"Is Cannabis finally entering a postT-THC era? "

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Cannabis sativa L. is prolific producer of structurally unique secondary metabolites belonging to three major biogenetic classes, namely meroterpenoids (cannabinoids), stilbenoids (bibenzyls) and polyketide alkaloids. Despite this diversity of constituents, interest for the phytochemistry of *C. sativa* has almost exclusively focused on cannabinoids, and in particular on Δ^9–THC, a narcotic member of a class of over 200 different compounds with a point-like distribution in Nature outside cannabis. Δ^9–THC was instrumental in the discovery of the endocannabinoid system, but its clinical development both as a monomolecular compound and a synthetic analogue met with limited success. Conversely, cannabis preparations have been anecdotally used for a variety of ailments, providing a basis for the successful development of a 1:1 combination of two cannabis standardized extracts (Sativex) for the symptomatic management of multiple sclerosis. Another important development was the discovery of the clinical efficacy of the anti-narcotic cannabinoid cannabidiol (CBD) for some forms of epilepsy characterized by dysfunction of the neuronal voltage-gated sodium channel Na(V)1 like the Dravet syndrome. While the mechanism of the anti-epileptic activity of CBD is unknown, this compound and Δ^9–THC might well represent the tip of an iceberg of cannabis constituents whose biological profile is potentially interesting and worth investigation. This view will be discussed using cannabinoid quinols, cannabis bibenzyls and caryophyllene as examples. Cannabinoid quinols are unstable oxidative derivatives of resorcinyl-type cannabinoids, and various methods of synthesis and stabilization will be discussed. Bibenzyls like canniprene inhibit the production of pro-inflammatory eicosanoids, can be vaporized and presumably occur in cannabis and marijuana smoke, while caryophyllene is the only hydrocarbon known to bind in a sub-micromolar fashion a macromolecular target.

“Natural Treasures from the Malayan Flora; a collaborative exploration between ICSN (France) and UM (Malaysia)”

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The Malayan flora, one of the oldest and diversed flora in the world, is an abundant resource of rare plants containing a massive array of phytochemicals possessing various types of bioactivities. This phenomenon has led to a scientific collaboration between ICSN/CNRS of France and University Malaya of Malaysia since 1980’s. Both parties are interested to develop the potential of plants from this rich flora for the enhancement of research in the field of medicinal science notably drug discovery. The collaboration started by random collecting of plants and chemical studies prioritized on plants containing alkaloids. Today, plants studied were selected by bioactivities such as cytotoxity, acetylcholinesterase, Bcl-xL and Mcl-1 inhibitors. The fruitful collaboration between ICSN and UM has produced publications and postgraduates from both countries. In the move to strengthen and increase the visibility of this ‘scientific friendship’ the formation of IFM- NatProlab (Associated International laboratory under CNRS) was initiated and an MOA was officially signed in 2015. This presentation will discuss briefly the past achievements and recent findings which includes plants from Lauraceae (isoquinoline alkaloids), Meliaceae (limonoids) and Rubiaceae (indole alkaloids and polyketides).

“The application of bioinformatic methodologies for targeted diversification of recombinant natural product biosythesis pathways. -New avenues for the a la carte synthesis of non-natural bioactives”

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The industrial exploitation of bioactive natural products is commonly hampered by lack of sufficient yields from the natural source. More recently, the advent of genomics and metabolic engineering allowed reconstruction of heterologous biosynthetic pathways in genetically tractable microbial cells such as *E. coli* and *S.cerevisiae*. These biosystems approaches now provide an alternative route for the high yield generation of bioactive natural products, such as the sesquiterpenoid antimalarial drug artemisinin. While systems biology methodologies have significantly contributed to improve natural product yields in recombinant cells, it was the advent of structural biology in conjunction with sophisticated bioinformatics tools that now enable the diversification of natural biosynthetic pathways simply by altering the catalytic specificity of key enzyme systems. In this talk I will address our efforts to utilize molecular mechanic simulations to predict amino acids residues that modulate catalysis in diterpene synthases, which convert the aliphatic diterpene precursor geranylgeranyldiphosphate (GGPP) in specific olefinic macocycles. The resulting macrocyclic compounds form the core of many bioactive compounds, such as the anti-cancer agent taxol or the fragrance compound sclareol. Our *in-silico* simulation centered enzyme engineering platform opened new avenues for the production of designed bioactive products. Moreover, the challenges associated with functional tailoring of diterpene synthase derived macrocyclic compounds will be discussed.

"Discovery of lead compounds against *Madurella mycetomatis* – one of the most neglected tropical diseases"

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Mycetoma is a chronic, progressively destructive disease caused by bacteria (actinomycetoma) or fungi (eumycetoma) such as *Madurella mycetomatis.* The latter usually involve the skin, and then spreading to the subcutaneous tissue and bone but can also spread to more distant sites through the blood and lymph which eventually leads to destruction, deformity and loss of function [1]. *M. mycetomatis* is further characterized by tumefaction, draining sinuses, and the presence of grains. The disease is endemic in many tropical and subtropical countries and is currently treated with extensive surgical intervention involving local excision of distinct lesions to amputation of the infected limb [2]. Due to the burden of mycetoma and the multiple gaps in our present knowledge about this disease, the 69th World Health Assembly (WHA) adopted an important resolution in May 2016 adding mycetoma to the list of NTDs and calling on al actors to join forces to control the public-health impact if this disfiguring and life threatining disease.

Prolonged treatment of *M. mycetomatis* with any of the currently available antifungal agents proved to be of disappointing clinical efficacy and associated with severe side effects which frequently results in surgical interventions leading to amputation [2]. The high degree of the clinical failures accompanied these approaches coupled with the lack of studies to discover novel antimycetomal lead compounds warranted us to collect a dozen of clinical strains *M. mycetomati from Sudanese patients followed by their* culturing and profiling of their susceptibility against seven known antifungal agents representing five azoles (ketoconazole, itraconazole, voriconazole, posaconazole, fluconazole) besides the allylamine, terbinafine and the lipopeptide, caspofungin.

The validity of our previously established *in vitro* screening method against *M. mycetomatis* [3] was further consolidated by subjecting a series of plant secondary metabolites of diverse chemical structures belonging to the terpenoids, phenolic compounds and alkaloids. Among them the protoberberine alkaloid, berberine exhibited remarkable MIC ranging between 0.046 to 0.743 µM, whiles the β-carboline alkaloid, harmine showed activity ranging between 0.074 to 0.589 µM against the various clinical strains investigated in the present study using ketoconazole and itraconozole as standard drugs.

Keywords: Mycetoma, *Madurella mycetomatis,* susceptibility*, in vitro screening, secondary metabolites, lead compounds.*

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“Biodiversity as Source for Potential Therapeutic Drugs”

Dr Marc LITAUDON

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Nowadays, the search for new leads has become a formidable challenge if we consider the number of compounds discovered each year, increasing exponentially, while the number of new chemical entities (NCE as defined by the FDA, *i.e.* a drug that contain no active moiety that has been approved) decreases years after years. Nevertheless, since the early 90s, the emergence of high throughput screening (HTS) and the use of powerful robotized technologies have changed dramatically the "laboratory approach" of the drug discovery process. Pharmaceutical discovery has become a "numbers game" requiring the biological evaluation of thousands of synthetic or natural compounds.

However, as was wisely said by Lynn Caporale, "*When you have no idea where to begin in a drug discovery program, Nature is a good starting point*", biodiversity should be considered as an inexhaustible source of organic molecules, inspiring the natural product chemist in its quest for new drugs. L. Caporale focused attention on evidence that natural selection can act on mechanism that generates variation, resulting in a wide chemical diversity. Without assuming that chemical diversity is necessary synonymous with biological diversity, the fact remains that nature should be considered as an amazing smart factory of bioactive secondary metabolites, which have been designed from millions of years of "environmental high throughput screening". The biological activities of natural products have indeed been shaped to overcome environmental stress and provide defence against natural enemies.

In this context, exploring plant diversity, and more specifically tropical rainforests, which are believed to contain more than half of plant species on earth, has become a high priority, especially when one know that logging and cultivation practises have significantly contributed to huge deforestation. Thus, with the objective to discover new bioactive secondary metabolites, the "Institut de Chimie des Substances Naturelles" (ICSN-CNRS) has launched, in the early 2000s an extensive research program based on the systematic prospection of various dense forest areas for taxonomic-oriented collection of higher plants. A unique collection of 6500 tropical plant species (over 14 000 parts of plants), which represents approximately 2 % of the world's biodiversity has been gathered over the last 20 years by our group through a network of international partnerships.

To have at disposal more easily libraries of extracts representing this biodiversity, a methodology has been set up, referring to models originally defined for chemical libraries. The "ICSN-Extracts library" has been created to be submitted to biological screening on various targets. The positive results obtained through these screenings can orientate research projects to discover new active molecules that can be potential drug leads as enzyme or protein-protein interaction inhibitors, and subsequently used in human therapy.

The main elements of our strategy will be presented. We will show that the success does not only depend on the use of sophisticated technologies, but mainly relies on the originality of plant species studied and on the discovery and the use of new and relevant targets. Several examples selected from the biological screening on various targets of our extracts library will be shown.

“Structural diversity in Bioactive Metabolites from the Yucatecan Flora”

Dr. Luis-Manuel PEÑA-RODRIGUEZ

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The historical importance of natural products as new and better pharmaceuticals, or models for them, is evident when we mention products such as taxol, vincristine, or artemisinin. Plants, and particularly medicinal plants, still represent the most important source of bioactive natural products today. Mexico is considered one of the richest countries in terms of biodiversity and in terms of cultural diversity; more than fifty ethnic groups recognize and use more than 3000 plants in their practice of traditional medicine. However, it is believed that less than 5% of these species have been studied in terms of their production of biologically active secondary metabolites. The general objective of the research carried out in our laboratory is to promote the conservation of the biodiversity of the Yucatan Peninsula, by emphasizing its importance as a natural source of novel bioactive metabolites. This presentation will mention the different types of biological activities (*e.g*. antiprotozoan, antifungal, antituberculosis, antioxidant, analgesic, anti-inflammatory, etc.) that are currently used by our group to guide the purification of bioactive metabolites produced by native medicinal plants of the Yucatan Peninsula, and the structures of a number of recently identified bioactive metabolites will be presented.

“Inhibition of 5-lipoxygenase – new aspects of an old target”

Prof. Daniela SCHUSTER

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Five-lipoxygenase (5-LO) is an established anti-inflammatory target and inhibited by a wide range of natural products. For many years, the structural basis for 5-LO inhibitor binding remained unclear. The publication of the human 5-LO X-ray crystal structure in 2011 did not explain substrate or inhibitor binding, because the active site was closed. A further, mutated 5-LO structure published in 2012 was reported to catalyze a different reaction, making it unsuitable for mechanistic investigations. Therefore, we employed in silico molecular dynamics simulations and docking to post-process the first 5-LO X-ray structure, open the substrate binding site and predict the binding modes of the substrate and inhibitors. Surprisingly, some natural products such as garcinoic acid from *Garcinia kola* are simply too large to bind into the substrate binding site. This was confirmed by *in vitro* data showing that garcinoic acid is not competitively inhibiting 5-LO. Using pocket finder algorithms, we identified alternative binding sites for garcinoic acid and related compounds. These findings encourage the experimental evaluation of series of vitamin E analogs for 5-LO inhibition.

"Olive bioactive compounds: Chemistry and Biology"

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The olive tree, closely connected to the Mediterranean region has provided a wealth of goods. Research on the olive has started early but it has proven inexhaustible revealing mainly a vast array of nutritional and health properties. Apart from olive oil and table olives, the by-products coming from olive processing industry have been proven attractive materials for research. The aim of this communication is to present a holistic research strategy towards the multifaceted exploitation of the olive tree including activities such as extraction, fractionation, isolation, analysis of olive tree products as well as investigation of processes related to olive industry and valorization of by-products.

The main products of the olive tree, olive oil and table olives as well as by-products such as leaves, paste, mill wastes and table olive wastewater have been used as sources for the recovery of valuable secondary metabolites. This has been performed with conventional techniques and also by adsorptive resin technology [1, 2]. In addition standardized enriched fractions have been prepared with various techniques, such as MPLC, HPLC, and CCC. Isolation of promising lead compounds with emphasis to olive polyphenols [3] oleuropein (leaves), hydroxytyrosol & tyrosol (olive oil, by-products), oleacein & oleocanthal (olive oil) and lactones (by-products), has been achieved. Additionally advanced analytical techniques and methodologies (UPLC/HPLC-DAD, HPLC-DAD-HR/MS^n, and HPTLC) have been developed and applied for the qualitative and quantitative determination of secondary metabolites in all the above mentioned materials [4]. The lab scale processes have been also adapted to pilot scale systems. The biological profile and the therapeutic potential of olive extracts and compounds is explored and supported by several *in vitro* and *in vivo* studies while their possible application as nutraceuticals, dietary supplements and cosmetics is also investigated.

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"SFC – A promising analytical tool for natural products"

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When supercritical fluid chromatography was first developed more than 50 years ago, it was considered as a revolutionary separation technique. However, it never emerged from the shade of GC and HPLC and its reputation slowly ebbed over the years. Within the last decade, the revived interest of manufacturers and scientists for this complementary technique which combines some of the best features of LC as well as GC lead to tremendous improvement of the SFC instrumentation resulting in a stable analytical platform with many advantages over the existing chromatographic methods. SFC has been approved as versatile, cost-efficient, green, user friendly high resolution technique with multi-detector compatibility [1,2]. In the past SFC has mainly been used for the analytical or preparative separation of chiral compounds [3], but it is getting more and more attention in the area of achiral separation nowadays.
The growing selection of columns - SFC can be operated both as normal phase and as reverse phase chromatography -, the possibility to change the properties of the mobile phase by modifiers, as well as the use of different detectors like DAD, ELSD or MS allows the application for an extremely broad spectrum of substance classes. In the field of natural product analysis, numerous publications demonstrate the increasing popularity of SFC. Successful SFC separations have been reported for apolar substances such as fats and fatty acids [4,5], without derivatization required for HPLC and GC analysis, fat-soluble vitamins, essential oils, carotenoids, terpenoid substances, polyprenols and cyclic hexadepsipeptides but polar classes of substances such as xanthones, alkaloids or flavonoids are separable too [5, 6].

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Lichen symbioses: A promising source of active compounds

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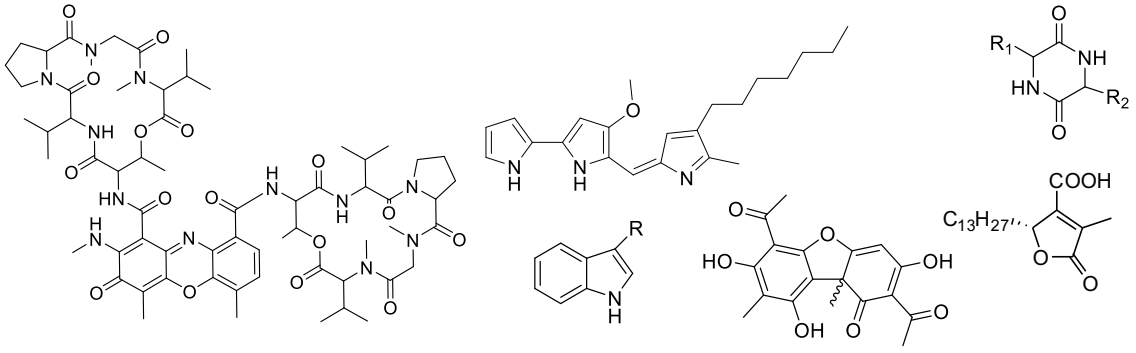
Lichens represent mostly commonly a mutualistic association between a mycobiont (fungus) and a photobiont (chlorophyte or/and cyanobacterium). This association is responsible for the production of original and active secondary metabolites [1]. The long-living lichen symbioses also provide an ecological niche for a high diversity of additional bacteria that were observed by culture-dependent [2] and independent approaches [3-5].These studies demonstrated that the bacterial communities associated with lichens are dominated by Alphaproteobacteria but also highlighted the presence of Actinobacteria [6].
Lichens have evolved effective means to control their inhabitants by producing metabolites acting as a communication and controlling system. Antibiotic effect of a number of lichen metabolites against gram-positive bacteria has been already reported [7]. The associated bacteria could themselves be an untapped source of highly effective new secondary metabolites (e;g. uncialamycin, cladoniamides, angucycline, aminocoumarines…) [8-11].
Lichens are an excellent model to study symbiosis and to evaluate the impact of their secondary metabolites in these symbiotic relationships via co-culturing experiments. We will see an overview of the results of our laboratory in the discovery of interesting bioactive compounds (cytotoxic, antibacterial…such as dibenzofuranes, diketopiperazines, phenoxazine, pyrrole, butyrolactones derivatives…) from lichens and from lichen-associated bacteria. The effects of lichen metabolites on the growth and the chemical production of associated bacteria will be also discussed.



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"Targeting protozoal diseases: Convergent approaches for pharmacological tools and lead finding from natural sources"

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Parasitic diseases are major cause of mortality and morbidity in the world [1]. The need for new drugs against tropical parasites such as *Plasmodium falciparum* and *Leishmania sp* is persistent since problems with resistance and toxicity are jeopardizing the currently available medicines. Morevover few validated molecular targets have been identified in this field limiting the discovery of new antiprotozoals with original mechanism of action. Natural products display a high chemodiversity and play a highly significant role in the drug discovery process against infectious diseases [2]. Previously, traditional remedies such as *Cinchona* bark or *Artemisia* aerial parts led to the discovery of the most potent antimalarials [3], bearing out that Nature is still an incredible source of original and bioactive compounds.

We combine different and convergent approaches allowing digging the terrestrial and marine plant biodiversity to rationalize the discovery of new pharmacological tools against two protozoan parasites (*Plasmodium* and *Leishmania*). Our goal is to untangle their mechanism of action to allow a better understanding of the biology of these parasites and to open new putative therapeutic opportunities.

Our strategy associates rational selection of novel plant species using ethnopharmacological, chemotaxonomic and phylogenetic criteria, bio-guided isolation, structural identification, medicinal chemistry and chemical biology. The strategy relies both on in-house skills and a network of international partnerships as well. The presentation will deal briefly with past achievements that served as starting points for recent findings. It will particularly focus on the discovery of a new promising antimalarial series inspired by the structural features of a biflavonoid isolated from a South American Anacardiaceae [4]. The lead compound is currently the fastest antimalarial ever described. The promising profile of the series stems from its rapid onset of action but also from its activity against all erythrocytic stages of the parasite including those resistant to the first line treatment artemisinin, its long half-life, and the absence of cross-resistance with current drugs. The *in vivo* activity is currently improved but our findings already make flavone derivatives a promising new class of antimalarials which could replace artemisinin which efficacy is threatened by increasing resistance.

Keywords: flavone, resistant malaria, *Plasmodium falciparum*, artemisinin resistance

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"Plant extracts and plant metabolites as potentiating agents for the development of new antifungal multi-component drugs"

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Systemic fungal infections are a major cause of morbidity and mortality in immunocompromised patients. Treatment is notoriously difficult because of the limited number of antifungal drugs in clinical use, whose efficacy is compromised by host toxicity, narrow spectrum of action or the emergence of fungal resistance. In addition, the number of new chemical antifungal entities introduced in the pharmaceutical market in the last decade has been very scarce. These drawbacks have prompted the search for better therapeutic strategies, such as using the antifungal agents in combination with other antifungal drugs in order to achieve synergism. This strategy is based on the believe that synergistic interactions produce an increase in effectiveness, a reduction of doses while maintaining the effect, a reduction in potential toxicity and a reduction or slowing of the resistance.

In recent years, there have been an increased interest in the study of new antifungal combinations formed by natural extracts (or metabolites) and antifungal drugs or by two or more plant extracts. Many examples in the literature show the potentiation of the antifungal effect of the antifungal drugs or an extract by natural products, that are appealing alternatives for the pharmaceutical development of a new antifungal agent. Although it appears simple to prepare antifungal mixtures that show enhanced activity in comparison with each partner alone, the expectations are often not followed by the appropriate methodology for measuring interactions which sometimes give controversial results. The results are highly dependent on the design used to test the mixtures, on the software used to interpret the results and also on the drug concentration used for the synergy assays.

In this lecture, synergism studies on antifungal mixtures involving plant extracts as well as metabolites, performed in our laboratory by using different designs and analyzed with different softwares, will be presented and discussed. In the different examples, either the Fractional inhibition Concentration Index (FICI), the Combination Index (CI) or the statistically-based Loewe Index (Lφ) were used for classifying the mixtures as synergistic, additive or antagonistic and to determine the Dose Reduction Index (DRI) as a measure of the interaction [1-3]. Among the obtained results, the statistical-based MixLow method allowed to find the composition of the most synergistic mixtures of *Zuccagnia punctata Cav with Larrea nitida Cav* against *Candida albicans* and *C. glabrata*; [1] the chequerboard design followed by the isobologram graphic and corroborated by time-kill curves led us to determine the type of interaction between *Phytolacca tetramera* Hauman with azoles against *Candida* spp.; in turn, the tridimensional design and the *CombiTool* software allowed us to find synergistic mixtures between *Baccharis* spp. and terbinafine against *Trichophyton rubrum* [2]. A Review on the patents disclosed on antifungal synergism evidenced the high interest of the scientific community in developing new antifungal combinations including natural products for overpassing the failures in the treatment of fungal infections [4].

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