibition of PBP2a. The methanol extract of *Topinanthus bangwensis* aerial parts improved only the activity of ampicillin (FICI 0.375-1), suggesting β-lactamase inhibition.

The methanol extract of *Holarrhena floribunda* aerial parts and the dichloromethane extract of *Spirospermum penduliflorum* aerial parts increased the activity of norfloxacin against strain SA1 (FICI 0.375-1) without modifying that of moxifloxacin, which suggests the presence of compounds inhibiting the efflux pump NorA.

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TWO NEW TRITERPENOID SAPONINS FROM THE ROOTS OF *WISTERIA FRUTESCENS*

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The knots of *Wisteria brachybotrys* SIEB. *et* ZUCC. (Leguminosae) have been used in Japanese folk medicine for the treatment of gastric cancer [1]. According to the previous works, the genus *Wisteria* of the Fabaceae family contained triterpenoid saponins [1,2,3,4] such as wisteriasapogenol A, B, C and soyasapogenol B. *Wisteria frutescens* (L.) is a woody perennial climbing vine which has never been investigated from a phytochemical and pharmacological point of view. The study of the roots of *W. frutescens* led to the isolation of two new triterpenoid saponins (**1**, **2**) and two known ones by using various solid/liquid chromatographic methods such as vacuum liquid chromatography (VLC) on normal and reverse phase RP-18 silica gel, medium pressure liquid chromatography (MPLC) and size exclusion chromatography on Sephadex LH-20. Their structures were established by a detailed 600MHz NMR analysis including 1D and 2D NMR (^{1}H, ^{13}C NMR, COSY, TOCSY, NOESY, HSQC, HMBC) experiments and mass spectrometry. Both compounds share the same oligosaccharidic sequence at C-3, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl] and differ by the aglycone, which was characterized as 22,28-diacetylolean-12-en-3β,16α,22α,28-tetrol (22,28-diacetylcamelliagenine A) in **1** and 22-acetylolean-12-en-3β,22β,24-triol (22-*O*-acetylsoyasapogenol B) in **2**.

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Evaluation of antiplasmodial and antileishmanial activities of herbal medicine *Pseudelephantopus Spiralis* (less.) Cronquist and isolated sesquiterpenoids

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Preparations of *Pseudelephantopus spiralis* (Less.) Cronquist (Asteraceae) are traditionally used for the treatment of various diseases including fever, malaria, and spleen or liver inflammations in Latin America [1], [2]. Aerial parts of *P. spiralis* were extracted with either ethanol or distilled water. Seven hirsutinolide-type sesquiterpenoids were isolated: 8-acetyl-13-ethoxypiptocarphol (**1**), diacetylpiptocarphol (**2**), piptocarphins A (**3**), F (**4**) and D (**5**), (1*S**,4*R**,8*S**,10*R**)-1,4-epoxy-13-ethoxy-1,8,10-trihydroxygermacra-5*E*,7(11)-dien-6,12-olide (**6**), and piptocarphol (**7**). Extracts and isolated compounds (**2, 3, 5-7**) were screened for their *in vitro* antiplasmodial activity against a chloroquine-resistant *Plasmodium falciparum* strain and antileishmanial activity against three stages of *Leishmania infantum.* Their cytotoxicities were also evaluated against healthy VERO cell lines and J774A.1 macrophages. Aqueous extracts showed a greater inhibitory effect than alcoholic extracts, with IC_{50} on *P. falciparum* of 3.0 µg/mL *versus* 21.1 µg/mL, and on *L. infantum* of 13.4 µg/mL *versus* >50 µg/mL. Both extracts were found to be cytotoxic on VERO cells (CC_{50}<3 µg/mL). Sesquiterpene lactones **2** and **3** showed the best activity against both parasites but failed in selectivity. The *in-vitro* antiplasmodial activity of aqueous extract of *P. spiralis* and of its main purified compounds **2** and **3** may underlie the use of this plant to treat malaria in South American folk medicine. Exploring the safety and antiplasmodial efficacy of this traditional remedy will require further toxicological and *in vivo* studies in the light of the cytotoxicity towards healthy cell lines displayed by the aqueous extract and compounds **2** and **3**.

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THE FORGOTTEN HISTORY OF TACAMAC RESIN.

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Fig 1: A view of the thematic showcase "Epices et plantes odorantes" (Author: X. Cachet)

One of the main activities of the active members of the "Société des amis du Musée François Tillequin" consists in the inventory of historical collections of the F. Tillequin Museum. This museum was originally founded in 1882, primarily as a "droguier". Each sample needs to be recorded and photographed in computerized database and in a second step, both scientific and historical data about samples are collected in order to introduce them during visits. In this context we focused our interest on several oleoresins designated under the vernacular names of "Tacamac", gathered and exhibited in several thematic showcases entitled "oléo-résines", "Epices et plantes odorantes" (Fig. 1), as well as in the "collection générale" and "collection géographique".

Once the inventory finalized, we were surprised to find no traces of these oleoresins in pharmacognosy books since the first half of the 20th century. Finally, on the basis of an extensive bibliographical research we are now able to trace the forgotten history of tacamac resin (or more precisely "resins", since about a dozen of substances have been described under the generic name of "Tacamac"). Brought back since the 16th century from West-Indies by Spanish colons, tacamac resin was originally used for medicinal purposes in Europe as substitute of other related oleoresins known under the common name of "élémi" and will eventually disappear in the early 20th while it was still used for the preparation of industrial varnish, ultimately replaced by synthetic polymers. This work emphasizes the problem of the transmission and broadcasting of scientific knowledge to descendants, particularly the choice of the storage media.

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« LE DROGUIER », A HERITAGE COLLECTION OF THE FACULTY OF PHARMACY OF LYON

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Faculty of Pharmacy has and hosts for years a heritage collection called "Le Droguier". For few years we work with ISPB students to value this collection: trying to trace its history, performing a computerized inventory of the collection to establish a database, making bibliographic research on the samples but also by making exhibitions and events for the general public to raise awareness of this collection.

Few documents on the history of this collection have been preserved; however, we were able to gather some preliminary information about its creation and enrichment. This collection was originally built up by a pharmacist from Lyon, Auguste-Antoine Dériard (1796—1873) and represented at that time several hundred samples from different geographic areas. It was later acquired by the former Secondary School of Medicine, and later located at the Faculty of Medicine and Pharmacy of Lyon. Then with the creation of the department of Botany, the curator of the collection Claude Abrial (1872-1945) has enriched it with gifts, exchanges with Institutes such as Royal Kew Museum, the Universal Exposition of Paris and Lyon, or private organizations such as Gignoux and Barbezat brothers or a Pharmacy in Macon… Recently, the collection of the Faculty of Pharmacy of Grenoble is deposited in Lyon. Thus the collection of Lyon includes almost one thousand samples, with a world-wide origin range, and belonging to minerals, animals, plants…

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ACCIDENTAL EXPOSURES OF CHILDREN TO ESSENTIAL OILS

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CONTEXT: Calls to the poison control center of Strasbourg concerning exposures to essential oils (EO) have tripled between 2008 and 2014.
OBJECTIVE: To determine the frequency and the severity of accidental exposures of children to EO.
METHOD: We reviewed all cases of EO poisoning of children younger than 12 years old listed by the poison control center of Strasbourg between January 2013 and December 2014. The aim was to collect all the information about these calls.
RESULTS: We collected 127 cases, including 69 boys and 58 girls (mean age = 1.6 years, between 7 days and 11 years). The main way of exposure was the oral way (72%). In 69% of the cases, children took the essential oil by themselves and put it in their mouth. Nonetheless, 25% of the cases were due to a therapeutic error involving the parents (mis-dosage, confusion of the flask…). Among the 127 phone calls, 24 (19%) children (12 boys / 12 girls; mean age = 1.7 years), exhibited symptoms. The most common symptoms found were oropharyngeal pain (27%), vomiting (17%) and eye pain (10%). Drowsiness, hypotonia and vomiting were also noticed on a child aged 30 months after ingestion of a small quantity of lavender EO which is known for its high tolerance. Although no severe intoxication was reported, 19 of the 127 exposed children (15%) were referred to an emergency department.
CONCLUSION: The increasing use of EO by households, or of aromatic product containing EO, leads to an increase of the risk of unintentional exposure to these products. This can be prejudicial, especially for frail people such as children. More than ever, the prevention of the toxicity risks of EO requires that the consumers are clearly informed, and maybe a better adaptation of the regulations.

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CINNAMIC ACID AMIDES AS POTENTIAL ARGINASE INHIBITORS

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Arginase is a metalloenzyme that converts L-arginine to L-ornithine and urea. Many studies performed these last years showed that excessive arginase activity in mammals is associated with cardiovascular and nervous system diseases, by reducing the supply of L-arginine needed by nitric oxide (NO) synthase to produce NO, a vasorelaxant factor, and by increasing production of L-ornithine, that leads to vascular structural problems and neural toxicity [1]. At the same time, interest for the development of arginase inhibitors has been growing. However, the most potent available arginase inhibitors are not fully satisfactory based on their pharmacokinetic and toxic properties [2]. New inhibitors are thus needed and such compounds may be inspired by natural substances [3]. Using a new *in vitro* test on purified bovine liver arginase, we recently showed that hydroxylated cinnamoyl moiety might constitute an interesting moiety to design new semi-synthetic arginase inhibitors [4]. We report here the synthesis of some cinnamic amide derivatives, their biological evaluation and molecular docking studies on a new homology model of bovine liver arginase. First results have shown that such compounds are able to decrease arginase activity. Among these candidates, compound **B7** possessed the best inhibitory potential (48% and 78% at 10 µM and 100 µM, respectively) and a docking score (-21 kJ/mol) predicting a binding affinity similar to N^{ω}-hydroxy-nor-L-arginine (nor-NOHA, reference arginase inhibitor, -22 kJ/mol). Our results suggest that cinnamic acid amides could be potential arginase inhibitors and valuable compounds for future possible pharmacomodulations that might increase the inhibitory arginase activity.

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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF AMIDOCHROMENES

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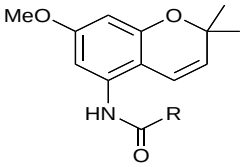
Numerous natural products having the 2,2-dimethylchromene (2,2-dimethylbenzopyrane) structural element show interesting biological activities such as, insecticidal (precocenes), cytotoxic and antitumor (acronycine), antibacterial (5-methyllupinifoliol, drummondine A), antiinflammatory (pongapinone A) activities or a selective protein kinase inhibition activity (robustic acid) *. Chromenes can be therefore considered as privileged pharmacophore.

Fifteen aliphatic and aromatic amides were synthesized from the 5-amino-7-methoxy-2,2-dimethylchromene synthon. The antibacterial, antifungal, antileishmanial and cytotoxic activities of all amidochromenes were evaluated. Neither antibacterial nor antifungal activities were observed. On the other hand 5 compounds had shown a significant antileishmanial activity with an excellent selectivity index.



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NATURE-DERIVED SYNTHETIC ANTIMALARIAL FLAVONES SPECIFICALLY TARGET EARLY *PLASMODIUM* BLOOD STAGES

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Malaria is the deadliest parasitic disease with almost 600.000 deaths every year [1]. The parasite (*Plasmodium sp.*) is transmitted by a female *Anopheles* mosquito during a blood meal. It undertakes then a complex life cycle in humans: first in the liver, then in the red blood cells. This erythrocytic cycle is responsible for the symptoms like fever, sweat and shivering. Death occurs in complicated cases due to severe anemia, kidney failure, or coma. Only a few drugs cure malaria, and on top of that the parasite is resistant to most of them. The first line treatment—artemisinin—is not an exception: in 2008, Noedl and colleagues reported the emergence of partial resistance to artemisinin in South-East Asia [2]. By the past, progress in decreasing the mortality has already been reversed because of resistance spreading from Asia to Africa. Artemisinin resistance seems to follow the same march: resistance has already spread to Myanmar near the Indian border [3]. To conduct its malaria eradication plan by 2050, WHO needs new fast acting drugs with original mechanisms of action. After the isolation of an active biflavonoid from *Campnosperma panamense* (Anacardiaceae, IC_{50} = 480 nM *in vitro* on *P. falciparum* K1 multi-resistant strain) [4], we developed novel simplified synthetic analogs (MR series) with improved pharmacological and pharmacokinetic profiles. Two of them (MR70 and MR87) exhibit a partial *in vivo* antimalarial activity. They reduce parasitaemia by 35% to 70% respectively on day 4 on a murine model (*P. berghei* ANKA, dosing regimen of 100 mg/kg for 4 days). But these compounds showed no significant improvement in terms of survival. MR70 is parasiticidal on early blood stages of *P. falciparum* in less than 30 minutes. Interestingly, these stages are specifically the ones that are resistant to artemisinin [5]. Further investigation is needed to optimize *in vivo* activity and to understand the underlying mechanism(s) of action of these compounds.

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DESIGN, SYNTHESIS AND EVALUATION OF THE ANTI-ANTI-AMYLOIDOGENIC ACTIVITY OF NOVEL HYDROXYTYROSOL DERIVATIVES

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Polyphenols are a wide family of compounds found in plant-based foods such as cranberries, grapes, olives, walnuts and have many diverse biological activities. Hydroxytyrosol, a simple phenol found in the olive leaves and pulp, shows a broad spectrum of biological properties due to its strong antioxidant activity. Hydroxytyrosol can slow down this process, known as oxidative stress, due to its high capacity for free radical scavenging activity. In addition, oxidative stress and generation of reactive oxygen species have implications in the aggregation of Beta-amyloid peptide (Ab) the major proteinaceous constituent of senile plaques that characterize Alzheimer's disease (AD). The aggregation of Ab is related with neurodegeneration, loss of cognitive ability and premature death and antioxidants such as hydroxytyrosol, may offer a protective or therapeutic alternative against amyloidosis.

Within this context, it was considered interesting to synthesize a series of novel hydroxytyrosol derivatives and to study their antioxidant activity and their ability to interact with Ab. Consequently, we describe here a convenient and facile synthesis of hydroxytyrosol esters and several analogues of hydroxytyroasol which could form noncovalent complexes with Ab peptide.

More precisely, the new compounds possess the hydroxytyrosol scaffold bearing modifications on the a-carbon of the catechol side chain. The activity of the new compounds was evaluated in vitro, by means of their interaction with Ab(1-28) with electrospray ionization mass spectrometry and circular dichroism.

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BOTANIC WITH SCENARI® - A NEW PRACTICE WITH DIGITAL SUPPORT

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French botanical digital literature on Internet exists but usually is not concerned with systematics. Even more, the aim is neither toxic, nor medicinal plants that our health students need. The present project is to (i) provide a written support to systematic botanical slideshows already present on "Université Numérique des Sciences Pharmaceutiques Francophone" (UNSPF), (ii) complete writing missing courses (like Monocots families), (iii) document iconography, (iv) create an interactive exercises bank, (v) broadcast the whole through the editorial software SchenariChain (Opale model), (vi) manage a series of check and validating step by the three co-authors as well as by members of "Association des Enseignants-Chercheurs en Sciences Végétales et Fongiques des Facultés de Pharmacie Francophones" (STOLON). So, scientifically proofed resources are matching the whole systematic botanical course of Pharmacy studies. 105 flowering plant families from which 49 are more elaborate and 56 smaller families only described in a single synthetic sheet with fundamental family and morphological features and a few pictures. These on line courses are written with APG III classification system. A lexicon and self-assessment exercises are also provided. Newly taking on colleagues may easily use this data supply and take it up to perform his own dynamic, interactive, inverse or hybrid pedagogy.

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PHYTOCHEMICAL STUDY OF *PORAQUEIBA SERICEA*

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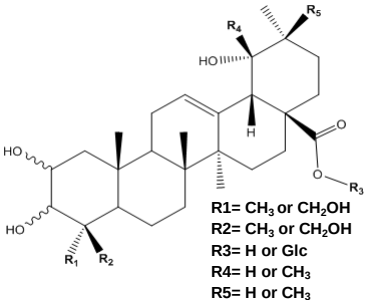

Our study concerns the exploration of plants of the Amazon rainforest (Peru) in search of new bioactive molecules. One of the studied plants is *Poraqueiba sericea* belonging to the family of Icacinaceae. From the methanolic extract of the stems of *P. sericea*, eleven products were purified and identified. Purification was carried out using chromatographic methods (flash chromatography, HPLC) and the structure elucidation was accomplished by consideration of NMR spectra 1D and 2D (1H, 13C, DEPT, COSY, HSQC, HMBC) and mass spectrometry. The purified compounds are 3 saponins [the niga ichigoside-F1, the trachelosperoside B1, and 4-epi-niga ichigoside-F1], 6 triterpenes [the trachelosperogenin B, the 19-α-hydroxyasiatic acid, the hyptatic acid, the myrianthic acid, the arjunolic acid, and the trachelosperogenin E], and 2 secoiridoids [the secoxyloganin and the secologanoside]. These compounds were evaluated for their cytotoxic activity (on fibroblasts) and antileishmanial activity (*Leishmani infantum* promastigotes) but no activity was revealed. Other biological activities are being evaluated.

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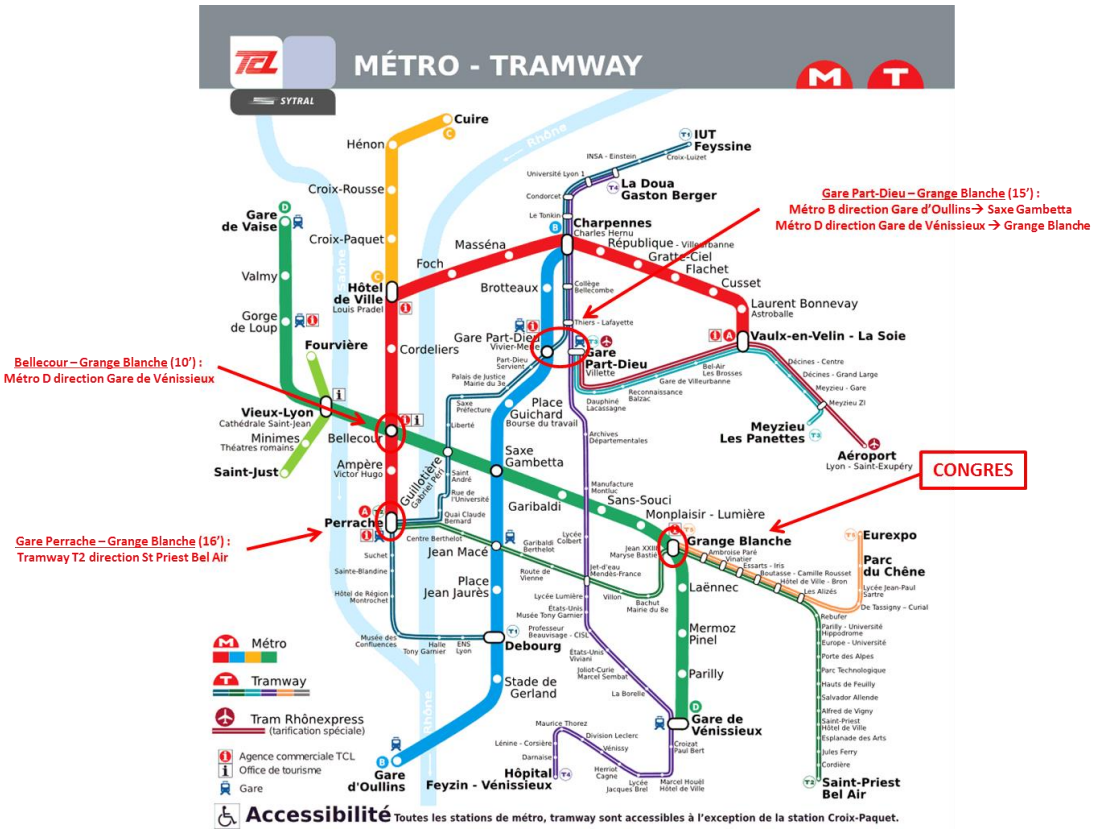

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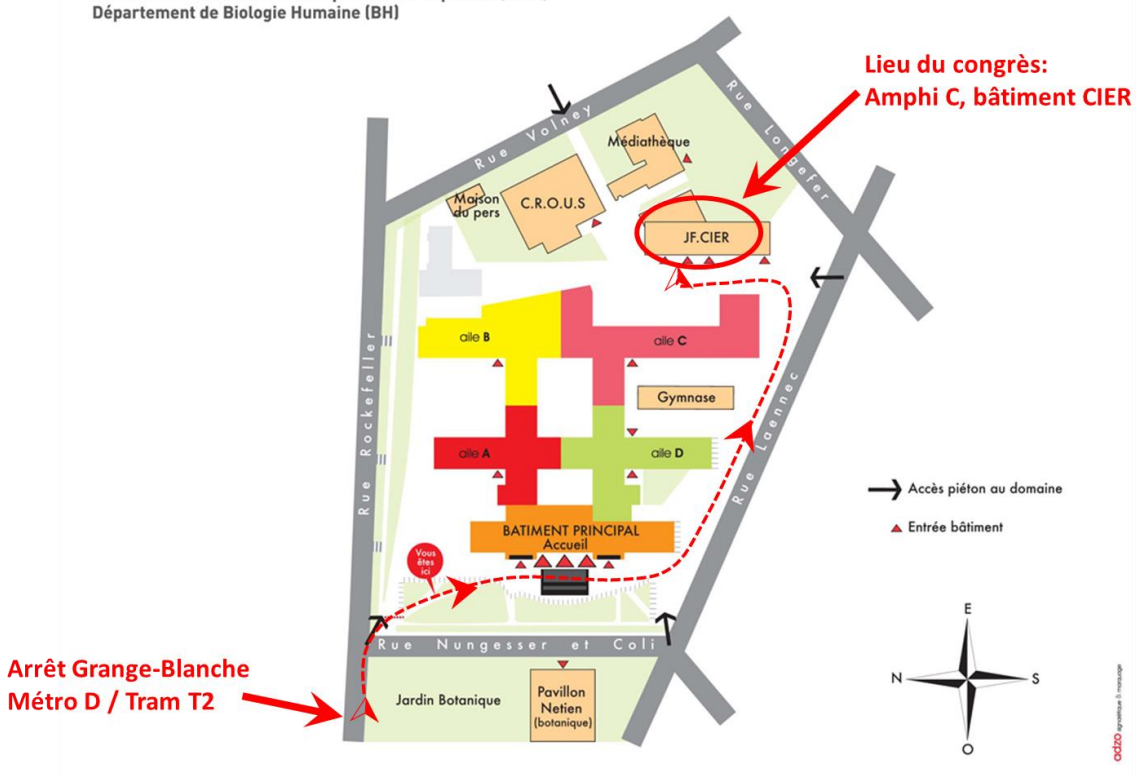


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