Franco-Brazilian Network on Natural Products

FB2NP

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ABSTRACTS

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1. THE FRANCO-BRAZILIAN NETWORK ON NATURAL PRODUCTS (FB2NP): A NEW NETWORK PROMOTING COOPERATION IN NATURAL PRODUCTS RESEARCH, IN LINE WITH THE STRONG TRADITION OF FRANCO-BRAZILIAN ACADEMIC PARTNERSHIP.

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The Franco-Brazilian Network on Natural Products (FB2NP) is a new international network of French and Brazilian academic researchers working in the field of natural products research, drug discovery and development. Created in 2019, it federates researchers interested in the isolation, purification, structure elucidation, synthesis and bioactivity of chemical compounds found in nature. The FB2NP has rapidly expanded and currently includes 30 academic teams with various and complementary expertises including organic, analytical and medicinal chemistry, pharmacognosy and phytochemistry, pharmacology, physiology, microbiology, phycology, toxicology, process engineering, molecular modelization and omics. The structuration of this network stands in the long tradition of the Franco-Brazilian scientific partnership and aims to stimulate scientific cooperation, organization of Franco-Brazilian scientific events (congresses, workshops, summer courses, visioconferences), exchanges of researchers and students, as well as dissemination of jobs and training offers. It should also favour the emergence of inter- and transdisciplinary approaches and stimulate exchanges of ideas and experiences. We invite all colleagues interested in joining the network to contact the FB2NP coordinators.

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Keywords

Natural products research; medicinal chemistry ; pharmacognosy ; phytochemistry, pharmacology ; physiology ; microbiology ; phycology ; toxicology ; process engineering ; molecular modelization ; omics, Franco-Brazilian cooperation.

2. EXPLORATION OF THE CHEMICAL AND GENETIC DIVERSITY OF WILD HOPS (*HUMULUS LUPULUS*) IN THE NORTH OF FRANCE WITH AN AIM OF LOCAL BREWING USE

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French Flanders is a historical region for beer production and for hop cultivation. Female cones are used in brewery for their bitterness and their flavours, as well as for their antimicrobial properties. Because of several factors, this local production is declining since the middle of the XX^{e} century. However, in recent years, a new dynamism is observed partly explained by a renewed interest in aromatic and craft beers. As a result, microbreweries are widely developing, using local and sustainable ingredients. To answer to this consumption shift, hops production is adapting. Especially hop growers are looking for hops suitable to local terroir, with good yields but above all, good chemical qualities for brewery. In addition, due to their chemical composition, hops have interesting biological properties, including oestrogenic, sedative, antimicrobial, antiproliferative and anti-inflammatory ones. In particular, hops produce prenylated chalcones including xanthohumol and desmethylxanthohumol as well as acylpholoroglucinol derivatives with alpha and beta acids [1]. The bitterness searched by brewers is due to alpha acids, whereas flavours come from volatile compounds.

In this context of varietal research, we are exploring the chemical and genetic diversity among fifty wild hops collected in September 2019 on several natural sites of the North of France. These fifty wild hops are going to be mapped and compared with ten commercial varieties and three old varieties. Genetic analyses are focused on the study of microsatellites regions [2-3]; the phytochemical characterization of hops is based on the quantification of their major secondary metabolites: prenylated phenolic compounds (xanthohumol and bitter acids) by UHPLC-UV and volatile compounds by GC-MS. Non targeted metabolomics analyses are also carry out to identify wild hops with an original chemotype which may interested in brewers. These analyses will be filled by morphological characterization of studied hops [4] and their organoleptic qualities. Multivariate data will be correlated by principal component analysis. This talk will especially present the first outcomes of the chemical characterisation of collected hops. Preliminary results show interesting levels of alpha acids in some wild hop comparable to those of commercial varieties.

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3. PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF THE SPECIES *VARRONIA DARDANI* (CORDIACEAE)

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The species *Varronia dardani* is endemic to the caatinga of Brazil, being distributed in states of the Northeast of the country as: Alagoas, Sergipe, Bahia, Ceará, Paraíba, Pernambuco, Rio Grande do Norte. Previous chemical studies by *V. dardani* reported the presence of some classes of secondary metabolites, mainly terpenoids and flavonoids.

For this preliminary study, the use of the species was registered in the database of the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen-Brazil) under protocol A0E7358. The aerial parts of *Varronia dardani* was collected in Serra Branca - Paraíba, on January 25, 2016 and the voucher is catalogued in the Herbarium Prof. Lauro Pires Xavier (JPB) of the Center for Exact and Nature Sciences - UFPB, with the code JPB 29509 being identified by the botanist Prof. Dr. José Iranildo Miranda de Melo of the Department of Biological Sciences - UEPB.

This initial phytochemical study of the aerial parts of *V. dardani*, led to the isolation of some compounds that were analyzed by ^{1}H and ^{13}C NMR techniques, and identified as isosakuranetin, naringenin, astragalin, rosmarinic acid, besides other compounds that are still in the identification phase.

This study also showed that the crude ethanolic extract presents a non-selective spasmolytic activity in relation to the different organs and agonists tested, presenting a greater relaxing power in the uterus of rats. Besides, in the tonic organs (aorta and trachea of rats) its relaxing action mechanism seems not to involve the participation of the relaxing factors derived from the endothelium/epithelium, already in the phasic organs, the extract may be exerting its spasmolytic effect in a common step to the contractile agents used, for example, by blocking the influx of Ca^{2+} through the Ca_V.

Therefore, the present work contributes to the chemical and biological knowledge of the genus *Varronia*, through the study of the species *V. dardani*. It is worth noting, that more studies are being conducted.

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4. INTEGRATION OF BIOGUIDED FRACTIONATION AND MOLECULAR NETWORKING TO IDENTIFY ANTI-MYCOTOXINS FROM GRAPEVINE

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According to Food and Agricultural Organization, 25% of the world's crops are affected by mycotoxins each year[1]. In Europe, cereals are the most impacted crops, mainly by *Fusarium graminearum* which produces type B trichothecenes (TCTB)[2]. Deoxynivalenol, the predominant TCTB and most common contaminant of cereals, is responsible for digestive disorders such as vomiting due to its strong emetic effects after consumption, because it is transported into the brain, where it runs dopaminergic receptors[3]. In order to substitute synthetic fungicides and durably control toxigenic fungal infections, we aimed to exploit the biological activity of phenolic compounds from grapevine by-products. In this study, an extract of grapevine canes was investigated for its inhibitory potential towards *F. graminearum* mycotoxin production. A bioassay-guided fractionation of this extract using centrifugal partition chromatography was performed together with a UPLC-MS^n-based metabolic profiling of raw extract and each fraction obtained. In vitro assays (TCTB quantification in fungal culture media) and dereplication with molecular networking revealed that fractions containing stilbenoids are particularly effective. These results show that grapevine canes are a promising feedstock of candidates able to control *F. graminearum* mycotoxin production.

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5. DEVELOPING ANTI-INFLAMMATORY VITAMINE E ANALOGUES STARTING FROM RENEWABLE RESOURCES

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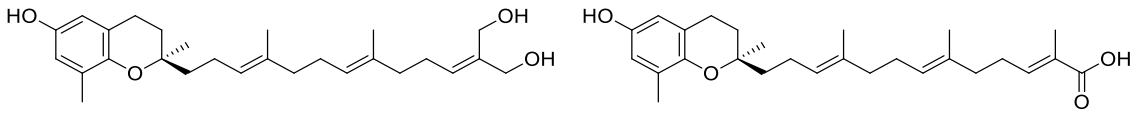
SONAS is a laboratory based at the University of Angers. Our team includes about 15 permanent staff, 7 lecturers and 3 professors, focusing their research interests on natural products, and teaching organic chemistry, analytical chemistry, pharmacognosy and phytochemistry at the school of pharmacy (Faculty of Health Sciences).

SONAS has a long term expertise in natural products chemistry starting with sourcing of strategic plant materials, thanks to collaborations with ICSN (CNRS, Gif sur Yvette). Most of the plants studied at SONAS belong to the *Clusiaceae* family. As an example, the phytochemical study of *Garcinia amplexicaulis* has led to the isolation of fifteen new tocotrienolic derivatives, with δ-amplexichromanol (δ-AC, figure 1) as a major secondary metabolite, along with already known δ-garcinoic acid (δ-GA).[1] An *in silico* pharmacophoric study identified 5-lipoxygenase (5-LO) as a potential biological target for both compounds. This enzyme is involved in the first steps of the arachidonic acid cascade leading to the synthesis of pro-inflammatory leukotrienes. These preliminary results were later confirmed through *in vitro* assays, using purified 5-LO or polymorphonuclear leukocytes (PMNL).[2]



δ-amplexichromanol (δ-AC) δ-garcinoic acid (δ-GA)

Figure 1.

Over the last few years, several series of analogues were semisynthetized to investigate the anti-inflammatory potential of this class of oxidized vitamin E analogues.[3-5]

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6. NUCLEAR MAGNETIC RESONANCE AND MASS SPECTROMETRY APPLIED TO THE METABOLOMIC AND DEREPLICATION STUDY OF *PASSIFLORA CINCINNATA* (PASSIFLORACEAE)

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The Caatinga biome represents the fourth largest area in vegetation cover in Brazil, covering approximately 60% of the territory of the Northeast region. *Passiflora* species, popularly known as passion fruit, have been widely cultivated in Brazil, especially in the Caatinga biome, due to the production of edible fruits as well as their medicinal use. The objective of this work was to investigate the chemical profile using nuclear magnetic resonance (NMR) and mass spectrometry (MS) techniques of the species *Passiflora cincinnata*, species found in regions of Caatinga in the São Francisco Valley. The species *P. cincinnata* is endemic to the Caatinga and this work represents the starting point for the implementation of a new line of research in our university/laboratory. Aerial parts of *P. cincinnata* (fruit peel, flower, leaf, seed, and stem) were collected in the city of Uauá (Coordinates: S 09°43′12.4″; W 39°39′33.3″), State of Bahia, Brazil. The samples were identified by a botanist, and a voucher specimen (#22870) was deposited at the Herbarium Vale do São Francisco (HVASF) of the Federal University of San Francisco Valley (UNIVASF). All procedures for access to genetic patrimony and associated traditional knowledge were carried out and the project was registered in SisGen (Register #A1D7010). The extracts were obtained with solvents of different polarities (chloroform, ethyl acetate, acetone, ethanol, ethanol 70% and water) in order to evaluate the efficiency and selectivity of the solvents in the extraction of the largest amount of metabolites by means of ^{1}H spectra in NMR and select the best extraction method. The obtained extracts also were submitted to chromatographic methods such as column chromatography and thin-layer chromatography for isolation of the chemical constituents. The isolated chemical constituents had their structures identified through NMR (uni and bidimensional) and MS techniques. The extracts also were analyzed by modern chromatographic methods such as HPLC-DAD, HPLC-DAD-MS and HPLC-DAD-MS/MS analysis, leading to the identification of fourteen secondary metabolites in *P. cincinnata* extracts, mainly derivatives of vitexin, isovitexin, orientin and isoorientin. The chemical composition of the plant was confirmed by NMR studies. It is hoped to develop a simple method of qualitative and quantitative analysis of substances that allows the identification of chemical markers and that can serve as support in the quality control of this species.

Keywords: *Passiflora*, flavonoids, metabolomic, dereplication, chemometric analysis, Caatinga.

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7. CYCLODEXTRINS-TERPENES INCLUSION COMPLEXES: A PROMISING APPROACH OR A FOOLISHNESS?

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Terpenes (TPs) constitute the most abundant group of secondary metabolites in higher plants with around 30,000 compounds. It is a diverse family of natural products (NP) derived from C5 isoprene units joined in a head-to-tail fashion. They represent a class of NP that provide a great number of possible solutions to different human-health issues[1]. Several therapeutic properties of TPs are known to be one of the most appreciable family of chemical compounds due to their pharmacological role in various systems and diseases. TPs are especially used in pain and inflammation treatment, immune system modulation, cytokines modulation, among other activities which are essential for controlling the symptoms of several diseases, especially chronic ones[2]. In contrast, the lipophilic profile of TPs that help the pharmacological properties are also a limiter for the therapeutic use, as well as their effects are usually more fleeting, due to short duration who seem to be directly related to bioavailability[3].

The formation of inclusion complexes with cyclodextrins (CDs) has been able to improve drug characteristics, such as bioavailability, solubility, stability, biological activity and efficacy[3]. Currently, more than 35 drugs available on the market are complexed with CDs to improve the therapeutic effects being able to reduce effective dose, improve patient safety and optimize therapeutics[4]. Moreover, the use of CDs associated with TPs and essential oils (EO) has been practiced by the cosmetics and food industry for some time, but its applicability to optimize the pharmaceutical properties is not stablished seeking use in chronic diseases. Several researchers groups around the world have published a consistent number of papers suggesting that for some TPs- or EO-complexed with CDs can be a promising approach for pharmacological application, since the results clearly demonstrate an improvement in chemical characteristics that compromise the use of these lipophilic compounds. Thus, our research group has concentrated efforts to evaluate the benefits or not of these inclusion complexes (TPs- or EO-CDs) focusing on preclinical models of pain and inflammation, including for the management of chronic pain.

The figure 1[8] shows in “A” the theoretical results obtained through molecular modeling corroborate with the NMR results, indicating that limonene (LIM), a terpene from Citrus sp, was not totally complexed inside the cavity of CDs. The green circle in the shows the space in the cavity of CD after interaction with LIM. The LIM-CD complex has been shown, by our group, to have an analgesic, inflammatory, and immunomodulatory improved profile, which appear to be enhanced after the complexation of LIM into CDs[8,9]. “B” and “C” demonstrated other effects of improving the pharmacological profile after inclusion of TPs with CDs, but all of these studies have the limitation of not demonstrating how these promising effects may be related to the change in the pharmacokinetic profile[8].

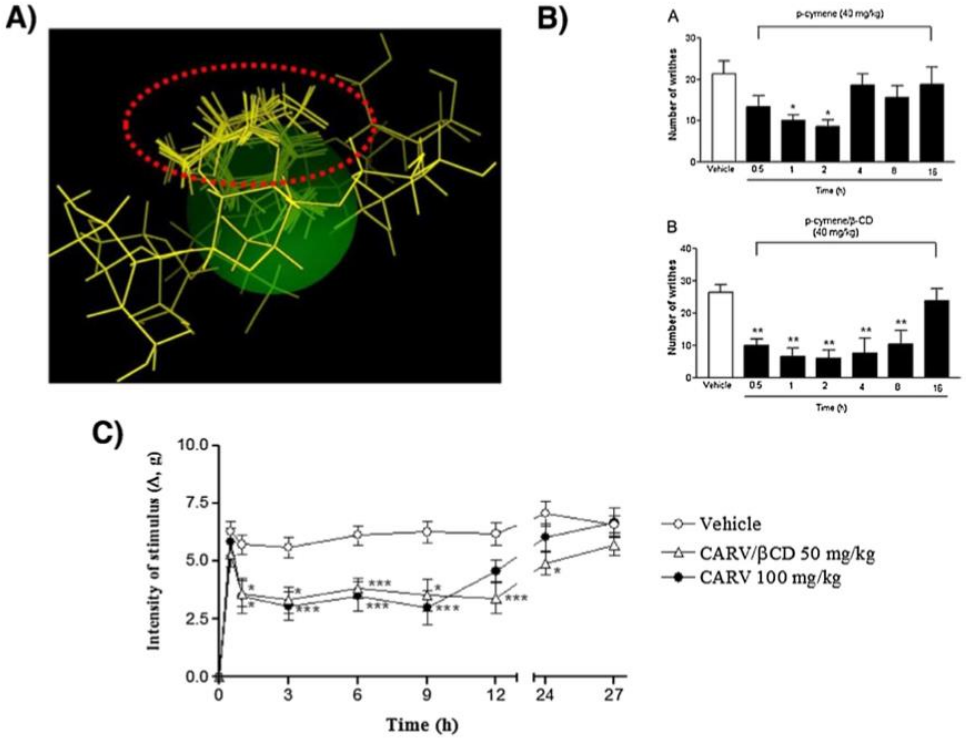

Figure 1. (A) Ten possible interactions of LIM and CDs obtained through molecular modeling. The green space represents the CD cavity (Adapted from Menezes et al., 2016). **(B)** Time response curve for the antinociceptive effect of (A) p-cymene or (B) p-cymene/β-CD complex on acetic acid-induced writhing response in mice. Writhings were counted over 20 min following i.p. administration of acetic acid (0.65%). p-cymene or p-cymene/β-CD (40 mg/kg) was administered p.o. 0.5, 1, 2, 4, 8 or 16 h before acid acetic injection (0.65%). Control animals received an injection of vehicle by p.o. route. Each column represents mean ± S.E.M. (n = 8, per group). *p < 0.05 or **p < 0.001 vs. control (ANOVA followed by Tukey's test) (Adapted from Menezes et al., 2017[5]; Quintans et al., 2013[6]). **(C)** Effect of carvacrol/β-cyclodextrin complex (CARV/β-CD) on the mechanical hyperalgesia induced by S180. Time-Effect Curve of CARV/β-CD (50 mg/kg) and CARV (100 mg/kg). *p < 0.05, **p < 0.01 and ***p < 0.001 vs. the control group (vehicle) (ANOVA followed by Tukey's test) (Adapted from Guimaraes et al., 2015[7]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article). This figure was taken from the article published by Lima et al, 2016[3].

A consistent scientific production has supported that CDs can be beneficial for the improvement of several TPs, especially alcoholic TPs, as suggested in our review published by Lima (2016)[3] due to the presence of hydroxyl group which is suggested that straight and branched chain alcohols enter the cavity of CDs alkyl end first, and the hydroxyl group hydrogen bonds to the outer oxygen ring of CDs. Although alcoholic TPs-CDs have shown more promise profile, at least from the point of view of assessing pain and inflammation, other TPs groups complexed with CDs have also shown promise pharmacological approach. However, the most studies fail to demonstrate how this improvement may be occurring, thus, pharmacokinetic studies and more molecular studies are required in order to better characterize this approach.

Finally, the fact that the growing number of pharmaceutical products is increasingly available on the market for the treatment of various diseases associated with CDs corroborate that this approach remains promising and current. According to Oliveira et al (2015)[10], Diniz et al. (2017)[11] and Gandhi et

al (2020)[12] the growing number of patents involving TPs and required CDs are a strong indicator that new therapeutic proposals must be placed on the market or at least that this approach follows the innovative profile required by the pharmaceutical market.

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8.BIOECONOMICS & BIOACTIVE COMPOUNDS FROM PLANTS FOR MATERIAL OR BIOLOGICAL APPLICATIONS

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Four major challenges present themselves to us for this 21st century. First, an increase in the world's population, leading to an increase in food and energy needs, all coupled with global warming. By 2050, the population is expected to increase by more than 50%, which will imply a demand of more than 70% in food needs and more than 100% in energy. Oil has allowed an explosion in technological and human development, so we need to prepare for after oil. The challenge now is to ensure economic development with renewable resources while limiting the impact on the environment and people. Plant chemistry, the pillar of green chemistry, can answer this. This context has the direct consequence of the development of the use of renewable resources to replace those of fossil origin. This is particularly the case for plant resources, such as plants whose use is motivated by their great abundance, their diversity, their renewable nature and their richness in compounds of interest.

Three major axes are emerging for plant chemistry: i) The challenge of natural reserves with the widening supply of renewable raw materials for the chemical industries, ii) Integration towards a bio-based economy with the creation of bridges between the different sectors and a balance between economy, environment and social component, iii) Consumer expectations with the improvement of current properties of formations and materials and the improvement of life cycle analysis.

The supply of raw materials is a challenge. We must ensure the secure supply of biomass, the lower cost of renewable raw materials and also the development of supply chains. This requires mobilizing additional areas and biomass, increasing agricultural areas and profitability per hectare, and improving the efficiency of the systems. Plant chemistry uses around 30 million tonnes of crops, or 6 million hectares, which represents less than 0.5% of the total arable land.

Within our research unit, we are exploring ways to enhance plants by using fractions (fatty acids, glycones, rhamnolipids, polyphenols, etc.) or biomolecules (chlorogenic acids, specific fatty acids) extracted for industrial or biological applications. These valuations can be by direct use of the extract or after formulation / chemistry in the context of sustainable chemistry. Among the applications we develop, we can cite the use of;

- Carbohydrate and lipid fractions for obtaining amphiphilic antibacterial, antitumoral compounds,

- Rhamnolipid fractions with antimicrobial, pesticide / elicitor properties (notion of bio-control),

- Chlorogenic acids, quercetine for their antioxidant property in particular

- Specific fatty acids such as nervonic acid and its involvement in certain neurodegenerative diseases.

- Biopolymers to biomaterials as bioplastics, biocomposites

It also addresses the fact that these valuations / uses must be integrated into a bio-based economy, the bio-economy; sustainable production from biomass of products for the food, animal nutrition, industrial and energy markets. With the necessity of creating bridges between the different sectors and a balance between economy, environment and social component.

9. CHEMICAL COMPOSITION OF PLANT PARTICLES BY PHYSICAL AND CHEMICAL APPROACHES

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Since thousands of years lignocellulosic biomass like hemp have been cultivated. At the beginning, the culture was based on fibers to produce garments or ropes for shipbuilding sector. Later on, culture was extended to produce new high molecules for pharmaceutical sector, second-generation biofuels and more recently bio-based materials for building engineering[1]. These kinds of bio-based product have interests according to their insulation capacities, low carbon impact or air quality increasing. Moreover, these products contribute to increase local employment and by the way, decrease impact carbon link to importations.

Thus, current research topics are focus on plant valorisation into bio-based material process like particle boards or mortars. However, plants still are a variable matter depending on pluviometry, culture condition or species, in compared to conventional building materials submitted to rules and international standards. Thus, a deeper understanding of plant composition is required to understand the physico-chemical interactions of molecules released by aggregates during process. Therefore, this could upgrade their quality.

Chemical approach following by physical characterization of lignocellulosic biomass is investigated. Van Soest method is a destructive chemical approach, mostly used in lignocellulosic biomass, in order to quantify cell wall compounds[2]. The literature suggests that polar molecules like hemicelluloses and some soluble compounds which represent less than 40% of biomass, are easier to extract by hot or basic pH water, than other cell wall compounds. That mean they might directly have an impact in particles interactions into binderless particle boards or mortars rigidifications by interacting with other compounds[3,4]. Also, a non-destructive version of Van Soest method was carried out to produce samples without some cell wall compounds and analysed by Thermal Gravimetric Analysis (TGA). The results obtained from these technics will be analysed and compared to conventional Van Soest method.

At first, Van Soest method was performed to quantify cell wall compounds. Non-destructive version of Van Soest method allowed us to separate each fraction derived from this method. All these fractions have been analysed by TGA to quantify and identify molecules in samples. The results obtained from these technics are analysed and compared. Analyses suggested that hemp shiv contain 51% of cellulose, 22% of hemicellulose, 19% of soluble compounds (pectins, lipids, oses, ashes, proteins…) and 8% of lignin. TGA results showed different thermal stabilities depends on considered fraction, which be link to cellulose interactions with lignin and hemicellulose. By this physical technic we provide another method to quantify cell wall molecules.

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10. FRANCE-BRAZIL COLLABORATIVE PROJECT FOR THE DISCOVERY OF AZAHETEROCYCLIC COMPOUNDS AS ANTI-*TRYPANOSOMA CRUZI* AGENTS TO FIGHT AGAINST CHAGAS DISEASE

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Neglected Tropical Diseases (NTDs) affect mainly underdeveloped or developing countries located in Africa, Asia and Latin America. Worldwide, there are more than one billion people affected by these diseases(1). These include Chagas disease, also known as American trypanosomiasis, a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). Besides side effects of the drugs currently used, the resistance of the parasite and late detection of the disease are concerns to address. Therefore, the discovery of new substances for the treatment of Chagas disease is extremely necessary. Thus, we developed a medicinal chemistry programme dealing with the synthesis of 5,6,7,8-tetrahydroimidazo[1,2-*a*]pyrazine based thiosemicarbazones as antiparasitic agents(2-5) (Figure 1).

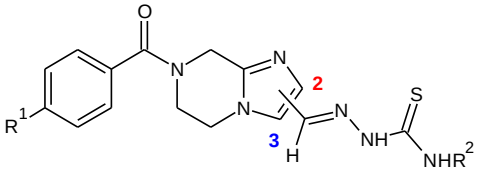

Figure 1. General structure of target compounds.

The first developments and biological results of this novel series of molecules will be discussed.

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11. HETEROCYCLIC COMPOUNDS INSPIRED FROM NATURAL PRODUCT CERCOSPORAMIDE: DESIGN, SYNTHESIS AND EVALUATION OF FLUCONAZOLE SUSCEPTIBILITY RESTORATION

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(-)-Cercosporamide was originally isolated in 1991 as an antifungal agent and phytotoxin from a fungal plant pathogen of cassava, *Cercosporidium henningsii* (Figure 1) [1].

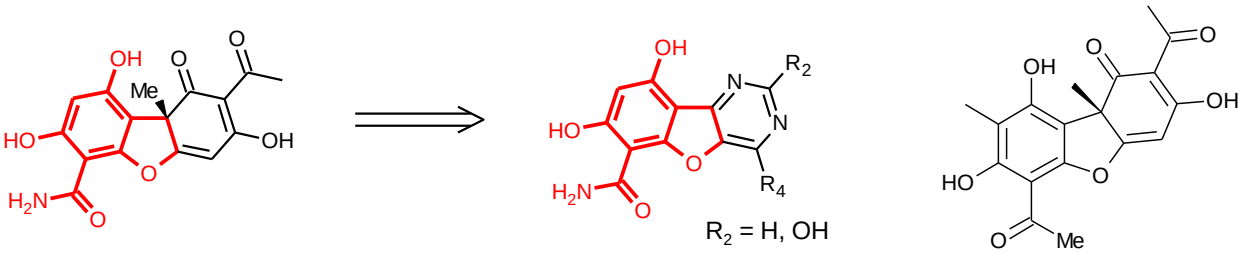

Figure 1. Structure of target compounds

Its antifungal effect on *Candida albicans* strains results from its selective and potent inhibition of fungal PKC-like 1 kinase (Pkc1), which is central to cell wall integrity (IC_{50} = 44 nM for *Candida* Pkc1) [2]. Cercosporamide inhibition of human PKC isoforms PKCα, β and γ is less efficient (IC_{50} = 1.02, 0.35 and 5.8 µM, respectively). Moreover, *Ca*Pkc1 is supposed to be involved in the mechanism of antifungal drug resistance [3]. The biological properties associated with cercosporamide, as a *Ca*Pkc1 inhibitor, are not found for its structural analogue usnic acid (Figure 1) [4], suggesting that the western part of cercosporamide is the pharmacophore of the molecule.

To design new molecules, this part was conserved and tricyclic analogues, bearing a pyrimidine moiety, were synthesized (Figure 1) [5]. To investigate the biological interest of these new molecules, *in vitro* inhibition of fungal kinase activity has been evaluated first. Then, MICs have been determined following an EUCAST modified procedure, on a collection of *Candida albicans* strains selected for their sensitivity or resistance to fluconazole (reference compound). Moreover the potential restoration of fluconazole susceptibility has also been investigated on resistant strains.

In the present work, the synthetic routes and the first results of the biological evaluation will be discussed.

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12. MOLECULAR DOCKING AND CHARACTERIZATION OF CARDIOVASCULAR EFFECTS OF FARNESOL AND ITS INCLUSION COMPLEX WITH B-CYCLODEXTRIN

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Background: Farnesol (FAR) is an alcoholic sesquiterpene with many therapeutic effects including cardioprotective, antioxidant and antiarrhythmic. However, due to its high volatility, drug incorporation system such as with cyclodextrins, have been proposed to improve pharmacological properties. **Objective:** The aim of this study was to evaluate and characterize the cardiovascular effects of FAR isolated and to investigate antihypertensive effects of the FAR complex with β-cyclodextrin (FAR+β-CD) in rats. **Methods:** Mean arterial pressure (MAP) and heart rate (HR) were direct measured before and after intravenous administration of FAR (0,5; 2,5; 5 and 7,5 mg/kg) in normotensive rats[1]. Dose-response curves were also obtained in animals pre-treated with atropine (AT), hexamethonium (HEXA), L-NG-Nitro arginine methyl ester (L-NAME) or indomethacin (INDO). To assess the antihypertensive effect, hypertensive rats were treated orally (200 mg/kg) with FAR and FAR+βCD complex. All procedures were approved by the Animal Research Ethics Committee of the Federal University of Sergipe, under number #56/2017. **Results:** In normotensive animals, FAR induced dose-depended hypotension associated with bradycardia. These effects were not affected by pre-treatment with L-NAME or INDO, but was partially attenuated by atropine. Pre-treatment with HEXA only attenuated hypotension. In hypertensive rats, FAR/βCD complex was able to improve FAR effect by decreasing MAP at 30 and 48h after administration. Molecular docking experiments demonstrated for the first time that FAR have affinity to bind in M3 and M2 muscarinic, and nicotinic receptors through hydrogen bonds in the same residues as known ligands. **Conclusion:** Our results demonstrated that FAR alone induced hypotension associated with bradycardia (i.v.) possibly through muscarinic and nicotinic receptors. The inclusion complex FAR+βCD potentialized antihypertensive effects of FAR, which can be relevant for treatment of cardiovascular diseases, such as hypertension.

Keywords: sesquiterpene, inclusion complex, hypertension, cyclodextrin, docking.

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13. INCLUSION COMPLEX OF D-LIMONENE AND HYDROXYPROPYL-B-CYCLODEXTRIN: PHYSICOCHEMICAL CHARACTERIZATION AND CARDIOVASCULAR EFFECTS

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Background: D-limonene (D-LIM) is a natural monoterpene main constituent in the essential oil of citrus fruits. Our previous studies have shown that D-LIM presents bradycardic, antiarrhythmic, and cardioprotective effects in rats. Despite these important pharmacological effects, its high volatility and low water solubility, prevent its wider use. **Objective:** This study aimed to make an inclusion complex between hydroxypropyl-β-cyclodextrin (HP-β-CD) and D-LIM, and to evaluate its cardiovascular effects in rats. **Methods:** The inclusion complex was prepared by the slurry complexation technique[1] and characterized using high-performance liquid chromatography (HPLC), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and nuclear magnetic resonance (^{1}H NMR). The hemodynamic effects were evaluated *in vivo* in normotensive and hypertensive rats, and the antiarrhythmic effect was assessed in a model of arrhythmia induced by Bay K 8644. All procedures were approved by the Animal Research Ethics Committee of the Federal University of Sergipe (#13/2016 and #80/2018). **Results:** The physicochemical characterization showed that the D-LIM + HP-β-CD complex formation had a complexation efficiency of 79.96 ± 0.24 %. In pharmacological essays, D-LIM (1, 5, 10, 20, and 40 mg/kg, i.v., n = 6) induced hypotension and bradycardia in normotensive rats, and the D-LIM + HP-β-CD complex (400 mg/kg) induced bradycardia by oral administration only in hypertensive rats. Furthermore, the complex D-LIM + HP-β-CD (10 mg/kg, i.v.) was able to prevent ventricular arrhythmias, decrease arrhythmia index (from 17.2 ± 2.4 to 5.3 ± 2.4 a.u; n = 4) and the duration of arrhythmias (from 51.6 ± 15.5 to 6.8 ± 5.8 sec; n = 4). Interestingly, D-LIM alone (10 mg/kg, i.v.) also reduced arrhythmia index and prevent tachycardia at the same magnitude of the complex, even though the complex have only around 7% of D-LIM. **Conclusion:** These results suggest the improvement of the pharmacological properties of D-LIM when complexed with HP-β-CD. Thus, the complex can be used for the treatment of cardiovascular diseases, mainly arrhythmias. However, clinical studies are needed to better assess its effects on humans.

Keywords: monoterpene, inclusion complex, arrhythmia, cyclodextrin.

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14. URIAGE NATURAL SPRING WATER: AN EFFICIENT INHIBITOR OF HOST-MEDIATED SKIN BACTERIA VIRULENCE

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The thermal spring water from Uriage-les-Bains (UTW), originating from French Alps, has a unique composition in natural minerals and trace elements. Isotonic with blood, it is recognized since 1877 by the French academy of medicine for its activity against inflammation, and particularly acne. The cutaneous microbiota plays a key role in skin homeostasis and depends of complex skin-bacteria interactions. We have shown that hormones and neuro-hormones present in skin and sweat, such substance P, calcitonin-gene-related-peptide (GCRP), natriuretic peptides and catecholamines (adrenalin and noradrenalin) can convert commensal microorganisms into opportunistic pathogens and stimulate the virulence of skin-associated bacteria such as *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus cereus, Pseudomonas fluorescens* and *Cutibacterium* (former *Propionibacterium*) *acnes*[1]. The efficiency of UTW was tested in this context. We observed that UTW inhibits the increase of virulence induced by Substance P on *S. epidermidis, S. aureus*, and *B. cereus*[2]. In fact, because of its ionic charge (11.000 mg/L), UTW should probably act through chelation of cutaneous peptides and neuropeptides. However, the effects of UTW are not limited to inhibition of peptidic mediators. Recent studies demonstrated that UTW can affect the growth kinetic of *S. aureus* and acneic strains of *C. acnes* by increasing the generation time and reducing the biomass formation[3]. UTW has also a marked anti-biofilm activity on both bacterial species. These effects should be associated to bacterial surface rearrangement and shifts of polarity[3]. Adrenalin and noradrenalin, well known mediators of stress, have a differential effect on acneic and non-acneic strains of *C. acnes* suggesting that the bacterium should serve as a relay between stress and acne[4]. As observed by confocal microscopy, the effects of UTW on *C. acnes* biofilm formation, which is essential in acne-associated inflammation, appear more important on acneic than non-acneic strains[4]. These results can explain the remarkable efficiency of UTW for the treatment of cutaneous diseases.

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15. REGIOSELECTVE REDUCTION OF α, β, γ, δ - UNSATURATED HALO-KETONES BY ENEREDUCTASES FROM *Penicillium citrinum* CBMAI 1186

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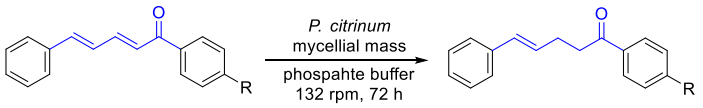
Species of fungi that belongs to the genus *Penicillium* are presents in several habitats around the world, the production of different metabolites and enzymes explains the success of the adaptation, and as well the attention of studies for these microorganisms. For example, there is the enereductase, an oxirreductase that catalyse the stereoselective reduction of α, β, γ, δ - unsaturated compounds with electron accepting groups of the C=C double bond[1]. Owing to the positive results that already was obtained with the use of enereductase from the fungus *Penicillium citrinum* CBMAI 1186 in our Laboratory[2], this specie was chosen for this work.

Thereby, to confirm the action of the enereductase from fungus native, reactions with specific substrates was done following these steps[2]: firstly, $1x10^7$ spores were inoculated in Erlenmeyer flasks with 100 ml of culture media (malt and synthetic sea water); and, 7 days later, 2.5 g of mycelial mass was separated and transferred to a flask with 50 mL of a buffer solution with 25 mg of the halogenated ketones dissolved in dimethyl sulfoxide. The biocatalytic reaction was maintained in orbital shaker (132 rpm) during 72 h at 32 ˚C.

Preliminary reduction of Knoevenagel adduct (2-benzylidenemalononitrile) was investigated with the *P. citrinum* in our Laboratory[2]. The use of this substrate it was to direct the experiments and confirm the results which already was obtained. As expected, more than 95% of the compound was reduced after 24 h. Then, three α, β, γ, δ - unsaturated halo-ketones were synthetized and tested with the fungus (Scheme 1). The % of reduction relative was calculated from the areas of the peaks for each substrate obtained by CGMS analysis.

Scheme 1. Regioselective reduction of α, β, γ, δ -unsaturated halo-ketones by enereductases from P. citrinum CBMAI 1186.



R = F (92%), Cl (85%), Br (77%)

The enzymes from *P. citrinum* promoted elevated reduction of halogenated ketones (Scheme 1). In the future of this study, to avoid the use of the whole microorganism because of the limitations, such as long periods for growth of the fungus and extensive time of reaction, the gene for the enereductase of two different species of *Penicillium* (*P. steckii* and *P. antarcticum*) will be synthetized and cloned by PCR for expression in bacteria *Escherichia coli* (BL21(DE3)). These species were selected because *P. steckii* is closely related to *P. citrinum*[3] and *P. antarcticum* is a fungus that grown in an inhospitable ambient, so the enzyme can present a structure more stable and yours use can be facilitated. It is expected to obtain a great amount of the enzymes to promote biocatalytic reaction in scale-up for α, β, γ, δ – unsaturated compounds.

Hence, as the enereductase from the species of *Penicillium* proved to be very useful in the reduction of
α, β, γ, δ – unsaturated compounds, the use of technology of recombinant DNA becomes essential to improve the productivity and, consequently, to increase the use of this important class of enzymes upon different compounds, such as flavonoids and chalcones.

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16. ANTIMICROBIAL ACTIVITY OF BIOSURFACTANT, *Apium graveolens* OILRESIN AND LIMONENE AGAINST *Bacillus cereus*

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The world Health Organization estimates that 600 million people fall sick and 420 thousand die every year from eating contaminated food[1]. In Brazil, bacteria, which include *Bacillus cereus*, are responsible for 95% of outbreaks involving foodborne diseases[2]. *B. cereus* is a Gram-positive spore-forming bacteria widely known for causing food poisoning and economic losses to food industry[3,4]. Development of natural and environmental-friendly methods to control microbial growth are increasing demands of food processing industry. Within this context, we propose the application of a rhamnolipid (RL) biosurfactant and an oilresin (OR) from *Apium graveolens* seeds as a natural alternative to control *Bacillus cereus*. The antimicrobial potential of compounds, both individually and in combination, was assessed by Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) assays. When used alone, RL was capable to inhibit growth and cause cell death at concentrations as low as 9.8 µg/ml. OR did not show antimicrobial effect (MIC and MBC were higher that 40000 µg/ml) however, when combined with RL, OR were capable of inhibit cell growth with 1000µg/ml OR + 62.5 µg/ml RL. Cell death was achieved when using a combination of 2000µg/ml OR + 125 µg/ml RL. OR components were identified by GC-MS and it showed that its main component was limonene (LN). Therefore, concentrations of LN alone and in combination with RL were tested for they antimicrobial effect. When accessed alone, LN showed a MIC value of 1250 µg/ml and MBC higher than 40000 µg/ml. When combined with RL, growth inhibition values were 1250 µg/ml LN + 7.81 µg/ml RL and cell death occurred with the concentration of 2000µg/ml LN + 125 µg/ml RL. These results suggest that RL can be explored to improve antimicrobial activity of natural oils and its components once RL amphiphilic nature helps increasing solubility of natural hydrophobic compounds.

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17. NEW INSIGHTS INTO THE CHEMICAL NATURE OF MARENNINE

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Marennine is a blue-green water-soluble pigment produced by the marine diatom *Haslea ostrearia* (Figure 1-A). It is responsible for the natural greening of oyster gills in Western France (Figures 1-B and 1-C), thus increasing the palatability and market value of bivalves (fines de claires vertes)[1]. Recent studies showed that marennine displays allelopathic, antioxidant, antiviral and antibacterial activities[2]. These properties combined with the scarcity of natural blue pigments could make marennine an ideal candidate to replace synthetic pigments for certain applications. However, despite years of research on the subject, marennine chemical composition and structure remain unresolved, impeding many applications in the cosmetic and food industry.

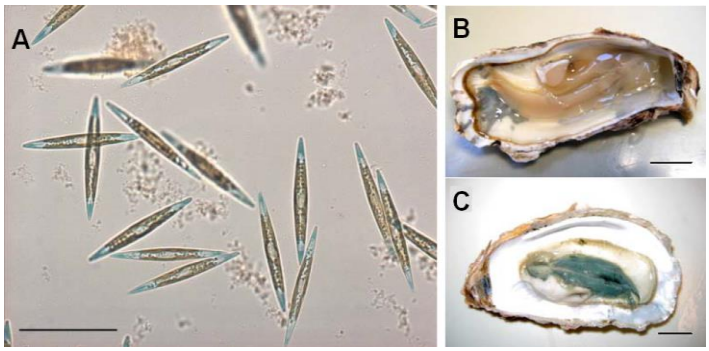

Figure 1 – A-*Haslea ostrearia* with marennine accumulated in the apical areas of the cells. Scale = 50µm, B-white and C-greened oyster: scale = 1cm.

A newly developed patent-pending method allows the efficient recovery and concentration of the marennine from *H. ostrearia* supernatant. This method was used to collect samples and investigate the chemical nature and the properties of marennine. Color changes depending on the pH or the redox state of the molecule were studied, revealing interesting features for different usages of the pigment. Several analytical techniques confirmed that marennine is a complex macromolecule composed of an aromatic chromophore linked to a polysaccharidic backbone. Acid hydrolyses were carried out to deconstruct the molecule. The composition of the polysaccharide was partially determined using anion exchange chromatography, size exclusion chromatography and nuclear magnetic resonance (NMR) analyses. The nature of the chromophore was studied using NMR and mass spectroscopy.

The elucidation of the composition of marennine is still under progress. However these recent advances give new insights for different applications of this natural blue pigment.

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18. MARINE FUNGI NATURAL PRODUCTS: FROM CHEMODIVERSITY TO CHEMICAL ECOLOGY AND APPLICATIONS

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Microorganisms in the marine environment (fungi, bacteria, microalgae) live in perpetual interactions whether in the open sea, in deepsea sediments, or associated to plants or animal hosts such as molluscs or sponges. These interactions are mediated by exchanges of chemical signals that new analytical and statistical tools allows to discover, which has seen the emergence of a new scientific field: chemical ecology. What are these signals? How to study them? What lessons can be learned from their study and what developments can be expected, in particular for research of new drugs? The MMS laboratory of the Faculty of Pharmacy of Nantes has developed since 15 years an expertise in LC-HRMS/MS analyzes and development of dereplicative tools (automated annotation[1], search for halogenated metabolites[2]) and biochemometrics[3], in order to study the metabolites of marine fungi, their chemodiversity, their biosynthesis[4], their bioactivities and their involvement in biotic interactions within the Atlantic coastal environment. This expertise has resulted in the creation of a platform for metabolomic analyses of marine microorganisms: "Corsaire-ThalassOMICS". As an example, the joint use of metabolomic studies and molecular networks has recently led to the discovery of new analogs of rare meroterpenes, miniolutelides produced by a *Penicillium ubiquetum*[5]. These approaches applied to studies of fungal strains in “ecological” culture media have also allowed the isolation of original pyran-2-ones potentially inhibitors of the quorum-sensing, compounds overexpressed by a *Penicillium restrictum* in the presence of its environmental host, the blue mussel *Mytilus edulis*. Other projects are underway, focusing on chemical interactions between fungi and sponges, and on dialogues between fungi and benthic microalgae, a couple never studied before.

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19. BLUE BIOTECHNOLOGY IN BRITTANY

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The Laboratory of Marine Biotechnology and Chemistry LBCM is a laboratory of the Université Bretagne Sud (UBS) (University of South Brittany) which is related to the Higher Education and Research Unit in Sciences and Engineering Sciences. LBCM was focused on biofilms, microbiome and blue biotechnologies, combining biological and chemical approaches. The laboratory is composed of around 40 members including 21 professors and assistant professors, 12 PhD students, 3 post-doctorate researchers. The blue biotechnology group has also patented several molecules and was involved in national (ANR Biopaintrop; ANTECOL, Save-C, Corsair) and international projects (ECOS North Mexico; PHC Nusantara Indonesia; OPUS, Poland). The purpose of the conference aims to show one thematic of research developed in LBCM about the concept of seaweed biorefinery. Marine macroalgae represent an excellent raw material for the production of bioactives, adsorbents, plant biostimulants, soil fertilizers and biogas. The success in the exploitation of seaweeds depends on their characteristics, and the approach used to separate their specific active components. In the context of circular economy, invasive species are a good candidate for exploitation, and biorefinery a key valorization technique.

20. FUCOXANTHIN CONCENTRATION IN CLONAL DIVERSITY OF TISOCHRYSIS LUTEA

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In the French National Resarch Dynalgue project, the genetic association approach (GWAS) has been used to identify genetic polymorphisms associated to variation of some target phenotypic traits which are important for the successful use of microalgae as food or feed source. We used *Tisochrysis lutea* in this project, because improvement programs are conducted in our laboratory for its current use in aquaculture and food and we expect to expand its use for other farm animal's markets. Specific carotenoids and xanthophylls such as fucoxanthin and echinenone has been measured in *Tisochrysis lutea* (1) and it provides significant antioxidant activities besides other interesting biological activities in cancer research (2).

Working from 15 strains of *Tisochrysis lutea* isolated from different ecosystems in the ocean, we cytometrically isolated 100 clones from these strains on the basis of their size, their fluorescence and their lipid content. We characterized each clone individually through two controlled culture conditions, nitrogen-limiting and phosphorus-limiting. During this webinear we will present the description of the pigment composition of each *Tisochrysis lutea* clones and first result of GWAS to decipher genes which play a role in fucoxanthin and echinenone content.

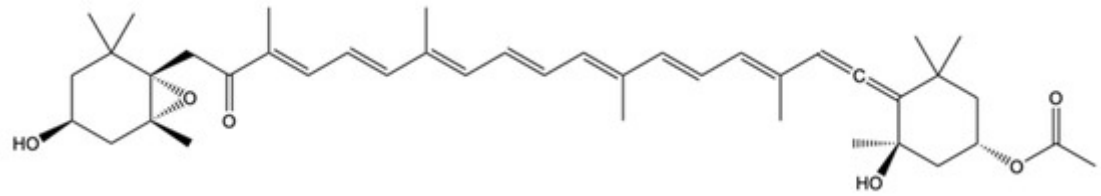

Figure 1. Chemical structure of Fucoxanthin.

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21. ENVIRONMENTAL SEM: THE BEST METHODOLOGY TO OBSERVE ORIGINAL, UNPREPARED NATURAL SAMPLE SURFACES

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We propose an optimized methodology using Scanning Electron Microscopy (SEM) in environmental mode (ESEM) to study the surface characteristics and the internal structure of natural samples[1]. Water vapor pressure in the 0.9 – 2.0 mbar range is introduced in the SEM specimen chamber during analyses to improve the electrical and thermal conductivity in the surroundings of the specimen, and to preserve it from damage. The main advantage of this methodology is that no preparation is required and, significantly, no dehydration is performed nor metallic coverage is deposited on the surface of the specimen, thus preserving its original morphology. In particular, it avoids introducing preparation artefacts, which could modify the surface morphology and shape, and mask information concerning important feature like porosities, roughness, etc.

This methodology has been used to observe plants like strawberry, rosemary, stevia, etc., dried by different methods. Fig. 1 shows French maritime pine bark waste from which essential oils were extracted by two different methods: classical hydrodistillation (HD) and the Solvent Free Microwave Extraction (SFME)[2]. Fig. 1A shows the original, untreated pine bark, where external surface is smooth and contains many folds. After extraction by HD (Fig. 1B) some ruptures and perforations appeared on the leaf surface and the folds are still present. After extraction by SFME (Fig. 1C), perforations and creation of canals are clearly observed and the surface appeared completely disrupted because of the strain induced by a rapid rise in temperature in SFME extraction and subsequent change in the surface tension of the glandular wall.

Chemical contrast is preserved in Backscattered Electron (BSE) images in environmental mode. As demonstrated for biopolymer capsules having a core constituted of an inorganic salt covered by type A gelatin or hydrogenated vegetable oil, BSE images allow visualizing the internal organization of biopolymer capsules, the quality of the envelope etc[1]. For some simple fatty molecules like stearic acids, Energy Dispersive Spectroscopy (EDS) analyses have been successfully performed in to obtain the relative concentration C/O.

Studies by ESEM/EDS involving Sulfideproducing Bacteria and their metabolic activity, responsible for the microbiologically influenced corrosion of the metallic parts of wood shipwrecks maintained in wet and anoxic conditions for long periods, as well as the precipitation of iron sulfides on the wood, will also be presented.

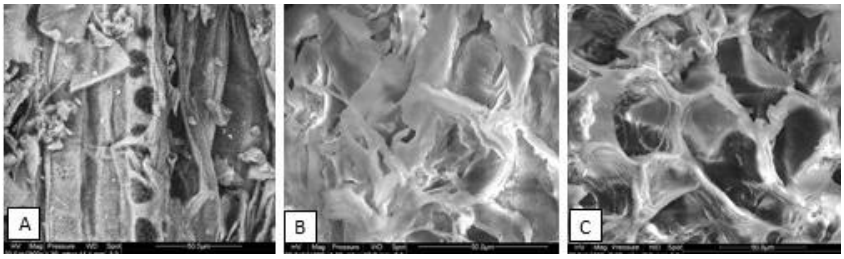

Fig. 1 : ESEM images of maritime pine bark waste. (A) Untreated; (B) after HD extraction and (C) after SFME extraction.

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22. CENTRIFUGAL PARTITION CHROMATOGRAPHY METHOD OPTIMIZATION FOR THE ISOLATION OF ANTIBACTERIAL COMPOUNDS FROM THE FRUITS OF *PISTACIA LENTISCUS*.

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Herbal preparations from several parts of *Pistacia lentiscus* L. (Anacardiaceae) such as resin (mastic gum), fruits, leaves and oil have been traditionally used in the Mediterranean basin for more than 2,500 years for their medicinal properties[1]. Mastic gum of this species harvested from Chios Island (Greece) contains antibacterial triterpenes (24-*Z*-masticadienonic acid derivatives MAD) but their isolation is tedious, due to the presence of a myrcene polymer[2]. The chemical composition of the fruits, considered as a waste of mastic production, was never extensively studied. Thus, we focused on this part of the plant as a potential source of bioactive metabolites.

In a preliminary step, small amounts of MAD and salicylic acid derivatives (SAD) isolated from a fruit extract using silica gel chromatography have strongly inhibited the growth of Gram+ aerobic and aerotolerant bacterial strains.

Centrifugal Partition Chromatography is a fast technique with feasible scale-up, based on the partition between two immiscible liquid phases. However, because of close polarity, SAD partially co-elute with triterpenes. A solvents combination including some percent of ammonia solution was then optimized in order to provide a better retention of the salicylates and an efficient separation of all of the targeted compounds in only one run with a good yield.

These results could lead to the valorisation of fruits and of their constituents as natural preservatives for food and cosmetic industry.

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23. NATURAL PRODUCTS ATTENUATING *PSEUDOMONAS AERUGINOSA* VIRULENCE

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The LMSM (Laboratory of Microbiology Signals and Microenvironment, University of Rouen Normandy) is involved in understanding the molecular mechanisms leading human opportunistic bacterial pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*…) to adapt to their environment and to exacerbate their virulence. The Lab is involved in developing new strategies against bacterial pathogens, searching for natural compounds displaying anti-bacterial, anti-biofilm, anti-virulence activities, as well as conventional antibiotics new adjuvants.

Pseudomonas aeruginosa is capable to deploy a collection of virulence factors that are not only essential for host infection and persistence, but also to escape from the host immune system and to become more resistant to drug therapies. Thus, developing anti-virulence agents that may directly counteract with specific virulence factors or disturb higher regulatory pathways controlling the production of virulence armories are urgently needed. In this regard, this study reports that *Pistacia lentiscus* L. fruit cyclohexane extract (PLFE1) thwarts *P. aeruginosa* virulence by targeting mainly the pyocyanin pigment production via interference with 4-hydroxy-2-alkylquinolines molecules production. Importantly, the anti-virulence activity of PLFE1 was associated to membrane homeostasis alteration through the modulation of SigX, an extracytoplasmic function sigma factor involved in cell wall stress response. A thorough chemical analysis of PLFE1 allowed to identify the ginkgolic and hydroginkgolic acids as the main bioactive membrane-interactive compounds responsible for the observed increased membrane stiffness and anti-virulence activity against *P. aeruginosa*. This study delivers a promising perspective for the potential future use of PLFE1 or ginkgolic acid molecules as an adjuvant therapy to fight against *P. aeruginosa* infections.

Tahrioui et al., 2020. Membrane-Interactive Compounds From *Pistacia lentiscus* L. Thwart *Pseudomonas aeruginosa* Virulence. Front Microbiol. 11:1068. doi: 10.3389/fmicb.2020.01068.