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PLENARY LECTURES

AUTOMATED GLYCAN ASSEMBLY AS BASIS FOR LIFE SCIENCE AND MATERIAL SCIENCE

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Rapid preparation of polysaccharides by automated glycan assembly (AGA)[1] using a synthesizer[2] provides access to diverse glycans as large as 151-mers.[3] Accelerated microwave-assisted synthesis methods[4] are now used to prepare ever more complex glycans including cis-linked polysaccharides[5] are enabling fundamental investigations into the structure and function of polysaccharides.
Synthetic glycans in combination with single molecule imaging,[6] molecular modelling and other physical methods to characterize carbohydrate structure [7-9] allow us to address fundamental questions of carbohydrate structure, folding and material science. [10, 11] Recently, we described the design, synthesis, and characterization of the first stapled oligosaccharides with increased enzymatic stability and cell penetration. [12]
Synthetic glycans are the basis for the development of vaccines against different bacteria [13] that are currently in clinical evaluation. Monoclonal antibodies and nanobodies against glycans are the basis for a program aimed at developing novel diagnostics and therapeutics. [14]

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MARINE CHEMICAL ECOLOGY IN A CHANGING OCEAN

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Marine natural products can have important functions in the ecology and behavior of marine organisms. They serve as chemical defenses against herbivores and predators and can prevent overgrowth by fouling organisms and inhibit disease-causing microbes. Examples of these natural functions will be presented in this talk, and many studies have now been published; however, little is known about the ecological roles of most marine natural products. At the same time, the oceans are changing due to climate change, ocean warming, hypoxia, overfishing, invasive species and other anthropogenic factors. These changing environments can lead to harmful algal and cyanobacterial blooms and higher incidences of marine diseases. Marine filamentous cyanobacteria are rich sources of natural products, and proliferation of cyanobacteria can negatively impact the environment because they can overgrow and kill other benthic organisms. Their diverse natural products can play a role in these competitive interactions.

Studies of the chemical ecology of coral diseases can provide insights into beneficial and pathogenic host-microbe interactions. For example, the natural products known as looekeyolides from the black band disease cyanobacterium *Roseofilum reptotaenium* were isolated and characterized to try to determine their natural functions in this coral disease. Recently, we discovered cryptic variation in the chemistry and genomes between *Roseofilum* strains that appears to be coral host specific. The devastating stony coral tissue loss disease outbreak has resulted in nine years of extensive coral mortality in over 20 Caribbean coral species. Bacteria were cultured from coral fragments resistant to disease and then screened for antibacterial activity to isolate protective bacteria (probiotics). In aquarium studies, disease progression could be slowed in fragments of *M. cavernosa* after exposure to *Pseudoalteromonas* sp. McH1-7, which also prevented infection of pretreated coral fragments. The most effective probiotic strains are being used to treat diseased corals in the field with some success in slowing disease progression.

FROM RAINFOREST TO CLINIC: A CASE STUDY OF THE SCIENTIFIC AND DEVELOPMENT CHALLENGES IN COMMERCIALISING NATURAL PRODUCTS AS PHARMACEUTICALS

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Small molecule natural products have historically played a major role in the pharmaceutical industry, either directly as drug substances or as inspiration for synthesis, or semi-synthesis, of novel derivatives and analogues. Compared to libraries of conventional synthetic molecules, natural products have significant advantages in providing both an unmatched diversity of molecular scaffolds and molecular structures that have been 'optimised' by evolution to function in biological systems (e.g., interaction with membranes). However, natural products also come with technical and commercial challenges especially associated with the current paradigms used by the pharmaceutical industry and drug regulatory authorities. These challenges include (i) the frequent incompatibility of activity-guided isolation approaches with the pharmaceutical industry's current target-based screening, (ii) obtaining access to sufficient quantities of raw material for isolation and structural elucidation of actives, (iii) the ability to protect intellectual property related to naturally-occurring compounds, (iv) non-conformance with Lipinski's rule of five, (v) difficulties in deconvoluting molecular targets, and, if the compound is not synthetically tractable on a commercial scale, (vi) the need to demonstrate reliable supply and manufacturing methods that produce drug substance of consistent quality.

Here we describe our experience in the discovery, development and commercialisation of the diterpene ester tigilanol tiglate, a structurally-complex oncology drug discovered from the Australian rainforest tree *Fontainea picrosperma* (Euphorbiaceae), that is now registered as a veterinary pharmaceutical for treatment of canine mast cell tumours by regulatory authorities in Europe (EMA), the United Kingdom (VMD), the USA (FDA-CVM) and Australia (APVMA) and which is currently in Phase II human clinical development for treatment of a range of solid tumours including head and neck cancers and soft tissue sarcomas. In particular, we outline the major pitfalls and challenges we encountered in the development and commercialisation process, as well as emphasising the clinical benefits that natural products with multiple modes of action can confer in treating cancer and other diseases with complex underlying aetiologies.

TETRODOTOXIN: CHEMISTRY, HISTORY, NATURE, OPPORTUNITY

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Tetrodotoxin (TTX) is a fascinating natural product initially isolated from pufferfish (*Tetraodontidae*) in 1909. In spite of being one of the most studied molecules for over 100 years, including by several Nobel Prize laureates and at this very own symposium (3rd ISCNP in 1964), TTX and its presence in nature not only garner unabating mystique and attraction from conventional chemists, toxicologists, pharmacologists and microbiologists, also reach the unexpected disciplinaries in ecology, evolution biology, genetics, anthropology, and herpetology. In this review, sciences and histories interweave across multiple dimensions to exemplify the diversity and convergence connected by a single chemical entity. TTX's compact dioxa-adamantane and cyclic guanidine structure poses a formidable challenge to organic chemists, who, for half a century, have continued conjuring new reactions, catalysts, and methods to achieve precise stereochemistry and efficient synthesis. The ubiquity of TTX in remarkably biodiverse marine and terrestrial species hides its natural origin in plain sight. Advancements in metagenomics and microbiomics are shedding light on the elusive Biosynthetic Gene Cluster (BGC), which will unleash the power of enzymatic machinery. The adaptability of TTX tolerance and capability of harnessing TTX-producing symbiont bacteria enable a growing number of non-indigenous species (NIS), propelled by increasing sea temperature, to invade, establish and spread in suspectable habitats lacking top predators owing to overfishing and depleted resources. These invasive species threaten the marine ecosystem, coastal communities and food safety at large. In this complex context, it is the nanomolar potency of blocking cellular sodium channels that presents the greatest opportunity for TTX. The lack of effective pain management therapies and the ongoing worldwide opioid epidemic urgently demands a safe and non-addictive pain medicine. Combinatorial chemistry and drug design have yet to produce a suitable candidate without off-targeting on Central Nervous System. TTX has demonstrated potential safety and efficacy in moderate and severe pain with minimal side effects where some patients experienced pain relief of up to 30 days and longer with a single cycle of treatment. TTX continues to be developed as the ideal analgesic to address the global need for a non-opioid and non-addictive analgesic and has to date, been tested in over 700 people in over 15 clinical trials.

BIODIVERSITY: OUR PATH TO A SUSTAINABLE FUTURE

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Over the past 50 years extraordinary increases in global economic output and life expectancy have occurred. The human population has doubled, the global economy has quadrupled, and more than 1 billion people have emerged from extreme poverty. Human activities have altered 75% of terrestrial environments and 66% of marine environments. About 25% of plant and animal species are threatened by human actions, with a million of species facing extinction, many within years or decades. Biodiversity is the main victim of global changes, as well as the main resource to solve them. It supports human and societal needs, including food and nutrition security, energy, fresh water, medicine, and pharmaceutical development. It underpins economic opportunities and leisure activities, contributing to overall well-being. The key role of biodiversity in creating a sustainable future has been recognized by governments and Institutions. Europe has developed the long-term plan Biodiversity Strategy 2030, to protect nature and reverse the degradation of ecosystems. This strategy aims to put Europe's biodiversity on the path to recovery by 2030, including specific actions and commitments. Being a central element of the European Green Deal, it will also support a green recovery following the COVID-19 pandemic. The value of biodiversity has also been acknowledged by Italian politics. Italy is among the countries with the highest biodiversity in Europe and with one of the highest rates of endemism in the Mediterranean basin and a mosaic of different habitats ranging from insular and coastal environments to mountain habitats and aquatic biotopes. With the amendments to articles 9 and 41 of the Constitution, the protection of biodiversity has been introduced among the fundamental principles of our Country. Biodiversity has been set as one of the main priorities of the National Recovery and Resilience Plan, as shown by the creation of the ambitious project coordinated by the National Research Council, i.e. the National Biodiversity Future Center (NBFC), which attracts a network of 48 partners and provides funding of over 320 million euros for the first three years (2023-2025), with the involvement of over 1300 researchers from partner institutions and a few hundred new hires.

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TOTAL SYNTHESIS AND FUNCTIONAL EXPLORATION OF MACROCYCLIC NATURAL PRODUCTS

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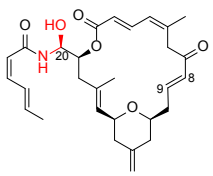
Macrocyclic secondary metabolites are a diverse group of bioactive natural products and many of these compounds have been, and continue to be important leads for drug discovery and development. In this talk, I will discuss key aspects of the synthetic chemistry and SAR of three bioactive macrocyclic natural products, the marine macrolide zampanolide (**1**), a microtubule-stabilizing agent (MSA), the myxobacterial metabolite disorazole Z (**2**), a tubulin assembly inhibitor, and the cyclic depsipeptide pyridomycin (**3**), an antitubercular agent.



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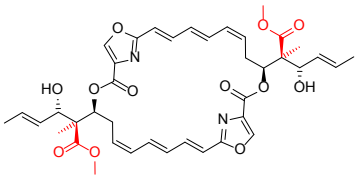

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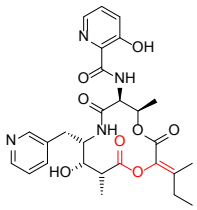

3

Each of these compounds incorporates a unique structural element (highlighted in red) that is rarely found in other natural products and whose importance for biological activity has been addressed either through synthesis of the natural product itself or of specific analogs. It will be shown that structural simplification of these natural products is possible without substantial loss in bioactivity.

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ADVANCED ANALYSIS OF LIPIDS IN FOOD SAMPLES USING INNOVATIVE CHROMATOGRAPHIC AND MASS SPECTROMETRIC APPROACHES

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The growing demand in natural products, representing a source of dietary and nutraceutical molecules, placed, as direct consequence, the urgent need for the development of suitable analytical methods able to provide a comprehensive characterization of both “conventional” and “unconventional” products.
In the last decades, lipidomic has emerged as a cutting-edge approach among omics- techniques, since lipids revealed to be essential molecules in the regulation of metabolic pathways. To this regard, the content of essential fatty acids (EFAs), as well as nutritional indices such as the levels of omega-3 and omega-6 fatty acids (FAs) and their ratio, are essential parameters to evaluate the beneficial properties of functional foods.
The aim of this research is the development of analytical strategies for the elucidation of lipids in different functional foods, including novel seed oils, as well as hemp derived products and the wastes of the fish industry according to a circular economy approach. There are two parallel and complementary analytical approaches commonly used in the lipidomic field: gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography-mass spectrometry (HPLC-MS). The former is essential for the detailed characterization of the total FA composition, being able to reliably identify the position of the double bonds along the carbon chain (omega nomenclature) and/or the configuration of the double bond (*cis*/*trans*), while the latter is employed for the elucidation of intact lipids (e.g., triglycerides, diglycerides, phospholipids, free FAs) as they are originally present in the sample, thus adding important information about storage conditions and how they will be metabolized. In both cases, particular emphasis is paid on the miniaturization and automation of the entire analytical workflow by using robotic workstations able to perform the sample preparation in a fully automated manner and online with the chromatographic system. Also, mass spectral library with embedded retention data (linear retention index, LRI) were built in both GC and HPLC methods to achieve a fast, reliable and automatic identification of lipid species through the development of a dedicated software, which applies a dual-filter identification strategy by exploiting the complementarity between MS and LRI data.

NATURAL PRODUCT BIOSYNTHESIS IN UNDEREXPLORED MICROBIAL NICHES

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Microbial natural products are a crucial source of therapeutics, particularly for anti-infectives and antitumor drugs. However, as resistance to currently used therapeutics increases, the traditional method of drug discovery, that is the screening of established producers, has become less and less effective. Analyses of whole genome sequences has shown that the biosynthetic capabilities of microbes are much higher than previously assumed, and that many genera (e.g. *Burkholderia* and *Clostridium sensu lato*) have been underexplored in terms of specialized metabolism. These 'neglected' microbes not only provide access to novel scaffolds, but also employ unusual biocatalysts for molecular assembly. Beyond bearing great potential for the discovery of novel lead structures, these overlooked biosynthetic pathways often play crucial ecological roles in specialized niches, which are of high relevance for agriculture and medicine. Selected recent works from our group will be presented in this talk.

DEEP-SEA MICROBIAL BIODIVERSITY AS A POTENTIAL SOURCE OF NATURAL PRODUCTS

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The deep sea encompasses the largest, most extreme and remote ecosystems on Earth. Deep seafloor ecosystems host a huge, yet largely unexplored biodiversity, and provide crucial services that are vitally important to the entire humankind. In this talk I will provide an overview of the current knowledge of deep-sea microbial biodiversity (from viruses to prokaryotes and fungi) to explore their potential contribution to the discovery of new molecules and natural products.

MARINE SYMBIONTS, A RICH SOURCE OF USEFUL CHEMICALS AND ENZYMES

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On the earth, ocean is the largest biological habitat characterized by high salt, low temperature and poor nutrient availability. In particular, the marine environment empowers some symbiotic microorganisms to express useful chemicals[1, 2] and unusual enzymes[3]. This talk will exemplify the clarification of biosynthetic steps of marine bacterial (*Streptomyces*) and fungal (*Talaromyces*) bioactives such as chartreusin and rugulosin A[4, 5, 6].

Chartreusin has a unique pentacyclic aromatic bilactone aglycone, and the active α-pyrone ring formation is catalyzed by an unprecedented dioxygenase (named ChaP) being a founding member of the vicinal oxygen chelate (VOC) enzyme superfamily. We provided insights into the molecular basis of the oxidative rearrangement that involves two successive C−C bond cleavage steps followed by lactonization using molecular and structural biology techniques. Furthermore, we developed a platform to alter the catalytic reaction trajectory of ChaP by directed evolution, verifying the unpredicted catalytic versatility and enzymatic plasticity of VOC enzymes with biotechnological significance.

Rugulosin A is a bisanthraquinone characterized by its distinct cage-like structure, and remains biosynthetically mysterious although characterized six decades ago. We have clarified the rugulosin A biosynthesis which is governed by the *rug* gene cluster. The formation of three C-C bonds between the two emodin-like monomers is achieved by the synergy of a cytochrome P450 monooxygenase (RugG) with an aldo-keto reductase (RugH). RugG appears to be substrate-promiscuous and thus valuable for expanding the bisanthraquinone chemodiversity. The work updates the understanding of rugulosin A biosynthesis and paves the way for synthetic biology accesses to cage-structured bisanthraquinones.

In aggregation, our data have shown that the network between marine symbionts' chemicals and enzymes is unique and may provide valuable inspirations for the discovery of drug leads and biocatalysts.

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NATURAL POLYPHENOLS AS VERSATILE MATERIALS FOR APPLICATIONS IN BIOMEDICAL AND FOOD SECTORS: POWERING THEIR POTENTIAL BY CHEMICAL MANIPULATION AND GREEN METHODOLOGIES

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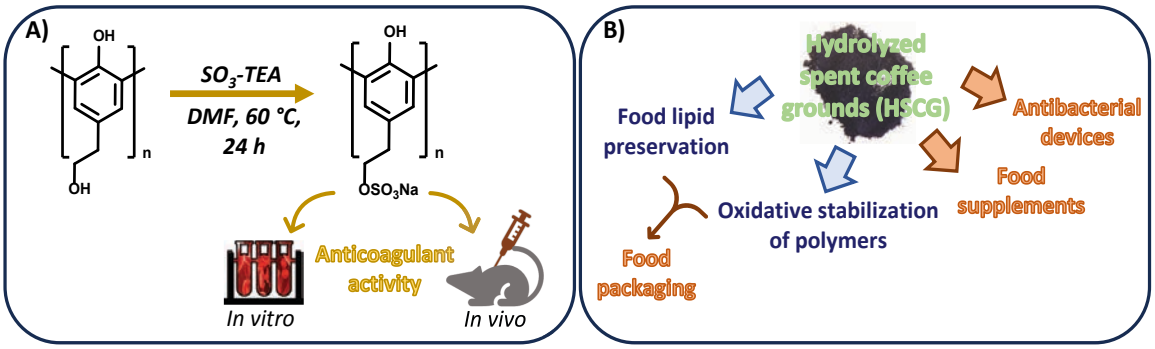
In the last decade the versatility of polyphenol chemistry and the unparalleled bioactivities exhibited by many of these compounds has increasingly been appreciated and largely translated into advanced biomedical and technological applications.
The activity of natural phenols is primarily associated to their antioxidant potential, but is ultimately expressed in a variety of biological effects. Molecular scaffold manipulation of this large class of compounds is a currently pursued approach to boost or modulate their properties. These strategies that include conjugation with biological thiols, selenylation, and polymerization will be presented [1,2] together with other examples showing how to impart biological activities to phenolic polymers, e.g. sulfation of polymers from tyrosol providing a new class of non-saccharidic heparin mimetics anticoagulants (figure, panel A). [3]
Nowadays, the remarkable antioxidant properties have also prompted the use of natural phenolic compounds not only as food supplements, but also as additives for the development of functional materials. The presentation will be therefore focused on relevant examples of how natural phenols can be exploited as multifunctional compounds in the food, cosmetic, and biomedical sectors. Particular attention will be paid to phenolic compounds from agri-food wastes such as spent coffee grounds (figure, panel B), exhausted woods from tannin industrial production, and nut shells, which are rich in phenolic polymers including lignin and tannins, endowed with very potent antioxidant properties. [4,5] The possibility to further improve these antioxidant properties through chemical manipulation will also be presented, together with efficient and green strategies for the recovery of phenolic compounds based on the use of ball milling and/or deep eutectic solvents (DES). [6]



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SUSTAINABLE SOURCES OF NATURAL PRODUCTS FROM MARINE INVERTEBRATES

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Sessile marine invertebrates, such as sponges and tunicates, are rich sources of bioactive natural products. However, drug development from these often is commonly prevented by low available substance amounts, the importance to preserve fragile marine habitats, and the chemical complexity of many substances that render total syntheses uneconomic. Previous work in our lab has shown that many sponge-derived compounds are biosynthesized by symbiotically associated bacteria.[1-5] An example are members of the candidate genus Entotheonella that produce a wide range of natural products in different sponges.[1-3] While these findings offer the prospect of biotechnological, bacteria-based production systems for rare substances, a challenge is that all producers identified to date are members of “microbial dark matter”, i.e., as-yet unculturable bacteria. This talk discusses the challenges associated with identifying hidden producers and their pathways in sponges and with creating sustainable production systems. Recent results will be presented on various strategies that allowed the production of the polytheonamide-[6] and phorboxazole-type compounds,[7] calyculins,[7] as well as new natural products from microbial dark matter.[8]

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INVITED LECTURES

ASSAULT, SIEGE OR TROJAN HORSE STRATEGY: USE OF NATURAL PRODUCTS TO FIGHT BACTERIAL INFECTIONS

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Multidrug resistant bacterial pathogens have become a major health concern. Especially infections by gram-negative bacteria are challenging, since their complex cell membrane architecture strongly impedes the uptake of drugs. Because microbial natural products continue to be a prime source to tackle these issues, we have investigated natural products as the basis for novel antibiotics. A metabolomics-based workflow for assessing the metabolic potential of recalcitrant marine and terrestrial producers will be briefly shown, followed by a deeper characterization of selected lead compounds.

The armeniaspirols represent a novel class of antibiotics with a unique spiro[4.4]non-8-ene chemical scaffold and potent activities against gram-positive pathogens. I will report a concise total synthesis of (±) armeniaspirol A and disclose their mechanism of action, that might be also valid for other chloropyrrole-containing, marine and terrestrial natural products.[1] A broad spectrum of gram-positive and gram-negative pathogens is addressed by cystobactamids, oligo-arylamids originally isolated from Cystobacter sp. Our efforts to optimize the properties of the cystobactamids by medicinal chemistry to leads with high in vivo efficacy will be presented.[2]

Beyond a classic 'assault' of bacteria with such antibiotics, the conjugation of natural products to targeting functions has been beneficial to improve their drug properties.[3] In the so-called Trojan Horse Strategy, antibiotics are conjugated to siderophores to hijack the bacterial siderophore transport system, and thereby enhance the intracellular accumulation of drugs.[4] We present novel artificial siderophores, characterize their transport and resistance mechanisms, and their efficacy when coupled to antibiotic natural products.[5] Finally, a novel approach for the selective bacterial targeting and infection-triggered release of antibiotic conjugates is introduced in the alternative siege concept, using the lipopeptide colistin as the antibiotic effector.[6]

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EXPLORING THE DIVERSITY OF MICROBIAL NATURAL PRODUCTS AND CURRENT CHALLENGES IN DRUG DISCOVERY

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Microbial natural products (NPs) are one of the most prolific sources of chemical diversity for the discovery of novel bioactive molecules to be developed as new drugs in response to emerging unmet medical needs. Their present a unique chemical space and architectural complexity, and their potency and selectivity are the result of an extended evolutionary selection to create biologically active molecules with the required properties to interact and potentially inhibit biological targets.

Less explored and untapped microbial communities have concentrated recent research efforts to identify new chemical diversity. Genome mining and cutting edge metabolomic approaches have become essential tools in any modern NPs drug discovery paradigm and omic tools are opening new opportunities to identify novel classes of compounds. New integrated NPs discovery approaches involving cultured-based strategies combined to high throughput phenotypic screening platforms are playing a key role in the identification of new molecules to be developed as new drugs.

MEDINA is a research center focused on the discovery of novel bioactive NPs with one of the richest and most diverse microbial collections that are at the origin of our collaborative drug discovery research programs in different therapeutic fields. As a result of these screening programmes we have identified different novel families of molecules with interesting new chemistry and biological activities that will be discussed in the context of current discovery efforts to address current needs in infectious diseases.

KEYNOTE LECTURES

AN OVERVIEW OF THE CHEMISTRY AND BIOACTIVITY OF THE FUNGAL GENUS *MALBRANCHEA*

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The fungal genus *Malbranchea* Sacc. (Onygenaceae, Ascomycota) contains about 30 species characterized by the production of alternate arthroconidia in branches from the vegetative hyphae. Most species are keratinophilic, while some are pathogens of humans and other animals.[1,2] The species have been isolated from the soil, caves, animal dung, decaying vegetation, and clinical samples.[1,2] *Malbranchea* represents a rich source of extrolites, including enzymes and specialized metabolites comprising many structures and interesting bioactivities. State-of-the-art techniques have enabled the isolation and characterization of benzoquinones, hydroquinones, and benzofurane with antimicrobial, cytotoxic, antiviral, or antioxidant properties;[3,4] phytotoxic, smooth muscle relaxant and antifungal terpenoids,[5-7] cytotoxic anthrasteroids,[8] vasodilating aromatic compounds,[9] as well as cytotoxic and potential antidiabetic polyketides and peptides.[10-16] Among them, a unique family of monoketopiperazines with vasorelaxant and calmodulin inhibitor properties, namely the malbrancheamides, have been obtained;[16-20] the groups of Williams and Sherman from the Universities of Colorado and Michigan, respectively, have elegantly investigated their synthesis and biosynthetic pathways.[21, 22, inter alia] In this presentation, relevant aspects of the chemistry and biology of this genus of fungi stemming from our work will be discussed.

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INVESTIGATIONS INTO THE PHARMACOKINETICS AND POSSIBLE NEUROPHARMACOLOGICAL MODES OF ACTION OF COUMARINS IN DIFFERENT MODEL ORGANISM

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Coumarins are an independent class of shikimate-derived natural products with a 2H-chromen-2-one core scaffold. In profiling studies, various coumarins show a surprisingly broad range of biological activities in vitro and in vivo. In addition, different coumarins, both synthetic and natural, have demonstrated effects on the central nervous system (CNS) in preclinical in vivo experiments, suggesting their penetration through the blood–brain barrier (BBB). However, the lack of critical insights into the translatability related to missing knowledge about tissue concentrations in vivo which is a serious limitation. In this presentation. examples of correlation of central effects with the concentration of coumarins and its major metabolites in mouse brain tissue and zebrafish body will be discussed.
Applications of advanced chromatographic techniques in pharmacological studies will be pointed out, such as countercurrent separation as efficient tool for the isolation of bioactive compounds from complex plant extracts. Furthermore, data on the employment of LC–MS/MS-based targeted metabolomics for the evaluation of neuropharmacological effects of coumarins on the brain lipidome associated with the endocannabinoid system (ECS), amino acids and neurotransmitters levels will be presented.

MARINE FUNCTION MOLECULES: FROM CHEMICAL RECOGNITION TO TARGET DETECTION

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Secondary metabolites from marine invertebrates and associated fungi are rich sources of biologically active natural products. While the chemistry of these metabolites is still a challenge considering their structural novelty and complexity, increasing attentions are drawn in understanding the biomedical functions and correlated action targets of these molecules, aiming to explore their potential application in various areas of biomedicine.

Our ongoing screening of bioactive compounds from marine sources resulted in the isolation of molecules against TSZ induced cell death and IFN-γ-induced PD-L1 expression. The molecules have frameworks of sesquiterpenoid and steroidal saponin. The bioactivities of the molecules were evaluated *in vitro* and *in vivo*. Their action mechanisms and protein targets were investigated. The cell death involves both apoptosis and necrosis. Attempt of understanding of their signal pathway revealed a possibility of a new mechanism. The PD-L1 suppression of molecules is due to targeting JAKs to inhibit the downstream activation of STATs and subsequent transcription of PD-L1. A silic docking was conducted to show the possible interaction of active compound and target proteins. The bilogical petential of these molecules are discussed. This research gives a paradigm for functional molecule discovery by integration of natural product science with molecule biology and chemical biology.

BIOAVAILABILITY AND METABOLISM ASPECTS OF NATURAL BIOACTIVES
THE CASE OF PHENYLALCOHOLS AND SECOIRIDOIDS

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The disclosure of the decisive role of gut microbiome in disease onset, prevention, and health maintenance, repositioned active small molecules found in food products as a unique class of natural products with high impact to human health[1]. It is evident that gut microbiome is one of the major factors affecting the metabolic fate and therefore bioavailability of orally administered compounds. Furthermore, scientific dialogs globally over-comments the correlation of biological and/or pharmacological functions of natural products with their *in vivo* bioavailability and metabolism, as well as highlight the lack of relevant information and solid data[2]. Amongst these lines, there are limited and scattered data related to pharmacokinetics and especially metabolism of olive oil biophenols (OBs). The most characteristic chemical classes of OBs are phenylalcohols and secoiridoids widely known for their significant health beneficial effects[3]. As expected, their bioactivity is directly related to their metabolism and biotransformations in human organism demonstrating their mechanism of action, efficacy and safety. Aiming towards the exploration of OBs bioavailability aspects, a multi-arm workflow is established involving *in vitro* investigation[4], animal experiments as well as human studies[5]. LC-HRMS & HRMS/MS-based metabolite profiling and dereplication approaches with the aid of public and in-house databases framed this workflow in a holistic and interactive crosstalk. Several biomarkers and biotransformation patterns revealed under the established metabolomics approach giving better insight into OBs biological mechanism of action.

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SOLVING THE SUGAR CODE: STRUCTURE AND IMMUNE RECOGNITION OF GLYCANS

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The evaluation of the structure and host recognition of glycoconjugates is key to two interconnected pillars: i) to provide structural insights into the mechanisms governing glycans' recognition and binding by host receptors and ii) to evaluate their contribute to the cell survival as well as their interaction with host receptor(s). The combined use of complementary approaches, including NMR spectroscopy, computational techniques and biophysical methodologies is essential to unravel the structure, conformation and molecular recognition features of glycoconjugates as well as their interaction with eukaryotic host.
The detailed (bio)-molecular characterization of microbial glycoconjugates (as Gram-negative LPS) and molecular insights into the mechanisms that govern their interaction with host receptors is of primary importance to "tune" the bacterial cell surface initiation or suppression of inflammatory response. Various examples will be here discussed, with description of the advantages and drawbacks of the application of the different methods and techniques.

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CONTROLLING G4 DNA TOPOLOGY WITH SMALL MOLECULES: TOWARDS THE DEVELOPMENT OF NOVEL THERAPEUTICS

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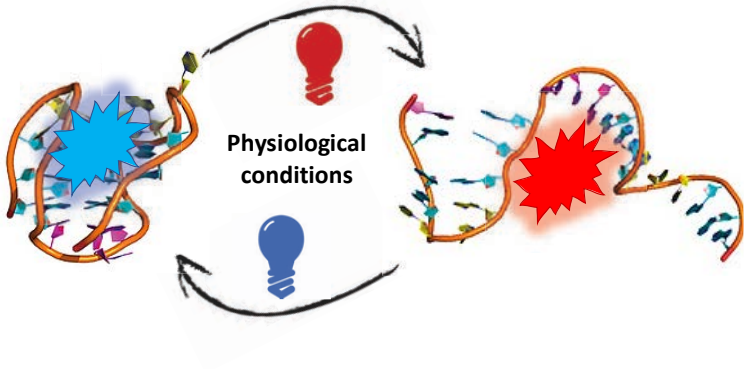
G-quadruplexes oligonucleotides (G4) are a fascinating class of nucleic acid structures formed from the self-association of guanine-rich sequences. This kind of four-stranded structures have potential applicactions in biological chemistry and responsive nanotechnology that may be exploited for therapeutic effect. While many examples of ligands that are able to stabilize G4 sequence are reported in the literature, those ligands do not induce reversible and controlable structural perturbations such as the re-folding of the G4 to an alternative topology or the unfolding of the G4 structure through binding modes at physiological pH. In this sense, light offers high spatiotemporal precision for the regulation of oligonucleotide structure.[1] During this lecture I will describe recent examples of photoresponsive ligands for G4 DNA regulations developed within our research group. From stiff-stilbene ligands which are capable of unfolding G4 DNA in physiological conditions in a reversible manner[2]
to dithienylethene chromophores with inherently superior photoresponsive properties for the study of G4-binding properties which can be used for the photo-reversible control of ligand binding mode and oligonucleotide folding employing exclusively red and blue visible light.[3]



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SULFUR-CONTAINING GLYCOLIPIDS, THE ACCESS KEY FOR TRIGGERING AND STUDY THE RESPONSE OF INNATE IMMUNE CELLS

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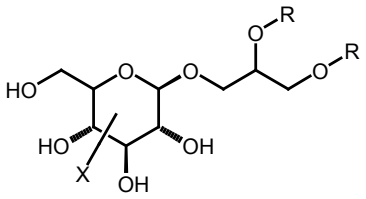
Glycolipids are ubiquitous molecules mainly present on cell membranes in which they play a structural and stabilizing function.[1] In recent years these substances have aroused huge interest for their ability to chemically interact with proteins and glycoproteins [2] and their cell exposure make them particularly suitable to influence cell-cell interaction and signals transmissions. Glycolipids in fact play a pivotal role acting as ligands of cellular receptors and regulators of transduction signals, opening the way to numerous pharmacological applications, especially in the field of immunomodulation. As in fact these substances are highly expressed on microorganisms and cell aberrations, the immune cells are equipped with protein systems (Pattern Recognition Receptors) capable to recognize them.[3]. In the last years, we have focused our study on the immunological properties of natural and nature inspired sulfur containing glycolipids (**1**), able to induce an unprecedented immunological response capable of restoring the immune homeostasis after perturbative and dysfunctional cell events. This peculiar activity is related to the ability of these molecules to bind the trigger receptor expressed on myeloid cells-2 (TREM2), a single-pass transmembrane immune receptor, responsible for cellular well-being both in the central nervous system and in the periphery.[4] This communication summarizes our work about the synthetic challenges associated with preparation, immunological assessment and preclinical development of this class of molecules and their use as vaccine adjuvants and immunomodulators for the treatment of TREM-2 linked dysfunction diseases, such as neuro-inflammation and neurodegeneration.[5]



X= OSO_3^-, SO_3^-
R= Acyl, Alkyl

1

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GLYCOSCIENCE IN MICROBIAL WORLD: THE POWER OF SUGARS

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Microbial cell surface molecules, such as the lipopolysaccharide, are very important cell wall glycoconjugates that act as microbe associated molecular patterns in eukaryotic/microbe recognition. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Microbes differently from Eukaryotes have at their disposal an enormous array of monosaccharide structures/derivative with which they built up they external cell surface molecules and drive their recognition by any eukaryotic host. Therefore, the chemical study of such glycoconjugates involved as virulence or beneficial factors in animal or plant interactions is a pivotal pre-requisite for the comprehension at molecular level of the (innate) immunity mechanisms. [1]
Viral glycoproteins are usually meant to carry on eukaryotic glycans. Indeed, typically, viruses use host-encoded glycosyltransferases and glycosidases to add and remove sugar residues from virus glycoproteins. However, the more recently discovered large and giant viruses broke from this paradigm. Instead, these viruses code for an (almost) autonomous glycosylation pathway. Virus genes include the production of activated sugars, glycosyltransferases and other enzymes able to manipulate sugars at various levels. [2]
By this work, I will also show that structural Glycoscience of microbial world is a fascinating travel through astounding chemical structures with no parallel in any other kingdom.

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A GAME OF TERPENES: STRUCTURE, STEREOCHEMISTRY, AND BIOSYNTHESIS OF MARINE TERPENOIDS

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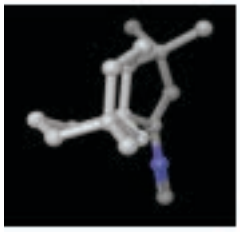
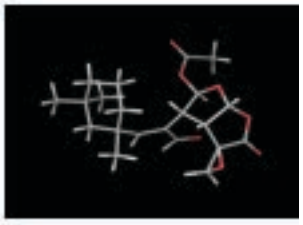
This talk will describe how an ecological study on chemical defense in nudibranchs led to the isolation and characterization of new terpene metabolites with extensively-rearranged carbon skeletons. Case studies that will be discussed include new isocyanoterpenes from phyllidid molluscs[1,2] and epoxy-substituted norditerpenes from *Goniobranchus* spp.[3,4] NOESY data run at 700 or 900 MHz, together with detailed conformational analyses informed by molecular modeling, DFT calculations, and in one example total synthesis in collaboration with a USA laboratory, enable assignment of individual configurations. The chemistry data are reviewed in an ecological and biosynthetic context.





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LABEL-FREE MS-BASED PROTEOMICS IN TARGET DISCOVERY OF NATURAL PRODUCTS

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Natural products (NPs), due to their peculiar chemical diversity and wide biological activities, can be considered an important source of potential hits in the drug discovery and development pipeline. Therefore, the analysis and discovery of the protein NPs targets is a fundamental demand in the understanding of their pharmacological and/or toxicological role.
The mechanisms of action of a potential drug are mediated by its interaction with proteins and other macromolecules. The most relevant approaches in the direct target identification of small molecules can be classified in *chemical labelling* and *label-free* methods.[1-3] Due to the drabacks of molecular labelling methods, several label-free protein target identification methods have been developed in recent years.
In this lecture, some of the main mass spectrometry-based label-free approaches will be discussed, along with their application on the target discovery analysis of several NPs belonging to diverse chemical classes. Moreover, combination of label-free MS-based chemical proteomics with
biochemical, biophysical and molecular modelling-based orthogonal techniques, for validation of the results or improvement of the target analysis, will be highlighted.

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CONTEMPORARY AND CLASSICAL APPROACHES FOR THE DISCOVERY OF BIOACTIVE SECONDARY METABOLITES

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The isolation of new bioactive natural products is currently a major challenge because of the large number of already known secondary metabolites.[1,2] As a consequence, the redundancy in finding already known compounds is increasing.[1,2] Therefore, strategies to overcome this major issue in finding new bioactive natural products are of much interest. One of such approaches is improving the dereplication step, that can be indispensable to avoid isolation of known metabolites. However, considering that dereplication procedures does not always indicate the exact structures of known compounds, it is frequently necessary to perform either clean-up, fractionation or even isolation of natural products for correct structure assignment. In this presentation it will be discussed strategies to accelerate clean-up, fractionation and isolation of natural products, including combining dereplication, MS- and bioassay-guided isolation, leading favourably to the discovery of new natural products. Examples will include metabolites from marine invertebrates and microbial sources, including fungi and bacteria, from both organic and aqueous fractions.

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UNRAVELING THE MARINE NATURAL PRODUCTS CHEMICAL SPACE

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Small molecules (SMs) not only dominate our ability to treat disease but can also advance our knowledge of biological processes. Bioactive molecular discovering is essential for drug discovery but it requires the ability to navigate the SMs chemical space effectively and efficiently, including previously unexplored regions. A large part of biologically relevant chemical space is occupied by Natural Products (NPs), which have a unique and vast chemical diversity and have evolved to effectively bind to biological macromolecules. NPs may also serve as biologically validated starting points for the design of focused libraries that might provide protein ligands with enhanced quality and probability[1]. Type 2 Diabetes Mellitus (T2DM) is one of the most common metabolic disorders worldwide; its development is primarily caused by defects in any of the molecular mechanisms involved in insulin synthesis, release, and response in tissues. In the state of metabolic disturbance, several major enzymes are abnormally expressed, and they could be interesting targets in drug development. Phosphoeleganin, a complex marine derived polyketide, is a dual-type inhibitor of PTP1B and AR enzymes[2,3], emerging targets for T2DM drug discovery. In order to render the exploration of its biologically relevant chemical space more tractable we used phosphoeleganin in a NP-informed Fragment-Based Ligand and Drug Discovery (FBLDD) campaign to enable the discovery of SMs that bind/modulate specific targets involved in the onset of T2DM and its chronic complications. The complex scaffold of phosphoeleganin was used as starting or guiding material for generation of fragments sets of diverse and unprecedented substructures that retain only certain parts of the MNP's structure while modifying other portions. The phosphoeleganin-based synthetic fragments libraries have been screening against the selected targets. The devised approach represents a novel opportunity to synthesize MNP-inspired compound libraries and to overcome limitations in the use of MNP in drug discovery because of a lack of accessibility and/or synthetic intractability.

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THE EUROPEAN GREEN DEAL: IMPACT FOR COSMETICS

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The European Green Deal (EGD) is a flagship initiative of the European Commission, an expression of its growth strategy and the guiding policy of the post-Covid Recovery Plan. Through this initiative, the EU aims to: become climate-neutral by 2050; protect human life, animals and plants, by cutting pollution; help companies become world leaders in clean products and technologies; help ensure a just and inclusive transition.

The EGD has led to a multitude of policy and legislative initiatives. Several of these are impacting the cosmetics industry, notably the Chemicals Sustainability Strategy (leading to a targeted revision of the Cosmetic Products Regulation, and the revision of two key chemicals Regulations: REACH[1] and CLP[2]), the Ecodesign for Sustainable Products Regulation, the Packaging & Packaging Waste Regulation, as well as the revision of the Unfair Commercial Practices Directive and a new Directive on Green Claims. These will impact cosmetic products and their packaging in terms of sustainability performance, sustainability information (including environmental labels and claims) and digital information.

The cosmetics industry fully supports the green growth agenda of the European Commission and has already taken action, including an ambitious voluntary initiative to support cosmetics companies in the EU towards the 'green transition'.

The presentation will provide insights into the concrete evolution of the regulatory landscape for cosmetics, with a focus on product-related information for consumers and other stakeholders, including the upcoming digital product passport.

1 Registration, Evaluation and Authorisation of Chemicals
2 Classification, Labelling and Packaging of Substances and Mixtures

REMIXING NATURE: THE NEW RENAISSANCE OF PLANT-BASED COSMETIC INGREDIENTS

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Since its inception in ancient civilizations until to the 20th century, the cosmetic field has used primarily naturally available compounds and materials, from mineral pigments to oils and waxes from plants and animals. However, as chemistry developed as a scientific and industrial field, a palette of economical petrochemical derived materials became available and synthetic ingredients have taken a primary role in formulation because of consistency, performance, cost, and overall convenience. Not incidentally, scientific progress led to safer products. Nowadays, great emphasis is given by users to sustainability, providing the pulse for a wave of new efforts aimed at the development of natural derived, highly performing ingredients replacing non-renewable counterparts. Plants synthesize a treasure trove of abundant and useful compounds for securing their structural integrity, store energy or manage water reserves (like carbohydrates and triglycerides): when appropriately manipulated by means of physical, chemical or fermentative processes, these materials may be used as “engineered extracts” or broken-down and transformed into useful building blocks (e.g. glycerine, fatty alcohols and fatty acids), and rebuilt at will to achieve whole new properties. In general, a profitable approach is emerging that melds plant-derived chemicals with polymer chemistry, leveraging abundant multi-source feedstocks to create stable, effective, and more sustainable cosmetic ingredients. This “renaissance” is field-wide and requires creativity, chemical mastery and an open mindset to recognize opportunities: in this quest, the principles of circular economy, eco-design and green-chemistry are truly put at work to deliver safe and industrially viable ingredients with high added value, at the forefront of sustainable chemistry.

PROTEIN GASTROINTESTINAL DIGESTION AND INTESTINAL SENSING FOR SATIETY

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Understanding the mechanisms that connect food gastrointestinal digestion, nutrient sensing at the gut, and satiety signalling are crucial to design foods with an adequate physiological response. Dietary protein is the macronutrient with the highest satiating effect compared to an isoenergetic intake of fat and carbohydrates. Products derived from protein digestion have a more potent effect on gastrointestinal-released hormones than other nutrients, but while the mechanisms of carbohydrate sensing are better understood, those for protein and lipid sensing are less well-defined. This presentation will deal about protein digestion and the study of protein digestion products as signalling molecules in the gastrointestinal tract. Since dietary protein digestion can be modulated by the technological processes applied to food, it would be possible to control hormonal response intake by regulating food digestion through different approaches. The study of *in vivo* digests obtained at different regions of the gastrointestinal tract (duodenum and jejunum) of milk proteins and hydrolysates will be illustrated. Protein digestion is also influenced by the interaction with other food components, such as polysaccharides; and some examples will be shown about developed polysaccharide-casein gel-like structures which have the ability to modulate protein digestion. Hormonal response of gastrointestinal digests and individual peptides in enteroendocrine cells and jejunal organoids will be illustrated. Peptides generated during gastrointestinal digestion act as potent hormone inducers in STC-1 cells, and the involvement of receptors of CaSR and GPR93 was demonstrated by the use of specific inhibitors, although our results pointed to the involvement of additional receptors and/or transporters.

CARBOHYDRATES IN FOOD: CHALLENGES AND OPPORTUNITIES FOR CLEAN LABEL STRATEGIES

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The arise of new methodologies of analysis of carbohydrates allows the revisiting of their structures and their understanding towards the development of new processing applications by food industry. The clean label concept applied to food products targets a total transparency of labelling, with less ingredients, minimization of the use of synthetic food additives, minimally processed foods, no allergen warnings, and no genetically modified organisms, promoting biodiversity. By a straight collaboration between academia and food industry, new quality related ingredients, formulations, and technologies are under development. This effort should result in healthy, tastier, affordable, and appealing processed foods based on natural products, following concepts like “right for me” personalized food, food oriented to specific genders, ages, or lifestyle markets. Examples are the development of polysaccharide films to produce sulfur dioxide free white wines [1, 2] and their extension to the application in cider or wine vinegar due to the presence of α-hydroxypolycarboxylic or α-cetopolycarboxylic acids [3]. Another example of the challenge and solutions achieved so far as a clean label strategy is the increment of soluble dietary fibre and oligosaccharides in apple and pear juices. Also, the brewers spent yeast glucans and mannoproteins can be used as emulsifiers in vegan mayonnaises [4], the inulin-rich tuberous root and derived fructooligosaccharides are able to replace sucrose added in processed jams for use in dairy products, and gelatinized starch combined with inorganic food grade derivatives can replace the recently forbidden use of titanium dioxide in candies and other white foods.

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FROM NATURAL PRODUCTS AND BIOWASTES TO ORGANIC RECYCLABLE ADDED VALUE APPLICATIONS

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Plastic packaging materials have become ubiquitous. They are widely used in the food and beverage, healthcare, cosmetics, consumer goods and home and garden industries. Most plastics start with hydrocarbons from crude oil. Our extensive collaborative work has turned that process on its head by developing biomass-based, biodegradable raw materials from among other sources food processing streams. We applied those natural products and biowastes to produce polyhydroxyalkanoate-based new organic recyclable packaging that rivals conventional petrochemical-based ones in technical characteristics and performance.
In this presentation, we will show several examples of new high performance packaging materials where the raw chemicals derived from for instance food processing side streams are converted, via a low footprint biochemical processing, into a portfolio of bio-based biodegradable building blocks enabling the realisation of complex packaging structures, including laminates and multilayer films, to match key functional requirements of commercial petrochemical plastics, such as gas/liquid barrier properties, mechanical resistance, cold temperature resistance, elasticity, hot-tack and super-repellency among others, while enabling the realisation of a full set of packaging items from rigid to semi-rigid and flexible by tuning the functionalisation of base resins through biosynthesis and traditional and innovative processing routes such as high throughput electrohydrodynamic processing.

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HYALURONIC ACID/GELATIN BASED, A.I. AIDED, SYNTHESIS OF 3D HUMAN TISSUE MIMETICS

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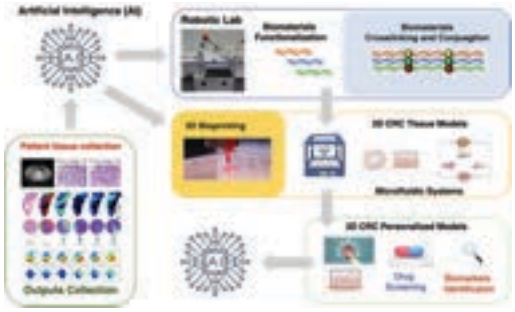
The development of human tissue mimetics is crucial in advancing theragnostic solutions for healthcare management through the generation of in vitro organs for drug screening and cell biology studies [1]. With the advent of automated manufacturing systems like 3D printing, it is now possible to control the formulation of biomaterials, limiting the combinatorial and artisanal chemical approach that is typically used to obtain 3D in vitro tissue models with tuneable properties [2]. In this study, patient-specific 3D gastrointestinal in vitro organoids were generated using also Artificial Intelligence algorithms, to reduce synthetic effort and predict the most efficient synthetic conditions to generate hybrid multifunctional biopolymers with selected properties and features. The methodology was developed exploiting natural products, such as hyaluronic acid and gelatin, functionalized with N_3-PEG-spacer arms and crosslinked with cyclooctine linkers, to generate a library of differently glycosylated bioinks by cell compatible SPAAC click chemistry [3]. The obtained bioinks were fully characterized and validated to generate gastric and intestinal tissue models from patient-derived organoids.



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LIFE IS CHEMISTRY? NOT ONLY

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In his last editorial for Nature, a journal he directed for decades, John Maddox made a bold statement: life is chemistry. Living matter is made of chemicals, but chemistry alone cannot account for the understanding of what is life. The diversity of life is based on emerging properties that go beyond the domains of physics and chemistry, even though living matter obeys the laws of both physics and chemistry. Biodiversity comprises 8-10 million species, but only 2 millions have been named: this lack of knowledge calls for substantial research efforts. The chemical diversity of biological diversity is much higher than the number of species, and amounts to billions of substances that might range from relatively simple ones, to genes regulating complex biological processes. The focus on model organisms has hindered the progress of knowledge of the diversity of biological phenomena, with the assumption that the study of just few species might reveal the secrets of life and even the exploration of biodiversity lost its appeal. This is far from true: every species has original features that might be important for our puroposes. At the Friday Harbor Laboratories, for instance, the study of bioluminecence in the hydromedusa *Aequorea victoria* led to the Nobel Prize in chemistry, due to the discovery of the Green Fluorescent Protein. The species is not easy to keep in the laboratory, but it occurs in the thousands at Friday Harbor, where scientists had easy access to abundant material. Another hydromedusa, *Turritopsis dohrnii*, has exceptional features, since it can go through ontogeny reversal: the adult jellyfish can de-differentiate its cells and re-differenciate them so as to form a polyp, the stage that preceeds the jellyfish in the life cycle. For these features, the species became famous as the immortal jellyfish. These two examples show that the exploration of biodiversity might lead to discover interesting (and profitable) features that might have important applications, but if we do not investigate the species, we will not find out their chemical properties. Biodiversity exploration, followed by its chemical exploration, is still an open frontier of research that is conducive to future important results.

THE POTENTIAL OF CHEMICAL ECOLOGY TO EXPLORE MARINE BIODIVERSITY AT ECOSYSTEM, SPECIES, AND GENETIC LEVELS

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As an interdisciplinary research area, chemical ecology arises from "a partnership between biologists and natural products chemists united by a shared vision and empowered by complementary skills"[1]. It is but one tool for exploring the vast variety of biotic interactions that are mediated by small molecules. Chemosensation actually provides all living organisms with chemical information regulating key biological processes, including competition, predation, nutrition, defense, and reproduction in a variety of environmental conditions[2-5]. In spite of the critical contribution of chemical ecology to evolutionary biology and to applicative sectors such as agriculture and medicine, limited research efforts are currently devoted to this field[6]. Moreover, the ecological roles and diversity of natural products are barely considered when proceeding to biodiversity assessments.
In the hope to bring new life to a discipline that should no longer be underestimated when approaching the study of the variety and variability of organisms living on Earth, in this communication I will use data from my personal experience in marine chemical ecology to show how chemically-mediated interactions affect all levels of biodiversity, from ecosystem and habitat diversity to species and genetic diversity.

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THE NEED OF HEALTHY OCEANS

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The concept of 'One Health' refers to the collaboration of multiple disciplines and sectors working locally, nationally and globally to contribute to the optimal health for humans and the environment. The marine system is a crucial component of the 'One Health' framework since it largely contributes to a wide range of ecosystem services with strong and direct links to human well-being. Increasing human impacts can challenge the possibility of reaching a sustainable use of our ecosystems with enormous consequences on the possibility to reach the targets set by the One Health Agenda. Here, I will document potential solutions coming from the conservation, management and restoration of marine systems stressing the need for urgent, large-scale interdisciplinary approaches to reverse present trajectories of change.

FROM BIODIVERSTIY TO PHARMACODIVERSITY: HARNESSING MOLECULAR MECHANISMS FOR FUTURE DRUG DISCOVERY

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Natural products represent a fascinating reservoir of highly diverse bioactive molecules that have co-evolved within biological matrices and biochemical contexts. This unique feature has made them an inspiring source for drug discovery even in the present day [1]. Advancements in technology over the past few years, particularly in reverse chemical genetic and proteomic approaches, have enabled us to decipher the mechanism of action (MOA) and targets of these natural products.

To fully utilize the potential of bioactive natural products, it is crucial to comprehend their pharmacological properties and to engage in biochemical analyses. Proper categorization requires a comprehensive understanding of their MOA, molecular targets, and the underlying biological processes they influence. Unfortunately, a major challenge that hinders progress in this field is the limited data on the bioavailability and metabolic stability of these compounds.

In my presentation, I will demonstrate how analytical methodologies, when combined with biochemical assays, can play a pivotal role in enhancing our understanding of the workings of natural products and that biodiversity significantly contributes to pharmacodiversity. By illustrating the workflow from in vitro assays to proof-of-concept studies conducted *in vivo*, I will showcase selected examples related to cannabinoids, coumarins and non-proteinogenic amino acids [2]

Through this presentation, we aim to shed light on the untapped potential of diverse bioactive natural products and how their understanding can be furthered with the application of cutting-edge analytical techniques and biochemical assays. By bridging the gap between in vitro and in vivo studies, we hope to accelerate the discovery of novel therapeutics inspired by nature's bounty.

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LICHEN BIODIVERSITY IN TERRESTRIAL ECOSYSTEMS: AN EVOLUTIONARY, BIOGEOGRAPHIC, AND ECOLOGICAL PERSPECTIVE

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Lichens are defined as miniature ecosystems in which several different organisms interact, the two main components being the mycobiont belonging to Ascomycota, and photobionts belonging to green algae or cyanobacteria. The symbiotic life style of fungi evolved several times among Ascomycota and therefore lichens are not a monophyletic group. In general, lichenization is an obligate lifestyle, even if in some cases it is facultative, depending on environmentaò conditions.
While the first fossil records date back to the Devonian period, most of the current lichen diversity evolved since the last mass extinction at the Cretaceous-Paleogene transition when several hyper-diverse groups started their radiation, as in the case of Parmeliaceae.
Lichens are widespread all over the world, in all terrestrial ecosystems, from warm-arid deserts to rainforests, high elevation habitats as well as polar regions. A small group of mainly cryptic species in the Verrucariaceae is typical of freshwater habitats and tidal line of rocky shores. While lichens have in general a wide distributional range, some geographically restricted taxa are also known, as in the case of the amphibian Lobothallia hydrocharis (Poelt & Nimis) Sohrabi & Nimis known only from Sardinia so far.
Lichens are poikilohydric organisms, thus extremely sensitive to climate conditions. For this reason, many species are endangered by climate change that may impact lichen diversity at the taxonomical, functional, as well as phylogenetic level. This may imply negative effects on ecosystem functioning, since lichens paly several ecological roles and provide relevant ecosystem services that are also related to their rich and peculiar secondary metabolism.
Several new species are described each year indicating that a lot effort is still needed to better define the picture of lichen diversity. This would provide the basis for conservation activities that are still in their infancy despite the fact that several species may be soon lost before they are known.

PROTEOMICS-AIDED INSIGHT TO DISCLOSE NATURAL PRODUCTS CELLULAR TARGETS

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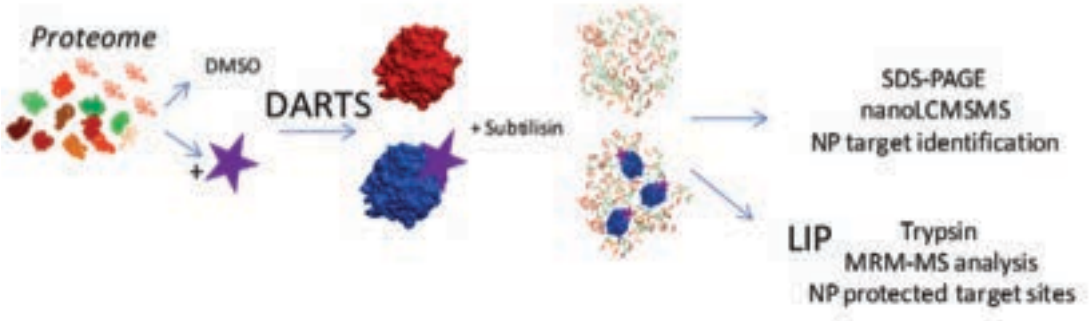
Actually, the identification of the potential primary cellular targets of bioactive natural products (NPs), along with their off-targets, is an important concern. Currently, affinity purification targets identification methods based on mass spectrometry, called AP-MS, are the most reliable ones [1]. However, they are limited by the NPs chemical modification: to overcome this constraint, a simple, universally applicable approach based on the direct binding of an unmodified compound to its targets, termed DARTS (drug affinity responsive target stability), can be performed. DARTS takes advantage of a reduction in the protease susceptibility of the target protein upon drug binding. This phenomenon allows the drug target(s) to be revealed by a gel-based approach, useful for protein bands visualization showing different protease accessibility, and proteomics identification [2]. Then, LiP-MRM approach couples limited proteolysis (LiP) with targeted mass spectrometric tools, exploiting the sensitivity and background filtering capabilities of multiple reaction monitoring (MRM) experiments [3]. As previously described for DARTS, LiP involves the use of a broad-specificity protease, such as subtilisin, under controlled conditions such that primary cleavage sites are dictated by the structural conformation of the protein which can be modified by drug interaction. Altered LiP patterns can be measured directly in a complex proteome matrix pointing out which protein regions are masked by NPs. Several examples will be discussed.



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RECENT ADVANCES IN COMPUTATIONAL NMR METHODS IN THE STRUCTURAL ELUCIDATION OF NATURAL PRODUCTS

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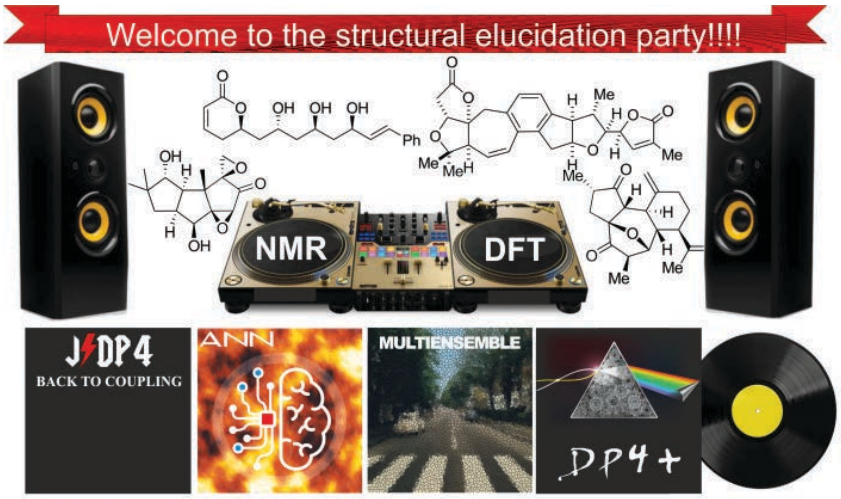
Nuclear magnetic resonance (NMR) is undoubtedly the most important spectroscopic technique for the structural and stereochemical elucidation of natural products. However, despite the impressive advances that have been made in this field, it is striking the large number of structures erroneously assigned based on spectroscopic data.[1] The use of quantum NMR calculation routines to facilitate the 3D elucidation has grown exponentially in last years, consolidating as an ideal complement to experimental NMR studies. The introduction of sophisticated strategies of data correlation and computational procedures paved the way for the development of new and exciting methodologies during the last decades, which proved to be extremely valuable in different areas of chemistry, including natural products discovery, medicinal chemistry, and organic synthesis.[2]

Our research team has made important contributions in this field,[2] developing popular methods such as ANN-PRA,[3a] DP4+,[3b] *J*-DP4,[3c] ML-*J*-DP4,[3d] and MESSI.[3e] In this lecture, I will discuss our recent advances, and the application of these methodologies in facilitating the structural elucidation of natural products.



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FROM STRUCTURAL STUDIES OF NATURAL PRODUCTS TO THE DESIGN OF NOVEL BIOACTIVE DERIVATIVES BY NMR AND COMPUTATIONAL TECHNIQUES

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Natural Products (NPs) extracted from marine and terrestrial organisms are an unlimited source of bioactive compounds.[1] They are characterized by a huge diversity of 3D- scaffolds, biochemical specificity, and other molecular features, making them a valid starting point for lead generation in drug discovery. The correct elucidation of the structural features of already reported natural products ("old") and unprecedented ("new") ones represents the early key step for accelerating their complete biochemical and pharmacological characterization. In this regard, the combination of NMR spectroscopy with modern computational techniques represents one of the most effective approaches to achieve this task. The DFT/NMR integrated method[2] and its applications can be employed to elucidate the structural features of NPs and, accordingly, as a proper support to total synthesis as well as to understand their mechanism of action. Indeed, the comprehension at a molecular level of their interactions with specific targets involved in pathologies represents a cornerstone for the successive stages of drug discovery. The final step of this process concerns the design and identification of novel molecular platforms[2] through computer-aided modifications of their original active skeletons, aimed at the development of semi-synthetic/synthetic derivatives with enhanced potency and selectivity towards specific targets involved in the pathological events, e.g., inflammation and/or cancer. Furthermore, prompted by the interest in discovering the potential interacting targets of the "new" and "old" NPs, the Inverse Virtual Screening (IVS) approach[3] can also be useful for achieving the drug repurposing task.[4] In this way, IVS can quickly orient the subsequent biological investigations, such as the specific binding assays of the investigated compounds with the identified putative interacting proteins as well as subsequent specific pharmacological tests. In summary, here we have reported the application of several orthogonal *in silico* tools, developed and optimized by us,[5] to reduce the number of false positives during the "hit identification" process to disclose natural, nature-inspired and novel synthetic molecular platforms.

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FOSTERING INTERDISCIPLINARY CONVERSATIONS FOR A SUSTAINABLE RESEARCH

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Sustainability science has emerged over the last two decades as a vibrant field of research and innovation. Like agricultural science and health science, sustainability science is defined by the problems to be addressed rather than by the disciplines it employs. In particular, the sustainability science seeks to facilitate the transition toward the sustainable use and exploitation of the earth resources. To this end, circular and sustainable use of biomass resources, such microorganisms, is becoming the fundamental key step, because of their considerable annual volumes and high economic potential.

Cyanobacteria are ubiquitous photosynthetic microorganisms, living in almost all phototrophic aquatic environments. Over the past two decades, worldwide attention has been given to the ecotoxicological aspects of cyanobacteria blooms and their exploitation as a source of bioactive compounds. Indeed, they represent a not yet fully explored source of new lead compounds for drug discovery.[1] Eutrophic conditions allow cyanobacteria to bloom, producing large green mats covering water surfaces and producing cyanotoxins giving rise to a serious problem for public health.

The study of cyanobacteria blooms and their use as biomass for drug discovery has been approached trough a multidisciplinary strategy for the early detection and constant monitoring of cyanobacterial blooms and their toxins based on combined remote/proximity sensing and MS-based molecular networking (FDS). The strategy has been validated in several case studies [2-4]. FDS for cyanobacterial blooms and associated cyanotoxins let to attribute the red colour to a bloom of *Planktothrix rubescens*, a toxin-producing cyanobacterium. In addition, our study allowed the detection and identification of 14 anabenopeptins. The same toxins were detected in water sea and bivalve samples collected from the outlet of the channel of Lake Avernus in the sea. Molecular networking was used to detect the cyanotoxins in the extracts, avoiding the purification process and, contributing to sustainable research aiming to reduce chemical waste and to use a cost-effective and low-energy-consuming approach.

Sustainability science needs an integration of knowledge across disciplines. Multidisciplinary research is often essential to develop an integrated approach that allow the understanding of complex environmental issues and provide data for decisionmakers world-wide. Multi-disciplinary research has the potential to develop new approaches to defining and analyzing a research problem that more closely represents the reality in which such problems are situated.

In this communication, the focus is on the interdisciplinary research project on cyanobacteria, where scientists from different disciplines share methods and data to work towards a common project goal.

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ORAL PRESENTATIONS

INVESTIGATION OF THE MECHANISM OF ACTION OF THE PROPOLIS-*PUNICA GRANATUM* MIXTURE AS AN ADDITIONAL THERAPEUTIC SOURCE FOR RHEUMATOID ARTHRITIS TREATMENT

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Rheumatoid arthritis (RA) [1] is a chronic autoimmune disease characterized by the production of diverse inflammatory factors. Since prolonged use of classical anti-inflammatory drugs exert several side effects, the identification of natural products capable of relieving RA symptoms could pave the way for new therapies for this disease. We have therefore devised and assayed a herbal preparation based on a traditional formulation from Campania region that has long been used against local and systemic inflammation, and composed by propolis and pomegranate peel. Firstly, propoli-pomegranate peels mixtures (PB) in different ratios were prepared and tested for their anti-inflammatory ability to reduce IL-6 secretion on human synoviocytes, a cell line suitable to study RA disease. Mixture PB 1:2 (w/w) was identified as the preparation exerting the most effective anti-inflammatory activity (IC_{50} = 25 µg/mL). In order to identify the compounds responsible for the observed effect, this mixture was subjected to chromatographic separation, collecting eight fractions (PB/1-8), whose anti-inflammatory activity was assayed at 12.5, 25, and 50 µg/mL by ELISA assay. Fraction PB/8, the most active one, was able to induce a significant and dose-dependent inhibition of IL-6 secretion (IC_{50} = 50 µg/mL). Therefore, a phytochemical characterization of this fraction was performed, leading to the identification of pinobanksin and phenethyl caffeate as the most representative constituents. To investigate the biological activity of PB/8, firstly its effect on the expression of inflammatory proteins was analyzed, observing that synoviocytes incubation with 25 µg/mL PB/8 caused STAT-3 cleavage and a significant over-expression of inducible-COX-2. It is noteworthy that the expression level of the above markers was restored when PB/8 concentration increased to 50 µg/mL. To elucidate the molecular mechanism underlying these effects, a chemical-proteomic approach, Drug Affinity Responsive Target Assay[2], was performed on PB/8 and its major constituents. Obtained results suggested several potential proteins belonging to MAPK signaling as putative targets. Finally, the anti-inflammatory activity of these components was further investigated in 3D-model of synoviocytes cells.

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SYNTHESIS AND PHYSIO-CHEMICAL PROPERTIES OF SULPHATED TAMARIND (*TAMARINDUS INDICA L.*) SEED POLYSACCHARIDE

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Tamarind seed polysaccharide (TSP) is a galactoxyloglucan isolated from seed kernel of Tamarindus indica. It is widely used in the industrial fields, due to its physical, chemical, and biological properties [1]. It is a neutral and water soluble polysaccharide with a high viscosity which makes its solubilization difficult. The absence of charges on the chain makes electrostatic interactions not possible with small molecules and large biomolecules. For this reason, a chemical modification of this biopolymer is carried out adding sulphated groups on the TSP chain. The presence of negatively charged groups may allow a better solubilization of the polysaccharide and also specific binding to proteins or receptors, giving new biological properties to TSP [2]. The sulfation reaction of TSP was performed in one-step process, using dimethylformamide as a solvent, and sulphur trioxide pyridine complex as reagent. Characterization of the chemical-physical properties of the sulphated products are carried out through different analytical approaches to verify the successful synthesis. Studies of viscosity, morphology, chemical structure and molecular weight distribution are conducted to obtain the complete characterization of the derivatized products. Sulphated TSP products have a molecular weight in the range of 400-1000 kDa, while the substitution degree determined by potentiometric titration is 5-50% of all the hydroxyl groups present within the repetitive unit of TSP. Further study of the sulphate groups distribution was performed by NMR and LC-MS after enzymatic hydrolysis of sulphated TSP with cellulase. Sulphated derivatives show higher solubility and exhibit lower viscosity than unmodified TSP.

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MASKWIO'MI: INVESTIGATION OF MEDICINAL PROPERTIES OF A CANADIAN INDIGENOUS TOPICAL SKIN REMEDY MADE FROM BIRCH BARK

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Birch bark has been used in cultures around the globe for medicinal purposes. Indigenous knowledge in Atlantic Canada is rich but at risk of being lost as Mi'kmaq (L'nu) elders pass away and the oral knowledge of how it is used is forgotten. One such traditional Mi'kmaq remedy is maskwio'mi (maskwi = birch bark; o'mi = oil) which is a viscous extract produced from paper birch (*betula papyrifera*) and is a skin ointment with exhibits excellent alleviations of a variety of skin issues such as eczema, psoriasis, acne, sun burn, rashes and mosquito bites. The Bierenstiel research group is working closely with the local Mi'kmaq First Nation community of Membertou in Sydney, Nova Scotia using 2-Eyed Seeing methodology, i.e. balancing science and Indigenous knowledge, for the preservation and understanding of Mi'kmaw remedies.[1]

Birch bark is made of up to 25 wt% (dried) betulin, a pentacyclic triterpene. The biological activities of betulin, betulinic acid (1°OH group oxidized to COOH) and their derivatives have been reported to include anti-inflammatory, antibiotic, antimalarial, anti-viral, antineoplasic, anti-tumor, analgesic, and astringent properties. [2,3]

UPLC-QToF-MSMS and GC-MS analysis of the raw extract show the presence of over 200 compounds. We postulate that the medicinal properties of maskwio'mi are based on derivatives of betulin that are produced in a thermal reaction during the extraction process. Our research group developed proprietary technology mimicking the fire pit conditions for accurate experimental reproducibility and commercial scale-up capability. The bark extracts showed excellent broadspectrum antibiotic properties against strains of Gram+ and Gram– strains with disc diffusion assays and MIC experiments. We have preliminary results on anti-inflammatory properties. Additionally, we could link an ancient creation myth of the Mi'kmaq people about a secret hunter for coding of birch bark being beneficial to bad skin as part of linguistic analysis of an oral tradition culture.

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IN VITRO AND IN VIVO PHARMACOLOGICAL CHARACTERIZATION OF A POTENT INDOLE-BASED SOLUBLE EPOXIDE HYDROLASE INHIBITOR

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Soluble epoxide hydrolase (sEH) emerged as a novel potential therapeutic target for the treatment of several pathologies associated with inflammatory disorders, such as pancreatitis,[1] rheumatoid arthritis,[2] cardiovascular[3] and kidney diseases.[4] In this work we describe the design, synthesis and pharmacological characterization of novel sEH inhibitors. Starting from a previous work, where we identified a potent indoline-based 5LOX/sEH dual inhibitor, we decide to further explore the structural requirements to obtain sEH selective inhibition.[2] The *in-silico* studies suggested that an increase in rigidity of the central scaffold would have strongly modified compounds selectivity in favour of sEH enzyme. For this reason, in the novel series we replaced the indoline scaffold with indole and carbazole nucleus, investigating also the effects exerted by different substituent at N-1 and C-5 positions. The synthetized molecules underwent to extensive in vitro analysis revealing compound **28** as the most promising one (IC_{50}= 2.0±1.1 nM against isolated sEH). This is why derivative **28** was challenged for its anti-inflammatory activity in an acute pancreatitis (AP) in vivo murine model. The performed analysis showed that **28** significantly reverse the cerulein-induced injury, exhibiting a protective effect as evidenced by reduction of edema, cell infiltration and neutrophils number. The efficacy of **28** in the AP murine model, also relies on its suitable pharmacokinetic properties, as assessed by *in vivo* pharmacokinetic studies. Collectively, compound **28** displayed remarkable in vivo anti-inflammatory properties, highlighting its potential as anti-inflammatory agent for the treatment of AP.

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BIOACTIVE NEOLIGNANS MAGNOLOL, HONOKIOL AND OBOVATOL AND THEIR SYNTHETIC ANALOGUES AS DIGESTIVE ENZYMES INHIBITORS

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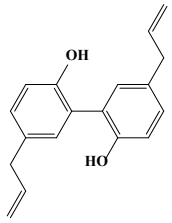
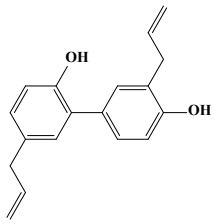
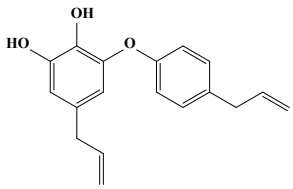
Obesity is a complex disease defined as an excessive amount of body fat. It is considered a risk factor for chronic diseases and disabilities such as cardiovascular diseases, cancer, osteoarthritis, and hypertension. [1] The incidence of obesity is frequently associated with the incidence of type 2 diabetes, a metabolic disorder characterized by insulin hormone dysfunction and, as a result, by high blood glucose levels. It is also noteworthy that hyperglycemia associated with type 2 diabetes is characterized by an increase in the production of reactive oxygen species, causing oxidative tissue damage. Several strategies have been developed to inhibit the enzymes involved in the dietary disease: pancreatic lipase, the enzyme responsible for the hydrolysis of free fatty acid; α -amylase and α-glucosidase, carbohydrate hydrolysing enzymes, whose inhibition is a well-established strategy to manage hyperglycemia. Orlistat and Acarbose are, respectively, approved anti-obesity and antidiabetic drugs, showing, however, some side effects.[2] For this reason, natural products and their analogues have been studied to find new and safe enzyme inhibitors with no or low side effects. The phytochemical studies of the genus of *Magnolia spp*. have provided a few compounds with different structures and biological activities. Among them, the neolignans magnolol, honokiol have been extensively investigated for their wide range of biological effects [3,4], while obovatol has attracted little attention. The present work reports our recent efforts to evaluate the inhibition properties of the three natural polyphenols and their synthetic analogues as promising α -amylase, α-glucosidase and lipase inhibitors.



Magnolol



Honokiol



Obovatol

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CROSSIELLIDINES A-F, UNPRECEDENTED PYRAZINE-ALKYLGUANIDINE METABOLITES WITH BROAD-SPECTRUM ANTIBACTERIAL ACTIVITY FROM *CROSSIELLA* SP.

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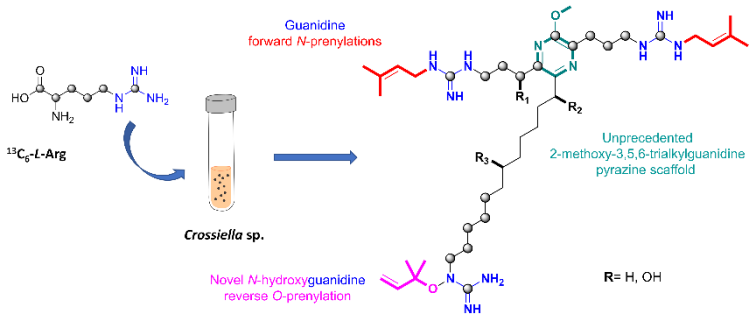
Unexplored bacterial taxa are a potential gold mine for the discovery of new bioactive compounds. Crossiellidines A-F, a family of novel antibacterial pyrazine-alkylguanidine metabolites, were isolated from the minor actinomycete genus *Crossiella*.[1] The intriguing structures of these new natural products were determined by 2D NMR spectroscopy and shown to be derived from an unprecedented 2-methoxy-3,5,6-trialkylguanidine pyrazine scaffold, further decorated with highly unusual “on-heteroatom” prenylations and varying hydroxylation degrees. The novel substitution pattern of the 2-methoxypyrazine core inaugurates a new class of naturally occurring pyrazine compounds, whose biosynthetic implications will be discussed in this talk. Stable isotope-guided metabolomics combined with genome analysis allowed us to propose a biosynthetic pathway to these metabolites from arginine. Crossiellidines displayed remarkable, broad-spectrum antibacterial activity, against relevant Gram-negative pathogens such as *Escherichia coli* and *Klebsiella pneumoniae,* and against methicillin resistant *Staphylococcus aureus*.[2]



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DIARYLHEPTANOIDS FROM THE SEAGRASS *ZOSTERA MARINA*: AN INTRIGUING HISTORY

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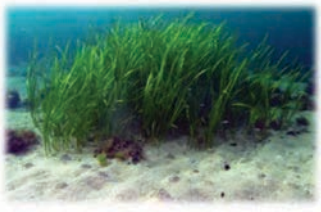
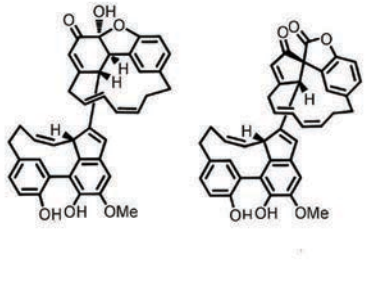
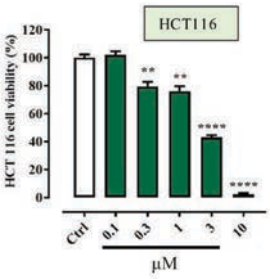
Natural products are a great source of inspiration due to the wide variety of chemical structures with potential pharmaceutical, food and industrial applications, and this is even more true when their natural source is abundant and accessible.[1] For this reason, the widespread seagrass *Zostera marina* is an ideal potential source of natural products.

Indeed, *Z. marina* was shown to contain a set of structurally intriguing cyclic diarylheptanoids, including two tetracyclic diarylheptanoids, zosteraphenols A and B,[2] and three diarylheptanoids heterodimers, zosterabisphenones A, B and C.[3,4] These compounds are structurally unique, featuring keto tautomers of catechol or rearranged benzene rings. Their characterization was made challenging by the coalescent NMR signals shown by all of them because of conformational equilibria and was achieved with the aid of quantum mechanical studies. In addition, zosterabisphenones were shown to be selectively cytotoxic against HCT116 colorectal human cancer cells, and this prompted us to an in-depth study on their mechanism of action and their *in-vivo* activity antitumor effects using the xenograft mouse model of colorectal cancer.

The presentation will give an overview of the structure of these unique compounds and of the methods used in their challenging structure elucidation, along with the most recent results on their *in vitro* and *in vivo* antitumor activity in colorectal cancer.







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EXPLOITATION OF LIGURIAN ROSEMARY ECOTYPES FOR A SUSTAINABLE DEVELOPMENT

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Salvia rosmarinus (L.) Spenn. [1] is a Mediterranean aromatic shrub widely cultivated for its aromatic, medicinal and ornamental uses. The species is growing in a large diversity of habitats and from sea level up to 1500 meters [2]. *Salvia rosmarinus* "Eretto Liguria" ecotype is widespread in Northwest Italy, and it is commonly used by farmers for cuttings and for marketing. To develop local agriculture, the chemical-biological characterization of this ecotype in comparison with other cultivars or ecotypes is attractive in terms of sustainability and low environmental impact. A targeted NMR metabolomic approach highlighted the influence of the different geographical locations on the composition and relative content of bioactive metabolites in rosemary extracts. Considering a single geographical area, the genetic factor becomes relevant. Multivariate statistical analysis and self-organizing maps (SOMs) showed that the accessions of "Eretto Liguria" appeared well characterized compared to the others and had a good content in the bioactive carnosic acid. Recently, the enhancement of plant waste is attracting more attention. Therefor, aim of the present study was also to investigate the quali-quantitative profile of these rosemary ecotypes to identify new possible applications in the agro-food chain. Soft rot diseases belonging to *Pectobacterium* genus represent a serious problem in potato culture. Rosemary methanolic extracts showed ability in reducing the pectolytic activity of a strain of *P. carotovorum* subsp. *carotovorum* on potato tissue. The bacterial soft rot assay, performed with the pure abietanes that mostly characterize the extracts, showed that isorosmanol, carnosol and 7-*O*-methylrosmanol, at the concentration of 1000 ppm, completely inhibited the bacterial soft rot.

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SEARCH FOR DENGUE ANTIVIRAL COMPOUNDS FROM *GONIOTHALAMUS LANCEOLATUS* Miq., AN INDIGENIOUS MALAYSIAN PLANT

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In our continuing search for bioactive compounds, we scrutinized the chemical constituents present in the leaves of *G. lanceolatus* Miq., an endemic plant from the rainforest of Sarawak, Malaysia. As these leaves are traditionally used by the indigenous population as a mosquito repellent and to treat fever, we were inspired to examine the plant for potential anti-dengue activity. Preliminary screening showed that at a concentration of 50 µg/ml, the dichloromethane extract of the leaves was able to inhibit 90.9% of Dengue Virus Type-2 (DENV-2). Dose- dependent plaque assays gave an IC_{50} of 4.16 µg/ml with a selectivity index (SI) of 5.82. Chemical profiling of the active fraction using high-resolution mass spectrometry (UHPLC-ESI-Orbitrap), via data-dependent MS/MS experiments dereplicated nine styryllactones from reference standards. Eighteen more styryllactones were further annotated by a molecular database search. Seven styryllactones were isolated from this active fraction. Bis-styryllactone goniolanceolatin A was further evaluated using quantitative reverse transcription qRT-PCR to determine the viral RNA level. The qRT-PCR data showed that the IC_{50} value for the compound was 5.07 µg/mL, and its corresponding SI value of 5.30. Docking studies of goniolanceolatin A showed that it can form binding interactions with crucial amino acids of the Envelope (E) of DENV proteins.

PLUMOSIDE, A NOVEL CYSTEINE-RICH PEPTIDE HYDROGEL FROM THE IRISH SPONGE *PHORBAS PLUMOSUS*

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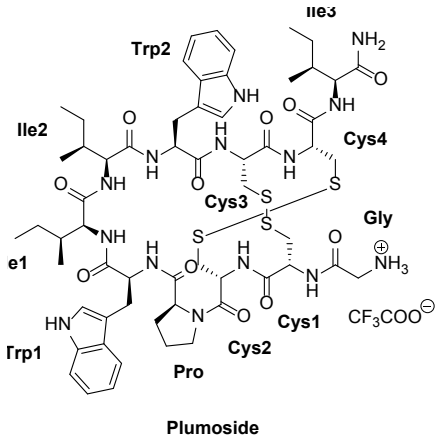
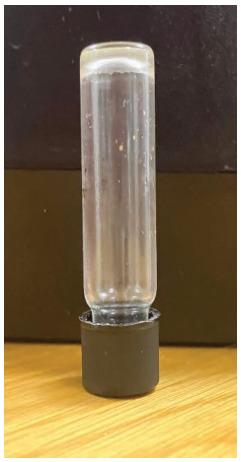
With a large underexplored marine habitat in Ireland, including the much of the coastline and the deep sea, we have conducted a number of extensive collections to investigate its chemical diversity. Interestingly, through our investigations we have identified a large number of samples that contain highly stable small peptides. The presence of peptides in such a diverse range of organisms throughout Ireland suggests that the temperate waters of the North Atlantic may be a good source of peptide-based molecules for various biological roles.

This led us to investigate the sponge, *Phorbas plumosus*, collected from the tidal areas of Galway bay, Ireland. Following HPLC purification, extensive NMR and MS/MS elucidation allowed the characterisation of a novel eleven residue cysteine-rich bicyclic peptide, plumoside. This peptide contained a large proportion of hydrophobic residues and a C-terminal amide posttranslational modification. Reduction and MS/MS experiments allowed the assignment of two disulfide bonds in a rare parallel tetracysteine ring (4CR). The only marine peptides with this 4CR structure reported are all part of the conotoxin T-1 family.[1,2]

While performing various structural elucidation steps with the purified peptide it was observed that the addition of water with the peptide formed a hydrogel complex. With peptide hydrogels being of interest for numerous applications including drug delivery, agricultural additives and food production we are conducting an extensive investigated into the physical and biological properties of this hydrogel.



Plumoside



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CHEMISTRY AND LEISHMANICIDAL ACTIVITY OF *ARTEMISIA* SPP. FROM GREECE

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According to WHO, one billion people are at risk of *Leishmania* infection while it is estimated that there are 12 million infected people worldwide[1]. Current chemotherapy for treating leishmaniasis exhibits numerous limitations, therefore there is an urgent need to develop new antileishmanial therapy treatment. As *Artemisia* spp. have been used as traditional medicine since ancient times for various purposes[2,3], we sought to investigate the antileishmanial activities of five different species of *Artemisia*, collected from different regions of Greece. Ethyl acetate and hydroethanolic extracts were prepared, analysed by LC-HRMS, and tested against promastigote *Leishmania donovani* and *Leishmania infantum*. The ethyl acetate extracts of *A. absinthium, A. vulgaris* and *A. umbelliformis* subsp. *eriantha* had the highest leishmanicidal activity against *Leishmania infantum* promastigotes with an IC_{50} of 78.6, 22.8 and 12.22 μg/mL respectively. Moreover, the active extracts had a low inhibition on THP-1 macrophages. We proceeded to the phytochemical investigation of *A. umbelliformis* subsp. *eriantha*. The active ethyl acetate extract was analysed to reveal a high content of eudesmane type sesquiterpene lactones, methylated flavonoids and polyacetylenes, while all isolated-compounds were characterised by means of NMR and HRMS. Chromatographic fractionation by Countercurrent Partition Chromatography (CPC), column chromatography, preparative HPLC and preparative TLC yielded four cis-fused C-8 lactones (telekin, epitelekin, umbellifolide, 5-deoxy-5-hydroperoxy-telekin) and one methylated flavonoid (eupatilin) previously isolated from *A. umbelliformis* [4, 5, 6] , five compounds found for the first time in *A. umbelliformis* subsp. *eriantha* and one novel eudesmane type sequiterpene lactone.

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MS-GUIDED DISCOVERY OF NOVEL TRICYCLIC GUANIDINO COMPOUNDS FROM TETRODOTOXIN-BEARING NEWTS AND NEW ANALOGUES OF ANTIMALARIAL PHOSPHOTRIESTER SALINIPOSTIN FROM ACTINOBACTERIA

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Mass spectrometry (MS)-guided screening method has been developed into a practical and common technique in the field of natural product chemistry. In this study, two compound classes containing highly unusual structures were screened using an MS-guided screening strategy. 1) Tetrodotoxin (TTX, **1**), a potent neurotoxin, occurs in a wide range of marine animals and terrestrial amphibians such as pufferfish, bivalves, newts, and frogs. Despite high interest in this unique toxin, TTX biosynthesis remains enigmatic. We have identified a number of TTX analogues and possible biosynthetic intermediates to elucidate the biosynthetic pathway of TTX.[1] Here, new *N*-hydroxy type TTX analogues[2] and cyclic guanidino compounds such as novel skeletal tricyclic compound **2**[3] were discovered from the toxic newts using LCMS. These structures were elucidated using NMR spectroscopy, including a long-range HSQMBC (Fig. 1). Based on the structures of new compounds, the biosynthetic/shunt pathways of TTX in terrestrial environments were proposed. 2) Salinipostin has a highly unusual phosphotriester structure and potent antimalarial activity.[4,5] New analogues of salinipositn (**3**–**5**) were discovered from actinobacteria via MS-guided screening focusing on their characteristic fragment ions (Fig. 2). They exhibited potent inhibitory activity against monoacylglycerol lipase (MAGL),[6] a key target protein of salinipostin.

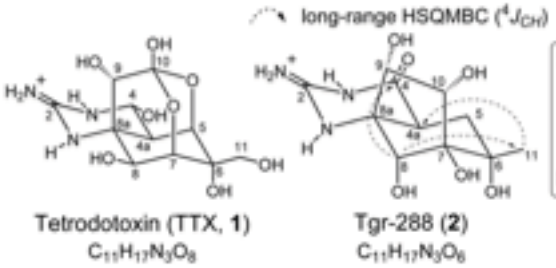

Fig. 1. Structures and molecular formulae of TTX and **2** with long-range HSQMBC correlations.

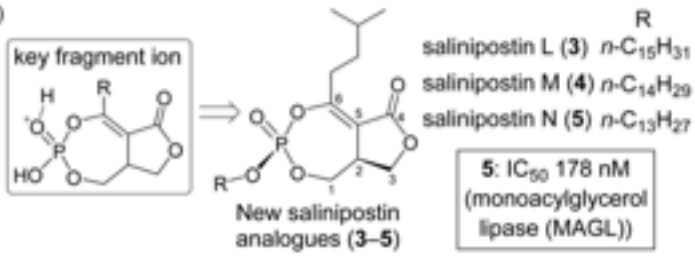

Fig. 2. Structures of salinipostins and key fragment ion for MS-guided screening, and MAGL inhibitory activity of **5**.

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STRUCTURAL DETERMINATION, TOTAL SYNTHESIS, AND BIOLOGICAL ACTIVITY OF IEZOSIDE, A HIGHLY POTENT CA^{2+}-ATPASE INHIBITOR FROM MARINE CYANOBACTERIA

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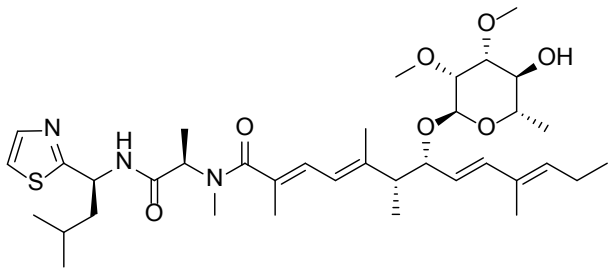
Natural products are abundant and powerful resources to discover novel bioactive compounds, and natural products produced by marine cyanobacteria are especially attractive. As a result of a series of investigations to discover new drug leads from marine cyanobacteria collected in Okinawa, Japan, we have isolated a unique compound with a novel chemical scaffold and extremely potent biological activity from the marine cyanobacterium *Leptochromothrix valpauliae*, which we named iezoside (**1**). In this presenation, we report our comprehensive analysis of iezoside (**1**), which covers its isolation, structural characterization supported by density functional theory (DFT) calculations and statistical analysis, total synthesis, and clarification of the mode of action of its potent antiproliferative activity (IC_{50} 6.7 ± 0.4 nM against HeLa cells).[1]



Iezoside (**1**)

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PATHWAY RETROFITTING YIELDS INSIGHTS INTO THE BIOSYNTHESIS OF ANTHRAQUINONE-FUSED ENEDIYNE

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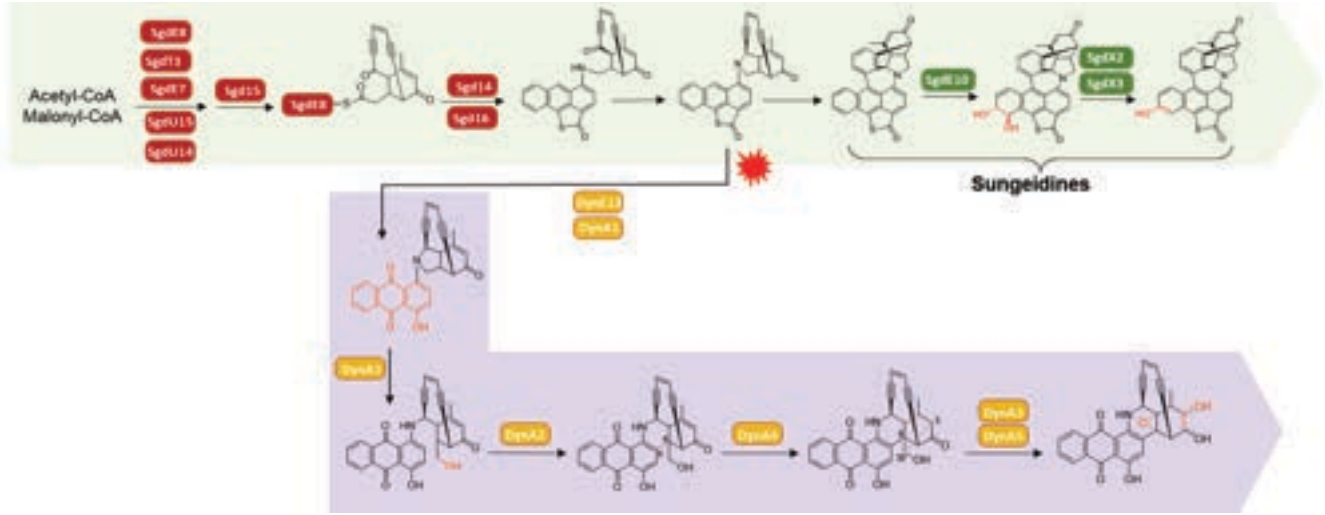
Anthraquinone-fused enediynes (AQEs) are renowned for their distinctive molecular architecture, reactive enediyne warhead and potent anticancer activity. Although the first members of AQEs, i.e., dynemicins, were discovered three decades ago, how their nitrogen-containing carbon skeleton is synthesized by the microbial producers remains largely a mystery. We recently discovered a non-canonical AQE pathway (sungeidine pathway) that contains the upstream enzymes for AQE biosynthesis. Retrofitting the sungeidine pathway with genes from the dynemicin pathway not only restored the biosynthesis of the AQE skeleton but also produced a series of novel compounds likely as the cycloaromatized derivatives of chemically unstable biosynthetic intermediates. The results suggest a cascade of highly surprising biosynthetic steps leading to the formation of the anthraquinone moiety, the hallmark C8-C9 linkage via alkyl-aryl cross-coupling, and the characteristic epoxide functionality. The findings yielded unprecedented insights into the late stage of AQE biosynthesis.



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OPTIMIZATION OF URSOLIC ACID EXTRACTION IN OIL FROM ANNURCA APPLE TO OBTAIN OLEOLYTES WITH POTENTIAL DEPIGMENTATION ACTIVITY

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Ursolic acid (UA) (3β-hydroxy-urs-12-en-28-oic-acid) is a pentacyclic triterpenoid carboxylic molecule widely distributed in fruits, especially in apples. In recent years, UA has attracted considerable attention due to its functional properties, such as antioxidant, antitumor, anti-inflammatory, neuroprotective, antibacterial, and especially for its depigmentation activity [1].

The main aim of this study was the optimization of the UA extraction process from Annurca apple (AA), using sunflower oil as a lyophilic food-grade solvent, by applying a Response Surface Methodology (RSM) statistical approach. Then, we moved to the evaluation of the nutraceutical potential of the obtained extract for the management of skin-pigmentation disorders. The results of RSM analysis showed that the maximum UA yield of 784.40 ± 7.579 (μg/mL) was achieved under the following optimized conditions: sunflower oil, as extraction solvent; 68.85 °C, as extraction temperature; 63 h, as extraction time. The HPLC-DAD-HESI-MS/MS analysis performed on the extract obtained under these optimized conditions, named Optimized Annurca Apple Oleolyte (OAAO), led to the identification of twenty-three phenolic and terpenoid molecules, and the quantification of eight of them.

Physiologically, melanin synthesis protects human skin from UV radiation; however, abnormal melanin production could lead to several skin diseases, including acquired hyperpigmentation, melasma, and age spots. Melanogenesis is mediated by the activity of the tyrosinase enzyme and the combination of several modulators such as tyrosinase-related protein (TRP) ½ and microphthalmia-associated transcription factor (MITF) [2-3]. Thus, the OAAO activity on the melanin production, and on the expression of MITF and (TRP) ½ was evaluated in the melanoma cell line. Our results showed that OAAO was able to inhibit tyrosinase with a calculated IC_{50} of 286.42 mg/ml. Additionally, treatment of melanoma cells with OAAO (30 mg/ml) resulted in a relevant reduction in melanin content (-20% vs CTR, $p < 0.0001$) and statistically decreased the expression of the melanogenic modulators TRP-1 (-60% vs CTR, $p < 0.0001$), TRP-2 (-15% vs CTR), MITF(-30% vs CTR, $p < 0.05$).

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ABSOLUTE CONFIGURATION AND CONFIGURATIONAL STABILITY OF CANNABICHROMENE (CBC), A SCALEMIC PHYTOCANNABINOID

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Cannabichromene (CBC) is unique within Cannabis phytocannabinoids in terms of biosynthesis and glandular storage [1]. CBC is chiral, and its absolute configuration (AC) has so far been established only by conversion to diastereomeric analogues, and not by direct chiroptical methods [2]. The different values of enantiomeric excess reported in the literature [2-4] imply that the biosynthesis of CBC occurs with an apparent variable degree of enantioselectivity, or, alternatively, that CBC has a matrix- and/or time-related configurational stability. These considerations provided a rationale to investigate the configurational of CBC in plant material by an enantioselective chromatographic method, additionally evaluating its stability using on-column DHPLC and off-column techniques, and evaluating the racemization barrier and stability of the compound in acid and basic environments. CBC enantiomers were separated by semi-preparative enantioselective chromatography, and the absolute configuration of the enantiomers determined by comparing experimental and calculated electronic circular dichroism (ECD). Our results provide a necessary base to optimize the biological potential of CBC, currently under evaluation in clinical trials and in a various pre-clinical settings.

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THE CRUCIAL ROLE OF SOIL LESS CULTIVATION TECHNIQUES IN NATURAL PRODUCT-BASED INDUSTRIAL APPLICATIONS: THE PROJECT "BOTANICALS"

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As well known, biosynthesis and accumulation of secondary metabolites in Plant Kingdom depend on genetic, environmental (climatic change, biotic and abiotic stresses), and agronomic factors (plant chemotype, growth conditions and phenological stage of harvested just to cite a few)[1]. This multifactorial regulation leads inevitably to a high variability in both quantitative and qualitative composition of the secondary metabolite content representing one of the main issues for the industrial use of vegetable matrices, particularly for medical purposes. Several strategies including high technological approaches based on soil-less cultivation in controlled environments (hydroponics, aquaponics and aeroponics) have been developed to overcome this intrinsic problem [2].

"Botanicals" (Biofitosanitari OTtenuti mediante l'impiego di ANIdride CArbonica supercritca da officinaLi coltivate con Sistemi in aeroponica per la definizione di nuove strategie di difesa biologica ed integrate), funded by Regione Campania, is a multidisciplinary project that, taking advantage of aeroponic cultivation system, is directed to the development of natural products with bioacide activity for the prevention of crop infections.

The "Botanicals" approach makes use of a green and sustainable multi-step strategy, that starting from the aeroponic cultivation of selected officinal plants and their extraction using supercritical carbon dioxide, aims at developing innovative phyto-extract based formulations by the identification and quantification of their active metabolites as to determine the cultivation conditions that ensure the more effective phytochemical composition.

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TRITERPENOID PYRIDINES AND PYRAZINES AS POTENTIAL ANTICANCER AGENTS

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Triterpenoids are natural compounds with various biological activities. Our research group is mostly focused on their cytotoxicity.[1] Triterpenoid pyridines and pyrazines, such as **1a** and **1b** had high cytotoxicity of IC_{50} 0.5 – 1.5 μM in leukemic cell lines (CCRF-CEM, K-562).[2] In addition, they had higher activity against daunorubicin and taxol resistant cells (CEM-DNR, K562-TAX) which makes them promising alternatives for the treatment of resistant leukemias.[2]

First of all, we investigated the mechanism of action of the most interesting compounds. Cell cycle studies, Western blot analysis of apoptosis and cell cycle - related proteins combined with the visualization of the cellular damage using fluorescent and electron microscopy proved that **1a** and **1b** trigger apoptosis *via* intrinsic pathway.[2] We found, that the compounds accumulate preferentially in the resistant cells, although the reason for that has to be uncovered yet. Unfavorable pharmacological parameters of the parent compounds motivated us to prepare two sets of prodrugs (e.g. **2**, **3**). This improved the pharmacological parameters and in addition, medoxomil-type prodrugs **3** surprised us by an extreme selective cytotoxicity against K-562 cells with IC_{50} 26 – 43 nM.[2]

Prepared triterpenic pyrazines and pyridines have a great potential in possible treatment of resistant lymfoblastic leukemia or may be useful in the development of the treatment for myeloid leukemia. Synthesis, mechanism of action, influence of prodrugs and SAR will be discussed.

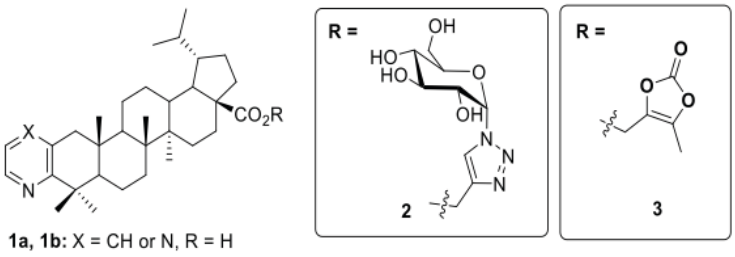

Fig 1: General formula of pyridines, pyrazines and their prodrugs

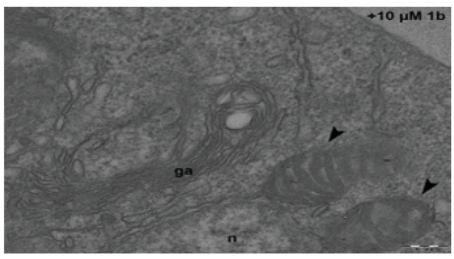

Fig 2: EM analysis of mitochondria after treatment with 1b

This work was supported by Czech Science Foundation (21-00902S); EXCELES - Next Generation EU (no. LX22NPO5102).

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THE CHEMICAL TRANSFORMATION OF STEMODIN INTO BIOLOGICALLY ACTIVE ANALOGUES

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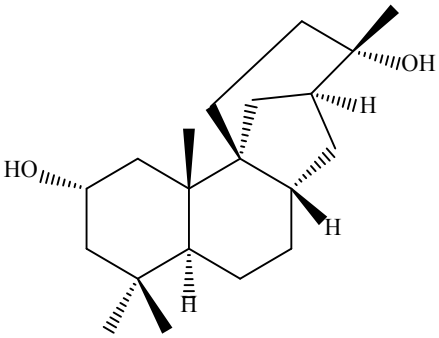
Stemodia maritima is a perennial herb, found in saline and brackish areas in some parts of the Caribbean and South America. In Caribbean folklore it is used to treat dropsy, stomach ache, body pain, swellings and venereal diseases.[1] Stemodin (1), the most abundant natural product isolated from this plant, has a unique tetracyclic stemodane core.[2] The diterpene has attracted significant interest because of its structural similarity to aphidicolin, which possesses antiviral and antitumour activity.[3] Stemodin is an excellent candidate for natural product research, because of its relative abundance within the plant and the ease with which it is isolated. Stemodin also exhibits antiviral and antitumour activity.[4,5] Chemical transformations were undertaken to determine how functional group changes in the structure affected its bioactivity. The potency of the analogues against a prostate cancer cell line will be reported.



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CHLORINATED ATROPISOMERS FROM CYANOBACTERIA IN THE WEST OF IRELAND

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Cyanobacteria are credited for producing a diverse array of secondary metabolites, with increasing occurrences due to our changing climate [1]. Though some cyanobacterial accumulations are harmless, others produce toxic compounds with detrimental effects to their surroundings. As part of an ongoing project investigating the chemical diversity of benthic freshwater cyanobacteria in Western Ireland, and their (eco)toxicology, collections of benthic cyanobacteria were subjected to a biological and chemical screening process. A large cyanobacterial accumulation proliferating regularly at Urlar Lough, Co. Mayo was selected for further chemical analysis owing to the presence of several polychlorinated metabolites detected by LC-MS in the methanolic fraction, which displayed cytotoxic activity.
Herein, we present the identification and structure elucidation of bisindole alkaloids possessing structural similarities to the established neurotoxin aetokthonotoxin (AETX) [3]. The planar structures of these tetrachlorinated analogues were deduced using HRMS and MS2 analyses, 1D and 2D NMR techniques. Compounds 1-5 display axial chirality arising from restricted rotation around the single bond linking both indole rings, whose absolute configurations were assigned through comparison of experimental and calculated ECD spectra. Efforts are ongoing to evaluate the biological activity and potential environmental effects of these discovered metabolites.

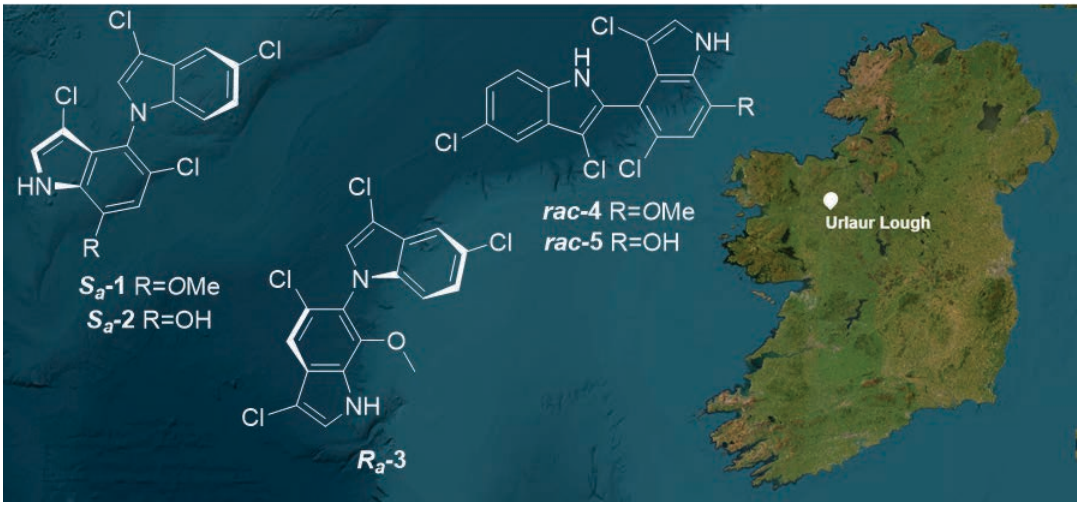

Figure 1: Bisindole alkaloids **1-5** from cyanobacteria

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ANTI-HIV MACROCYCLIC DAPHNANE ORTHOESTERS FROM *EDGEWORTHIA CHRYSANTHA*

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Macrocyclic daphnane orthoesters (MDOs) are a class of 1-alkyldaphnanes with a macrocyclic ring spanning the diterpene skeleton, and characteristically distributed in the plants of the Thymelaeaceae family. MDOs have attracted much attention for drug discovery because of their significant antineoplastic and anti-HIV activity. *Edgeworthia chrysantha* Lindl. (Thymelaeaceae), also known as Oriental paperbush, is a deciduous shrub and has distinctive three-pronged branches. It is mainly distributed in Japan, China, and Nepal. As part of our research for searching anti-HIV diterpenoids from the Thymelaeaceae family, herein, we reported LC-MS/MS analysis, isolation, structural determination, and anti-HIV activity of MDOs from *E. chrysantha*.

Each MeOH extract from the flower buds, flowers, leaves, and stems of *E. chrysantha* was partitioned between EtOAc and H_2O, respectively. The EtOAc fractions were subjected to LC-ESI-MS/MS analysis using UHPLC-Q-Exactive-Orbitrap HRESIMS, and seven MDO peaks were detected across all four parts of the plants by calculation of the molecular formula by high-resolution accurate mass and analysis of the MS/MS fragmentation. Among these peaks, five were identified as edgeworthianins A–E (**1**–**5**), which were isolated from the flower buds of *E. chrysantha* in our previous study.[1] The other two peaks exhibited MS/MS fragmentation pattern similar to **1**–**5**, but with different molecular weights, suggesting they were MDOs that have not been isolated so far. Further isolation of the extracts from the flowers and stems, which had higher content of the two MDOs, were carried out using ODS and silica gel column chromatography as well as preparative HPLC, and afforded edgeworthianins F and H (**6** and **7**). Their structures were determined by various spectroscopic analyses, including MS, ECD, and NMR. Edgeworthianins A–H (**1**–**7**) are characterized by MDOs with an unusual macrocyclic ring structure that originates from a C_{14} unsaturated aliphatic chain. The anti-HIV activity of MDOs (**1**–**7**) were evaluated against HIV-1 infection of MT4 cells and three MDOs (**5**, **4**, and **2**) exhibited significant anti-HIV activity with EC_{50} values of 2.9, 8.4, and 29.3 nM, respectively.

In conclusion, the study highlights the discovery of anti-HIV MDOs from *E. chrysantha*, thereby expanding the repertoire of potential anti-HIV drug candidates derived from the Thymelaeaceae family.

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METABOLOMICS APPROACH FOR THE RAPID IDENTIFICATION OF CYTOTOXIC CYCLOARTANE SAPONINS FROM *ASTRAGALUS* SPECIES

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Plant species belonging to the *Astragalus* genus (Fabaceae) are promising sources of bioactive compounds, since many of them have been used for millennia for treating various diseases, including cancer.[1] In a preliminary study, aimed at the screening of several Mediterranean Fabaceae species, extracts from *Astragalus boeticus* showed a remarkable anti-proliferative effect on drug-resistant colon cancer cell lines. [2] It was suggested that this bioactivity could be due to the cycloartane saponins identified in the extract. [2] Based on the preliminary promising results, two further species were also analysed along with *A. boeticus*, namely *Astragalus hamosus* and *Astragalus glycyphyllos*, using a metabolomics approach. Metabolite profiling showed the presence of saponins in the *A. boeticus* and *A. glycyphyllos* extracts, which were therefore further studied. The isolation of the metabolites was performed by using different chromatographic techniques, while their chemical structures were elucidated by nuclear magnetic resonance (NMR) (1D and 2D) and electrospray-ionization quadrupole time-of-flight (ESI-QTOF) mass spectrometry. The cytotoxic assessment on several cancer cell lines was performed *in vitro* by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays.

From *A. boeticus*, five saponins were isolated. All of the compounds were characterized by a 3-O-β-d-xylopyranosylcycloastragenol unit which was then further substituted with other sugars and/or organic acids. From *A. glycyphyllos*, six saponins were isolated. These compounds were characterized by a higher structural complexity as compared to the ones isolated from *A. boeticus*. The sapogenin was identified as cycloastragenol for two saponins and as cyclolanostanol for four compounds. These saponins differed also for the number and type of sugar moieties and for the presence/absence of organic acid moieties. Two of the saponins isolated from *A. boeticus,* namely 6-O-acetyl-3-O-(4-O-malonyl)-d-xylopyranosylcycloastragenol and 6-O-acetyl-3-O-β-d-xylopyranosylcycloastragenol preferentially inhibited cell growth in colorectal cancer cell model resistant to epidermal growth factor receptor (EGFR) inhibitors. Further studies will be aimed at understanding the mechanism of action of these promising compounds.

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PPARg/a MODULATION OF NEW AMORFRUTINS FROM *GLYCYRRHIZA FOETIDA*

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Among the different targets involved type-2 diabetes mellitus and Metabolic Syndrome, widespread pathological conditions in Western world, PPARγ has always been challenging for medicinal chemistry. Thus, it is an attractive perspective to discover new PPARγ agonists, considering that this target plays a crucial role in these disorders and the only available drugs are the debated thiazolidinediones. Amorfrutins are a novel family of natural compounds that could be interesting leads for this purpose [1]. This intriguing class of secondary metabolites were recently isolated from *Glycyrrhiza foetida*, a less known but promising species of genus *Glycyrrhiza*, from Northern Africa, whose phytochemical features have not been investigated yet. Therefore, within the framework of our research activity in exploring natural compounds active on Metabolic Syndrome [2], we have devoted our studies on amorfrutins characterization from *G. foetida* aerial parts. After a LC-MS^2 analysis, a series of chromatographic separations were performed, leading to the isolation of 16 pure amorfrutins. Ten of them, belonging to pentyl and phenethyl series, were unprecedent in literature, thereby completely characterized through 2D NMR and HR-MS analysis. All the pure compounds were tested on PPARγ and PPARα, exhibiting encouraging pharmacological activities and allowing us to deepen in their structure-target interaction relationships. Beside the known effect of amorfrutin A, we have identified a selective PPARα agonist and a new dual activator, leading the way on new possible lead compounds active of this crucial targets.

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CATCH, ENRICH AND RELEASE NATURAL PRODUCTS FROM COMPLEX MATRIX

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The discovery of novel bioactive natural products (NPs) has been crucial for the development of the human health system.[1] However, the field suffers from the high cost of the isolation and extraction process and the rediscovery of known compounds.[2] To overcome those issues, several technologies have emerged, such as using well-designed derivatization agents to target a class of NPs specifically.[3,4] This approach led to a fast identification of a target class of compounds while using only a small amount of sample. A downside of this methodology stands in obtaining a derivatized NP. An ideal protocol would use a chemical reaction that can be reversed at the end of the process to obtain the NP as an underivatized compound. Herein, we present a chemoselective approach that catches and enriches amine-containing NPs and releases them as underivatized compounds.[5]

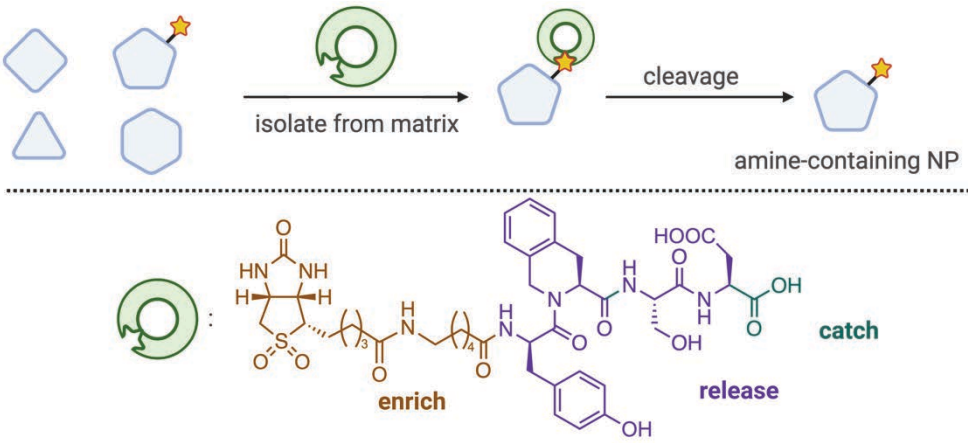

Figure 1: Catch, enrich, and release strategy for amine-containing NPs and structure of the chemical probe.

The amine-containing NPs react with the chemoselective probe using a standard amide coupling procedure (**catch**). Next, the matrix composed of unreacted compounds is removed by exploiting the high affinity of streptavidin for biotin (**enrich**). In this case, a derivative of biotin, biotin sulfone, was used to ease the removal of the modified probe from the resin. Finally, the underivatized amine-containing NPs are obtained by selective cleavage of the amide bond by the protease legumain (**release**).

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MUCUS OF THE PHOTOSYNTHETIC SEA SLUG *ELYSIA CRISPATA*: PROTEOME AND ANTI-BACTERIAL ACTIVITY

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Elysia crispata (Sacoglossa, Gastropoda) is a tropical sea slug that can incorporate functional chloroplasts from various macroalgae, a mechanism termed kleptoplasty[1]. This sea slug, which can be easily reared in the laboratory, produces mucus, a viscous secretion that serves multiple purposes such as lubrication, protection, and aiding in locomotion[2]. In this study, we present a comprehensive analysis of the mucus proteome of *E. crispata* using gel electrophoresis and HPLC-MS/MS techniques and probe its potential anti-bacterial activity. A total of 306 proteins were identified in the mucus secretions of *E. crispata*, despite the limited information available for this species in the Uniprot database. By employing Gene Ontology for functional annotation, the mucus proteome of *E. crispata* was shown to include proteins involved in various functions, such as hydrolase activity (molecular function), carbohydrate-derived metabolic processes (biological processes), and cytoskeletal organization (cell component). Notably, a considerable proportion of the identified proteins in *E. crispata* mucus possessed enzymatic activity, indicating potential biotechnological applications. The presence of a significant number of hydrolases further suggests potential antimicrobial properties leading us to perform preliminary tests to evaluate its antibacterial activity. Initial results demonstrated strong inhibitory activity against several bacteria, including *Pseudomonas aeruginosa*, a species that causes high-risk infections due to its frequent multidrug resistant profile[3]. Further investigations will help unravelling the full range of applications associated with the mucus of this unique marine organism.

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SECHIUM EDULE GENOTYPES AS A SOURCE OF NATURAL PRODUCTS WITH PHARMACOLOGICAL ACTIVITY

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In order to derive projects for the use of underutilized plant genetic resources, the hybrid of *Sechium edule* H387-07 was obtained by genetic improvement, whose phytochemical characterization and biological activity showed to be relevant as a source of natural products (secondary metabolites) for pharmacological use. It was analyzed for three years, eight flavonoids, eight phenolic acids and four cucurbitacins were recorded. This hybrid was studied through assays *in vitro* and a murine model. Its antiproliferative effect was evaluated in malignant cell lines P388, J774 and WEHI3, in addition to normal mouse bone marrow cells. The doses (0.07, 0.15, 0.3, 0.6, 1.2, 2.5 and 5.0 µg mL^{-1}) compared with Cytarabine (Ara-C®), induced apoptosis in P388, J774, and WEHI-3 leukemic cell lines and mononuclear cells (MNC) from bone marrow treated with or without IC_{50} of the extract H387-07, or Ara-C®). The extract induced fragmentation of the analyzed DNA by agarose gel electrophoresis (10,000 to 250 bp). The control cells and those treated with 5 mM Ara-C®, as well as those treated with the IC_{50} of the H387-07 extract indicated a statistical difference with respect to the control (Tukey $p \leq 0.05$). The extract induces apoptosis in leukemia cells, but not normal ones, and its intraperitoneal administration in mice increases the mitotic index in bone marrow cells, which indicates that it is not myelotoxic, and the levels of proinflammatory cytokines in serum as a factor of tumor necrosis alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) are reduced. The expression of the TNF-α and MCP-1 genes is induced by the transcription factor nuclear factor kappa B (NF-kB), so there is the possibility that metabolites such as terpenes and flavonoids present in the extract of H- 387-07 inhibit the activation of NF-kB, a crucial transcription factor in the progression of acute lymphoblastic leukemia (ALL), which is the most common cancer in children.

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MANGIFERIN MODIFIED SELF-FUNCTION OF BONE MARROW MESENCHYMAL STEM CELL TO EXERT NEUROPROTECTION FOR CEREBRAL ISCHAEMIA REPERFUSION INJURY THERAPY

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Background and purpose: To establish the model for high-throughput targeted screening of efficient inducers of bone marrow mesenchymal stem cells (bMSCs)", a plasmid vector containing luciferase reporter gene was constructed using NGF (Nerve Growth Factor) as the promoter, and the host cells were transfected with lentivirus to obtain stable cell lines as described.[1-3] Such a system facilitated our identification of mangiferin (MGF) as a promising hit. The work aimed to determine whether MGF can regulate the proliferation, homing and paracrine functions of bMSCs and to address why the combination of MGF and bMSCs enhanced the neuroprotective effect on cerebral ischemia reperfusion injury.
Experimental: Flow cytometry, U-CFU assay, Scratch assay, Transwell chamber, Western blot, Immunofluorescence, Elisa and qPCR were used to detect the effect of MGF on the self-function of bMSCs. The combined efficacy of MGF@bMSCs, MGF-embedded bMSCs, was evaluated by in vivo imaging, neurological function score, Brdu staining, TTC (Triphenyltetrazolium Chloride) staining, immunofluorescence staining, and brain histopathology.
Key Results: MGF enhanced the proliferation, migration and homing function of bMSCs, and improved the neuroprotective efficacy of MGF@bMSCs.
Conclusion and Implications: The precise regulation of bMSCs by exogenous molecules is therefore a feasible way of alleviating cerebral ischaemia–reperfusion injury. MGF enhances neurotrophic factor expression in bMSCs and neuroglia cells, inhibits the inflammatory expression of microglia, and thus promotes the cell migration. The work showed that MGF targets the pathological microenvironment of cerebral ischaemia injury via the CXCR4/SDF-1α axis.

Keywords: Mangiferin; Bone marrow mesenchymal stem cells; Neuroprotection; Cerebral ischaemia reperfusion injury

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LANOSTANE-TYPE TRITERPENES FROM *INONOTUS OBLIQUUS* EXERT CYTOTOXICITY AGAINST DOG BLADDER CANCER ORGANOIDS

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Inonotus obliquus (Chaga) is an edible mushroom often used as the natural supplement for cancer treatment. Compared to chemotherapy, natural supplements usually show less side effects on patients. Various studies have already shown that Chaga is cytotoxic against various of cancer cell lines including breast cancer,[1] lung adenocarcinoma[2] and colorectal cancer.[3] Recently, it was reported that Chaga reduced the cell viability of dog bladder cancer organoids (DBCO).[4] DBCO is a model for muscle-invasive bladder cancer (MIBC),[5] in which the cancer cells spread to the detrusor muscle of the bladder. In this study, we have isolated active substances from Chaga extract following the activity in the cell viability assay using DBCO. We identified four known lanostane-type triterpenes as the active components based on the MS and NMR spectral data. These four compounds reduced cell viability of DBCO at 10 µg/mL.

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DEVELOPMENT OF NATURAL-PRODUCT-BASED MOLECULAR GLUE FOR A SPECIFIC SUBTYPE OF PLANT HORMONE CO-RECEPTOR

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(3*R*, 7*S*)-jasmonoyl-L-isoleucine (JA-Ile) is a fatty acid-derived plant hormone that regulates many plant responses, including plant defense against pathogens and insects, secondary metabolite production, etc. However, JA-Ile also causes severe growth inhibition in addition to these responses. Thus, the dissection of growth and defense has been a critical issue. In *Arabidopsis thaliana*, JA-Ile functions as a molecular glue to cause protein-protein interaction between F-box protein CORONATINE INSENSITIVE 1 (COI1) and jasmonate-ZIM-domain (JAZ) repressor proteins. The *Arabidopsis* genome encodes 13 *JAZ* genes, thus giving rise to 13 COI1-JAZ co-receptor pairs. JA-Ile binds to these co-receptor pairs simultaneously, triggering the diverse responses described above.

We previously demonstrated that COI1-JAZ9/10-selective agonist enabled the dissection of the growth-defense tradeoff in the jasmonate signaling of *A. thaliana*.[1] Here, we present that screening of the chemical library harboring all the stable stereochemical isomers of JA-Ile mimic natural product (NP), coronatine, provided the agonists of COI1-JAZ9 selective affinity among the 13 COI-JAZ co-receptor pairs.[2] The agonist caused specifically JAZ9-dependent jasmonate signaling in *A. thaliana* and weakly activated plant defense against pathogenic infection without causing growth inhibition. Our COI1-JAZ9-selective agonist enabled the analysis of a unique gene expression pattern governed by a single JAZ repressor, JAZ9.

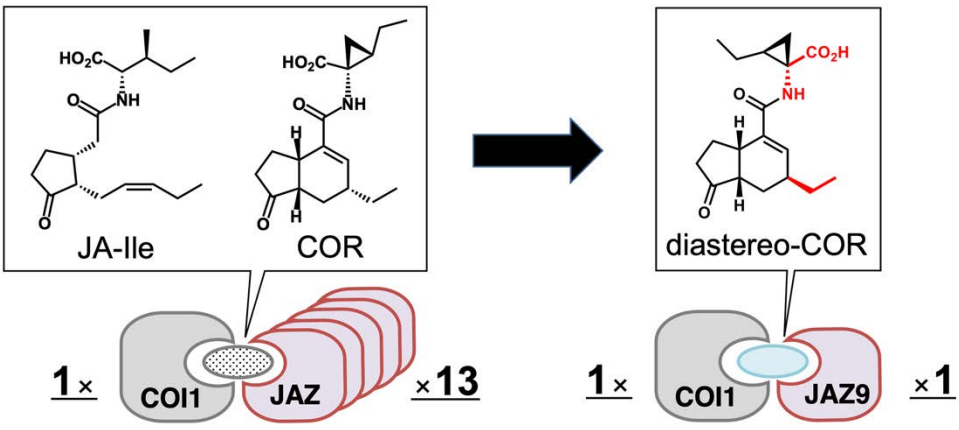

Figure COR-based subtype-selective agonist of plant hormone co-receptor

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LUPANE TRITERPENOIDS AND THEIR NEUROPROTECTIVE ACTIVITY IN SEVERAL NEURODEGENERATION MODELS

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Lupane triterpenoids are natural substances with interesting biological activities.[1] During our studies of compounds with anticancer activities, we found derivatives with low cytotoxicity and high neuroprotective activity that were synthesized from betulin and betulinic acid and evaluated in salsolinol (SAL)- and glutamate (Glu)-induced models of neurodegeneration in neuron-like SH-SY5Y cells. Betulin triazole ***I*** bearing a tetraacetyl-*β*-d-glucose substituent had the highest neuroprotective effect among the first set of screened compounds. To better understand which parts of the molecule is responsible for the activity, substructures of compound ***I*** were prepared (compounds ***II***, ***III***, ***IV*** in Figure 1), and evaluated independently. Second generation of compounds was prepared based on the results. Additional biological evaluations showed the potential of the best candidates for the treatment of Parkinson's and Huntington's disease, their structures and activity were patented.[2] Synthesis, activity, and detailed mechanism of action will be discussed.[3]

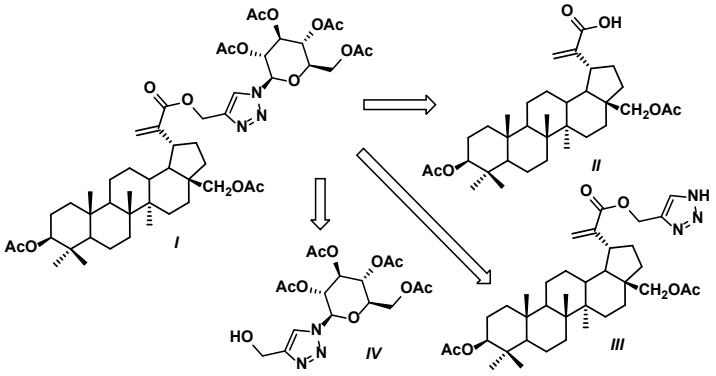

Figure 1. Neuroprotective lupane derivative I and substructures II-IV.

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ISOLATION AND CHARACTERIZATION OF GUAIANOLIDE-TYPE SESQUITERPENE LACTONES AND CHEMICAL ANALYSIS OF ESSENTIAL OIL FROM SICILIAN ENDEMIC *SILER SICULUM*

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Siler siculum (Spreng.) Thell is a plant belonging to the Apiaceae family, commonly known as Sicilian (Italy) “cow-parsnip”. It is endemic plant of Sicily and it has been traditionally used in folk medicine for its potential therapeutic properties.
This study reports the isolation and spectroscopical characterization of four novel guaianolide-type sesquiterpene lactones obtained from an acetone extract of *S. siculum*. The extraction was performed using standard acetone extraction methods, followed by purification through preparative chromatography techniques. The chemical structures of the isolated compounds were determined using mass spectrometry, and ^{1}H-NMR and ^{13}C-NMR mono- and bi-dimensional spectroscopy. The results confirmed that the four isolated products are guaianolide-type sesquiterpene lactones, never found in other *Siler* species: **(1)** 2-butenoic acid, 2-methyl-, 6-(acetyloxy)-2,3,3a,4,5,6,6a,7,8,9b-decahydro-8-hydroxy-3,6,9-trimethyl-2-oxoazuleno[4,5-b]furan-4-yl ester; **(2)** 2-butenoic acid, 2-methyl-, 3,6-bis(acetyloxy)-2,3,3a,4,5,6,6a,7,8,9b-decahydro-8-hydroxy-3,6,9-trimethyl-2-oxoazuleno[4,5-b]furan-4-yl ester; **(3)** 4-acetoxy-8-hydroxy-3,6,9-trimethyl-2-oxo-2,3,3a,4,5,6,6a,7,8,9b-decahydroazuleno[4,5-*b*]furan-6-yl 3-hydroxy-2-methylenebutanoate; **(4)** 6-acetoxy-8-hydroxy-3,6,9-trimethyl-2-oxo-2,3,3a,4,5,6,6a,7,8,9b-decahydroazuleno[4,5-*b*]furan-4-yl 3-hydroxy-2-methylenebutanoate.
Additionally, essential oil of *S. siculum* was always isolated from the aerial parts using hydro-distillation method, and its composition was analyzed using GC and GC-MS.
Furthermore, the essential oil analysis revealed that the main constituents belonged to the family of monoterpene hydrocarbons (90.19%). The primary metabolites identified were □-phellandrene (41.98%) and limonene (23.76%). Further studies are required to evaluate the biological activity of these compounds and their potential application as therapeutic agents.

A PIPELINE TOWARDS THE IDENTIFICATION OF NOVEL ANTIMICROBIAL COMPOUNDS DERIVED FROM THE MICROBIAL DARK MATTER

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Background. The emergence of drug-resistant fungal pathogens and the limited range of available antifungal drugs necessitate the discovery of novel compounds for effective treatment. Natural secondary metabolites have historically been a valuable source for drug development; however, compound rediscovery and associated costs have posed challenges in traditional discovery platforms. In this study, we employed a comprehensive approach to isolate novel strains and identify new antimycotic compounds.
Study. We isolated soil bacteria and fungi using standard plating and the iChip method for long-term *in situ* cultivation. After implementing the One Strain Many Compounds (OSMAC) approach, we identified 389 secretion broths that exhibited activity against the pathogenic fungus *Candida albicans*. Lead hits were further purified using bioactivity-based fractionation through HPLC, followed by tandem LCMS-MS analysis. The proposed structures of the compounds are currently being confirmed through ongoing validation using NMR.
Results. Our investigation led to the identification of several species that produce previously unreported antifungal secondary metabolites. Additionally, whole genome sequencing revealed novel species. The genera associated with our lead hits include *Pseudomonas, Tsukamurella, Paraburkholderia* (bacteria), and an unidentified member of the *Atheliales* order (fungus). The *Pseudomonas* species seem to produce variants of the antimycotic iron-chelating pyoverdine class. Our lead candidate, the unidentified species of the *Atheliales* order (a type of mold), exhibits both antifungal and antibacterial properties. The extract mixture derived from this candidate demonstrates activity against resistant strains of *Candida* species and several clinically relevant ESCAPE organisms, indicating its dual antifungal and antibacterial potential. Initial assessments through HPLC and LCMS suggest the presence of multiple distinct active compounds.
Conclusion. Overall, our study highlights the significance of exploring diverse microbial sources to uncover novel antimicrobial compounds. The identification of previously unknown species and their associated bioactive compounds expands the possibilities for drug development targeting drug-resistant fungal pathogens and clinically relevant ESCAPE organisms. Moving forward, future research will focus on advancing these promising leads towards the development of effective drugs.

NEW IRIDOIDS AND PHENOLIC SECONDARY METABOLITES FROM *NUXIA CONGEST* GROWING IN SAUDI ARABIA

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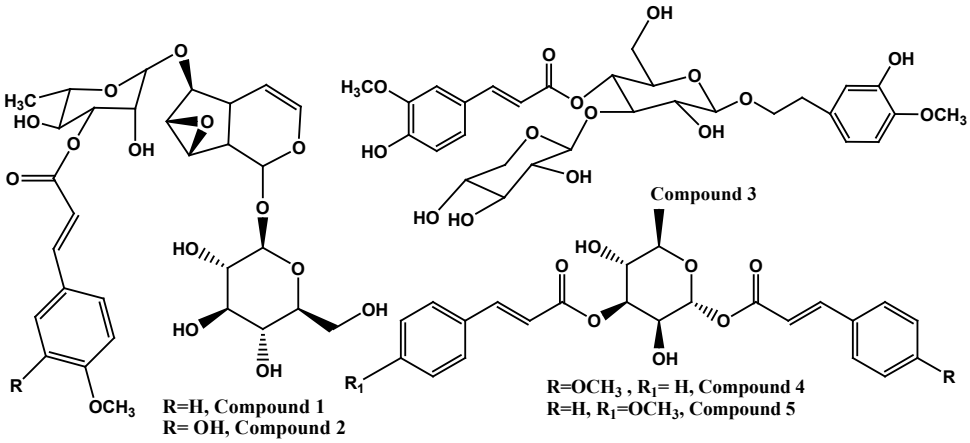
The genus *Nuxia*, family Stilbacea, is found in the Arabian Peninsula and across the Afrotropical areas[1]. *Nuxia* plants are, traditionally, used as laxatives and to treat venereal infections[2]. In Ethiopia and Madagascar, *Nuxia* is used to treat malaria[3]. This genus is represented in Saudi Arabia by two species, *N. appositifolia* and *N. congesta*[4]. Previous phytochemical studies led to the isolation of terpenoids and phenolic compounds from these two species[4-5]. These compounds displayed cytotoxic, anti-diabetic, anti-plasmodial, anti-malarial, and anti-inflammatory activities[6-7]. The titled plant *n*-butanol and ethyl acetate fractions were subjected to chromatographic purification and isolation. Compounds **1-5** have been identified and thoroughly described by various spectroscopic techniques, including HRESIMS, 1D, and 2DNMR. The obtained compounds will be biologically screened for anti-COVID-19 and scavenging activities. This study demonstrated Unedoside, an iridoid with eight carbon atoms, is found in the genus *Nuxia* which represents chemical and taxonomic significance for this genus.



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RATIONAL DESIGN OF LACTOFERRIN-DERIVED PEPTIDES ACTIVE TOWARDS INFLUENZA VIRUS: IDENTIFICATION OF A NOVEL POTENT TETRAPEPTIDE

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The effective treatment of Influenza virus infection is still an unmet goal. Even though some antivirals are available, the alarming increase in virus strains resistant to them highlights the need to find new drugs.[1] An ideal target for new anti-influenza therapy should be a viral component, whose function is essential for virus infection. In this contest, the influenza A virus hemagglutinin (HA) represents a very promising target. Previously, Superti et al. have demonstrated that bovine lactoferrin (bLf), in particular, bLf C-lobe, is able to bind and inhibit HA.[2] Successively, through truncation library, we identified the tetrapeptides, SKHS (1) and SLDC (2), derived from bLf C-lobe fragment 418-429, which were able to bind HA and inhibit cell infection in a concentration range of picomolar.[3]
Considering the above highlighted, the aim of this study was to synthesize a new library of peptides and peptidomimetics active towards influenza virus. In order to test their ability to bind HA, we carried out a preliminary screening by biophysical assays such as surface plasmon resonance (SPR) and the orthogonal immobilization-free microscale thermophoresis (MST) assays. Biological and computational assays on synthesized peptides were carried out.[4]
All applied methods agreed upon the identification of a novel potent tetrapeptide, SAHS, able to bind hemagglutinin with high affinity and inhibit influenza virus hemagglutination and cell infection at femtomolar concentration. This small sequence, with high and broad-spectrum activity, represented a valuable starting point for the design of new peptidomimetics. This work opens the way to new perspectives for the development of new anti-influenza drugs.

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DISCOVERY AND BIOSYNTHESIS OF THE CYTOTOXIC POLYENE TERPENOMCYIN IN HUMAN PATHOGENIC *NOCARDIA*

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Nocardia are opportunistic human pathogens that can cause a range of debilitating and difficult to treat infections of the lungs, brain, skin and soft tissues. Despite their close relationship to the well-known secondary metabolite producing-genus, *Streptomyces,* comparatively few natural products are known from the *Nocardia*, and even less is known about their involvement in pathogenesis. Here, we combine chemistry, genomics, and molecular microbiology to reveal the production of terpenomycin, a new cytotoxic and antifungal polyene from a human pathogenic *Nocardia terpenica* isolate. We unveil the polyketide synthase (PKS) responsible for terpenomycin biosynthesis and show that it combines several unusual features, including “split”, skipped and iteratively used modules and the use of the unusual extender unit methoxymalonate as a starter unit. To link genes to molecules, we constructed a transposon mutant library in *N. terpenica*, identifying a terpenomycin-null mutant with an inactivated terpenomycin PKS. Our findings show that the neglected actinomycetes have an unappreciated capacity for production of bioactive molecules with unique biosynthetic pathways waiting to be uncovered, and highlights these organisms as producers of diverse natural products.

IN VITRO ANTIOXIDANT, ANTIMICROBIAL AND ANTI-INFLAMMATORY SCREENING OF GREEK *AMELANCHIER OVALIS* MEDIK. LEAVES AND TWIGS EXTRACTS

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Amelanchier ovalis Medik. (Rosaceae), -commonly known as snowy mespillus or European juneberry-, is a shrub or small tree, native to central and southern Europe, north-western Africa, and south-western Asia. It is a rather overlooked species, despite the high nutritional and antioxidant value, as well as erythropoiesis-stimulant properties of its fruits. Moreover, the leaves and branches also exhibit antioxidant and antibacterial activity[1]. In the present study, various methanolic extracts from Greek cultivated *A. ovalis* leaves and twigs were screened for their total phenolic content (by means of the Folin-Ciocalteu method), radical scavenging activity (interaction with DPPH), inhibition of lipid peroxidation, antimicrobial activity against common bacteria (through the broth micro-dilution method), and anti-inflammatory activity (soybean lipoxygenase inhibition assay). Results showed that almost all extracts had a noteworthy radical scavenging activity (>90%), correlated to the high phenolic content (ranging from 9.32 ± 0.39 to 155.93 ± 1.41 mg GAE L^{-1} extract) - however, they exhibited weak or moderate anti-inflammatory activity (<50% in most cases). As far as their antimicrobial activity is concerned, the extracts exhibited strong and moderate antibacterial activity for *S. aureus* and *E. faecalis* and weak for *E. coli* and *S. enteritidis*. In almost all assays, the twigs extracts proved to be more potent compared to the ones obtained from the leaves. In conclusion, our study contributes to providing new data regarding the antioxidant, antimicrobial and anti-inflammatory activity of Greek A. ovalis. Further assessment of its chemical compounds will possibly help to better understand its properties.

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FROM NATURE TO CANCER THERAPY: EVALUATING THE RTK-INHIBITING POTENTIAL OF *STREPTOMYCES CLAVULIGERUS* SECONDARY METABOLITES

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Receptor Tyrosine Kinases (RTKs) are vital signalling molecules that play a critical role in regulating several cellular processes, including cell growth, differentiation, migration, and metabolism. Disruptions in RTK expression, such as mutations, overexpression, or gene amplification, have been linked to multiple types of cancer. Therefore, targeting RTKs or their signalling pathways provides a unique opportunity for developing pharmacological treatments for cancer therapy. The study aimed to evaluate the protein kinase inhibitory potential and cytotoxicity activity of Streptomyces clavuligerus extract. Additionally, all the secondary metabolites produced by this strain were identified and characterized. Their potential to inhibit RTKs was evaluated by molecular docking, simulation, MM/GBSA calculations, and free energy approach. The formation of a whitish bald zone by Streptomyces clavuligerus extracts indicated the presence of protein kinase inhibitors. The cytotoxicity activity of Streptomyces clavuligerus crude extract was assessed through Sulforhodamine B assay on MCF-7, Hop-62, SiHa, and PC-3 cell lines. The results demonstrated that the GI50 value of extract was lowest for the MCF-7 cell line followed by the PC-3 cell line showing potent growth inhibitory potential against human breast cancer and human prostate cancer cell line. HR-LCMS identified ten tripeptides and multiple secondary metabolites from the aqueous and organic extracts of Streptomyces clavuligerus, which were not previously documented in this strain. Computational studies revealed the superior inhibitory potential of secondary metabolites Dodecaprenyl phosphate-galacturonic acid (DPGA) and Epirubicin (Epi) against Fibroblast growth factors receptor (FGFR). Additionally, Epi exhibited more excellent inhibitory activity against the Platelet-derived growth factor receptor (PDGFR), and DPGA effectively inhibited the Vascular endothelial growth factor receptor (VEGFR) than the FDA-approved drug Pazopanib. Further, HPLC analysis validated the presence of the anticancer drug epirubicin in the Streptomyces clavuligerus extract.

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EXPLORING BOTANICAL EXTRACTS FOR SUSTAINABLE COSMETICS: ENHANCING AGRONOMIC HERITAGE AND ANTIOXIDANT STABILITY

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The present study is a part of the broader "Valpharmarecchia" project, funded by the Emilia Romagna Region,[1] aimed at evaluating the potential cosmetic applications of botanical extracts derived from mushrooms and medicinal herbs from the Marecchia Valley.
Due to the increasing consumer interest in natural and environmentally sustainable cosmetic products, there is a growing need for plant-derived raw materials in the cosmetics industry.[2] Among the various plant species growing wild in the Marecchia area, *H. italicum* and *H. stoechas* were selected for their potential as sources of biologically active molecules.[3]
In this study, extracts obtained from *H. italicum*, *H. stoechas* and *G. lucidum* were analyzed for polyphenol content using the Folin-Ciocâlteau assay. The antioxidant activity of the extracts was assessed through PCL, FRAP and DPPH analysis, providing valuable insights into their potential cosmetic applications. In addition, convergent analytical methods, such as XRF, EA-IRMS, ICP-QQQ-MS and genetic analysis, were employed to create unique extracts and enhance the agronomic heritage of the Upper Marecchia Valley. Geochemical and isotopic characterization of soils and flowering tops of *H. italicum* from various localities within the Valmarecchia territory were conducted. Genetic variability of twelve Helichrysum samples from different origins was evaluated using ISSR markers, allowing for a deeper understanding of the differences between native and commercial Helichrysum. To demonstrate the cosmetic application of the obtained extracts, O/W emulsions incorporating extracts with high antioxidant activity were formulated. The emulsions underwent a six-month stability study, coupled with PCL analysis, to evaluate the long-term antioxidant stability of the extracts within the cosmetic formulations. The project aims to serve as a useful model that can be extended nationwide, promoting circular economy approaches and sustainable practices in the cosmetics industry.

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GREEN CONCEPT AND TECHNOLOGIES FOR MICROALGAL BIO-BASED COSMETICS

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Interest in natural-based cosmetics continues to rise as consumers prioritize healthy lifestyles, sustainability, wellness, and well-being based on informed choices. The concept of "bio-based cosmetics" reflects the aim to replace conventional ingredients with natural-based, safe, and effective materials [1]. Our main interest was the search for bio-active ingredients from renewable bio-based alternatives, like plants and algae, for the development of "greener" cosmetics. We focused on eco-sustainable extraction technologies for recovering bioactives from microalgae, optimization of extraction procedures and assessment of their suitability as cosmetic ingredients. Recently, we studied cyanobacteria and microalgae such as commercial *Artrospira* (H) and lab- produced *Chlorella* (CHL) in a photobioreactor (PBR). Biomass production yield (1kg/month wet weight and 300g/month spray-dried) was attractive. A sustainable and environmentally friendly extraction method was developed using green solvents (water, ethanol), and the impact of pre- treatments and temperature on yield was evaluated. From spray-dried CHL cells, extracts rich in polysaccharides (CHL-P) and carotenoids (CHL-V) were produced studying process variables and optimizing extraction methods. Polysaccharides content was determined spectrophotometrically and lutein, as the extract's marker, by HPLC-DAD. From *Artrospira*, novel bioactive peptides, particularly from the hydrolyzed highly water-soluble extract (HSE)[1] were isolated. Our way to transform extract in cosmetic ingredients requires the production of engineered microparticulate powders, more stable and efficiently manufacturable showing better sensory characteristics. CHL- V and HSE were carried using spray-drying in Inulin/OSA-modified starch and Chitosan/Mannitol matrices, respectively, giving the powders (PURIL-CHL and CM-HSE) with good process yield and encapsulation efficiency)[2]. Microencapsulation enhances the stability, solubility, and ease of formulation, offering exciting possibilities for the cosmetic industry. Incorporating these powders into cosmetics preserves their antioxidant property, suggesting beneficial properties in the management of one or more target functions in the skin [2,3].

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2 Siti et al., 2021, Materials Science and Engineering. Vol. 1011. No. 1 3 Bartolini et al., 2022, Food Chemistry: Molecular Sciences, 100134

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UPCYCLING SQUALENE AS PENETRATION ENHANCER IN DERMATOLOGICAL AND COSMETIC FORMULATIONS

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Squalene is a triterpene, one of the main components of skin surface lipids. It is widely used in cosmetic formulations like antioxidant, moisturizer and emollient. Recovered from shark liver in the past, it is recovered from vegetable sources like olive oil, or microorganisms like *S. cerevisiae*. The aim of the work was the optimization of the extraction method of squalene from white wine lees, rich in *S. cerevisiae*, the green catalytic hydrogenation in squalane, and the evaluation of both squalene and squalane as penetration enhancers. We apply ultrasound-assisted extraction (UAE) to isolate squalene from wine lees and *n*-hexane like solvent, in an ice bath to avoid the overheating of the sample. The frequency of the sonicator was 20 kHz and the energy input was 97% of the total energy of 500 W. A duty cycle with an active interval of 8 seconds was used and 4 different extraction times were tested: 10, 15, 20 and 29 min. The quantification of squalene in the lipidic extracts was carried out with HPLC-DAD [1]. The purification of the squalene was made using a column chromatography, and a green Palladium-Mediated catalytic hydrogenation was made, using 3 different clays: montmorillonite, palygorskite and sepiolite. Samples were characterized by ^{1}H-NMR. Franz cells were used for testing the variability of quercetin permeability in the presence or not of either squalene or squalane. Several squalene-based formulations were then tested. The quantification of squalene with HPLC-DAD demonstrated that increasing the extraction time it's possible to extract an higher amount of squalene. The collected solution after the hydrogenation resulted in colourless oil with a yield of 70% for montmorillonite and 60 and 90% for palygorskite-metal and sepiolite-metal, respectively. The ^{1}H-NMR confirmed the hydrogenation of squalene in squalane, where the signals detected from 0.5 to 2 ppm were related to the structure of squalane. Membrane permeability test with Franz cells showed an increase of the permeability of quercetin in the presence of either squalene or squalane. Wine lees is an ethical source of squalene. With a novel and easy process of extraction and catalytic hydrogenation, it is possible to obtain a high quantity of squalane. Its ability to promote permeation of active compounds through membranes make the squalane an excellent ingredient for dermatological and cosmetic formulations.

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BIO-BASED FILM-FORMERS: THE FUTURE OF LONG-WEAR MAKEUP

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The cosmetics industry is rapidly shifting towards replacing fossil fuel-derived polymers and materials with bio-based alternatives to reduce environmental impact and promote sustainability.
The objective of this study is to explore the potential of bio-based film-forming materials as sustainable and effective solutions for long-lasting makeup. The research focuses on the formulation of new bio-based polyurethane polymers with high Natural Origin Index (NOI)[1], derived from selected natural-based polyols. These polymers are designed to create self-consistent films on the skin, exhibiting distinguishable rheological characteristics and compatibility with common cosmetic ingredients. Building upon previous research experiences with alkyl tartrate in combination with HDI and/or IPDI diisocyanates[2,3,4], which have shown advantageous no-trasfer and film-forming ability, the synthesis process needs to be redesigned.
The aim is to select alternative diols with similar functionality and aliphatic branching, offering an overall comb-like structure associated[5]. The formulation process involves optimizing the polymer composition, molecular weight, and other parameters to achieve desired film-forming properties, including adhesion, flexibility, and water resistance.
The research emphasizes the development of bio-based polyurethane polymers with high NOI as a sustainable solution for film-forming applications in cosmetics, addressing the increasing demand for long-wear makeup products with reduced environmental impact.

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BIOLOGICAL ACTIVITY ASSESSMENT OF ACTIVE INGREDIENTS

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The skin represents the interface between body and environment and so for its same nature is continuously working in interaction between itself and external or internal agents [1]. Arterra Bioscience spa is focused on the discovery and production of active compounds for biotechnological application. For discovery of actives with cosmetic application the Arterra researchers in the last 15 years had developed an extensive platform based on *in vitro* and *ex vivo* test to study the effect of natural ingredients on skin cells and tissue. To identify and understanding the biological events that occur following active exposure of skin cells different output can be monitored. Analysing changes in gene expression, in protein synthesis or in signal transduction it is possible to understand if a substance or a natural extract could be effective in inducing skin benefit at different levels. Some examples [2,3] reported that different *in vitro* test performed on specific skin cells could give information on effect of active ingredients in skin cell rejuvenation, in inducing extracellular matrix proteins or in reducing sebum release from skin cells but every molecular target related to a specific cosmetic claim could be investigated.
Following the validation of results from cell-based assays, we used *ex vivo* skin model to translate the efficacy at the cellular level into consumer relevant effects on the skin. The skin specimens are analysed using immune-histological methods to visualize the effect and therefore provide scientific evidence of efficacy. as necessary for the successful launch of a cosmetic active ingredient [4,5].
Recently we are also developing a platform to verify the potential usage of natural extracts as hair care promoting agents based on *in vitro* model composed by human follicle dermal papilla cells, 3D model based on spheroids and *ex vivo* model of human follicles.
In vitro and *ex vivo* therefore represent reliable and robust systems to characterize active ingredients for their biological activity and to better finalize the following clinical test as necessary for the successful market launch of them.

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GREEN APPROACH FOR THE ACTIVATION OF POLYPHENOLIC COMPOUNDS FOR DERMATOLOGICAL AND COSMETIC APPLICATIONS

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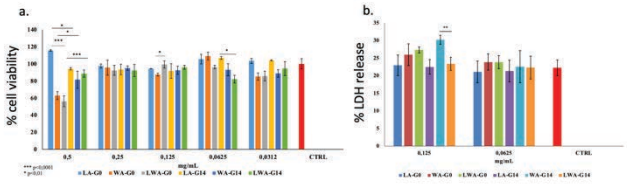

Figure 1. MTT (a) and cytotoxicity (b) assays.

Food waste is a major waste management and environmental issue in our society. These wastes may have beneficial components like antioxidant molecules that may be recovered and valorized. This study focuses on fruit peel, an abundant source of antioxidant molecules, to develop a sustainable extraction process that allows for the recovery of these valuable molecules. We developed a novel and eco-friendly method to prepare fermented extracts from fruit peel, exploiting the potential of enzymatic sources to promote the bioconversion of glycosylated molecules into their aglyconic forms [1]. Ultrasonication and enzymatic fermentation were combined to obtain the final extract reached in bioactive molecules. The peel (1 g) was dispersed in 100 mL of solvent and it was sonicated using Ultrasound-Assisted Extraction (UAE) (frequency of 20 kHz, amplitude of 95% for the total energy of 500 W, 3 min.). The extracts were incubated with an enzymatic complex and kept at 37 °C in the incubator for 14 days. All samples were then subjected to sonication, centrifugation, and subsequent analyses at three different time points, G0, G1, and G14, to monitor the samples over time. The evaluation of the formation of the bioactive molecules in the aglyconic form in the fermented extracts was carried out by High-Performance Liquid Chromatography (HPLC-DAD). The results allowed the identification and monitoring over time of the loss of signal related to glycosylated molecules, cyanidin 3-glucoside and pelargonidin 3-glucoside, used as standards. The antioxidant capacity (DPPH, ABTS, FRAP assays), Total Phenol Content (Folin-Ciocalteu assay), cell metabolic activity and cytotoxicity using human fibroblast (HGFs) were evaluated. We detected an increase in the total phenol content in the samples G0 and G14 which represent the fermented extracts. The antioxidant capacity showed a high antioxidant capacity in G1 and G14 fermented samples. The cell results (Figure 1) revealed that all tested sample doses appeared to be perfectly tolerable by HGF cells, as the cellular vitality rate always exceeds 85%. A statistical reduction in cell toxicity when treated with samples of fermented extracts was also detected. These promising results open up new opportunities in the development of functional products derived from fruit peel, contributing to the sustainable recovery of valuable food resources.

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PROTECTIVE EFFECT OF *CAMELLIA SINENSIS* L. EXTRACTS ON UVB-IRRADIATED FIBROBLASTS TO USE IN COSMETIC FORMULATIONS

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Skin aging is a complex process that may be caused by different factors such as ultraviolet (UV) radiations that represent one of the main sources of skin damage and characterizes a process known as photoaging. Based on the wavelength, UV spectrum can be divided into three segments: UVA (320-400 nm), UVB (280-320 nm) and UVC (100-280 nm)[1]. In this study, the attention was focused on UVB radiations that represent only the 5% of total UV radiations that reach the Earth, but they are considered extremely damaging because of their high energy. These are the radiations responsible for summer tan, but an over exposure can cause oxidative stress, burns, erythema, and other severe damages to the skin involving degradation of extracellular matrix and excessive production of melanin which is responsible for several dermatological problems. Cells have developed antioxidant defence and repair mechanisms to reduce the UV-induced genotoxic damage. Recently, several molecules have been studied for their ability to enhance these defences, and considerable attention is given to compounds of natural origin. White tea (WT), green tea (GT), black tea (BT) and decaffeinated black tea (DBT) are obtained from different fermentation processes of *Camellia sinensis* L. leaves. Given their known antioxidant power[2], the present study aimed to evaluate their potential anti-aging properties. The phytochemical profile was analysed by spectrophotometric assays determining the total content of flavonoids, phenols and condensed tannins[3], and evaluating the quantitative of theaflavins and thearubigins[4] by HPLC-PDA/UV-ESI-MS/MS. The extracts were analysed for the Sun Protection Factor (SPF), and for their ability to inhibit enzymes involved in the aging process of the dermis, such as tyrosinase and elastase. BT and DBT showed the highest SPF (23.47 and 23.15) and good inhibition against enzymes which can be ascribed to the high presence of theaflavine and thearubigine. The anti-photoaging effect of the extracts was studied in UVB (100 mJ/cm^2)-irradiated normal human dermal fibroblasts (NHDF)[5]. WT and GT were able to prevent the UVB-induced loss of viability at lower concentration (50 µg/mL) than BT (200 µg/mL), while DBT was ineffective at the tested concentrations. The photoprotective properties of green tea are widely known among the scientific community, but considering the promising results on black tea obtained for the first time in this study, its use in the cosmeceutical field could be considered.

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INTEGRATED ANALYTICAL TOOLS AND BIOASSAYS TO ELUCIDATE THE PROTECTIVE ROLE OF A NOVEL SPIRULINA, GANODERMA AND MORINGA COMBINATION IN A DOXORUBICIN MEDIATED CARDIAC DAMAGE MODELS

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Anthracyclines are essential adjuvant therapies for a variety of cancers, particularly breast, gastric and esophageal cancers. Whilst prolonging cancer-related survival, these agents can induce drug-related cardiotoxicity. Spirulina, Reishi and Moringa are three nutraceuticals with anti-inflammatory effects that are currently used in cancer patients as complementary and alternative medicines to improve quality of life and fatigue. We hypothesize that the nutraceutical combination of Spirulina, Reishi and Moringa (Singo) could reduce inflammation and cardiotoxicity induced by anthracyclines. Firstly, we investigated the chemical composition of Spirulina platensis, Moringa oleifera and Ganoderma lucidum extracts. To determine the protein composition, a label-free based liquid chromatography–mass spectrometry (LC–MS/MS) proteomic approach was carried out. The analytical platform allowed us to determine the distribution of the molecular weight, protein sequence coverage and the profile of lengths for all identified peptides. The polysaccharide content of extracts was determined by the phenolic–sulfuric acid method while the characteristic functional groups were identified by FT-IR spectroscopy. Female C57Bl/6 mice were treated with short-term doxorubicin and Singo. In preclinical models, Singo significantly improved ejection fraction and fractional shortening. Reduced expressions of myocardial NLRP3 and NF-kB levels in cardiac tissues were seen in DOXO–Singo mice vs. DOXO ($p < 0.05$). The myocardial levels of calgranulin S100 and galectin-3 were strongly reduced in DOXO–Singo mice vs. DOXO ($p < 0.05$). Immunohistochemistry analysis indicates that Singo reduces fibrosis and hypertrophy in the myocardial tissues of mice during exposure to DOXO.
In conclusion, in the preclinical model of DOXO-induced cardiotoxicity, Singo is able to improve cardiac function and reduce biomarkers involved in heart failure and fibrosis [1].

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HEMP SEEDS: A SOURCE OF POTENTIAL BIOACTIVE NATURAL COMPOUNDS

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From ancient times seeds, as legumes or cereals, represent a source of different natural components such as proteins, essential amino acids, dietary fiber, essential fatty acids, polyphenols, etc., which are important nutrients in human diet. Hemp (*Cannabis sativa*) seeds contain lipids (25 – 35%) composed of high percentages of polyunsaturated fatty acids, among which linoleic and linolenic acids are the most representative. In addition, hemp seed is a good source of proteins (20%–25%) that contain essential amino acids, and dietary fiber (about 30%)[1]. Moreover, also polyphenols are present in *Cannabis sativa* seeds, exerting antioxidant properties[2].

Hemp seeds, that represent a by-product of the cultivation of industrial hemp mainly destined to the production of textile fiber, can be processed to obtain oil and flour. In the last few years, hemp flour, a naturally gluten free flour, is becoming an interesting ingredient for bakery products as it is a rich source of proteins, essential amino acids, fiber, and essential fatty acids[3].

In this contest, the present study is aimed at evaluating the potential use of hemp flour to fortify bread formulations to improve the nutritional profile. The first step of the work was the chemical characterization of hemp flour, obtained from seed of hemp plant destined to the production of textile fiber. Macro-component (water, lipids, proteins and fiber) content was determined, and then also the profile in essential amino acids, fatty acids, and phytosterols was investigated. In addition, total phenolic content (TPC), and antioxidant activity were determined. The same parameters were then studied in bread samples fortified with hemp flour. The results underlined a significant increase ($p<0.05$) in protein and amino acids, lipid and fatty acids, TPC and antioxidant activity in hemp flour enriched samples if compared to the typical white bread.

In conclusion, hemp flour could be considered a good source of bioactive compounds, and this promote its use in human diet.

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FECAL METABOLOMIC FINGERPRINTING OF BREASTFED LATE PRETERM INFANTS VERSUS POSTBIOTIC-SUPPLEMENTED FORMULA MILK

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In contrast to conventional foods, functional foods have demonstrated physiological benefits and can reduce the risk of chronic disease beyond basic nutritional functions, including maintenance of gut health[1]. Exclusive breast milk is suggested from birth to 6 months of life as normative standard for infant's nutrition[2]. Human milk contains a number of nutritional and bioactive molecules including microorganisms such as *Bifidobacterium* spp. and *Lactobacillus* spp., which colonize the intestine of the newborn, and their metabolites. However, if breast milk is not available or insufficient, formula milk is used as a substitute. Infant formula can be supplemented with postbiotics to promote maturation of immune, metabolic and microbial components, similar to breast milk. Postbiotics are preparations composed of both microbial constituents and their metabolites, produced during fermentation.
The aim of the present study was to detect the differences in the fecal metabolome of the newborns fed with breast milk, standard formula milk or formula milk supplemented with 0,5 mL of SMART D3 MATRIX, through the use of untargeted and targeted MS-based metabolomics followed by multivariate data analysis.
A prospective single-center observational cohort study was conducted from March to December 2022 at Buon Consiglio Fatebenefratelli Hospital (Naples, Italy). 27 late preterm newborns with body weight appropriate to gestational age were enrolled in this study. The patients were divided into 3 groups: group A (feeding with formula + SMART D3 MATRIX, 7 patients), group B (feeding with standard formula, 9 patients), group C (breast feeding, 11 patients). Stool samples for metabolome study were collected at T0 (5-7 days after birth), T1 (1 month old) and T2 (3 months old) giving rise to 81 collected samples, and analyzed by liquid chromatography coupled to high resolution mass spectrometry (in duplicates). T0, T1 and T2 LC-MS raw data were processed using the software MZmine 2.53 and SIMCA P 17.0 (Umetrix AB, Umea Sweden) for Principal Component Analysis (PCA) and Partial Least Square-Discriminant Analysis (PLS-DA).
The plot obtained with these projection methods showed a good separation between stool samples (T0, T1 and T2), highlighting similarities between fecal metabolome of breast and formula plus postbiotic fed infants. On this basis, this new formula milk could be considered as a good alternative to breast milk.

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MEXICAN PURPLE CORN: A PROMISING SOURCE OF ANTHOCYANINS

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Anthocyanins are secondary metabolites that in recent decades have been of great interest to both the scientific community and consumers, who seek to take advantage of their bioactive properties and qualities as a natural colorant[1]. Its importance is such that new sources of anthocyanins are continuously sought among plant diversity. In our country, we have identified that Mexican Purple Corn is a plant genetic resource with a natural potential to produce anthocyanins in the kernels, corn husks, and corncobs, conferring them a purple shade. At Colegio de Postgraduados we have studied, preserved, and improved its agronomic attributes and grain yield[2, 3]. At this moment, we count on outstanding genotypes that express a higher frequency of corn ears of a dark and intense purple color. To size the productive potential of anthocyanins of the Mexican Purple Corn, we quantified the content of total anthocyanins (TAC, mg of TA 100 g^{-1}) in the kernels, corn husks, and corncobs from six genotypes (from Pop1 to Pop6) obtained in the Pigmented Corn Improvement Program of our institution. The results endorsed that anthocyanins are synthesized in all the organs of the corn ear and, that the concentration is contrasting between them ($p < 0.05$). Corncobs accumulated the highest quantity of anthocyanins (1295.7 mg of TA 100 g^{-1}), followed by the corn husks (752.7 mg of TA 100 g^{-1}), and kernels (96.8 mg of TA 100 g^{-1}). According to the maximum concentrations spotted in each organ, we suggest that it is possible to obtain higher TACs; this variant of corn is capable to produce up to 2497.2, 1618.3, and 183.1 mg of TA 100 g^{-1} of corncobs, corn husks, and kernels, respectively. From the studied genotypes, the ones with the higher TACs were Pop5 (1537.7 mg of TA 100 g^{-1} of corncobs, 961.8 mg of TA 100 g^{-1} of corn husks, and 112.9 mg of TA 100 g^{-1} of kernels) and Pop3 (1468.9 mg of TA 100 g^{-1} of corncobs, 786.1 mg of TA 100 g^{-1} of corn husks, and 108.7 mg of TA 100 g^{-1} of kernels). With the above, it is evident that Mexican Purple Corn is a sustainable and promising source of anthocyanins due to its pigment content and because of the use of organs that usually are considered agricultural waste.

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EVALUATION OF *IN VITRO* WHEY PROTEIN DIGESTIBILITY IN A PROTEIN-CATECHINS MODEL SYSTEM MIMICKING MILK CHOCOLATE

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Flavonoids are largely present in plant food such as cocoa and derived products. These compounds can interact with proteins inherently contained in the food matrix and/or the proteolytic enzymes involved in gastrointestinal digestion. The flavonoid/protein interaction might hamper protein bioaccessibility and digestibility, affecting the nutritional quality. However, information on the digestion fate of proteins in food matrices containing both proteins and flavonoids is limited. The aim of this work was to evaluate the interaction between proteins and flavonoids and verify the potential effects of this interaction on protein digestibility. Taking milk chocolate as model, first a simple whey proteins/catechins mixed system was evaluated, and then the effects on digestibility were also verified in a real sample of commercial milk chocolate. The effects of the catechins/whey proteins interaction in the model system were evaluated by optical and chiro-optical spectroscopy, outlining a slight protein structure modification upon interaction with catechins. The digestibility of the protein fraction both in the model system, with and without catechins, and also in milk chocolate, was then determined by the application of INFOGEST in vitro digestion method: the bioaccessibility was evaluated in terms of protein hydrolysis and protein solubilisation, and major peptides generated by the digestion were also determined by LC/HR-MS. Despite the slight interaction with proteins, flavonoids were found to not hinder nor modify protein solubilization, protein hydrolysis and peptide profile by digestive enzymes. Also protein digestibility in milk chocolate, evaluated by SDS-PAGE, was found to be complete. The present data clearly indicate that the interaction of the proteins with the flavonoids present in the cocoa matrix does not to affect protein bio accessibility during digestion.

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CATIONIC GUAR: FROM A NATURAL SOURCE TO A SUSTAINABLE COSMETIC INGREDIENT

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According to current Personal Care market trends, respect for environment and people are of great importance. At the same time performance, safety and sensorial appeal need to be taken into account**.** For this reason, when developing new cosmetic ingredients, it is important to combine three essential pillars: nature, ethics and science. In this work, this goal is achieved through the use of a suitable renewable raw material, which can be conveniently modified through responsible chemistry, acquiring specific properties in line with Personal Care latest requirements.
In the wide range of natural polymers, guar gum is one of the most known and used polysaccharides. It is a high molecular weight polygalactomannan, composed of beta-linked poly-mannose linear chains with galactose alpha 1-6 ramifications, obtained from the seeds of *Cyamopsis Tetragonolobus,* a *Leguminosae* annual plant (1,2).
Among guar derivatives, cationic ones are widely used in hair care applications, especially conditioning shampoos, to impart improved combability, frizz control, enhanced deposition of water-insoluble ingredients and hair shine (3). Cationic guar properties, together with shampoo composition, influence final formulation performances. Molecular weight (MW) and degree of cationic substitution (DS) are demonstrated to be important parameters to fine-tune conditioning effects (4), targeting specific consumer's needs.
Usually, industrial productions of this kind of guar derivatives require a highly water-demanding purification step, for the removal of dangerous by-products; these processes lead to relevant amounts of wastewater. In this work we present a new low-water demanding process for the manufacture of high-quality cationic guar for Personal Care. Thanks to this procedure, therefore, a significant reduction of the environmental impact is achieved. Thus, cationic guar obtained through this technology represents a good example of personal care ingredient in line with modern trends.

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UTILIZATION OF OLIVE AGRICULTURAL RESIDUES FOR THE DEVELOPMENT OF HIGH ADDED VALUE PRODUCTS FOR THE WOOD PROCESSING INDUSTRY

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The wood processing industry is a key pillar of the global economy. A shortage of timber is observed, mainly due to deforestation, inadequate management and utilization, and the growing demand for wood for pulp and biorefinery production. On the other hand, the agro-industrial sector is responsible for the production of a huge amount of liquid or solid wastes, the exploitation and proper environmental management of which is a major challenge [1]. The present work involves the manufacture of innovative and ecological wooden boards with the use of waste from olive processing and new adhesives. By-products of the olive processing were used, specifically olive leaves, olive branches and olive pomace. The samples were extracted according to predefined conditions. The extracts produced were rich in secondary metabolites and they were analyzed with HPLC-DAD and HRLC-MS. Samples before (initial raw materials) and after (residual materials) extraction were characterized by means of infrared spectroscopy (FTIR), thermogravimetry (TGA), scanning electron microscopy (SEM-EDS) and X-ray diffraction analysis (XRD), to identify their chemical structure and elemental analysis, thermal behavior and morphology. In continuation, the woody materials (olive branches) were used to replace virgin wood for the manufacture of chipboards, while the remaining non-woody materials were pulverized and used as components in the manufacture of adhesives. New protein adhesives were developed meeting market demands for green products. This work has revealed new materials from agricultural waste biomass that can be successfully used in the manufacture of wooden boards and offer technology for new, sustainable, and “green” products in the context of the circular economy.

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ECO-FRIENDLY FERTILIZER PRODUCTION THROUGH SUSTAINABLE NUTRIENT RECOVERY FROM WASTEWATER EFFLUENT USING VACUUM-ENHANCED MEMBRANE DISTILLATION

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Municipal wastewater treatment has traditionally involved aerobic processes, with the final effluent being released into water bodies. Nevertheless, the energy-intensive nature of the aerobic method has led to the exploration of more efficient alternatives. [1] One such solution involves employing an anaerobic digestion process alongside membrane filtration, resulting in what is known as an anaerobic membrane bioreactor (AnMBR). This innovation has garnered attention due to its potential for energy neutrality or positivity.[2] Despite its advantages, the AnMBR effluent contains NH_4^+ and PO_4^{3-}, which cannot be directly discharged into natural water sources. However, proper recovery methods can transform these compounds into valuable nutrients. In light of this, the current study focuses on the concentration and recovery of NH_4^+ and PO_4^{3-} from AnMBR effluent, achieved through a vacuum-driven membrane distillation (MD) process. The MD process utilizes submerged hydrophobic membranes with a contact angle of 118 degrees being operated under vacuum conditions. Initial AnMBR effluent resulting from synthetic municipal wastewater treatment exhibited NH_4^+ levels of approximately 44 mg/L and PO_4^{3-} levels of 2.8 mg/L. The MD process concentrated NH_4^+ and PO_4^{3-} up to a factor of 10 at an acidic pH level of 5. The resulting concentrate holds promise as an environmentally friendly fertilizer. However, challenges arose at neutral and alkaline pH levels (pH 7 and 9) due to precipitation reactions, particularly involving substances (e.g., $CaCO_3$). These reactions led to significant fouling in the MD process. In contrast, no notable fouling was observed at pH 5. In summary, using a vacuum-enhanced MD process, this study successfully demonstrated the recovery of highly concentrated nutrients (NH_4^+ and PO_4^{3-}) from AnMBR effluent within an acidic environment. The National Research Foundation of Korea supported this study through grants (No. 2020K1A4A7A02108858 and 2022K1A3A1A25081808).

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ACCESSING THE HIDDEN TREASURE OF BROMINATED EXOMETABOLITES RELEASED BY TWO MEDITERRANEAN SPONGES

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Marine sponges are known to produce a wide variety of structurally diverse specialized metabolites that are extensively studied by natural product chemists, working both in drug discovery and chemical ecology.[1] Through their filter feeding and metabolic activities, sponges can recycle organic matter, subsequently releasing diverse metabolites (*i.e* exometabolites) in their environment, and possibly including their specialized ones.[2-3] *Aplysina cavernicola* (Vacelet, 1959) and *Agelas oroides* (Schmidt, 1864) are two emblematic and relatively well-studied Mediterranean sponges known to produce bromo-spiroisoxazolines (*e.g.* aerothionin) and bromo-pyrroles (*e.g.* oroidin) alkaloids, respectively.[4-5] The present study evaluates the presence, structural diversity, and relative concentration of brominated alkaloids in the seawater surrounding each sponge species. Replicated experiments were conducted both in aquaria and *in situ,* where complementary exometabolite enrichment set-ups were deployed. MS-based metabolomics, dereplication through molecular networking, and *in silico* structural annotation were implemented in order to characterize the exometabolite chemodiversity.[6] Consequently, new compounds were structurally identified for each species, which also displayed unique exometabolite fingerprint. *Aplysina cavernicola* was found to release a greater diversity of metabolites. Some of them were more abundant in the concentrated seawater extract than in the sponge crude extract. The collected results open new questions pertaining to the structural stability of compounds when released in seawater, but also to their functionalities in benthic ecosystems. Overall, the presentation will illustrate that new chemical insights can be gathered, even on already well investigated sponges, when considering the organisms in their environnement and from the lens of marine ecologists.

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LINKING GENOMICS AND METABOLOMICS TO DISCOVER BIOACTIVE NATURAL PRODUCTS IN MARINE EXTREMOPHILES

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Extremophiles are living organisms able to survive and proliferate in environments with extreme physical (temperature, pressure, radiation, pollution) and geochemical parameters (salinity, pH, redox potential), with the vast majority of them belonging to Archaea and Bacteria. So far, marine extremophilic bacteria have been acknowledged as a prolific factory of bioactive natural products. Network analysis of microbial genomes and metabolomes has emerged as a valuable strategy to enhance the bioassay-guided fractionation workflow and enable discovery of novel natural products from bacteria, as reducing the rediscovery rate, prioritizing isolation of new chemical entities and giving access to the biosynthetic tools to create sustainable sources of rare bioactive compounds.

Herein, we will show how linking genomics and metabolomics led to the identification of a) cyclolipopeptides from the deep-sea *Rhodococcus* sp. I2R, b) imidazolium-containing alkaloids from *Shewanella aquimarina* sp., and c) lassopeptides from *Novosphingobium* sp. PP1Y.

The biosynthetic ability of *Rhodococcus* sp. I2R to produce cyclolipopeptides was inferred by genome mining, shedding light on the presence of a multimodular non-ribosomal peptide synthetase (NRPS) gene cluster which allowed to predict the chemical structures of the encoded metabolites.

Combining the OSMAC approach, the tandem mass spectrometry molecular networking analysis and the bioassay-guided fractionation unveiled the presence of novel imidazolium-containing phenethylamine derivatives from *Shewanella aquimarina.*

In silico genome analysis led to the detection of the biosynthetic pathway of a family of new lassopeptides from *Novosphingobium* sp. PP1Y., paving the way for the heterologous expression of these metabolites.

INVESTIGATING THE ROLE OF CYANOBACTERIA PROTEASE INHIBITORS ON MODULATING THE EXO-METABOLOME IN THE MARINE MICROBIAL LOOP

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Countless natural products (NPs) from numerous cyanobacteria genera including *Microcystis, Dichothrix, Lyngbya, Nostoc* and *Oscillatoria*, are known to inhibit protease action [1, 2]. Most interest in these molecules have been in the context of drug discovery, where protease inhibitors represent an important class of pharmaceutical mode of action, with multiple block-buster drugs on the market. However, protease inhibition is a key process in many ecosystems, such as predator - prey biochemical warfare, and organic matter turnover, as proteases are among the key enzymes in the enzymatic cascade of the Microbial Loop, and the foundation of marine food webs [3].

To capture and assess the unknown space of protease inhibitors in complex environmental samples, we developed a native metabolomics workflow that combines non-targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) and parallel protease binding assays, which we leveraged to identify a rang a protease inhibitors from *cyanobacteria* [4].

To assess the effect of *cyanobacteria* protease inhibitors on the ocean microbiome, their community metabolome and turnover, we performed incubation studies with bulk dissolved organic matter from the pacific surface ocean as well as exo-metabolomes from *cyanobacteria*, under the presence of serine proteases and protease inhibitors. We tracked the chemical transformation of the myriads of metabolites via non-targeted LC-MS/MS, feature-based molecular networking and our recently developed chemical proportionality approach. Our preliminary results indicated a multitude of biotransformations that are directly modulated by the presence of the protease inhibitors tested. This work provides first insight on the role of protease inhibitors in modulating organic matter turnover in the surface ocean which may have important implications for microbial community function and global carbon cycling. Beyond important new biogeochemical insights, our methodological framework contributes to the systematic investigation of the role of natural products in the environment and how chemistry shapes our planet.

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AN ALIEN METABOLITE AND AN ANTHROPOGENIC CHEMICAL HAZARD COMPARED ON *MYTILUS GALLOPROVINCIALIS*

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Bioactive natural products from marine invasive species may dramatically impact native communities, while many synthetic pharmaceutical drugs are also released into the marine environment and have long-lasting harmful effects on aquatic life. The present report focuses on the alkaloid caulerpin (CAU) from the green alga *Caulerpa cylindracea*, which is highly invasive in the Mediterranean, and on the synthetic lipid-lowering drug fenofibrate (FFB) that is widely considered hazardous to the aquatic environment. Since CAU and FFB share similar mechanisms of action performing as agonists of peroxisome proliferator-activated receptors (PPARs)[1], concerns about the ecotoxicological potential of CAU have been raised in analogy with FFB. Here, we compared the effects of the two compounds on the mussel *Mytilus galloprovincialis* as an ecotoxicological model, through biochemical, metabolomic, and histopathological analysis. Mussels were fed with food enriched with CAU or FFB (1mg/g dry food) under laboratory conditions. After treatments, biochemical markers revealed metabolic capacity impairments, cellular damage, and decrease in acetylcholinesterase activity in mussels fed with FFB-enriched food. Metabolomic results also showed significant alterations in FFB-treated mussels. In addition, FFB produced morphological damage in the mussels' gills and digestive tubules. Conversely, dietary treatment with CAU did not affect the mussels' physiology. Overall, our results support a sustainable valorisation of the biomass from the highly invasive *C. cylindracea* as a source of CAU, a metabolite of interest in drug discovery[2].

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STEROL SULFATES AS DEADLY MEDIATORS IN MARINE DIATOMS AND TRIGGERS IN INNATE IMMUNE SYSTEM

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Diatoms are unicellular photosynthetic microalgae responsible of the 40% of the marine primary productivity and of the 25% of the carbon fixed on earth. Large scale production of diatom biomass and organic compounds has been a topic of industrial interest for decades. These organisms are infact considered a huge source of natural products for the development of drugs, energy, biomaterials. Peculiar characteristic of some of these microalgae is the rapid development of short-term large populations after a rapid exponential growth referred as algal bloom or flowering.

Research in the last years has pointed out as also chemical signals derived from interspecific and intraspecific biotic interactions can shape complex community structure in aquatic systems. In this context we characterized sterol sulfates (StS) as inhibitory molecules from a marine diatom *Skeletonema marinoi*, acting as intracellular mediators in death signal transduction pathway[1,2]. The StS pathway provides another view on cell regulation during bloom dynamics in marine habitats and opens new opportunities for the biochemical control of mass-cultivation of microalgae.

Contrary to well understood biochemistry of sterols in plants, animals and fungi, the knowledge of this process in diatoms remains fragmentary. We defined the sterol biosynthesis in diatoms as a hybrid pathway with very peculiar characteristics that combines plant and animal reactions[3].

Starting from the eco-physiological role in diatoms, further investigation outlined the specific capacity of StS to trigger the response of human innate immune system. The results obtained led to the investigation about the use of sterol derivatives as ligands of a specific member of Pattern Recognition Receptors.

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THE POTENTIAL OF MARINE DIATOMS FOR BIOACTIVE SECONDARY METABOLITES PRODUCTION: ECOLOGICAL AND BIOTECHNOLOGICAL IMPLICATIONS

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Diatoms are one of the major and most diverse group of eukaryotic microalgae, accounting for up to 40% of annual productivity at sea. They are widely distributed in all aquatic environments, and to thrive in these very diverse habitats they have developed different strategies to adapt to different conditions through the perception of environmental signals[1, 2] and to fight against competitors and grazers[3, 4]. Although the growing interest in phytoplankton chemical ecology, there are still several open questions, and for some secondary metabolites a definitive identification of their role is still lacking. Our recent exploration of the biosynthetic potential of some diatom species led to the identification of key enzymes involved in the production of bioactive secondary metabolites still poorly investigated in these microalgae. The transcriptomic analysis of *Thalassiosira rotula* allowed the identification of pathways involved in the production of bioactive molecules, i.e. secologanin, polyketides, and prostaglandins (Pgs)[5], compounds that often display biological activities of possible interest for various biotechnological applications. The gene expression of the key enzymes involved in this pathways was assessed along the growth and under different nutritional stresses showing that secologanin synthase was significantly up-regulated in silica limitation while other biosynthetic pathway, as for prostaglandin biosynthesis, were significantly down-regulated in this condition. The Pgs biosynthetic pathway has been further investigated and we showed a peak of expression of PgE-synthase and PgF-synthase at the end of the exponential growth phase while the expression rate-limiting enzyme cicloxygenase increased at the stationary phase, when associated bacteria population thriving on organic matter released by dead diatom cells increases, suggesting a possible role of these molecules in controlling the associated bacterial community[6].

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CHEMICAL INTERACTION AND COMMUNICATION IN INSECT HOLOBIONTS

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Environmental microbes are an under-exploited resource for the discovery of bioactive compounds. Plants are the main sources of natural products and have contributed to the discovery of drugs for the treatment of various human diseases[1,2]. However, the arsenal of specialized metabolites has been recently expanded to include those of microbial origin and, among others, those associated with plants and insects[3,4]. Insects are symbiotic with a wide variety of microscopic life forms, including bacteria, fungi, protozoa, and nematodes. The study of these holobionts requires the isolation and identification of the microorganisms. To this end, we first developed a tool for the identification and dereplication of environmental strains by MALDI-TOF MS fingerprint comparison[5]. Then, in order to identify microorganisms naturally producing chemical weapons against different pathogens (such as entomopathogens), the chemical diversity of microorganisms was explored based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and molecular networks (with MetGem software based on t-SNE visualization developed in our institute)[6]. The search for biological activities is guided by the environmental function of these microorganisms. The combination of different methods such as OSMAC (One Strain Many Compounds), metabolomics and genomics for the production and annotation of secondary metabolites implemented in this work allow to significantly accelerate the chemical study of extracts of microorganisms for the isolation of new bioactive compounds whose use is potentially transferable in human health[7].

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UNCOVERING PHYTOTOXIC METABOLITES FROM *COLLETOTRICHUM* SPP. INVOLVED IN LEGUME DISEASES BY OSMAC-METABOLOMICS APPROACH

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Anthracnose disease, caused by various species of *Colletotrichum* fungi, poses a significant threat to major legumes worldwide, resulting in substantial economic losses. The disease is characterized by dark, sunken lesions on leaves, on stems, or on fruits. *Colletotrichum* species have produced a wide range of biologically active and structurally unique metabolites that could be involved in the infection process. In this study, we aimed to uncover the phytotoxic secondary metabolite profiles produced by three isolates of *Colletotrichum* spp. involved in legume disease. We investigated the metabolites produced by two isolates of *C. truncatum* and *C. trifolii* employing the One Strain Many Compounds (OSMAC) approach integrated with targeted non-targeted metabolomics profiling. Four substrates were selected for in vitro growth: PDA, PDB, Richard's medium and rice. Additionally, we evaluated the phytotoxicity of crude fungal extracts on their primary host and related legumes, investigating possible correlation with the metabolite profiles generated under different cultural conditions. Our results showed that the phytotoxicity of organic extracts varies depending on legume species, fungal isolates, cultural conditions, and tested concentration. The highest phytotoxicity was found in Richard's medium extract. The metabolomics analysis and the chemometrics analysis pinpointed 84 discriminant metabolites. As a general result, these secondary metabolites produced by *Colletotrichum* species belong to numerous natural product classes, including alkaloids, terpenoids, coumarins, chromones, xanthones, polyketides, quinones, peptides, phenols, and macrolides lactones. Furthermore, our data show that their production heavily depends on the selected cultural media. The chemometric analysis of the untargeted metabolomics profiles of the organic extracts showed that the selected cultural media influence the production of specific secondary metabolites, even though the upregulated or downregulated compounds depend on the *Colletotrichum* species. In conclusion, studying secondary metabolites, particularly phytotoxic ones, is essential to gain information on the pathogenicity or virulence of a specific pathogen to develop more sustainable control methods. Indeed, this research could be beneficial for investigations dealing with fungal chemotaxonomy, for host-pathogen interaction, searching for potential fungal biomarkers or, more in general, for investigations dealing with genomics, transcriptomics, and proteomics, to provide a more comprehensive understanding of fungal metabolism and physiology.

THE UNIQUE CUTICULAR AND DEFENSE CHEMISTRY OF COLLEMBOLA

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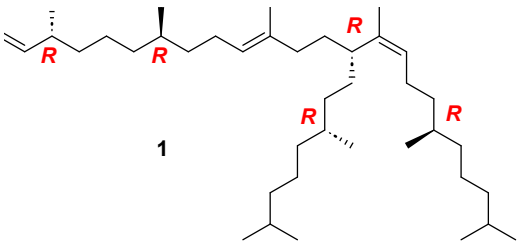
Collembola or springtails (Hexapoda) are a sister class of the Insecta with around 10,000 described species from which they diverged already 400 million years ago. These tiny arthropods have a surprisingly different chemistry compared to insects. Access to biological material is limited due to their tiny size, often less than 1 mm. As is typical for arthropods, the collembolan cuticle is covered by an epicuticular wax layer, which is often superhydrophobic, non-fouling and self-cleaning. In contrast to insects, which use long-chain fatty acid-derived alkanes as cuticular compounds, many Collembola are covered by terpene hydrocarbons such as viaticene (**1**).[1] The first total synthesis of this unique [6^{14}+2^{1}]-terpene uses cross-coupling as key steps to arrive at different steroisomers, establishing its *all-R*-configuration. Another example a of cuticular terpene is socialane of *Hypogastrura socialis*, the first cyclic, fully regular head-to-tail connected [9]-terpene. The structure of socialane was postulated from GC/MS and NMR data. The relative configuration of the naturally occurring isomer was elucidated by total enantioselective synthesis of several of the 128 possible stereoisomers. But even the fatty acid-derived hydrocarbons used by some Collembola show unique features, as alkanes with cyclopropane units or as highly branched long-chain esters. These structures were proven by synthesis. Chemical defense is widespread in Collembola, replacing or supporting the primary defense mechanism of jumping. Sigillin A (**2**) is a Collembolan insecticide and insect deterrent.[2,3] New derivatives occur in unrelated species, suggesting widespread occurrence within the Collembola. The class of the collembolan-specific pyridopyrazines such as (**3**)[4] is extended by imiddazopyrazine **4**, surprinsingly a new natural product.



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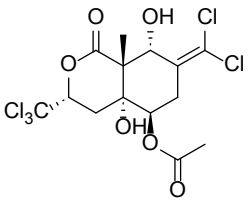

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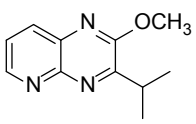

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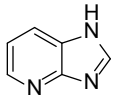

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CHEMICAL DIVERSITY AND CHEMOTAXONOMY OF NEO-TROPICAL XYLARIALES FUNGI

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Fungi of the order Xylariales belong to the class Sordariomycete in the Ascomycete division. Most can form large fruiting bodies and produce a high diversity of specialized metabolites, including some with significant antibiotic activities. However, the majority of these compounds were extracted and identified from their microscopic form.(1) In this work, we will focus on the macroscopic form of Xylariales fungi to study their metabolic production and its potential relationship with taxonomy.
Thirty-eight fungal specimens were collected from French Guiana and morphologically identified. To further refine their identity, an environmental strain replication method developed at ICSN was employed, lipidic fingerprints were obtained by MALDI-ToF-MS, data processing was achieved using R(v4.1.2) and spectral comparison using MetGem.(2,3) Results showed a good match for replicates of six species from genus *Xylaria*, *Phylacia*, *Thamnomyces* and *Camillea.* However, no match was obtained within the *Kretzschmaria* genus despite several replicates of *K. clavus* and *K. deusta.*
To complete this chemotaxonomic study, specialized metabolite production was also explored. Ethyl acetate extracts of each specimen were analyzed by RP-LC-HRMS/MS. The data was used to generate a molecular network to annotate each metabolome. We detected compounds commonly produced by genera in the Xylariaceae family. Among these compounds, we focused on a family of metabolites abundant in genus *Thamnomyces* that could not be annotated using databases. Several compounds were isolated to characterize the structure of new or non-dereplicated molecules from the databases.

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IDENTIFICATION OF CYSTEINE-KNOT PEPTIDES IN PHILIPPINE PLANT SPECIES

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Cystine-knot miniproteins or knottins are small proteins with 30-50 amino acids. These molecules are extremely stable due to the presence of a 'knot' from three disulfide bridges. Knottings from plants are very stable even in the presence of mutations and peptide grafts thus making them potential drug scaffolds. LC-MS screening of 180 plant species revealed 6 Philippine plants that produce disulfide-rich peptides that are structurally related to knottins. Further analysis of these plants using transcriptome screening showed the presence of knottin-like peptides similar to previously reported Apocynaceae alpha amylase inhibitors in Alstonia scholaris, Wrightia pubescens and Tabernaemontana pandacaqui. Given the novelty of their sequences, it is possible that these peptides possess novel functions as well. This is the first report of cysteine knot peptides from *W. pubescens* and *T. pandacaqui.*

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TINTENSTRICH COMMUNITES: A FIRST INSIGHT INTO ALPINE CYANO-LICHEN

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Subaerial and often dark-colored biofilms, termed *Tintenstrich* (from German ink stripes) communities (TCs) extend on rock surfaces in semi-aquatic environments. TCs are widely distributed in high mountain areas, including large parts of the Alpine region. They are predominantly composed of free-living cyanobacteria and cyano-lichens (cyanobacteria associated with lichen-forming fungi). Cyanotoxins pose a major concern for environmental and public health and the World Health Organization defined limits for four cyanotoxins in drinking water and recreational water quality guidelines[1]. Despite that, their occurrence and role at the soil-water interface remains unknown. The primary objective of this work is to provide initial insights into the presence of cyanobacteria (16S rRNA gene sequencing), toxin-producing genes (PCR), and metabolite profiles (onlineSPE-LC-HRMS/MS) from TCs in the Swiss Alps. 209 TC specimens exhibited the presence of cyanobacterial strains, with 45% containing genes encoding for microcystin, nodularin, anatoxin, and/or cylindrosperopsin production. Suspect-screening against a metabolite database CyanoMetDB[2] confirmed the presence of a range of toxins and other secondary metabolites including microcystins, anabaenopeptins and nodularins. We further analyse isolated cyanobacteria from the TCs to verify the toxin-encoding genes (Sanger sequencing) and to obtain more complete metabolite profiles to characterise these habitats.

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INSECTICIDAL ACTIVITY AGAINST STORAGE PESTS OF THE ESSENTIAL OILS OF FOUR AROMATIC SPECIES OBTAINED UNDER DIFFERENT EXTRACTION CONDITIONS

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Tribolium castaneum and *Sitophilus zeamais* are the main pests that affect stored products. To control these insects, toxic substances are used, making it necessary to find new safe, and effective substances for their control [1]. In this sense, the QuiProNaB research group has found that the essential oils (EOs) of *Tagetes zypaquirensis, Anethum graveolens, Satureja viminea,* and *Minthostachys* sp. exhibit promising insecticidal activity [2,3]. The objective of the work is to determine the best extraction conditions to obtain the EOs of the four aromatic species with insecticidal activity. For this purpose, the production of EOs was evaluated by steam distillation and microwave-assisted hydrodistillation from dry and fresh plant material. The chemical composition was determined by GC-MS and the insecticidal potential was evaluated by the fumigant toxicity test. The results indicated that the best EO extraction conditions for *T. zypaquirensis* were microwave-assisted hydrodistillation with fresh plant material; for *A. graveolens* steam distillation using fresh material, and for *S. viminea* and *Minthostachys* sp. steam distillation from dry plant material works best. The major components of the EOs were: dihydrotagetone (45%) and β-myrcene (15%) for *T. zypaquirensis;* R-pulegone (32%) and p-menth-3-en-8-ol (35%) for *S. viminea*; α-phellandrene (24%) and dill ether (15%) for *A. graveolens* and piperitone oxide (51%) and menthone (15%) for *Minthostachys sp.* The LC_{50} values against *T. castaneum* and *S. zeamais*, respectively were for *T. zypaquirensis:* 49.98 ppm and 102.59 ppm; for *A. graveolens*: 91.31 ppm and 346.56 ppm; for *S. viminea*: 8.53 ppm and 51.20 ppm and for *Minthostachys* sp.: 5.23 ppm and 30.46 ppm. The EOs obtained under the best extraction conditions are those that present the best insecticidal effects on the two pests of interest. This work was developed with resources from the BPIN 2020000100342 project, financed by the SGR of Colombia and supported by the Agreement on Access to Genetic Resources and Derivative Products No. 121, Addendum No. 21, and within the framework of the amnesty established in the Law 1955 of 2019.

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EXPLORING THE COMPLEXITY OF NATURAL PRODUCTS WITH ASSEMBLY THEORY & MASS SPECTROMETRY

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The ability to produce complex molecules in abundance is a feature exclusively associated with living systems and this can be used as an agnostic life-detection tool. We are developing a new approach to quantify complex molecules and identify unknown Natural Products using mass spectrometry and a new classifier (the Molecular Assembly Index, MA) from Assembly Theory, a new theory which helps determine quantitatively if a molecule is the product of selection and evolution. This project aims to expand and validate this approach to complexity for Natural Products, which have been the focus of medical and pharmacological research because of their tendency to feature a vast range of bioactivities.[1] In this regard, the development of analytical methods has been focusing on software and algorithms for the efficient classification of chemical species known to feature determinate bioactivities[2-4]. Using Assembly Theory, we have developed the new molecular complexity measure, Molecular Assembly (MA), which employs a construction algorithm to determine a value of complexity and assembly pathway for a given chemical species. This method represents an agnostic measure of the likelihood of a given species to be assembled in any detectable concentration (not to be a random occurrence)[5]. The theory quantifies the constraints required to produce a molecule by measuring the minimum number of steps to produce its molecular graph[6]. This new approach yields the potential to determine the nature and origin of unknown unknowns, rendering it a resourceful tool in life detection endeavours exploring for new life forms and the products of evolutionary processes in the environment[7].

Here we will present a LC-MS/MS method that was developed for the exploration of secondary metabolites. The intention is to extrapolate an MA value from the MS data of mixtures obtained as fractions from plant/ bacterial/ fungal crude products, and to create a database that would classify such species based on the experimentally determined MA value. With the central thesis that molecules with a high MA value are unlikely to be produced abiotically, we propose that there is a direct correlation between the biotic formation of complex chemical species and their MA. MA can hence be employed as a tool to explore mixtures for novel compounds, by focusing separation and isolation efforts on the fractions featuring fragmentation patterns associated with a “living range” of MA values.

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IDENTIFICATION OF CHEMICAL DEFENSES OF A TROPICAL TREE *SEXTONIA RUBRA* USING MOLECULAR NETWORKS FROM GC-MS, LC-MS/MS AND MALDI-MS/MS IMAGING DATA

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Sextonia rubra is a tropical tree endemic to the Guiana Shield and the Brazilian Amazon. It is known for its heartwood natural durability that comprises numerous lactone derivatives[1]. However its fruits have not been studied chemically. Here we propose analytical methods to explore the chemical diversity of these fruits using SPME-GC-MS, LC-HRMS/MS and MALDI-FT-ICR MS imaging. For the first time, GC-MS and MALDI-MS/MS data were analyzed using molecular networks for efficient annotation using MetGem software[2].

The most abundant volatile compounds identified by SPME-GC-MS in the hydrolate were eucalyptol (13.5%), α calamenene (7.4%), β caryophyllene (7.4%), α copaene (7.3%) and δ cadinene (5.8%). The antimicrobial activity of the hydrolate was tested against pathogens with a relative MIC equal to 5%.

In parallel of chemical analysis, wood decay resistance was assessed using long-term soil bed tests. Ethyl acetate extracts of different parts of the tree were tested against 6 Glutathione-S-Transferase (GST) of *Trametes versicolor*, corollary of their anti-fungal activity[3].

Our results suggest that for pith and heartwood, the higher the concentration ratios of lactones towards alkaloids, the higher the reactivity of GSTs, and the more the natural durability against wood-decaying fungi is positively affected. Although the involvement of lactone derivatives in the natural durability of this species was known, the involvement of alkaloids is new and suggests that *S. rubra* specializes its chemical defenses according to the tissue.

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NEW INSIGHTS IN CHEMICAL STRUCTURE DETERMINATION OF WOOD-DERIVATIVE POLYMERS

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Hypromellose is a non-ionic cellulose ether derived from pulp paper cellulose as raw material. It's a non-toxic white powder, odorless, and tasteless. In cold water, it swells into a clear or slightly turbid colloidal solution. Hypromellose is widely used as a food additive, emulsifier, thickening or suspending agent and excipient in oral tablet and capsule formulations[1], where, depending on the grade and precise chemical structure, it works as drug controlled release agent. Important molecular variables that control efficiency of hypromellose are the nature of the monomers and monomer linkers, monomer sequence distribution along chains, average molecular weight and molecular weight distribution and molecular architecture. Due to the broad source of cellulose in nature, the properties of hypromellose are highly impacted by the molecular structure. The most impacting microstructural parameter of hypromellose is the density of grafting groups on polymer chains, namely the degrees of substitution of the polymer chains. The complexity of hypromellose structure and resulting properties is due to the presence of two grafting groups, the methoxy group (OCH3) and the hydroxy-propyl group (OC3H6OH). The goal of this work is to present an original way to characterize the structure of hydroxypropyl methylcellulose (HPMC) on the basis of ^{13}C Nuclear Magnetic Resonance (NMR). In order to propose a precise characterization of hypromellose polymers, preliminary analyses were done using model polymers intervening in the chemical process of hypromellose synthesis, like cellulose, methyl cellulose and hydroxypropyl cel-lulose. These three polymers have an increasing complexity in term of lateral substituents. These substitutions are described by the degree of substitution (DS), i.e. the number of methoxy groups attached on a glycosidic unit, and the molar substitution (MS) i.e. the number of moles of hydroxypropyl group per mole of anhydroglucose in the chain. On the basis of ^{13}C NMR associated to Cross-Polarization Magic Angle Spinning (CP-MAS) technique, a quantitative determination of the degrees of substitution for anhydroglucose in positions 2, 3 and 6 as well as total substitution and molar substitution is achieved. Moreover the weight percent of substituents and reactivities of the substituted carbons are also determined, indicating that C2 and C6 are the most reactive positions.

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SUSTAINABLE EXTRACTION PROTOCOLS FOR CHESTNUT WOOD VALORIZATION

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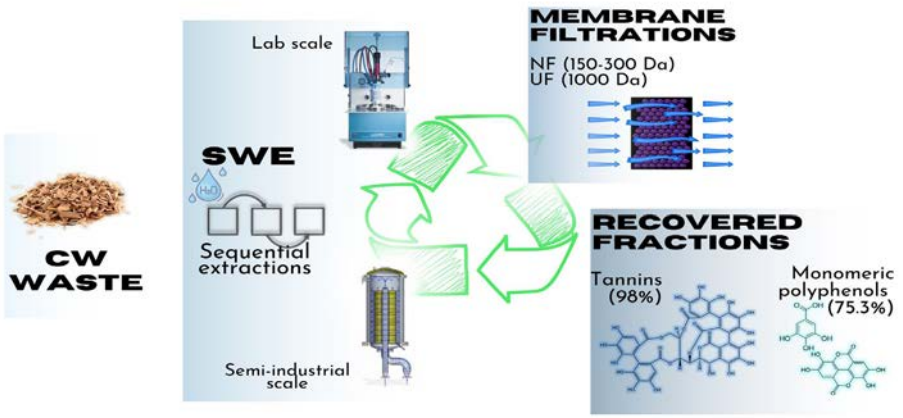
In recent decades, a new trend has emerged based on the concepts of circular economy and green extraction[1], aiming at the recovery of value-added compounds from plant feedstocks. This approach can be a safe, affordable, and environmentally friendly solution to recover bioactive compounds that can be used as nutraceuticals and cosmeceuticals.
Chestnut wood (*Castanea sativa*, Mill.) has been shown to be very rich in various active metabolites that have different effects such as antimicrobial, antioxidant and anticancer activity. In this work, the possibility of obtaining enriched fractions of certain classes of metabolites using sustainable and environmentally friendly protocols was investigated. In particular, CW residues were subjected to microwave-assisted subcritical water extraction (MASWE), and together with green and easily scalable downstream technologies, the isolation of two enriched extracts was successfully performed. Finally, the whole valorization protocol was scaled up to a pilot plant (up to 13 kg matrix and 150L), demonstrating the industrial feasibility of the process[2].
Specifically, pre-fractionation was performed using a two-step sequential extraction strategy. Then, to further increase selectivity, the use of membrane filtration (ultrafiltration and nanofiltration, UF and NF) for downstream was investigated. The retentate of the UF fraction contained more than 98% tannins, while the permeate after NF concentration contained up to 75.3% monomeric polyphenols. This case study demonstrates the promising features of a fractionation strategy developed in synergy between extraction and downstream, demonstrating the feasibility of sustainable recovery of bioactive compounds, thus leveraging the results of scientific research on the extraction of natural products.



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MACHINE LEARNING-INTEGRATED NATURAL PRODUCT CHEMISTRY RESEARCH

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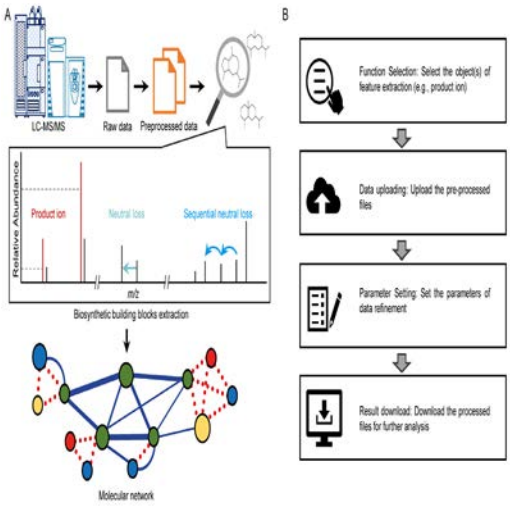
Natural products play an important role in drug discovery due to the fact that many new drugs are derived from natural products or their derivatives. The challenge of how to rapidly discover new natural compound structures has always been a problem in the field of natural product research.
With the advancement of detection and purification methods, the focus of natural product chemistry has shifted from traditional random purification to systematic and targeted isolation of compounds of interest. Molecular networking is a powerful tool to detect known and unknown compounds according to the MS/NS analysis. In our previous investigation, two new disesquiterpenoids were isolated from *Ainsliaea macrocephala* using molecular networking.[1] However, redundant data and complex data processing discourage chemists. In this study, we established a Building Block Extractor, an MS/MS data mining program with a user-friendly interface that automatically extracts user-defined specified features, and applied it for locating sesquiterpene lactone dimers in extracts of *Aetemisia heptapotamica*. The strategy significantly reduces data redundancy in MN analysis. Besides, the characteristic ions, their relative abundance and the sequential neutral loss features were integrated as building blocks for the first time. Eventually, nine undescribed sesquiterpenoid dimers were discovered and isolated. The compound artemiheptolide I were found to suppress the infection of Influenza A/Hongkong/8/68 (H3N2) *in vitro* with IC_{50} of 8.01±6.19 μM.



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DEVELOPMENT AND APPLICATION OF NEW TECHNOLOGIES TO ELUCIDATE THE TRANSCRIPTIONAL REGULATORY NETWORKS CONTROLLING SECONDARY METABOLITE PRODUCTION

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Actinomycetota are one of the bacterial phyla known for producing a wide variety of chemical compounds as secondary metabolites. Although they encode a myriad of secondary metabolites, only a small fraction of these metabolites are produced when cultivated in laboratories. This is due in part to the complex transcriptional regulation controlling expression of biosynthetic gene clusters (BGCs). *Streptomyces coelicolor* A3(2), a model *Streptomyces* species that is capable of producing more than 25 secondary metabolites, encodes more than 800 transcription factors (TFs). The complexity of the transcriptional regulatory networks governed by this large number of TFs hinders activation and functional characterization of many BGCs. Only a small fraction of these TFs have been characterized and their target genes identified to date. Characterization of TFs *in vivo* is resource intensive and difficult to scale. Here, we demonstrate development and application of new high throughput technologies, DAP-seq, and, RIViT-seq, for systematic identification of target genes of transcription factors [1, 2]. DAP-seq determines binding sites of transcriptional activators and repressors *in vitro* and easily scalable. RIViT-seq enables systematic identification of regulons of sigma factors by combining an *in vitro* transcription assay and RNA-sequencing. Using DAP-seq and RIViT-seq, target genes of 36 transcription regulators and 11 sigma factors were identified, respectively. The DAP-seq and RIViT-seq data expanded the transcriptional regulatory network in *S. coelicolor* A3(2), discovering new regulatory cascades, crosstalk between transcription factors and binding motifs of several transcription factors. Additionally, several TFs likely controlling expression of silent BGCs were identified. Further implementation of DAP-seq and RIViT-seq for other TFs in streptomycetes and other actinomycetes will advance our understanding of transcriptional regulatory networks in these industrially-important bacteria and facilitate discovery of new secondary metabolites.

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SYNTHESIS OF FLUORESCENT OR BIOACTIVE AZAPHILONES

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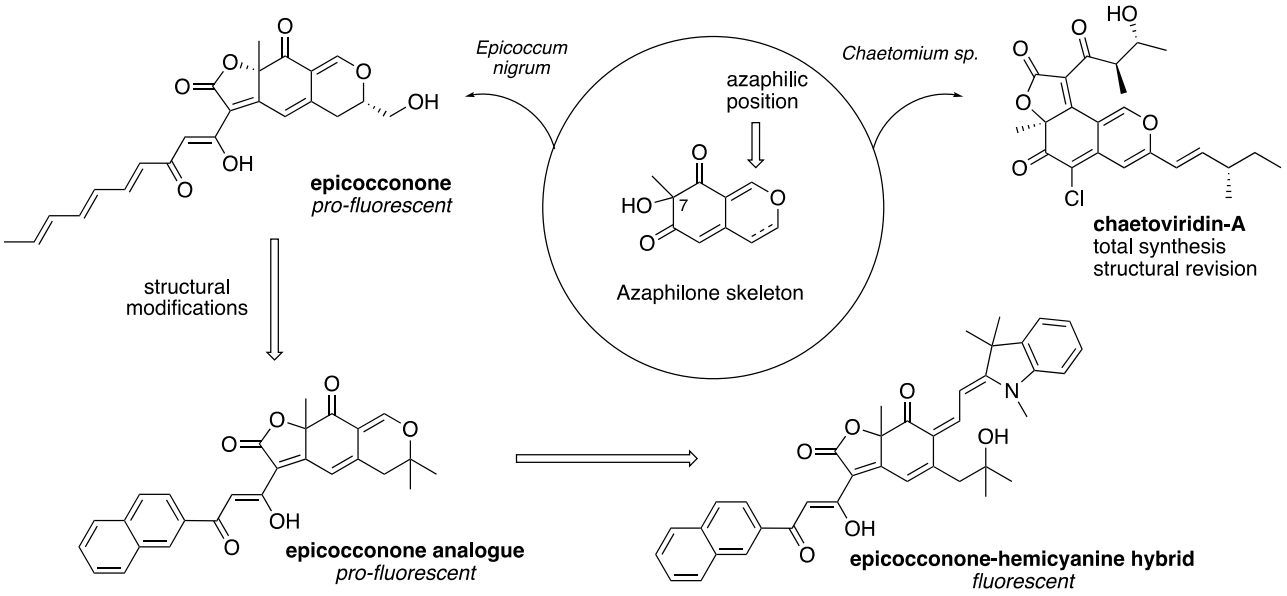
Summary
Azaphilones are a family of fungal polyketide metabolites possessing a highly oxygenated pyranoquinone bicyclic core, named isochromene, and a quaternary oxygenated carbon center at C-7. They belong to a large group of fungal pigments, turning red in the presence of primary amines due to an exchange of the pyran oxygen for nitrogen. With more than 600 members, they present an extremely large structural diversity associated with a wide biological activity spectrum.[1]
In this family of azaphilone, we picked-up two Natural Products named epicocconone and chaetoviridin-A, for their unusual structure, fluorescence properties when bound to proteins,[2] or biological activity.[3]



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BEYOND NATURE: DEVELOPMENT OF A BETALAIN-FLAVYLIUM DYE

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Plants produce pigments for various purposes, such as attracting pollinators, functioning as antioxidants, and protecting the photosynthetic apparatus from photodegradation. Betalains and anthocyanins are antioxidant pigments found in most flowers and fruits, but they do not coexist in the same species.[1] Betalains naturally occur in specific plant families of the Caryophyllales order, in certain basidiomycete fungi, and in unique bacterium species.[2]

The utilization of these pigments largely hinges on their characteristics, including color, stability, and antioxidant properties. Nevertheless, the extensive use of natural products can become challenging when these components are needed in large quantities. Developing functional bio-inspired products might overcome the complications linked to seasonality, variability in properties, and scalability of employing secondary metabolites.[3] These biomimetic compounds also present the prospect of creating new substances derived from natural molecular structures, possibly providing advantages over their natural equivalents.

Here, we describe the design and semisynthesis of a dye that merges the chromophores of both betalains and anthocyanins. This betalain-flavylium dye, synthesized using an intermediate in betalain biosynthesis, was named “WineBeet blue” due to its deep blue color in hydroalcoholic solutions. Differently from BeetBlue, a bioinspired safe blue dye,[4] exposing WineBeet blue to oxygen at room temperature leads to a more stable magenta compound, named “WineBeet rosé”. Both WineBeets exhibit high antioxidant capacity, significantly outperforming ascorbic acid, and exhibit tinctorial power. While their safety remains to be validated, these WineBeets compounds demonstrate potential as redox mediators and biocompatible dyes.

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NOVEL ASPECTS IN MOLECULAR RECOGNITION OF NATURAL PRODUCTS WITH SERUM ALBUMIN AND THE ANTI-APOPTOTIC PROTEIN BCL-2 WITH THE COMBINED USE OF NMR AND DOCKING CALCULATIONS

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Ligand-observed NMR techniques in combination with docking calculations have been a fruitful approach for investigation of protein-natural products interactions not only with isolated proteins in buffer solution, but also at the cellular level [1]. In this lecture we will summarize our recent research results in the field of: (a) Ligand-macromolecular interactions, with the use of saturation transfer difference (STD) NMR, intermolecular transfer NOEs for PHArmacophore Mapping (INPHARMA) NMR, in combination with docking calculations [2-5]. The method was successfully applied in the case of: (i) proteins with multiple binding sites, such as in human serum albumin, with competition experiments with drugs of known binding sites [2-5] and (ii) the anti-apoptotic protein Bcl-2 [6]. (b) Ligand-macromolecular interactions in living cells without the need of isotope labeling techniques [6,7].

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DEEP SPATIAL MULTI-OMICS PROFILING OF CHOLANGIOCELLULAR CARCINOMA PATIENTS

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Cholangiocarcinoma is a highly heterogeneous and metastatic cancer with a poor prognosis, characterized by a 5-year survival rate of only 7-20% and a 2% mortality rate[1]. The use of traditional omics techniques in its analysis often results in a loss of spatial information due to homogenization. To counteract this, spatial omics has emerged as a direct in situ detection technique that preserves spatial location information. In particular, it is highly applicable in the analysis of tumor heterogeneity[2].

In this study, we utilized spatial transcriptomics and metabolomics to analyze the molecular features of different niches and tumor cell subclones in cholangiocarcinoma tissue. Twelve fresh tissue samples were collected from ten patients, and spatial transcriptomic and metabolic analyses were performed using the 10× Visium platform and Tims-TOF MALDI 2 platform on adjacent slices.

Using spatial metabolomics, we analyzed the metabolic levels of different niches in cholangiocarcinoma tissue. Based on precise analysis of H&E images and spatial transcriptomic data, we successfully differentiated between various pathological area structures and established a metabolic feature library of spatial pathological regions for cholangiocarcinoma. Spatial multi-omics analysis revealed the existence of two subclones differentiated into distinct tumor phenotypes and further exposed metabolic hierarchy differences dependent on tumoral subclonal distance. Additionally, we characterized the metabolic status of stroma, including cancer-associated fibroblasts and immune cells, and surrounding subclones of different cancer cells based on distance and computed the correlation between different distances and specific metabolic accumulation.

Our study explored the first mechanisms of metabolic feature changes in different subclones of cholangiocarcinoma and microenvironments using spatial multi-omics technology, revealing the metabolic-driven evolution process of cholangiocarcinoma. This can provide a theoretical basis for diagnosis and treatment of cholangiocarcinoma in the future.

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RECONSTRUCTION OF PALEOLITHIC BACTERIAL GENOMES ADDS TIME AXIS IN NATURAL PRODUCT DISCOVERY

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Microbial natural products are evolutionary drivers that orchestrate interactions between microorganisms and with their abiotic environment (1). Their intrinsic biological activity and evolutionary diversification makes natural products ideal candidates for medicinal lead structures. While todays biosynthetic potential is mined by prospecting entire ecosystems (2) or a wealth of individual organisms (3, 4), past genomic records have not yet been harnessed for natural product discovery. Although computational tools for *de novo* assembly of bacterial genomes from complex metagenomes have developed, their application to highly degraded ancient DNA (5) has been challenging. We have overcome the limitation to modern metagenomes and reconstructed 459 bacterial metagenome-assembled genomes (MAGs) from the dental calculus of Neanderthals and anatomically modern humans spanning the past 100,000 years (6). After screening for biosynthetic gene clusters (BGCs) that enable microbial generation of natural products, we synthesized a novel BGC present in seven Pleistocene-era bacterial MAGs for function elucidation. Heterologous expression of the biosynthetic genes in live host bacteria resulted in the production of functional enzymes that generated ancient natural products. We can now make use of past genomic information to discover novel natural products from ancient microbiomes with potential applications in biology and medicine, and gain insight into microbial evolution within paleoecological frameworks.

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INNOVATIVE PROCESSES FOR NATURAL ACTIVE INGREDIENTS EXTRACTION AND THEIR ONLINE FUNCTIONALIZATION USING FLOW CHEMISTRY TECHNOLOGY

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Natural products extracted from biomass are an abundant source of bioactive compounds with high potential for therapeutic or cosmetic applications. Flavonoids compounds, for example, are frequently found in fruits, plants or wood, and offer antioxidant, anti-tumorous, anti-inflammatory or anti-allergic effect[1]. Yet, flavonoids often suffer from their lack of stability or solubility to be properly valued as active ingredients[2].
To overcome those limitations and offer environment-friendly industrial applications, flow chemistry has risen as a green technology for innovative process alternatives. Its many advantages provide more efficient and reliable reactions, and facilitate coupling to further functionalization or online analysis[3].
In this context, our interest has been focused on the development of an integrated process including biomass flow extraction of natural ingredients and their online derivatization in order to enhance their properties.
We implemented the online extraction and functionalization of *Robinia Pseudoacacia* wood, an abundant tree which embodies a valuable biomass source of flavonoids. After optimization, the continuous extraction cell was coupled to various types of structural modification, including mild-condition and selective enzymatic pathways. Innovative new ingredients have been produced from this modular online system, embedded in a responsible and industrializable approach.
This work provides a transferrable methodology of online extraction/structural modification to other biomass and extractible bioactive molecules.

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POSTER SESSION 1

ST1 BIOACTIVE NATURAL PRODUCTS AND DRUG DISCOVERY

PO1-PO44

DEVELOPMENT AND IMPROVMENT OF A FRACTIONATION PLATFORM BASED ON ORTHOGONAL SPE METHODS FOR IDENTIFICATION OF IMMUNOGENIC PRODUCTS

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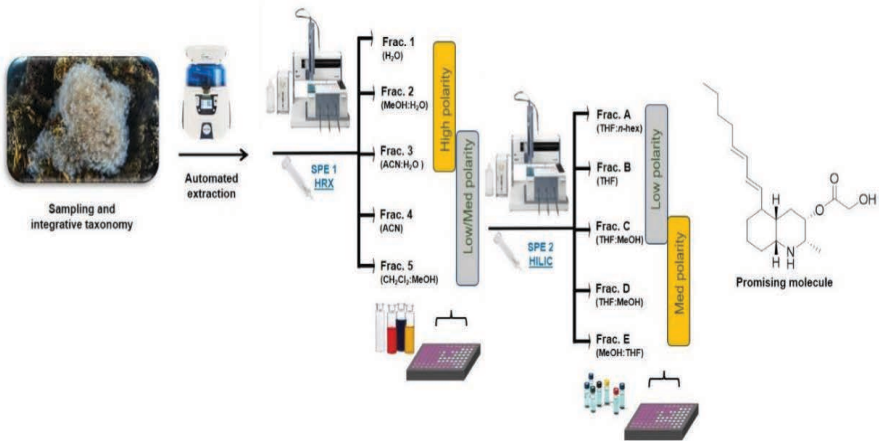
Marine Natural Products are a traditional source of molecules with relevant pharmacological potential. For this purpose, we have recently developed a bioassay-guided screening platform [1] including a pre-fractionation of crude extracts by Solid Phase Extraction (SPE) on a spherical, hydrophobic polystyrene-divinylbenzene resin that allows simple loading of the crude material in water, desalting and enrichment of the minor active components in chemically homogeneous fractions. Recently, the screening platform has been further improved with a second fractionation step based on an SPE protocol using hydrophilic interaction liquid chromatography (HILIC-SPE method).[2] In this stage, the HRX-SPE active fractions were further fractionated starting from an eluent of low polarity, providing a chromatographic resolution that is based on chemical interactions that are opposite to the hydrophobicity-driven fractionation achieved by polystyrene-divinylbenzene resin. Among the various relevant results of the screening-platform, there was the isolation of Lepadin A, from the tunicate *Clavellina Lepadiformis* [3], as potential inducer of Immunogenic Cell Death (ICD). The combination of the sequential hydrophobic and hydrophilic phases following an orthogonal chromatographic approach allows quick dereplication of complex mixtures of small scale and a validation of the biological activity.



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EVIDENCE OF TIME-DEPENDENT INHIBITION OF RAT LIVER MICROSOMAL CYTOCHROME P-450 2C11 ISOENZYME ACTIVITY BY IMPERATORIN

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Imperatorin, reported to have therapeutic properties, is a linear furanocoumarin commonly found in numerous plant-based foods and used in traditional medicines. Previous studies have shown Imperatorin as a potent inhibitor of numerous drug-metabolizing cytochrome (CYP) P-450 isoenzymes. However, to our knowledge, the biological impact of Imperatorin on CYP2C11 isoenzyme activity has not been reported yet. The effect of Imperatorin on CYP2C11 was determined by radiolabelled bioassays containing mature male Sprague-Dawley rat liver microsomes with labelled [N-methyl-14C]-diazepam, used as CYP2C11 probe substrate. We showed that Imperatorin potently inhibited diazepam demethylation with a half-maximal inhibitory concentration (IC50) determined to be 0.25 μM. Imperatorin-inhibited CYP2C11 activity occurred in a time- and concentration-dependent manner, suggesting a mechanism-based enzyme inhibition. The experimental inactivation parameters of KI and kinact were estimated to be 14.56 μM and 0.058 min-1, respectively. Of note, the rat liver CYP2C11 isoenzyme is a functional homologue to human CYP2C9. Moreover, a molecular docking study was conducted to predict the interactions between Imperatorin and CYP2C9 binding site. Thus, the crystal structure of CYP2C9 was used to dock Imperatorin and revealed that the furan ring formed π-π interactions with HEM 500 in CYP2C9 active site. Additionally, the in-silico predictions for pharmaceutical properties of Imperatorin including Toxicity, ADME, CYP isoenzymes inhibition, and Target prediction suggested moderate toxicity, high oral bioavailability, and CYP1A2, CYP2B6, and CYP2C9 inhibition. Taken together, these findings caution health practitioners and consumers against the concomitant consumption of Imperatorin-rich natural products and of CYP2C9 substrates, such as anti-depressants (e.g., benzodiazepines), anticonvulsants (e.g., phenytoin), and anti-coagulants (e.g., S-warfarin) treatments, which might lead to pharmacokinetic interactions with possible significant overdose risk for vulnerable patients.

STABILIZATION OF THE BIOSYNTHETIC PRECURSOR OF BETALAINS

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Betalains are antioxidant pigments that have become valuable due to their biocompatibility and their potential uses as food colorants, antioxidants, pharmaceuticals, and redox mediators. Betalamic acid, a metabolite of L-tyrosine found in most plants from the Caryophyllales order, some types of fungi, and the bacterium *Gluconacetobacter diazotrophicus*, is a key player in the biosynthesis of betalains.[1] Schliemann and colleagues has proven that a spontaneous reaction between nitrogen nucleophiles and betalamic acid can take place both *in* and *ex vivo*, opening up the potential for semisynthesizing betalains.[2] Unfortunately, betalamic acid is susceptible to autoxidation, hydrolysis and photodegradation;[3] therefore, it can only be preserved for a few months under specific conditions – inert atmosphere, darkness, and in ethyl acetate solution.

Here we show that the protection of the aldehyde group of betalamic acid using sodium bisulfite produce a stable adduct, which can be purified by selective precipitation, and can be stored for several months as a solid if kept in the dark. This adduct can then be used to regenerate betalamic acid *in situ* for the semisynthesis of amino acid betaxanthins, a yellow betalain class with marked antioxidant properties.[2] This method, which is simple, cost-effective, and quick, has the potential to revolutionize the semisynthesis of betalains and to facilitate the elusive structural characterization of betalamic acid. It optimizes the isolation of betalamic acid from beetroot juice and enables the storage of a stable adduct that can be used to scale up the production of betalains and their analogs.

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APOPTOTIC AND CYTOTOXIC EFFECTS OF HERNIARIN ISOLATED FROM TARRAGON ON SEVERAL CANCER CELL LINES

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Cancer is a serious public health problem and responsible for more than 70% of all deaths worldwide [1]. Herniarin is a coumarin derivative found in plants and the methoxy analog of umbelliferone. In this study, the cytotoxic and apoptotic effects of herniarin on colon, lung and bladder cancer cells were investigated. Herniarin was isolated from the dichloromethane extract of tarragon (*Artemisia dracunculus* L.) by silica gel column chromatography [2]. **HT-29** colorectal, **H1299** lung, **HTB9** metastatic bladder and **RT-112** non-metastatic bladder cancer cell lines were cultured and WST-1 test was applied to find the appropriate herniarin dose. Flow cytometry analysis was performed to determine the rate of cell viability and apoptosis with and without herniarin. According to the results, the appropriate doses of herniarin were 340.7 µM, 316.6 µM, 315.6 µM, and 330 µM, for HT-29, H1299, HTB9 and RT-112 cells at the 24th hour respectively. After herniarin application, cell viability decreased from 95.82 % to 56.97% in HT29 cells ($p<0.0001$), while it decreased from 96.32% to 19.20% in H1299 cells ($p<0.0001$). After herniarin treatment in HT29 cells, apoptosis increased from 4.01% to 41.9% ($p<0.0001$), while it increased from 2.18% to 80.28% in H1299 cells ($p<0.0001$). For metastatic and non-metastatic bladder cancer cells, cell viability decreased from 95% to 20% in HTB9 cells ($p<0.0001$), while it decreased from 93.21% to 64.91% in RT112 cells ($p<0.001$). After herniarin treatment in HTB9 cells, apoptosis increased from 2.51% to 79.21% ($p<0.0001$), while in RT-112 cells it increased from 6.27% to 28.45% ($p<0.01$).

According to these findings, it can be suggested that herniarin is effective on colon, lung and bladder cancer cells. These results of the study are prominent in terms of elucidating the mechanism of action of herniarin in different cancer types and it is a prominent study. This mechanism can be explained in more detail with the application of herniarin in different cancer cell lines.

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EPIPHYTES OF LAMINARIA: A PROMISING SOURCE OF ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS

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The harvested *Laminaria hyperborea* contributes to approximately 3.3 million tons of epiphytes discarded as waste despite containing organisms such as red algae and bryozoa, known to produce a range of bioactive compounds. Our research goal is the valorization of these epiphytes by investigating methodically their cytotoxic and antimicrobial potential and developing a purification procedure by using bioassay-guided flash fractionation. We evaluated the cytotoxic activity of extracts and fractions of this biomass against a range of cancer cell lines, leukemia (MOLM-13), prostate (PC3), and breast cancer (MCF-7), through WST-1 proliferation assay after 72h exposure. The antimicrobial activity of epiphytes was evaluated by determining the MIC against a range of pathogens. Six extracts showed considerable cytotoxic activity against MOLM-13 dose-dependently, while the hexane extract was selective when compared to non-tumorigenic cell lines. Five extracts showed considerable effect on the viability of MCF7 and PC3 with acetone being the most active against PC3 cells. The multistep purification procedure of the methanolic extract resulted in higher potency of the fractions. Particularly, the flash fractions displayed a selective-activity pattern depending on the elution ratio. The cytotoxic activity was concentration-dependent; two flash fractions were significantly cytotoxic towards all cell lines and two were notably selective towards MOLM-13 and PC3 lines. The hexane and ethyl acetate crude extracts were more promising for antimicrobial activity and the methanol fractionation enhanced the activity in line with the cytotoxic activity. The results show that the epiphytes of Laminaria hold potent anticancer and antimicrobial substances thus, further separation is needed for their characterization.

A NOVEL QM/NMR "FOCUSED" DATABASE APPROACH FOR THE STRUCTURAL ELUCIDATION AND STEREOCHEMICAL DETERMINATION OF NATURAL AND SYNTHETIC COMPOUNDS

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Structural elucidation and assignment of the relative/absolute configurations are key steps in accelerating the identification of novel natural and synthetic compounds and subsequent characterization of their chemical and pharmacological properties. NMR spectroscopy and computational approaches can be proficiently coupled to solve structural issues, comparing experimental data (e.g.,$^{13}C/^{1}H$ chemical shifts) with those predicted at the density functional theory (i.e., DFT/NMR combined approach).[1] Combined QM/NMR approach is based on a general workflow composed of several steps that can, for each case-study molecule, facilitate the appropriate identification of the correct three-dimensional structure: 1) building of the input structures (all the accounted stereoisomers); 2) conformational search; 3) geometry and energy optimization of the sampled conformers; 4) computation of NMR data; 5) comparison of all the experimental/predicted data and elaboration of the results.[2] This "all data" approach, although highly performing, is sometimes not sufficient to solve more complicated situations. With this aim, we developed a novel computational "focused" method, based on modulating the number of bonds starting from the stereocenters, in order to vary the dataset to be considered for the calculation of the statistical parameters, e.g., the mean absolute error (MAE). In order to validate this new approach, 55 molecules and their related stereoisomers (218), featuring two or more stereocenters and for which the correct isomer is already known, were investigated. For each of them, starting from the geometries obtained at the MPW1PW91/6-31g(d) level, GIAO NMR calculations were performed considering the MPW1PW91/6-31g(d,p) functional/basis set. The two approaches were compared through the analysis of the obtained MAEs values, which highlighted a comparable overall performance in predicting the correct isomers, namely 33 and 34 out of 55 molecules through the "all data" and "focused" methods, respectively. Currently, other functionals/basis sets (B3LYP, M062X, B972 functionals and 6-31g(d,p) basis set) have been recently considered for additional NMR chemical shift calculations, and the analysis of the related data is ongoing. Furthermore, we are implementing a similar approach considering a distance cutoff starting from the stereocenters, rather than the number of bonds, for modulating the datasets. Finally, we have automatized the entire process through a package of scripts, which will be released on our website (www.computorgchem.unisa.it).

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NATURAL CANNABICHROMENE SHOWS DISTINCT SCALEMICITY GRADES AND ENANTIOMERIC DOMINANCE IN *CANNABIS SATIVA* STRAINS

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Cannabichromene[1] (CBC, see Fig. 1) occurs in *Cannabis sativa* as a scalemic mixture with enantiomeric excess and enantiomeric dominance that is strain-dependent. Herein, the chirality of CBC, an oily compound, was shown not to be significantly affected by standard conditions of isolation and purification. Enantiomeric self-disproportionation effects were minimized by carrying out the chiral analysis (NP-eUHPLC, using inverted chirality column approach (ICCA)[2]) on crude fractions obtained from ethanol extraction, rather than on purified products. The different enantiomeric state of CBC in *Cannabis* therefore seems to have a genetic basis, implying that the chirality of natural CBC in the plant is associated with the differential expression of CBCA-synthase isoforms and/or of associated directing proteins with antipodal enantiospecificity.[3] In this work, the two enantiomers of CBC have been analyzed.[4] The biological profile of each single enantiomer of CBC should therefore be investigated independently to assess the contribution of this compound to the activity of *Cannabis* preparations.

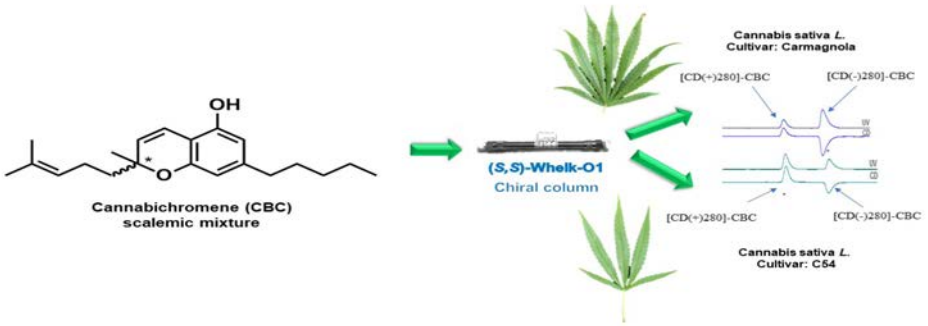

Figure 1. Structure of cannabichromene and chiral UHPLC analysis.

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PROTEOMIC EVALUATION OF THE INTERACTOME OF A NATURAL POLYKETIDE FROM A MARINE SPONGE

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Natural products (NPs) have been valuable in drug discovery due to their chemical diversity and drug-like properties. Their unique structures and biological activities make them promising starting points for developing new drugs. Investigating the protein targets of natural compounds is indeed essential for understanding their mechanisms of action and harnessing their therapeutic potential.
Limited proteolysis-based techniques, in combination with mass spectrometry, have become important tools in studying the interactions between small molecules (SM) and proteins. These techniques involve the controlled enzymatic digestion of proteins using proteases, generating a pattern of peptide fragments that, analysed through mass spectrometry, can gain valuable information about the interactions between the small molecules and proteins.
Gracilioether A (GE)[1] is a polyketide compound isolated from the marine sponges *Plakinastrella mamillaris*, found in the Fiji Islands. The exact biological functions and therapeutic applications of GE are still under investigation.
In this work the interaction profile of GE has been evaluated through two techniques based on the limited proteolysis: *Drug affinitive responsive target stability* (DARTS)[2] and *targeted Limited Proteolysis coupled to Mass-Spectrometry* (t-LiP-MS)[3].
DARTS is a technique used to identify the protein targets of small molecules or drugs based on changes in protein stability, while LiP-MS is a powerful technique used for studying protein-ligand interactions and protein conformational changes.
This approach has led to the identification of Ubiquitin carboxyl-terminal hydrolase 5 (USP5) as the most promising target for GE, providing valuable insights into the binding affinities, target stability, and proteolytic profiles of the protein targets when exposed to GE.

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PROTECTIVE EFFECTS OF *KAEMPFERIA PARVIFLORA* ON ADVANCED GLYCATION END PRODUCTS- INDUCED OXIDATIVE STRESS AND β-CELL DYSFUNCTION IN PANCREATIC RIN-M5F CELLS

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Kaempferia parviflora (KP) or Krachaidam, also known as black ginger, a traditional folk medicine in Thailand. It is popularly used for managing a variety of diseases, especially type 2 Diabetes mellitus (T2DM). *K. parviflora* and its main effective methoxyflavones, possesses a protective effect against the oxidative stress and advanced glycation end products (AGEs), which plays an important role as an adverse effect for diabetes. T2DM is a one of the most chronic metabolic diseases that are projected to increase due to food consumption, modern and inactive physical lifestyle.

It's characterized as a combination between a high blood glucose level (hyperglycemia) and insulin resistance. It causes defective and insufficient of insulin secretion from pancreatic β-cells. Importantly, persistence hyperglycemia leads to the reaction of reducing sugar and protein which gradually formed AGEs as a toxic compound to β-cells. AGEs alters the intracellular signaling by increasing of ROS generation and oxidative stress which consequently made progressive damage on the β-cell function and mass. Thus, protection the pancreatic β-cells against AGEs-mediated damage, activation of compensatory ROS mechanism and enhancing insulin secretion might be the promising therapeutic ways to delay the progression of T2DM. Interestingly, KP extracts not only counteracts AGEs effects but also enhances SIRT-1 activity which is the interesting antioxidant therapeutic target and increasing the expression of necessary factors for insulin synthesis (PDX-1 and Glut2). Activation of SIRT-1 can both attenuate the oxidative stress effect which unfavorable factors for T2DM and enhance the insulin secretion. Therefore, our study focusing on the protective effects and the underlying molecular mechanisms of KP extracts on AGEs-induced oxidative stress and β-dysfunction in pancreatic RIN-m5F cell via SIRT-1/AMPK signaling pathway. Whether enhanced SIRT-1 activity by using KP-treated can reduce intracellular oxidative stress and increase insulin production and insulin status of pancreatic β-cells. As a result, KP extracts shown high levels of antioxidant activity in DPPH and ABTS free radical scavenging assay. Cell viability was increased and corelated with a healthy morphology after treated with KP extracts. *K. parviflora* shown a protective effect on β-cells cytotoxicity by suppressing AGEs-induced ROS generation and decreasing the levels of intracellular ROS and ROS-related gene expression (NOX1 and NOX4). KP extracts also promotes the insulin production and secretion by enhance the expression of insulin-related gene (PDX-1, GLUT2, GCK, and PRE-INS) and increase insulin secretion in rat pancreatic cells (RIN-m5F). Furthermore, KP extracts improve the activity of SIRT-1 and stimulate insulin secretion by reduce the ROS level and attenuate the toxicity of AGEs-induced oxidative stress and β-dysfunction by enhance SIRT-1/AMPK signaling pathway. Thus, we consider *K. parviflora* as a potential alternative medicine that alleviate the β-cell dysfunction and delay the onset in T2DM.

IMPLEMENTATION OF THE 3D-BRD9 PHARMACOPHORE MODEL: FROM SYNTHETIC COMPOUNDS TO QUINAZOLINE ALKALOIDS AS NEW POTENTIAL BRD9 BINDERS

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Bromodomains are druggable protein modules capable of recognizing post-translational modifications on histones, and they are involved in various diseases, especially cancer and inflammation.[1] Thanks to the support provided by the AIRC Foundation for Cancer Research,[2] we are currently engaged in the discovery of novel compounds from both natural and synthetic sources targeting the BRD9 protein. The development of 3D structure-based pharmacophore models related to BRD9, along with virtual screening experiments, has provided a valuable *in silico* tool for the rapid selection of the most promising compounds, opening up a new avenue for discovering BRD9 ligands. In a recent work,[3] we reported the synthesis of a library of sixteen compounds based on 6-methylquinazolin-4(3*H*)-one chemical core and the subsequent identification of five items as new binders of the target protein. Considering the abundance of natural sources containing quinazoline alkaloids, such as the Rutaceae, Asteraceae, and Papaveraceae families, we are now shifting our focus to exploring quinazoline derivatives exclusively sourced from natural origins. By analyzing the binding modes and extrapolating chemical features from the previously synthesized sixteen 6-methylquinazolin-4(3*H*)-one-based compounds, we are integrating such information for further optimizing BRD9 pharmacophore models.[4] Additionally, starting from a collection of quinazoline alkaloids reported in literature, virtual screening experiments will be performed with the aim of selecting further compounds and to evaluate their potential binding affinity with BRD9. In the final step, AlphaScreen assays will be conducted to validate the computational predictions and, if necessary, small synthetic modifications may be applied to produce semi-synthetic derivatives with the aim of enhancing the binding towards the target.

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PRENYLATED ACYLPHLOROGLUCINOL ALCOHOLS AND PEROXIDES FROM *HYPERICUM CORIS* L.

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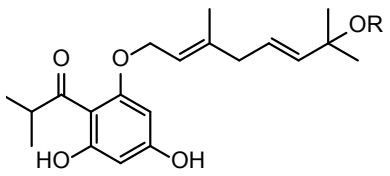
Hypericum Tourn. ex L. is the largest genus from the Hypericaceae family comprising over 500 species of small trees, shrubs, and herbs. Beside the well-known antidepressive St. John's wort (*H. perforatum*), several of these species have been used in traditional medicine as antibacterial, cytotoxic, antioxidant, anti-inflammatory, or antihyperglycemic agents. Previously, we have investigated various *Hypericum* species in the search of neuroactive, antibacterial, and cytotoxic secondary metabolites for specific medicinal applications. [1, 2] The chemical and biological screening of the crude extract from *H. coris* L. indicated antibacterial activity and the presence of species specific phloroglucinols. [3, 4]

For the phytochemical study, *H. coris* was extracted successively via Soxhlet with chloroform and methanol, and the extracts were further purified by various chromatographic techniques (CC in silica gel, sephadex LH-20, and semi-preparative HPLC). From the chloroform extract two yet undescribed prenylated acylphloroglucinols (hypercorisins A-B, **1**-**2**) together with their two corresponding peroxide derivatives, (hypercorisins C-D, **3**-**4**), also still undescribed, and the known 2-geranyloxy-1-(2-methylpropanoyl)-phloroglucinol were isolated. From the methanol fraction, six known compounds were isolated and identified as sucrose, vanillic acid 4-*O*-β-d-glucopyranoside, chlorogenic acid, neochlorogenic acid, shikimic acid, and quercitrin. All compounds were fully assigned by means of NMR analysis and HR-MS/MS. A fragmentation pattern under ESI conditions was proposed for the undescribed compounds.

Hypercorisins A-D were tested for their antibacterial activity against the gram-positive *Bacillus subtilis*, gram-negative *Aliivibrio fischeri*, and cytotoxic activity against PC-3 and HCT116 cancer cell lines. All compounds were active against *B. subitilis* at the highest concentration tested (100 µM) but showed no appreciable antibacterial effect on the gram-negative *Aliivibrio fischeri* nor cytotoxic activity on PC-3 or HCT-116 cancer cell lines. For compounds **1** and **2** IC_{50} values of 86 and 34 µM were determined against *B. subtillis*, respectively, whereas no IC_{50} values could be obtained for the peroxides **3** and **4**, likely due to instability and degradation during incubation.



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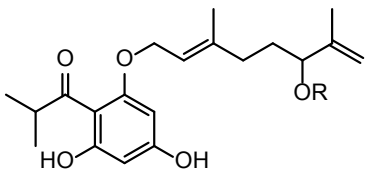
3 R = OH



2 R = H
4 R = OH

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CHEMICAL PROFILE AND BIOACTIVITIES OF TWO SPECIES OF *MENTHA* GENUS

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Mentha (Lamiaceae family), a genus of aromatic perennial plants distributed in temperate regions of Europe, Asia, Australia and South Africa, possess medicinal and commercial importance [1]. The aim of this work is to study the chemical profile and bioactivities of hydroalcoholic extracts of two species of the genus *M. spicata* L. and *M. pulegium* L. reported in literature for the treatment of several diseases [2,3]. The chemical analysis of the extracts, obtained from plants collected in Campania region and performed by LC-MS, revealed a higher number of compounds in *M. pulegium* than in *M. spicata* (Table1). Diosmin and rosmarinic acid were identified in both extracts, while luteolin and apigenin were found only in *M. pulegium*. The antioxidant activity of extracts is associated with the amount of TPC: the extract of *M. pulegium* showed a greater content of phenols than *M. spicata* extract (224.88 mg towards 116.66 mg GAE/g). Also, the antioxidant activity of *M. pulegium* extract, evaluated by DPPH and FRAP assays, was higher than *M. spicata* one. Finally, the foodborne strains tested, resulted to be sensitive to the action of the hydroalcoholic extract of *M. pulegium*; on the other hand, only *P. aeruginosa* resulted to be sensitive to action of *M. spicata*. From the results obtained, the extract of *M. pulegium* could be used in the food industry to increase the shelf life of food products such as meat and cheese.

Table 1. Chemical composition of hydroalcoholic extracts of *M. pulegium* and *M. spicata*.

Composto	RT(min)	Rapporto m/z	Addotti rilevati	Formula molecolare	*M. pulegium* L.	*M. spicata* L.
Luteolina-7-O-rutinoside	8.16	595,1642	$[M-H]^+$	$C_{27}H_{30}O_{15}$	X	
Luteolina-7-O-glucoside	8.33	449,1063	$[M-H]^+$	$C_{21}H_{20}O_{11}$	X	
Apigenina-7-O-rutinoside	8.48	579,1690	$[M-H]^+$	$C_{27}H_{30}O_{14}$	X	
Diosmina	8.59	609,1798	$[M-H]^+$	$C_{28}H_{32}O_{15}$	X	X
Acido rosmarinico	8.94	361,0910	$[M-H]^+$	$C_{18}H_{16}O_8$	X	X
Linarina	9.27	593,1849	$[M-H]^+$	$C_{28}H_{32}O_{14}$	X	
Didimina	9.38	595,2007	$[M-H]^+$	$C_{28}H_{34}O_{14}$	X	
Luteolina	9.82	287,0545	$[M-H]^+$	$C_{15}H_{10}O_6$	X	
Sideritiflavone	10.45	361,0910	$[M-H]^+$	$C_{18}H_{16}O_8$	X	X
Diosmetina	10.62	301,0699	$[M-H]^+$	$C_{16}H_{12}O_6$		X
Xantomicrolo	10.90	345,0958	$[M-H]^+$	$C_{18}H_{16}O_7$	X	
Timonina	10.92	361,0912	$[M-H]^+$	$C_{16}H_{12}O_6$		X
Diidrossi--tetrametossilavone	11.34	375,1067	$[M-H]^+$	$C_{19}H_{18}O_8$	X	X
Nevadensina	11.87	345,0960	$[M-H]^+$	$C_{18}H_{16}O_7$	X	
Idrossi-tetrametossiflavone	12.40	359,1120	$[M-H]^+$	$C_{19}H_{18}O_7$	X	

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ADVANCED *IN VITRO* SYSTEMS FOR HIGH THROUGHPUT SCREENING OF COMPOUNDS WITH THERAPEUTIC APPLICATIONS

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In vitro screening systems, characterized by low cost, convenience handling and high reproducibility are useful tools to screen new compounds with therapeutical applications. Society and scientific community call for more animal-free experiments; moreover, following the last legislation of 2022, new drugs need not be tested in animals to receive U.S. Food and Drug Administration (FDA) approval. *In vitro* models should be upgraded to better resemble human physiology and to allow a reliable transition to human studies. Complex *in vitro* models consist of co-cultures of different cell lines communicating each other directly or indirectly through synthetic supports [1]. Given the gut centrality in the development of pathologies, we will show a platform of high-throughput screening systems designed to reproduce the main components of human gut barrier, a complex structure that prevents the passage of dangerous molecules or microbes, while allowing the correct adsorption of nutrients. The gut barrier includes commensal microorganisms, mucus layer, intestinal epithelial monolayer and mucosal immune system; in its inner part there are also an endothelial system directly related to the transport of nutrients or inflammatory molecules (Gut Vascular Barrier), and neuronal cells that define the Enteric Nervous System (ENS). The epithelial monolayer is composed by several specialized cells, sealed together by proteins complex. The alteration of barrier integrity, also known as “leaky gut”, is related to the onset of several diseases [2]. On this basis, we have set up a leaky gut model consisted in a co-culture of differentiated intestinal epithelial cells communicating with a macrophage cell line. Macrophages stimulation with specific inflammatory stress leads to nitric oxide production that damages intestinal monolayer, reproducing a human intestinal barrier injury mechanism. Adding or modifying elements in the co-culture system, we reproduced several barrier models, such as mucus-secreting gut barrier model, vascularized gut model, enteric-nervous system model and hormone secreting gut barrier model. These models are robust, reproducible and extremely versatile tools that lead to a sophisticated characterization of tested compounds activity. The final goal would be to set up a more complex *in vitro* model of gut barrier that can include all the components described.

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ANTIOXIDANT PROPERTIES OF SECONDARY METABOLITES FROM *G. MANGOSTANA*: ISOLATION, MOLECULAR DOCKING, AND BIOLOGICAL EVALUATION

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Numerous physiological and physiopathological processes, both endogenous and external, that have an impact on cellular homeostasis are caused by oxidative stress[1]. An unbalance in the intracellular concentration of oxidants can lead to the alteration of biological macromolecules such as proteins, lipids, and nucleic acids. The most known regulator of antioxidant response is the transcription factor Nrf2, constitutively sequestered in the cytoplasm by its natural inhibitor Keap1. The tropical evergreen plant *Garcinia mangostana*, a member of the Clusiaceae family, is indigenous to the Sunda and Maluku Islands. The plant yields a reddish-purple fruit that is well-known for its nutritional benefits. More recently, a great deal of research has been done on the impacts of xanthone metabolites on diseases like cancer, inflammation, oxidative stress, infections, and other pathologies[2,3]. Prompted by these findings, we decided to extract and evaluate first *in silico* and then *in vitro* the capability of disrupting the interaction between Nrf2 and Keap1, inducing an antioxidant response, of 5 secondary metabolites of *G.mangostana*.

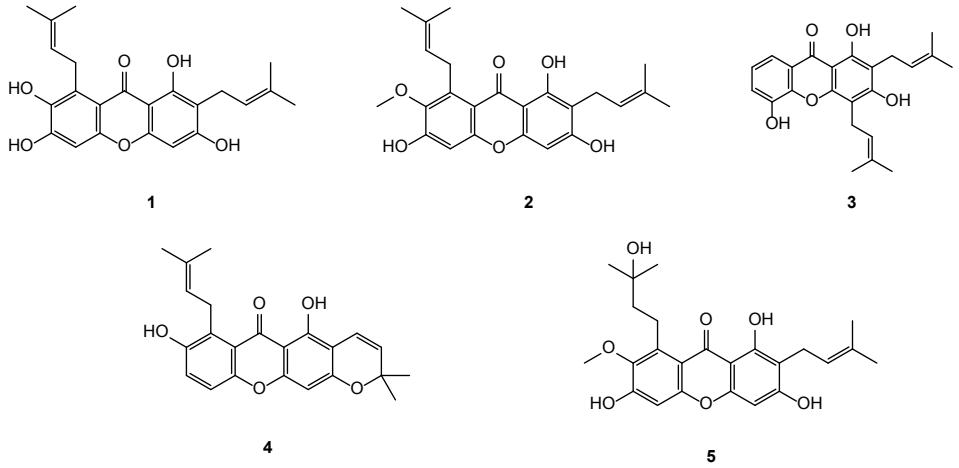

Figure 1. The secondary metabolites extracted from *G.mangostana*.

Among those molecules, demethylcalabaxanthone (**4**) showed the most promising results in biological tests in terms of cytoprotective effects against oxidizing agents. Moreover, Western Blot experiments confirmed a higher concentration of Nrf2 in the nucleus after treatment with compound **4**, corroborating the hypothesis of a disruption between Nrf2 and Keap1.

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BIOACTIVITY-BASED MOLECULAR NETWORKING-GUIDED IDENTIFICATION OF GUTTIFERONE J FROM *GARCINIA CAMBOGIA* AS AN ANTI-OBESITY CANDIDATE

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Obesity is increasing at an alarming rate worldwide. Adipose tissues can be classified into three types: white adipose tissue that stores energy in the form of triglyceride, brown adipose tissue that contains large amounts of mitochondria and dissipates lipids as heat by uncoupling protein 1 (UCP1) to maintain body temperature, and beige fat that arises as multilocular adipocytes and has a highly inducible thermogenic capacity upon stimulation[1]. Tens of synthetic or naturally occurring compounds have been identified to boost fat combustion. Loss of activity or failure to separate bioactive compounds is a common trap in bioactivity-guided fractionation workflow. Alternatively, a complementary workflow termed “bioactivity-based molecular networking” has been described to quickly identify bioactive compounds[2]. The Global Natural Products Social Molecular Network platform provides an open-access tool that uses an algorithm to compare large numbers of MS/MS spectra based on their similarities. The bioactivity score is calculated based on the statistical Pearson correlation coefficients between chemical features and observed bioactivity, which can be mapped out on the molecular network to predict potential bioactive compounds or chemical families and thus guide their targeted isolation[3]. The bioactivity-based molecular networking-guided isolation yielded several polycyclic polyprenylated acylphloroglucinols from the fruits of *G. cambogia* with lipid lowering effect on adipocytes, Guttiferone J reduced lipid accumulation in 3T3-L1 and C3H10T1/2 adipocytes, respectively. Furthermore, Guttiferone J increased the expression of the deacetylase Sirtuin 3 and activated it, which, in turn, reduced the acetylation level of PPARγ coactivator-1α to boost mitochondrial biogenesis, and promoted uncoupling protein 1 expression to enhance thermogenesis, resulting in browning of adipocytes. In high-fat diet-induced-obese mice, GOJ protected against adiposity, hyperlipidemia, insulin resistance and liver lipotoxicity, through boosting SIRT3-mediated browning of inguinal adipose tissue[4].

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DISCOVERY OF DEGLUCORUSCIN AS A NEW ARYL HYDROCARBON RECEPTOR AGONIST

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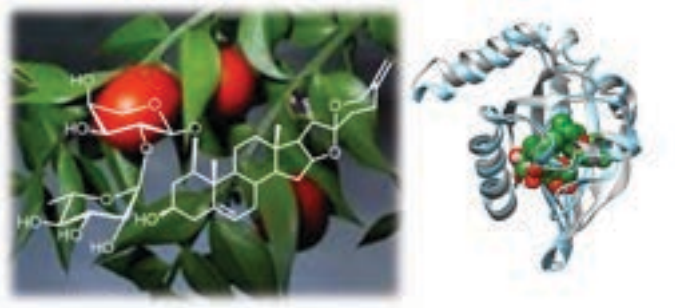
The aryl hydrocarbon receptor (AhR) is a transcription factor member of the basic helix-loop-helix Per-ARNT-SIM family that mediates many of the biological and toxicological actions of structurally diverse chemicals. The AhR can accommodate different compounds in its ligand binding pocket, with synthetic halogenated aromatic hydrocarbons as the best characterized high-affinity ligands. Nowadays, AhR is known as an emerging therapeutic target due to its involvement in different key processes including development, cellular differentiation and proliferation, immunity and inflammation, homeostasis, and metabolism.[1-3]
Within our interest in nuclear receptor modulators from natural sources,[4,5] we proceeded in the isolation and pharmacological decodification of ruscogenins from a commercially available preparation of *Ruscus aculeatus* extracts. This plant, known as butcher's broom, belongs to the Asparagaceae family and is a well-known distributed European plant. Extracts of rhizomes and roots of *Ruscus* species are used in traditional medicine as products against chronic venous disorders (CVDs) and are a rich source of steroidal saponins, called ruscogenins.[6,7]



In this poster we will report the isolation and structural elucidation of several furostanic and spirostanic glycosides. Furthermore, we demonstrated for the first time that deglucoruscin, the major metabolite of *Ruscus* extract, is capable to activate AhR and, utilizing a combination of molecular docking and dynamics simulations, we proposed its binding mode and mechanism of action. Overall, deglucoruscin represents a new naturally occurring AhR agonist potentially useful for the treatment of various immune and inflammatory diseases.

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COLOMBIAN FRUIT EXTRACTS REDUCE INDUCED OXIDATIVE STRESS IN RAW 264.7 MACROPHAGES

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Noncommunicable diseases (NCDs) are responsible for 74% of deaths worldwide, considered one of the leading causes of death. These pathologies are usually associated with diet, an important factor that must be considered when establishing treatment. Unhealthy diets are considered risk factors that trigger chronic inflammatory processes, characterized by increased cellular oxidative stress associated with signs, symptoms, and complications of NCDs. Among the recommendations for preventing and managing these pathologies is the implementation of a healthy diet, which includes fruits and vegetables in high proportions. In this work, 12 total ethanolic extracts obtained from fruit pulps of the Colombian flora were evaluated to explore the potential effect against oxidative stress induced by LPS in RAW 264.7 macrophages using the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay, a quantitative method for the evaluation of oxidative stress in cells. The results showed that all fruit extracts inhibited the production of reactive oxidative species (ROS) in LPS-activated macrophages at the tested concentrations (2000 and 1000 µg/mL). The fruit extracts that presented the highest percentage of inhibition of ROS production were those obtained with mamey (*Mammea americana*), soursop (*Annona muricata*), and dragon fruit (*Hylocereus megalanthus*). These fruits can be considered a source of substances that can neutralize the ROS generated in pathological states such as NCDs. Therefore, this important finding would drive their massive consumption to prevent and create NCDs therapy adjuvants.

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COMPOUNDS FROM GREEN COFFEE BEANS REGULATE POST-TRANSLATIONAL HISTONE MODIFICATIONS

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Post-translational modifications (PTMs) of histones, the major components of nucleosomes, are well known. PTMs are related to the alteration of the chromatin dynamics, thereby affecting gene expression.[1] Defects in the histone modifications are thought to cause chronic diseases,[2] e. g. reduction of the acetylation level of histone H4 Lysine 16 (H4K16ac) have been observed in cancer cells.[3] Therefore, we have searched for food ingredients altering histone modifications that may be expected to prevent chronic diseases.

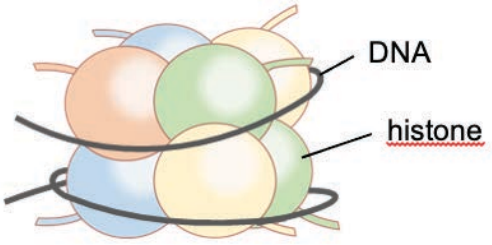

Figure. Schematic diagram of the nucleosome structure.

In this study, we focused on green coffee beans, that contain various functional components such as caffeine[4] and chlorogenic acid.[5] As the result, we have identified two compounds with the biological activity enhancing the level of H4K16ac. The structures of these compounds were elucidated by the spectral analysis to be coffee diterpenes.

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INVESTIGATING THE EPIMERISATION OF 5-OH IN TIGILANOL TIGLATE (EBC-46): A CASE STUDY OF OXIDATIVE-INDUCED REARRANGEMENTS

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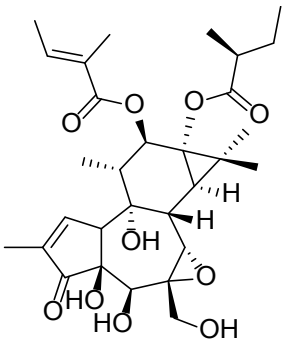
Tigilanol tiglate (EBC-46, **1**) is an epoxytigliane diterpenoid isolated from the kernels of *Fontainea picrosperma*. It was approved for the treatment of non-metastatic, canine mast cell tumours, and is currently in Phase 2 Human Clinical trials for head and neck malignancies (HMN) and soft tissue sarcoma (STS).[1] While tiglianes are widespread in euphorbiaceous plants, epoxytiglianes are very rare, and their structural hallmark (the epoxidation of the endocyclic double bond and hydroxylation at C-5) make them hybrid constructs of tiglianes and daphnane diterpenoids. As part of a study to reduce this dual structural difference into a point-like mutation, the chemical modification of the 5-hydroxyl was explored in reactions of acylation, methylation, oxidation, and deoxygenation.
An unusual reactivity profile emerged, characterised by the diotopic exchange of the substituents around the C4-C10 bond, a reaction already described but never investigated so far in terms of general relevance in compounds with a 1,3-dioxo-2-hydroxy moiety.



(1)

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EPOXY-TIGLIANE INDUCED DISRUPTION OF ESTABLISHED BIOFILM MATRICES FACILITATES NEUTROPHIL DIFFUSION INTO *ESCHERICHIA COLI* AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* BIOFILMS

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Chronic bacterial biofilm-associated infections represent an often unrecognised cause of morbidity and mortality. Within biofilms, bacteria are embedded in the charged, entangled extracellular polymeric substance (EPS) matrix that provides structural stability and acts as a diffusion-limiting barrier to the host immune cells and antimicrobial agents. Recently, a novel, non-antibiotic therapeutic strategy has been described for chronic skin wound infections, employing a semi-synthetic epoxy-tigliane ester (EBC-1013) derived from the Australian blushwood tree (*Fontainea picrosperma*)[1]. Here, we investigate the direct and indirect mechanisms by which EBC-1013 may induce neutrophil (PMNL) invasion and biofilm disruption *in vivo.*

We studied the effect of EBC-1013 on (a) established 96 h *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms alone and (b) in recombinant co-culture model (RCM) systems using differentiated promyeoloblast HL-60 cells (dHL-60). Confocal laser scanning microscopy, fluorescent labelling, COMSTAT and ImageJ analysis were used to quantify cellular and EPS matrix changes within the biofilms. The direct effect of EBC-1013 on the migration of dHL-60 cells was investigated using viability and chemotaxis assays. The direct/indirect role of reactive oxygen species (ROS) in mediating PMNL invasion and biofilm disruption was also studied.

These studies demonstrated that EBC-1013 (at 256 µg/mL) induced significant biofilm disruption (through reorganisation and increased porosity of the biofilm matrix) and resulted in increased neutrophil-like cell migration ($p < 0.05$). Although EBC-1013 stimulated the generation of the ROS in dHL-60 cells and RCM system ($p < 0.05$), it did not induce chemotaxis *in vitro*. Exogenous ROS, used at a concentration similar to that produced by activated neutrophils (≤ 50 µM), failed to disrupt the established 96 h *E. coli* and MRSA PMNL invasion of biofilms *in vivo* is independent of ROS or chemotactic effects of EBC-1013, but rather reflects structural reorganisation of the biofilm architecture and rearrangement of the biofilm EPS. Clinical trials are ongoing with EBC-1013 in the treatment of human biofilm-associated infections.

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ALPHA-GLUCOSIDASE AND 15-LOX INHIBITION ACTIVITIES OF SOME FRUIT AND VEGETABLE PEELS

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Solid vegetable and fruit wastes such as peels, pomace and pulp are generated during the production and manufacturing processes [1] and they end up in the dump site. This study is an attempt of waste utilization in a way that if found to exhibit pharmacologic properties, these fruit and vegetable peels will be put into good use and will no longer be considered waste by-products.

Methanol and hexane crude extracts of the peels of banana blossom (*Musa balbisiana*, Mb), red beetroot (*Beta vulgaris*, Bv), sponge gourd (*Luffa cylindrica*, Lc), miracle fruit (*Crescentia cujete*, Cc), and yellow passion fruit (*Passiflora edulis*, Pe) were evaluated for their antidiabetic and anti-inflammatory properties using α-glucosidase[2] and 15-LOX inhibition[3] assays. At 200 ppm, the hexane extract of Bv, Cc, and Pe inhibited α-glucosidase at 98.28, 98.24, and 96.95%, respectively. These results are at par with that of acarbose (98.32%) at the same concentration. The methanolic extract of Bv and Cc gave an inhibition activity of 42.87 and 36.10%, respectively. At 100 ppm, methanol extract of Pe inhibited 15-LOX with 43.14% inhibition followed by the methanol extract of Lc at 36.63%.

The study has shown that the hexane extracts of Bv, Cc, and Pe have antidiabetic potentials. Further confirmatory assays are recommended to corroborate these results. On the other hand, compared to nordihydroguaretic acid (NDGA), a moderate to weak anti-inflammatory activity, were shown by Pe and Lc. Other anti-inflammatory tests are recommended.

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BOEHMERIA NIVEA SUPPRESSES TARC AND MDC IN TNF-α/IFN-γ-INDUCED HaCaT CELLS

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Boehmeria nivea is widely used in Eastern Asia as a fiber crop, edible leaf, and herbal medicinal root. The *Boehmeria* genus contains various alkaloids, lignans, flavones, and catechins[1, 2] The ethanolic extract of *B. nivea* was partitioned into n-hexane, chloroform, ethyl acetate, n-butanol, and aqueous layers. Two phytochemicals were isolated using column chromatography on an ethyl acetate layer: kaempferol-3-*O*-rutinoside (**1**) and apigenin-7-*O*-glucoside (**2**). The structures of the isolated compounds were confirmed using NMR spectroscopy. Its anti-inflammatory effect was examined, and whether macrophage-derived chemokine (MDC/CCL22), thymus and activation-regulated chemokine (TARC/CCL17) levels were reduced was investigated in tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ)-induced human keratinocyte cell lines (HaCaT). These chemokines play a central role in the initiation of the innate immune system via pro-inflammatory cytokines[3] and are known to be associated with allergic disorders[4]. Moreover, the fractions suppressed TARC and MDC by regulating the MAPK and NF-kB pathways in TNF-α/IFN-γ-induced HaCaT cells. The results demonstrate that *Boehmeria nivea* has the potential to be developed as an ingredient to help reduce skin inflammatory responses.

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EFFECTS OF CALLUS FROM THE PULP OF *MALUS DOMESTICA* 'MELA ANNURCA CAMPANA' ON ANTIOXIDANT AND ANTI-INFLAMMATORY CELL MODELS

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Annurca Apple received a lot of attention among the various apple varieties. It is the most widely grown cultivar in Campania (Italy). Given the cultivar's importance to Campania's economy, the "Istituto Sperimentale di Frutticoltura" in Ciampino (Rome) launched a research program in 1970 to mitigate Annurca's flaws. These efforts have included research into its biology, breeding, and storage. This cultivar has also been proposed to the European Council for "Protected Geographical Indication" (PGI), a European project aimed at preserving local and distinctive agricultural commodities (CEE rule nr. 2081, 1992) [1,2,3]. Considering that the Annurca Apple is known to be a high-value apple to produce nutraceutical ingredients we set up an *in vitro* method to produce secondary metabolites, based on the induction of callus cultures from adult plant cells [4,5]. Annurca apple pulp was used as starting material and two callus cultures, one grown in the dark and the other in the light, were obtained. In the present study, hydroalcoholic extracts were obtained and analyses were performed to investigate their biological properties on cellular models of oxidative stress and inflammation represented by keratinocytes (HaCaT) and murine macrophages (RAW 264.7) cell lines respectively [6]. The results were always compared with peel and pulp hydroalcoholic extracts. Viability tests were conducted by the MTT assay, to exclude any cytotoxic concentrations. All the extracts tested concentrations were not cytotoxic. These concentrations were used to quantify the effect of the extracts on intracellular ROS production, measured at the basal level and after 30 and 60 minutes of H_2O_2-induced oxidative stress. Results from this analysis showed that both calli had no antioxidant activity at the basal level and only the callus in the light showed significant antioxidant activity after 60 minutes of H_2O_2 injury; peel and pulp showed an antioxidant activity both at basal level and after H_2O_2-induced oxidative stress. The anti-inflammatory activity of the extracts was investigated with Griess assay on lipopolysaccharide-stimulated RAW 264.7 cells. All the extracts showed significant anti-inflammatory activity and both calli presented greater anti-inflammatory activity when compared to peel and pulp.

Further research is in progress to evaluate the gene expression of various markers linked to the inflammation and oxidative process and to create cosmetic formulations with anti-inflammatory and antioxidant properties based on this callus extract.

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ISOLATION AND STRUCTURE DETERMINATION OF ANTITRYPANOSOMAL LIPOPEPTIDE; UKABAMIDE

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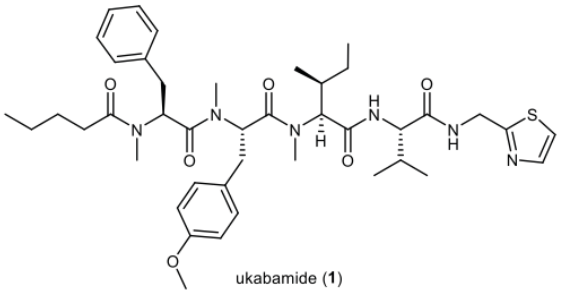
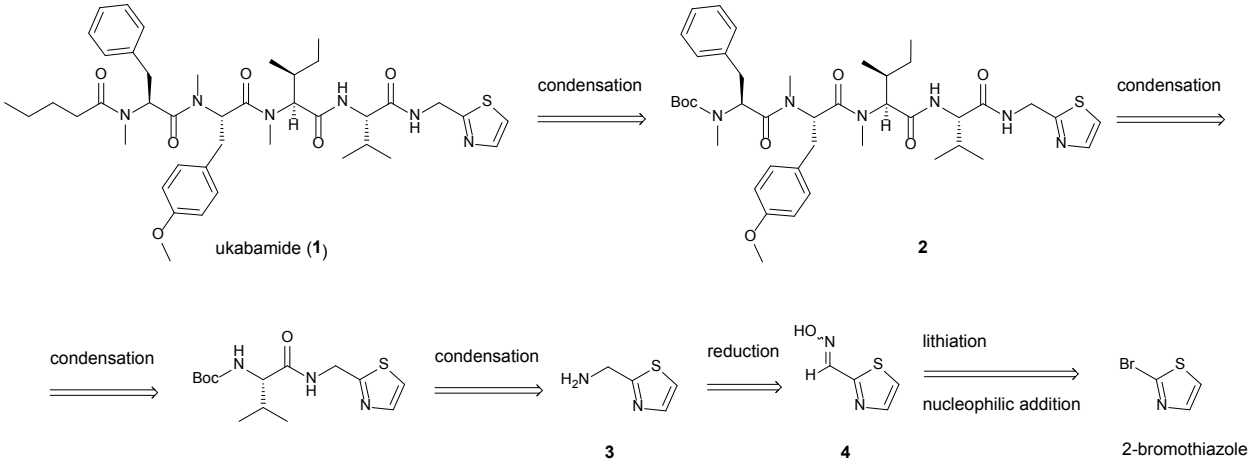

ukabamide (**1**)

Human African trypanosomiasis (HAT) is an endemic of sub-Saharan Africa caused by a protozoan parasite (*Trypanosoma brucei*; *T. b.*) transmitted by infected flies. Our group isolated a new linear lipopeptide ukabamide (**1**) from an unidentified marine cyanobacterium collected in Nanjo, Okinawa, Japan. Ukabamide (**1**) has some structural features: (1) three *N*-methylated amino acids (2) a terminal thiazole (3) a relatively rare odd fatty acid. The planar structure was determined by a combination of 2D NMR and MS/MS analyses. The absolute configuration was determined by chiral HPLC analyses of acid hydrolysate of **1**.

Regarding the biological activities, ukabamide (**1**) exhibited potent growth-inhibitory activity against *T. b. rhodesiense* (IC_{50} 91 ± 48 nM) without significant growth-inhibitory activity within 1 μM against human cervical carcinoma HeLa cells.

Currently, total synthesis studies are underway to develop a synthetic route for quantitative supply to drug discovery. Ukabamide (**1**) was planned to be synthesised by condensing valeric acid with hexapeptide **2**. The hexapeptide **2** could be obtained by sequential condensation of the appropriate *N*-methylated amino acids with amine **3**. Amine **3** was synthesised by reduction of oxime **4**, which was obtained by two nucleophilic addition reactions followed by lithiation of 2-bromothiazole. To date, the condensation of L-valine has already achieved.



IN VITRO ANTI-INFLAMMATORY STUDY OF LIMONOIDS ISOLATED FROM *CHISOCHETON* PLANTS

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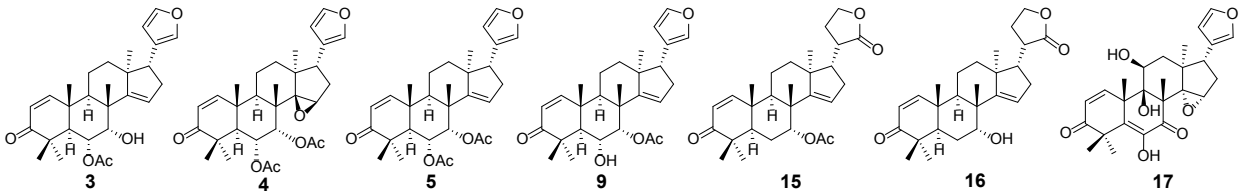
Chisocheton plants from the Meliaceae family have been used traditionally to treat several diseases although the scientific evidence is still limited[1]. The abundant chemical constituents found in this plant are limonoids known for their various biological activities, including anti-inflammatory[2]. In the previous studies, 17 limonoid compounds were isolated from *Chisocheton* plants[3-6]. The anti-inflammatory effects and underlying mechanisms of action of the constituents derived from the *Chisocheton* plants have not been fully explored. In this report, we evaluated the anti-inflammatory activity of the compounds mainly by measuring the inhibitory effects of pro-inflammatory cytokine production including TNF-α, IL-6, IL-1β, and MCP-1 in the LPS-stimulated THP-1 cells using ELISA assay. We found that some compounds showed a wide range of inhibition activity such as 6α-O-acetyl-7-deacetylnimocinol (**3**) and dysobinin (**5**) that exhibit moderate to strong inhibition to all evaluated pro-inflammatory markers and some compounds have selective inhibition on pro-inflammatory markers that mediated by MyD88-dependent pathways such as 6α-(acetoxy)-14β,15β-epoxyazadirone (**4**), nimonol (**9**), pentandricine B (**15**), pentandricine C (**16**) and pentandricine G (**17**). Collectively, these compounds may be useful as anti-inflammatory agents to treat inflammation by suppressing the inflammatory cytokine.



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GC-MS ANALYSIS OF THE TRITERPENOID CONSTITUENTS IN LUSITANIAN HEATHS

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The Lusitanian heaths, *Erica erigena*, *Erica mackayana*, *Erica stuartii* and *Daboecia cantabrica* belong to the Ericaceae family[1]. These plants have a limited geographical distribution and in Northwest Europe are localized to the west coast of Ireland in counties Galway and Mayo in particular[1]. As these plants are somewhat underexplored phytochemically the purpose of the study presented here was to determine their triterpenoid content. Thus, an ethyl acetate extract of the finely powdered leaves and flowers of these plants were prepared in triplicate and subjected to analysis by GCMS using BSTFA containing 1% TCMS as derivatizing reagent. Over twenty triterpenoids in free form were identified with examples from the lupane, ursane and oleanane classes being particularly dominant. Across all four species, ursolic acid was the dominant triterpenoid with an average content (20.18 mg/g) in the leaves of these plants. Lupeol (14.62 ± 0.10 mg/g), micromeric acid (6.31 ± 0.44 mg/g), oleanolic (7.06 ± 0.43 mg/g) and ursolic acid (23.09 ± 0.09 mg/g) were present in the highest concentration in *E. erigena* leaves. Coumaroyl triterpenes based on α-amyrin, β- amyrin and lupeol as well as micromeric acid are being reported for the first time from *E. erigena*. The leaves of the hybrid, *E. stuartii*, had a slightly higher level of ursolic acid (22.57 ± 0.22 mg/g) compared to its parent species *E. mackayana* (19.98 ± 1.26 mg/g) and *E. tetralix* (15.52 ± 0.30 mg/g). Total triterpenoid content is highest in *E. erigena* leaves (57.00 mg/g) while *D. cantabrica* flowers (12.99 mg/g) had the lowest content. In general, the highest total triterpenoid content was in the leaves of all studied plants. Across all four species minor amounts of α-amyrin, β-amyrin, lupenone, β-amyrenone, lupeol acetate, β-sitosterol, uvaol, erythrodiol, betulin, ursolic aldehyde and oleanolic aldehyde were present. In conclusion, these plants and in particular, *E. erigena*, represent a valuable source of biologically important triterpenes.

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SYNTHESIS OF BETULIN DERIVATIVES MODIFIED AT THE POSITION C-30 USING CYCLOADDITION REACTIONS

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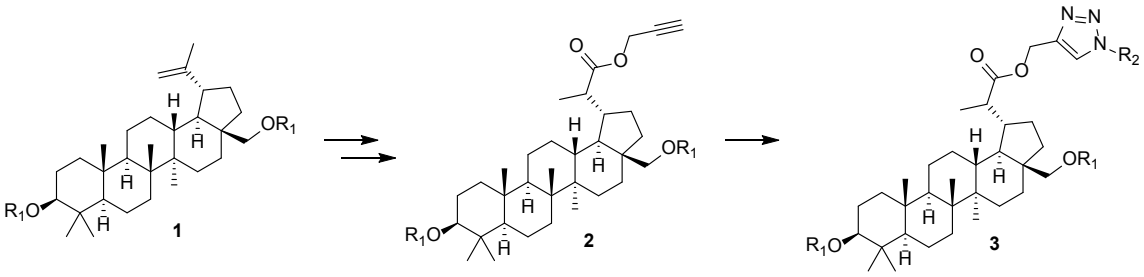
Pentacyclic triterpenoids are secondary metabolites from living organisms such as plants and fungi. They can serve as repellents or in general as antifeedants[1]. These compounds have been intensively studied as potential source of pharmaceutically relevant substances. Many of them have a large variety of biological activities such as selective cytotoxicity in cancer cells[2], antimalaritical[3], hepatoprotective[4].
Our research group is oriented towards the synthesis and development of compounds with anticancer and more recently with neuroprotective activity[5,6,7]. The presented work is focused on neuroprotective analogues of betulin **1** prepared by the modification of the position C-30 (Scheme 1). First of all, propargyl ester **2** was prepared by the catalytic hydrogenation of the double bond in betulin **1** followed by the allylic oxidation and alkylation with propargyl bromide. In the next step, Huisgen 1,3-dipolar cycloaddition with organic azides was used to prepare 1,2,3-triazole derivatives **3**. Based on the evaluation of the structure-activity relationships during our earlier research[7], we selected mainly heterocyclic and sugar substituents. This As a result, a library of 24 new triazole conjugates and several intermediates was obtained. Detailed synthetic procedures and results of biological activities will be discussed.



Scheme 1: Synthesis of target compounds.

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RECOVERY AND IDENTIFICATION OF HUMAN URINE METABOLITES

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Personalized nutrition and consumption of foods rich in bioactives (FBs) have emerged as beneficial approaches for human health maintenance and prevention of diseases. Bioavailability and especially metabolism play a critical role in the demonstration of the biological or pharmacological function[1]. However, studying FBs metabolism is regarded as a challenging task, due to low consumed dosages, foods/extracts' chemical complexity, and compounds extensive biotransformation. Advanced analytical methodologies like NMR and LC-HRMS metabolomics are usually being incorporated and metabolites annotation possesses the most significant challenge in such workflows. However, the unavailability of reference compounds is the major bottleneck for accurate metabolite identification and meaningful pathway interpretation.
Thus, the goal of the present study is the isolation of FBs-related metabolites from urine by embedding established chromatographic tools from the field of natural products chemistry and the unambiguous structural characterization thereof. The experimental workflow involved supplementing a healthy male volunteer with hydroxytyrosol capsules (15 mg/day) for one month and collecting daily 24-hour urine samples. The urine samples were pre-concentrated, extracted using XAD-7 resin, fractionated using Centrifugal Partition Chromatography (CPC), and finally purified using semi-preparative HPLC-UV. The proposed approach is a novel and robust methodology for the isolation of metabolites from human urine: i) in sufficient amount for detailed characterization by NMR ii) avoiding the cumbersome and expensive de novo synthesis of metabolites, iii) allowing the rapid purification of low-abundance metabolites, and thus, iv) could significantly contribute to accurate determination of metabolites and biotransformation monitoring in human organism.

Acknowledgment
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PREPARATION OF CHEMICAL PROBES FOR IDENTIFYING THE TARGETS OF THE MARINE NATURAL PRODUCTS KAPAKAHINES A AND F

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Marine invertebrates are known as the rich source of diverse chemical structures and biological activities. More than 30,000 marine natural products have been isolated[1], however only a limited number of compounds have been characterized for their mechanisms of actions.
Kapakahines A and F, isolated from the marine sponge *Cribrochalina olemda*, are marine cyclic peptides with a tetracyclic core containing an α-carboline skeleton[2]. All seven analogues sharing this skeleton are cytotoxic against P388 mouse myeloid leukemia cells at 5 μM[3,4]. In this study, fluorescent dyes and azide-terminal linkers were introduced into the amino group of kapakahines, respectively.The former fluorescent probes allowed us to monitor the subcellular localization and the latter to identify the target molecules. As the results, the fluorescent probe of kapakahine A was observed to localize in mitochondria or lysosomes. Pull-down experiments using magnetic beads (FG beads) were conducted and the following LC-MS/MS analysis, revealed mitochondrial inner membrane proteins, prohibitin 1 (PHB1), prohibitin 2 (PHB2), and adenine nucleotide translocator 2 (ANT2) as the candidate target proteins of kapakahines[5,6]. These results suggest that kapakahines may cause cytotoxicity through a mitochondria-mediated molecular pathway. In addition, we investigated the effects by kapakahines on the histone modifications.

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SEARCH FOR COMPOUNDS IN JAPANESE PEPPER THAT HAVE ACTIVITY IN REGULATING NEURAL STEM CELL DIFFERENTIATION

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Neural stem cells (NSCs) are pluripotent stem cells that can differentiate into neurons and glia cells. They continue to supply these cells throughout life in the adult central nervous system.[1] However, it is revealed that differentiation capacity of NSCs has been to decrease with aging and mental stress.[2,3] The decreasing capacity suspected to be associated with the development of neurodegenerative diseases and depression. Therefore, food ingredients that regulate NSCs differentiation are expected as supplements applicable to the prevention and treatment of such diseases.

Japanese pepper *Zanthoxylum piperitum* (L.) DC. is distributed in Japan and the Korean Peninsula, and its pericarps has long been familiar to the Japanese as a spice. It has been used not only for food, but also for medicinal purposes, such as in the traditional Japanese medicine Daikenchuto (TU-100). Hydroxy-*α*-sanshool in Japanese pepper is known to stimulate the sense of pain by activating the receptor TRPV1 expressed on sensory nerves[4] and to induce a unique numbness by inhibiting KCNK3, a target of anesthetic drugs.[5]

In this study, we have isolated four alkylamides, including a novel compound, from Japanese pepper pericarps extracts. Bioactivity of each compound was evaluated by in vitro NSCs differentiation assay using NSCs derived from mouse embryonic stem cells.[6] As a result, one of the compounds isolated in Japanese pepper was found to show the activity promoting astrocyte differentiation.

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ENZYMATIC TRANSFORMATION PRODUCTS OF MAJOR NEOLIGNANS IN *MAGNOLIA OBOVATA* BARK

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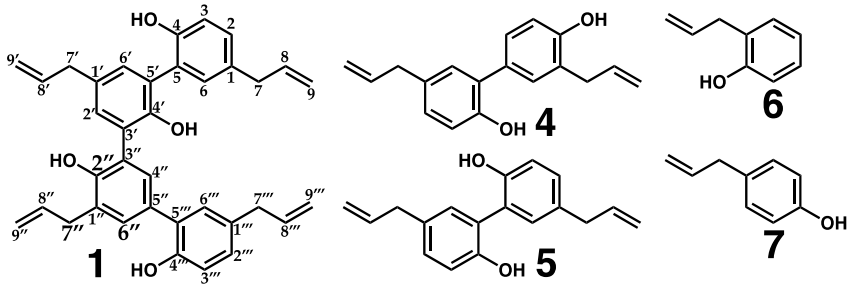
Honokiol and magnolol are the major neolignans in the bark of *Magnolia* spp.; exhibit diverse biological activities. Focusing on the C_6-C_3 unit of these neolignans, it can be pointed out that they have a characteristic structure with a hydroxy group and an allyl group attached on the aromatic ring. The aim of this study is to carry on enzymatic transformation of these neolignans [honokiol (**4**), magnolol (**5**): C_6-C_3 dimer] and C_6-C_3 monomers [2-allylphenol (**6**), 4-allylphenol (**7**)] and to elucidate the chemical structure and biological activity of the products. Present report describes the isolation and structure presumption of the product obtained by peroxidase treatment of the ethyl acetate soluble part (EtOAc Sol.) of the ethanol extracts of *Magnolia obovata* bark.

Ethanol solutions of EtOAc Sol. of the extracts of *M. obovata* bark (E)(600 mg), honokiol standard (4)(1 mg), magnolol standard (5)(1 mg), mixtures of 4 and 5 (4+5)(1 mg) were treated with aqueous solutions of peroxidase and H_2O_2. Enzymatic treated sample (E) was subjected to reversed-phase preparative HPLC; the most abundant three products (**1**-3) were separated. In the chromatograms of analyses for enzymatic treated samples, product **1** was formed in mixture (4+5) sample, however was not formed in the single sample (4 or 5). Therefore, **1** was presumed to be a linked product of honokiol and magnolol. Products 2 and 3 were formed in the single sample (4); they were presumed to be honokiol dimers. The most abundant product **1** was purified by normal-phase preparative HPLC; its NMR spectra (in $CDCl_3$, 400 MHz) were measured. Complete decoupling and DEPT ^{13}C NMR spectra of **1** showed 14 C, 14 CH and 8 CH_2 signals. ^1H NMR spectrum of **1** showed integral values of 10 protons in aromatic region. In the ^1H-^1H COSY spectrum of **1**, the existence of four allyl groups was suggested. HMBC spectrum of **1** showed a cross peak between an aliphatic CH_2 (H_2-7'') and an oxygenated quaternary carbon (C-2''); the result suggested the existence of a 2-allylphenol moiety. Long-range ^1H-^1H COSY spectrum of **1** showed cross peak between H_2-7'' and a proton on a four substituted aromatic ring (H-6''). These results suggested that **1** is a dineolignan possessed a biphenyl linkage between honokiol and magnolol. Products 2 and 3 are currently being purified.



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NETWORK PHARMACOLOGICAL ANALYSIS AND EXPERIMENTAL VALIDATION OF THE EFFECT OF *SMILACIS GLABRAE RHIXOMA* ON GASTROINTESTINAL MOTILITY DISORDER

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Gastrointestinal motility disorder (GMD) is a disease that causes digestive problems due to inhibition of the movement of the gastrointestinal tract and is one of the diseases that reduce the quality of life of modern people. *Smilacis Glabrae Rhixoma* (SGR) is a traditional herbal medicine for many diseases and is sometimes prescribed to improve digestion. As a network pharmacological approach, we searched the TCMSP database for SGR, reviewed its constituents and target genes, and analyzed its relevance to gastrointestinal motility disorder. The effects of the SGR extract on the pacemaker activity in interstitial cells of Cajal (ICC) and gastric emptying were investigated. In addition, using the GMD mouse model through acetic acid (AA), we investigated the locomotor effect of SGR on the intestinal transit rate (ITR). As a result of network pharmacology analysis, 56 compounds out of 74 candidate compounds of SGR have targets, the number of targets is 390 targets, and there are 904 combinations. Seventeen compounds of SGR were related to GMD, and as a result of comparing the related genes with the GMD-related genes, 17 genes (active only) corresponded to both. When looking at the relationship network between GMD and SGR, it was confirmed that quercetin, resveratrol, SCN5A, TNF, and FOS were most closely related to GMD. In addition, the SGR extract regulated the pacemaker activity in ICC and recovered the delayed gastric emptying. As a result of feeding the SGR extract to AA-induced GMD mice, it was confirmed that the ITR decreased by AA was restored by the SGR extract. Through network pharmacology, it was confirmed that quercetin, resveratrol, SCN5A, TNF, and FOS were related to GMD in SGR, and these were closely related to intestinal motility. Based on these results, it is suggested that SGR in GMD restores digestion through the recovery of intestinal motility.

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THE EFFECTS OF *CLERODENDRUM TRICHOTOMUM* THUNB. EXTRACT AND ITS CONSTITUENTS ON ADIPOGENESIS IN 3T3-L1 PREADIPOCYTES

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Clerodendrum trichotomum Thunb. is a member of the Verbenaceae family and is native to East Asia, including Korea, Japan, Taiwan, and China[1]. It contains various phytochemicals such as sterols, flavonoids, glycosides, and diterpenoids, which have antioxidant and analgesic properties[2, 3, 4]. The present study investigated the effects of *C. trichotomum* Thunb. extract (CTE) in 3T3-L1 pre-adipocytes. First, the effects of CTE on the viability and cytotoxicity of 3T3-L1 cells were evaluated using the MTT and LDH assays. No cytotoxicity was observed even at the highest concentration of 400 μg/mL. Based on the cytotoxicity evaluation results, treatment with 100, 200, and 400 μg/mL CTE inhibited lipid droplet accumulation during adipogenesis in a concentration-dependent manner. Phytochemical analysis of CTE resulted in the isolation of three compounds: acteoside (**1**), isoacteoside (**2**), and acacetin-7-*O*-diglucuronide (**3**). The structures of these compounds were elucidated using ^{1}H and ^{13}C NMR spectroscopy. The content and molecular weight of the isolated compounds in the extract were analyzed using HPLC and LC-MS, respectively. Additional anti-obesity studies of the separated compounds will confirm the possibility of developing functional materials for CTE.

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ENHANCING THE BIOACTIVE COMPONENTS IN *PSILOCYBE CUBENSIS* MYCELIUM: THE INFLUENCE OF SUBMERGED FERMENTATION CONDITIONS

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This study explores the submerged fermentation conditions that impact the accumulation of bioactive compounds, including psilocybin and psilocin, in the mycelium of *Psilocybe cubensis*. The research investigates the effects of various factors, such as fungi strain, nutrient media composition, pH, agitation, aeration, and mycelium disruption, on the accumulation of mycelial biomass and bioactive compound content. The best results show up to 20 mg/g (2%) of psilocybin in the dry mycelium within 13 days of growth, a value that is even higher than values that are documented for fruiting bodies. The findings have important implications for enhancing the bioactivity of *P. cubensis* mycelium and could lead to the development of new mushroom-based nutraceuticals and pharmaceuticals.

Psilocybe cubensis mycelium grown in submerged fermentation conditions

THE POTENTIAL OF LAKE KINNERET, ISRAEL, ALGAE TO SERVE AS A NEW SOURCE FOR MICROBIAL PESTICIDE

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Algae are known as a “green” source for antimicrobial substances. Nonetheless, little effort has been invested in searching for an algal source for use against plant diseases. In the present study, we report on the first examination of micro-algal isolates collected from the Sea of Galilee (Lake Kinneret) for their potential to serve as a source for new plant pesticides for agricultural applications. Cultures of 20 micro-algae species were grown under controlled conditions. Using a variety of extraction methods and Bioassay-guided fractionation, we were able to demonstrate the inhibitory effect of the extracts on different phytopathogenic microorganisms. The antimicrobial potential of the crude extracts was examined in vitro and on detached leaves against a wide variety of plant pathogens, including bacteria, fungi and oomycetes. Extracts from the P1 alga (code name because the species used is confidential) exhibited a minimal inhibitory concentration (MIC) of 2,500 ppm against the bacteria *Xanthomonas* sp., *Erwinia amylovora* and *Clavibacter michiganensis*, and a relatively low MIC of 187 ppm against the oomycete *Plasmopara viticola*. Extracts from two other algal cultures, S1 and C1, exhibited a MIC of only ~150 ppm against *C. michigenensis*. Polarity-based fractionation (using a silica gel column) of the crude extract from the S1 algal culture showed at least two active metabolites: one relatively hydrophobic fraction (eluted with 10% methyl-tert-butyl ether in petroleum ether) and a second, relatively polar, fraction (eluted with acetone). These fractions demonstrated a MIC of 100 ppm against *C. michiganensis*. The latter fraction was further fractionated and reached a MIC of 50 ppm. These results demonstrate the potential of local microalgae isolates as a source for new biopesticides. Future study will be focused on the identification of the active molecules using LC-MS and GC-MS.

ANTICANDIDAL PROPERTIES AND CHEMICAL COMPOSITION OF POLISH PROPOLIS

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Propolis is a sticky substance produced by the bees mainly from plant exudates mixed with beeswax. The propolis extracts are known for their high antimicrobial activity, which however is inconsistent for different samples which poses a challenge[1-2]. The ethanolic extracts of propolis from Poland exhibited relevant variations in activity against different *Candida* strains (*C. albicans*, *C. glabrata*, *C. krusei*, and *C. parapsilosis*). The minimal inhibitory concentration (MIC) of the most active extracts was 64 µg/ml, while for the least active it was higher than 256 µg/ml. The detailed composition of the samples were analysed using UHPLC-DAD-QqQ-TOF-MS, revealing flavonoids, phenolic acids and their esters as the most abundant components. To study the correlation between activity and chemical composition, the obtained data was investigated using multivariate data analysis. Orthogonal partial least squares discriminant analysis (OPLS-DA) model was created based on the LC-MS data and the activity against *Candida* spp. in terms of MIC. The analysis allowed identification of several compounds, mainly flavonoids (flavanones and flavonols) that are linked with elevated anticandidal activity of propolis and thus may be responsible for its antifungal potential.

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PHYTOCHEMICAL CONSTITUENTS FROM THE AERIAL PARTS OF *FORSYTHIA VELUTINA* NAKAI AND THEIR ANTI-DIABETIC ACTIVITY IN ZEBRAFISH MODEL

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Forsythiae Fructus, the fruit of *Forsythia* genus plant and a well-known traditional chinese medicine, has been widely used to treat pyrexia, inflammation, gonorrhea, carbuncle and erysipelas and other diseases[1]. *Forsythia velutina* Nakai is a Korean endemic species and belong to the family Oleaceae[2]. This study was conducted to investigate phytochemical constituents and biological activities of *F. velutina* in the course of searching for biologically active natural products. The aerial parts were extracted in EtOH, and then, the EtOH extract was partitioned consecutively with CH_2Cl_2, EtOAc and *n*-BuOH. Among these fractions, EtOAc and *n*-BuOH fractions were subjected to column chromatographic separation. Twenty-seven compounds were isolated from the EtOAc and *n*-BuOH fractions of the aerial parts of *F. velutina*. The structures of isolated compounds were identified as coniferin (**1**), matairesinoside (**2**), arctiin (**3**), acteoside (**4**), caffeic acid (**5**), rutin (**6**), quercitrin (**8**), ethyl β-D-glucopyranoside (**9**), liriodendrin (**10**), salidroside (**11**), forsythoside F (**12**), guaiacylglycerol (**13**), coniferaldehyde 4-*O*-β-D-glycopyranoside (**14**), forsythoside B (**15**), nicotiflorine (**16**), chlorogenic acid methyl ester (**17**), neochlorogenic acid methyl ester (**18**) by comparison of their spectral data with literature values. A new lignan glycoside named velutinoside A was determined as structure **7** on the basis of spectroscopic analysis and chemical evidence. Anti-diabetic activities of isolated compounds were evaluated by using diabetic zebrafish models for type 1 (alloxan-induced) and 2 (insulin-induced) diabetes. Many isolated compounds showed anti-diabetic activities for type 1 and 2 diabetes. Compounds **2**-**4**, **7**, **10**, **19**, and **12**-**18** revealed potent recovery effect on alloxan-induced pancreatic islet damage in zebrafish (type 1 diabetes). For the type 2 diabetes, compounds **1**-**4**, and **15** showed significant recovery effect on insulin-induced pancreatic islet damage in zebrafish. Among these compounds, forsythoside B (**15**) revealed the strongest ameliorative effect on pancreatic islet damage in zebrafish models for the type 1 and 2 diabetes.

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COUPLING THE POWER OF INVERSE VIRTUAL SCREENING AND 3D STRUCTURE-BASED PHARMACOPHORE SCREENING FOR ACCELERATING THE TARGET IDENTIFICATION AND REPOSITIONING OF NATURAL PRODUCTS

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Inverse Virtual Screening (IVS) approach represents a reliable computational method for quickly selecting the most promising interacting targets of natural products[1,2]. IVS is based on molecular docking calculations between the selected case-study compound(s) and a high number of target proteins to select the most affine targets of interaction. Protein panels contain up to thousands of macromolecules and they must be correctly prepared prior to docking experiments. The target preparation, if performed manually, may be costly in terms of time and efforts and it represents the bottleneck of the entire process. With this goal, we recently developed an automated workflow to shorten this time-consuming step in a fully automated fashion (PDB CI.O.E.)[3,4]. Once performed docking experiments, the selection of the most promising interacting targets represents the key challenging task, due to the occurrence of false positive/false negative results if the predicted binding affinities are considered as the only selection parameter. We overcome this issue applying a normalization of these data, which led to an improvement of the results[1,2]. Also, the careful analysis of the binding modes of the case-study compounds with the selected proteins may be highly beneficial for further filtering the most promising complexes. This task can be accomplished performing 3D structure-based pharmacophore screening for verifying whether the sampled ligand docking poses resemble the binding modes of known ligands for the investigated protein, the latter inferable from protein/ligand crystal structures available on the Protein Data Bank. With this aim, we recently developed PharmaCore,[5] a tool that generates 3D structure-based pharmacophoric hypotheses for a specific target in a fast and fully automated way. We show here the benefit of coupling IVS with 3D structure-based pharmacophore screening through PharmaCore, aimed at improving the selection of the most promising interacting targets of a query compound by joining both “scoring” (normalized predicted binding affinities) and “sampling” (compliance with 3D structure-based pharmacophore features) data from docking experiments.

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CHEMICAL CHARACTERIZATION AND POTENTIAL MECHANISM OF THE ANTI-ASTHMATIC ACTIVITY OF BLACK GINSENG EXTRACT

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Asthma is a chronic inflammatory lung disease that causes respiratory difficulties. Black ginseng extract (BGE) has preventative effects on respiratory inflammatory diseases such as asthma. However, the pharmacological mechanisms behind the anti-asthmatic activity of BGE remain unknown. Black ginseng is a new type of the Korean ginseng prepared from fresh ginseng with over 3 times steaming/drying cycles, which changes the ginseng color to black[1]. This repetitive heating process increases the content of nonpolar ginsenosides, which contributes to the enhancement of various physiological activities[2]. In this report, for the first time, we describe that standardized BGE and its major ginsenosides (Rg3, Rg5, and Rk1) possess effective anti-asthmatic activity by showing a relationship between PKCθ and asthma-associated transcription factors in PMA/ionomycin-stimulated EL4 cells or OVA-exposed mice with allergic airway inflammation. Further studies led to the isolation and identification of 33 ginsenosides, guided by ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS)-based rapid characterization of chemical constituents.

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BLACK GINSENG EXTRACT SUPPRESSES BENIGN PROSTATIC HYPERPLASIA IN VITRO BY INDUCING S-PHASE CELL CYCLE ARREST AND REGULATING THE INFLAMMATORY RESPONSE

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Prostatitis acts as the root cause of benign prostatic hyperplasia (BPH). Recently, non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, have been reported to be effective in suppressing BPH, and the potential of black ginseng extract as an anti-inflammatory drug has also been reported. In this study, we confirmed the effect of black ginseng 30% EtOH extract (BG30) on BPH in vitro. In RAW264.7 cells, BG30 inhibited lipopolysaccharide (LPS)-induced NO production and the levels of pro-inflammatory cytokines, such as TNF-α and IL-6. BG30 inhibited the proliferation of BPH-1, a prostatic hyperplasia cell line. In addition, BG30 significantly inhibited the expression of an androgen-related protein, such as androgen receptor (AR), prostate-specific antigen (PSA), and 5-alpha reductase 2 in BPH-1. Although further studies are needed, these results provide essential data that can partially explain that BG30 can inhibit prostatic hyperplasia-related inflammation, thereby inhibiting the induction and progression of benign prostatic hyperplasia. This study was supported by a grant from the Cooperative Research Program for Agriculture Science and Technology Development (No. PJ01419501 and PJ01419503), Rural Development Administration, Republic of Korea.

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UNNATURAL ALKALOID GENERATED BY PRECURSOR-DIRECTED BIOSYNTHESIS AND DISCOVERED BY LIGAND FISHING SCREENING (ACHEee)

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Plants produce a wide variety of pharmacologically active molecules called natural products, and in general, analogues of natural products to improve their biological activity. Precursor-directed biosynthesis strategy provide the opportunity to rationally design a broad variety of new natural products analogues. Among plants with therapeutic potential, the species of the genus *Tabernaemontana* (Apocynaceae) are widely used in traditional medicine due to several biological activities, including the neuropharmacological action. The iboga-type alkaloids found in the genus *Tabernaemontana* have great potential as AChE inhibitors and can be significant compounds in the study of biochemical interactions involving cholinesterases [1-3]. In this context, precursor-directed biosynthesis (PDB) strategies were conducted with adventitious roots from *T. litoralis* to obtain unnatural alkaloid derivatives. Adventitious roots (n=20) were incubated with 5-fluoro-tryptamine (1 mM) for 6 days. A control group of roots were prepared without incubation with the precursor analogue. After that, the crude extracts were prepared using methanol and both extracts were submitted to UPLC-MS analysis. Control and PDB extracts were screening using ligand fishing that target to ACHEee (Acetylcholinesterase from *Electrophorus electricus*) and an unnatural iboga-alkaloid was found (m/z 340; IA 340) in the PDB extract. Chromatography procedures were carried out to isolate the IA 340 and the identification of chemical structure was made by NMR 1D and 2D. A considerable number of studies have demonstrated that co-imobilization and magnetic bead-based approaches can quickly identify active compounds from complex mixtures [4-6]. Our results showed that precursor-directed biosynthesis strategies using 5-fluoro-tryptamine precursor produce successful the bioactive unnatural alkaloid in *T. litoralis*.

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SYNTHESIS OF NEW SUBSTITUTED 6-AZAINDOLES WITH CYTOTOXIC ACTIVITY

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Purine analogues are important therapeutic tools due to their affinity to enzymes or receptors that are involved in critical biological processes. With the aim to discover novel derivatives with potential cytotoxic activity, our research group is actively involved in the synthesis of numerous purine isosters and the subsequent evaluation of their cytotoxic activity. We have thus discovered compounds possessing IC_{50} values in the nM, or low μM concentration, against a variety of cancer cell lines of human origin. As a continuation of this project, we present here the design and synthesis of some new 6-azaindole derivatives, substituted with aryl groups at positions -3 and -7 of the scaffold. Commercially available 2-amino-3-nitro-4-methylpyridine was used as the starting material for the preparation of the target compounds. In total, 20 novel derivatives were synthesized and were evaluated for their potential to inhibit the proliferation of four cancer cell lines (A431, HT-1080, MCF-7, MDA-MB-231). Additionally, their effect on the proliferation of normal, non-cancer cells was examined. The cytotoxicity results revealed interesting SARs concerning the effect of the substitution pattern of the novel compounds on the antiproliferative activity. The analogues bearing the 2,4-dimethoxyphenyl group at position -7 of the 6-azaindole core proved to be the most potent, with IC_{50} values in the range 12-18 nM against the HT-1080 and MDA-MB-231 cancer cell lines. Notably, all analogues showed insignificant effect on the normal cell line AG01523, thus possessing great selectivity indices. In order to investigate the possible mechanism of action of the novel derivatives, a cell cycle analysis was performed for the most active compounds. The novel derivatives proved to cause a significant cycle arrest at phase G2/M when tested at the breast cancer cell line MDA-MB-231.

FORMULATION OF A PHYTODRUG AGAINST DERMATOPHYTOSIS BASED ON ESSENTIAL OILS FROM FOUR MYRTACEAE OF CAMEROON

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Dermatophytosis, caused by keratinophilic fungi called dermatophytes, has seen a surge in developing countries. Current chemical treatments have drawbacks, including recurrence, side effects, high costs, and prolonged durations. Our research aimed to formulate a natural phytodrug with antidermatophytic and antioxidant properties using essential oils from four Cameroon *Myrtaceae*, addressing the need for more effective and less restrictive control measures. The essential oils of dry flower buds and leaves of *Syzygium aromaticum*, leaves of *Syzygium guineense*, *Callistemon citrinus* and *Callistemon rigidus,* were extracted by hydrodistillation and the chemical composition was evaluated by GC and GC-MS. Additionally, antioxidant tests were performed using DPPH, ABTS, and FRAP methods. Furthermore, the inhibition of spore growth of *Trichophyton rubrum* and *T. soudanense* by the essential oils and their combinations (formulations) was assessed *in vitro* using microdilution in liquid medium. The most effective formulation's inhibition was tested *in vivo* on guinea pigs infected with *T. rubrum* and *T. soudanense*, and its impact on toxicity parameters was assessed. Results showed that, the predominant compounds in the essential oils were eugenol (87.62% and 87.13%) and β-caryophyllene (5.88% and 7.91%) for *S. aromaticum*'s buds (cloves) and leaves essential oils respectively; (Z) -β-ocimene (21.51%) and α-pinene (7.20%) for *S. guineense*; and 1,8-cineole (51.68% and 71.95%), and α-pinene (27.20% and 16.68%) for *C. citrinus* and *C. rigidus* respectively. Notably, *S. aromaticum*'s essential oils demonstrated superior antioxidant activity, surpassing the reference antioxidant BHT. The minimal inhibitory concentration (MIC) for cloves' essential oil was 2000 ppm against both pathogens, whereas leaves exhibited 2000 ppm against *T. rubrum* and 8000 ppm against *T. soudanense*. Other MICs exceeded 8000 ppm. Among formulations, F4 displayed superior *in vitro* activity (sporicidal at 2500 ppm). *In vivo*, it completely eradicated infections from the 9th day, without adverse effects on guinea pigs' organs at the tested dose. Consequently, F4 was used as the active ingredient in formulating a non-toxic 1% ointment named "MYCODERM." In conclusion, "MYCODERM" holds promise as a safe and effective drug against dermatophytosis linked to *T. rubrum* and *T. soudanense*."

Keywords: antioxydant, antidermatophytic, essential oils, Myrtaceae, phytodrug

COMPUTATIONAL SCREENING OF ALKALOIDS AND QUASSINOIDS ANNOTATED FROM *BRUCEA JAVANICA* (L.) MERR. AS POTENTIAL INHIBITORS FOR DENGUE VIRUS

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Brucea javanica (L.) Merr., belongs to Simaroubaceae family, is a tropical and subtropical plant widely distributed in various countries. Commonly known as Melada Pahit in Malaysia, it has been traditionally used for the treatment of fever. The therapeutic potential of *B. javanica* has been attributed to its alkaloids and quassinoids compounds. In previous study, the DCM roots extract displayed good dengue antiviral activity, with an EC_{50} = 0.30±0.08 µg/mL and CC_{50} = 2.99±0.99 µg/mL (SI of 9.96) in adenosine triphosphate (ATP) assay and plaque assay. With the aim to identify the dengue antiviral compounds, we explored the interaction between a set of seventy-eight chemical constituents annotated from *B. javanica* dichloromethane (DCM) roots with two proteases of the dengue virus (DENV); NS5 methyltransferase (MTase) and NS5 RNA-dependent RNA polymerase (RdRp). Using molecular docking analysis performed with the CDOCKER module in Discovery Studio® 3.1 (Accelrys, San Diego, USA), we hypothesized that, a ligand binding to the guanosine triphosphate (GTP) binding site of NS5 could potentially inhibit the 2'-O-methyltransferase activity, which is essential for viral replication. Among the tested chemical constituents, fourteen alkaloids exhibited good binding energies ranging from -34.82 kcal/mol to -9.10 kcal/mol for DENV Mtase, while twenty-four alkaloids ranging from -64.70 kcal/mol to -8.60 kcal/mol for DENV RdRp protein. Notably, 6-Hydroxymetatacarboline H and Metatacarboline C demonstrated a particularly strong binding affinity with a RdRp and Mtase, respectively. Moreover, results from in-silico Lipinski's rule and ADMET analysis indicated that these compounds fulfill the drug-likeness properties. These findings highlight the potential of *B. javanica* alkaloids as inhibitors of DENV replication by targeting NS5 MTase and RdRp proteins. Further investigations are warranted to elucidate the precise mechanism of action and evaluate the therapeutic efficacy of these compounds in the context of dengue viral infection.

POSTER SESSION 1

ST2 ROLE OF NATURAL PRODUCTS IN COSMETIC FORMULATIONS

PO45 – PO52

CHARACTERIZATION OF *H. CRENULATA* EXTRACTS AND QUANTIFICATION OF MARMESIN

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Introduction: *H. Crenulata* or thanaka is a tropical plant, commonly used by burmese people for making a paste used for skincare, especially for sun protection thanks to the presence of marmesin. This plant attracted the interest of cosmetic industries, but its chemical and biological properties are still mostly unknown [1]. The aim of this work was the full characterization of thanaka's extracts obtained from different sustainable methods of extraction.

Methods: Ten extraction protocols were applied by Soxhlet Extraction (SE) and Ultrasound Assisted Extraction (UAE) methods. Thanaka bark was ground into a powder and dried at 35°C for 24 h. Five extraction solvents were used: water:ethanol mixtures (28% vol., 67% vol., and 48% vol.), a mixture of ethanol and ethyl acetate 50:50 (V/V) and demineralized water. For the UAEs, the samples were sonicated in an ice bath at a frequency of 20 kHz for 5 minutes and with an amplitude of 95% of the total energy of 500 W. For SEs, the parameters (heat level and extraction cycles) of the extractor were changed based on the solvent that was used. The extracts were lyophilized and characterized for yield, antioxidant capacity (DPPH, ABTS, FRAP assays), Total Phenol Content (Folin-Ciocalteu assay) and anti-collagenase activity. Quantification of marmesin in the extracts was made with HPLC-DAD [2]. Human gingival fibroblasts (HGFs) cell line was used for cell proliferation assay, collagen release, and prostaglandin E2 release.

Results: The best extraction solvents in terms of antioxidant activity, phenol content and anti-collagenase activity were the hydroalcoholic solutions with 48% vol. and 67% vol., both for UAE and SE. The same extracts gave the best results also with the cell proliferation assay, the release of collagen and anti-inflammatory activity in HGFs. Comparing the quantity of marmesin found in these extracts, the one with the higher amount was the extract obtained with SE using the hydroalcoholic solution 67% vol.

Conclusion: The extractions allowed to obtain a powder with antioxidant and anti-collagenase activity, and with the potential to protect against UV light. The powder can be used for the formulation of anti-age and sun-protective face cream.

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OPTIMIZATION OF EXTRACTION METHODS, PHYSICOCHEMICAL CHARACTERIZATION AND PRE-FORMULATION STUDIES OF SERICIN OF DIFFERENT MOLECULAR WEIGHTS FOR COSMETIC AND DERMATOLOGIC FORMULATIONS

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Introduction: Sericin is a protein from silkworm with a molecular weight (MW) ranging from 10 to 400kDa, depending on the extraction method. Sericin of higher MW has been fully characterized, while there is a lack of data concerning the recovery of lower MW fractions and their characterization [1].
Methods: A Central Composite Design was applied to optimize the degumming of sericin from silk waste. Two extraction methods were evaluated, one under heating, and one under sonication. The independent variables were temperature (60-100 °C), extraction time (30-120 min) and solvent (water, urea 8M, Na2CO3 0.5% w/w) for the first, amplitude (20-90%), sonication time (3-15 min) and solvent (water, urea 8M, Na2CO3 0.5% w/w) for the latter. Among the dependent variables, the yield was calculated, and the MW was determined by SDS-PAGE. Sericin of different MW (<30; 30-100; <100kDa) were recovered with Centrifugal Filters (MW cut off: 30kDa and 100kDa), The three fractions were characterized by SEM, IR, X-ray Crystallography and DLS. Sericin of different MWs were formulated as hydrogels by storing 2% w/w sericin solution at 4 °C for different time intervals. The rheological behavior of the fractions was determined by a cone-plate rheometer.
Results: The DoE allowed to select the best method for the recovery of sericin of three fractions of different MW and different properties. The gelation of sericin occurred by the formation of hydrogen bonding between sericin molecules. It has been proved that the network formation is dependent on the temperature and MW [2][3]. Robust gels were recovered when the crosslinking occurred at 4 °C with MW >100kDa sericin. The gelation properties of the formulations were tested and confirmed by rheological measurement.
Conclusions: Sericin with MW <30kDa was used for its capacity to retain water and penetrate up to the stratum corneum. Sericin with MW >30KDa was used to formulate hydrogel.

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THE INFLUENCE OF MODIFICATION BY ACETYLATION OF PLANTAIN STARCH (*MUSA PARADISIACA)* IN ITS PHARMACEUTICAL PROPERTIES

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The pharmaceutical industry uses a wide variety of formulation auxiliaries, including starch, which due to its pharmaceutical properties, is very useful when preparing various pharmaceutical forms [1]. The use of starch is however limited by its poor functional properties of flow, compressibility and compatibility. Hence, starches are chemically, physically or enzymatically modified to improve the aforementioned properties in the development of new products [2]. In this study, acetylation was carried out in different degrees of substitution (GS) of plantain starch (PS) (*Musa paradisiaca* L. Family: Musaceae) with various volumes of acetic anhydride (Ac_2O) at 99 % purity. The chemical modification was confirmed using infrared (IR) spectroscopy, and the pharmaceutical technology properties were determined and compared against the corn starch (CS) used as a control in this investigation. The percentage of acetyls and DS increased with the increase in the volume of Ac_2O. The chemical modification caused an increase in swelling power, water absorption index, solubility index, gelatinization temperature, particle size, and bulk for both banana and corn starch. On the other hand, properties such as bulk density and tamped density decreased with acetylation in both starches. In properties such as the Hausner index, which measures the cohesiveness and degree of compression (results from 1.20 to 1.32 for both starches), and the Carr index, which measures the degree of fluidity (18.45 to 23 .72% for PS and from 16.72 to 21.54% for CS) [3]; no significant changes were found concerning for the modification. The changes in the properties of the native starch by acetylation improve its application at the pharmaceutical level. Further research studies will include the application of the modified starches as excipients in pharmaceutical and cosmetical formulations.

Keywords: Starch, acetylation, acetic anhydride, infrared, degree of substitution, bulk.

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ANTI-MELANOGENIC EFFECT OF *CANNABIS SATIVA* STEM FERMENTED WITH BENEFICIAL BACTERIA

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Cannabis sativa (CS) has been in the spotlight not only for its medical uses but also as a raw material for cosmetics. As fermented cosmetics are known to have various health benefits, research on them has been actively conducted. Here, we investigated the characteristics of CS stems fermented using various gut microbes. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay and melanin content analysis revealed that melan-a cells containing CS stems fermented by *Weissella parameseteroides* (CSWP) showed significantly reduced melanin content. Additionally, CSWP downregulated the expression of several melanogenesis factors, such as microphthalmia-associated transcription factor and tyrosinase-related protein-1. This study suggests that the antimelanogenic effect of CSWP could provide a new basis for the development of skin-lightening agents.

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A NEW CONCEPT OF CREAM FOR DRY SKIN FORMULATED WITH RED GRAPE-BASED EXTRACTS

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In the second half of the twentieth century was observed a new trend regarding cosmeceuticals preferred by consumers. These topical products, with an effect on both skin appearance and functioning, must be made of natural ingredients with active functions and valuable benefits on the skin. Natural polyphenols from red grapes, through their confirmed antioxidant activity relative to radical oxygen species, are of constant interest in the cosmetic field for antiaging purposes or for nutraceutical applications. Therapy with bioactive compounds associated with autochthonous red grape-derived products has been proposed in this study in terms of treatment of extrinsic skin aging processes. Given the general preference of consumers for natural products and the care given by international forums, regarding the prohibition of synthetic compounds in the composition of cosmetic products used daily/weekly, this study offers consumers a solution mainly related to chemophobia. In this respect, the aim of this study was to obtain an emollient cream for dry skin, pale pink in color, with a pleasant smell, based on natural products from organic vineyards (*Vitis Vinifera* L), with use in the cosmetic or dermatological field, mainly for anti-wrinkle purposes. Emollient cream for dry skin is characterized by the fact that it consists of a mixture of lanolin, cetyl alcohol, and sodium lauryl sulfate, in which has been incorporated a 7% (volume/mass) hydroalcoholic extract obtained from the red grapes, skin and seed, of Romanian Feteasca Neagră variety (without genetic modifications, a special chemical composition - resveratrol known as " elixir of youth" and piceid a stilbenoid glucoside). The combination of the components of the cream results in a product that deeply nourishes and prevents skin drying (moisturizing, revitalizing, antimicrobial effect), repairs and smoothes fine expression lines, and prevents aging (anti-wrinkle effect). Regarding the phytochemical profile, the results showed that on one hand, emollient cream with grape skin extract has a total content of polyphenolic compounds in the range of 2.50-3.50 mg-EAG/mL, a total flavonoid content in the range of 3.00-4.50 mg-EQt/mL and antioxidant activity in the range of 0.80-1.50 mg-EAA /mL, and on the other hand, emollient cream with grape seed extract has a total content of polyphenolic compounds in the range of 4.50-7.50 mg-EAG/mL, a total flavonoid content in the range of 4.20-7.70 mg-EQt/mL and antioxidant activity in the range of 10.00-12.50 mg-EAA. Several analytical investigations (i.e., electrochemical, vibrational, chromatographic) were used in this research to identify structural changes and to highlight the compounds with bioactive potential from hydroalcoholic extracts incorporated in the novel emollient skin creams. In addition, microbiological and clinical investigations were performed, as well.

MAIZE-DERIVED ZEIN/ACRYLIC HYBRID EMULSION FOR SUPERIOR SKIN ADHESION AND WATER RESISTANCE IN COSMETIC ADHESIVES

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Waterborne acrylic emulsions have been considered as suitable materials for cosmetic skin adhesives due to their desirable characteristics, including a safe water-based polymerization process and low emissions of volatile organic compounds (VOCs). However, a significant challenge in using emulsion adhesives lies in their inadequate water resistance, primarily caused by excessive surfactants retained at the emulsion particle interface during film formation. Overcoming this challenge is critical to the viability of using emulsion adhesives in the cosmetic industry. Zein, a protein derived from maize, is known for its hydrophobic amino acids, which impart exceptional water-repellent properties. Additionally, zein is expected to exhibit remarkable skin adhesion due to its abundance of amino acid functional groups[1, 2]. In this paper, we introduce a pioneering zein/acrylic hybrid emulsion that demonstrates remarkable skin adhesion and improved water resistance. The incorporation of zein facilitates the formation of a zein-surfactant complex[3], which enhances the unfolding of the zein chain, promoting skin adhesion while inhibiting surfactant migration to improve water resistance. To ensure effective complex formation and stable dispersion of the zein-surfactant complex, zein was introduced during the pre-emulsion step of emulsion polymerization. As anticipated, the prepared zein/acrylic hybrid emulsion exhibits favorable skin adhesion and noteworthy enhancement in water resistance. Our developed approach presents a promising avenue for the practical application of emulsion nanoparticles as eco-friendly cosmetic adhesives, offering excellent durability and skin adhesion through the utilization of naturally derived proteins.

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GRAPHENE AS FUNCTIONAL AND TEXTURIZING AGENT IN COSMETICS

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This research is focused on a natural agent of great industrial interest: **GRAPHENE**.
Graphene is an allotropic form of carbon and it's obtained from graphite by physical expansion: thus it is a product free from harmful chemical substances and it is generally classified as an eco-friendly product. Graphene has very particular properties: it is one hundred times stronger than steel, it has high elasticity, flexibility, high thermal conductivity and light/UV interaction. Graphene means, as it is well known, a material consisting of carbon atoms arranged in a monoatomic layer, with each atom bound to three neighbours in a honeycomb structure [1,2]. The term "graphene" is here to be understood strictly, i.e. it is not inclusive of chemical derivatives of graphene, such as graphane, fluorographene, graphene oxide, etc. This natural agent has been included in skin (make-up) [3,4] and hair (hair coloring and strengthening treatments) [5] products. There is a growing interest in multifunctional cosmetics capable of combining make-up with beneficial effects (deep hydration, water resistance, shine, photoprotection). As far as face make-up is concerned, it must be a very light and nude look coverage. The compositions obtained give texture characteristics, excellent spreadability, long lasting, shining and nude effect to the skin while retaining covering properties, with protection against photoaging and thermal dispersion, anti-pollution and antioxidant effects. The inclusion of graphene in lip products has given rise to products with a particularly high degree of sun protection, together with excellent rheological, physical, functional and sensorial properties, and high stability against the photoxidative damage. The research of graphene applications in hair products, through the new compositions has allowed the solution of the following problems: the excessive product permanence, the restoring of the original color of natural grey hair, the damage of excessive exposition to heat treatment. In the present research graphene nanoplatelets (3-6 nm thickness and 4±2 micron particle size) is used as a concentrated aqueous paste, a powder or as solid granules depending on the characteristics required by the formulation. The compositions are eco-friendly, free from aggressive agents in both cases (make-up and hair treatment) and they have shown interesting application properties.

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A PRELIMINARY PHYTOCHEMICAL AND FORMULATION STUDY OF *STACHYS PAROLINII*

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The genus *Stachys* L. comprises about 300 species and is one of the largest genera of the Lamiaceae family. In Greece, 56 species and subspecies of *Stachys* are distributed in parts of the mainland and/or insular Greece[1]. Several *Stachys* plants have long been applied in folk medicine to treat various diseases such as inflammations, healing of the skin, and stomach disorders[2,3]. In continuation of our research in the genus *Stachys*[2,4-7], we investigated *Stachys parolinii* Vis., which is found in North Peloponnese, Central Greece, and Ionian Islands (Kefalonia and Lefkada). This study focused on the phytochemical analysis of the *S. parolinii* methanol extract and the formulation of an oil-in-water microemulsion for cutaneous use to improve the low aqueous solubility of the extract for achieving optimal cutaneous administration and bioavailability. Overall, its secondary metabolites were categorised into iridoids, flavonoid glycosides, phenylethanoid glycosides, and phenolic acids. The microemulsion was prepared using Solutol HS 15 (surfactant), Transcutol P (co-surfactant), and Capryol 90 (oil), selected for the high solubility of the extract. According to previous studies, we fixed the surfactant/co-solvent ratio at 1:1, 2:1, and 3:1, and we constructed the corresponding pseudo-ternary phase diagrams by the titration method to investigate the microemulsion existence regions. The extract solubility in the final microemulsion was evaluated for those with the lowest polydispersity index (around 0.15) by the flask shake method for three hours. It was quantified in terms of isoscutellarein 7-O-[6‴-O-acetyl]-β-D-allosyl-(1→2)-β-D-glucoside concentration and resulted in about 25 mg/mL for all the samples without significant differences. Further studies will be focused on the full characterisation of the phytochemical content and optimisation of the microemulsion composition and technological features.

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POSTER SESSION 1

ST3 FOOD AND FOOD SUPPLEMENTS

PO53 – PO60

CHEMOMETRIC PREDICTION MODELING AS A TOOL FOR HERBAL PRODUCT IDENTITY INVESTIGATIONS

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Untargeted metabolomics greatly accelerates numerous aspects of natural product research, especially regarding botanical authentication and identity claims. Chemometrics combines the vast chemical datasets generated in untargeted studies with multivariate statistics and machine learning, offering an avenue to visualize and interpret metabolite variation and draw relevant conclusions. This study compares multiple supervised and unsupervised chemometric modeling approaches to investigate identity claims of dried *Ocimum* (Tulsi) products. 30 *Ocimum* varieties belonging to three species, *O. tenuiflorum*, *O. gratissimum*, and *O. basilicum*, were grown under identical greenhouse conditions before morphological authentication. Untargeted metabolite profiles were generated via UPLC-Orbitrap MS/MS and preprocessed in MzMine3. We first conducted PCA to visualize underlying sample variations before using Partial Least Squares – Discriminatory Analysis (PLS-DA) to build a classification model based on the correlation and covariance of samples' metabolomes. PLS-DA predicts the classification of a sample (in this case, as one of the three species) based on the similarities between the sample's metabolite profile and those of the samples in the model. A Random Forest (RF) predictive model, which uses multiple decision trees to classify a sample based on metabolite presence and intensity, was also constructed. Following PLS-DA and RF model construction and validation, we evaluated the identity of 16 commercially available *Ocimum* products from India and the United States. Overall, both models had similar product classifications, error rates, and validation scores, suggesting chemometric modeling is a consistent means to botanical authentication via multiple approaches.

NATURAL PRODUCTS FROM MARINE INVASIVE SPECIES AS FUNCTIONAL INGREDIENTS IN AQUACULTURE FEED

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In the last decade, there has been a growing scientific interest in the potential use of phytochemicals as dietary supplements to increase aquaculture farms' productivity and improve the welfare of farmed animals. This study focuses on bioactive substances that can be obtained from exotic species that have invaded the Mediterranean basin, whose enormous biomasses pose a critical threat to local biodiversity. Special attention has been paid to the flavone apigenin that is widespread in many terrestrial edible plants and in the invasive seagrass *Halophila stipulacea* [1]. Apigenin is renowned for its health-promoting effects, with countless nutritional and organoleptic features [2]. The study also focuses on the sesquiterpene caulerpenyne from *Caulerpa cylindracea,* a highly invasive alga voraciously eaten by native fish. This compound is already known for its antibiotic properties against gram-positive bacteria [3] and could be therefore of interest in aquaculture to fight this type of bacterial pathogens that cause economic losses in fish farming worldwide [4]. Both natural products have been added to fish feed and tested for their palatability in *Danio rerio* (zebrafish), which is one of the most widely used *in vivo* models to study physiology and nutrition in aquatic vertebrates. The effects of the phytochemicals on fish metabolism and intestinal microbiota composition have also been evaluated through metabolomics and metagenomic NGS approaches, respectively. Experiments have also been carried out on the marine shrimp *Palaemon elegans*, highlighting differences in the palatability of the two compounds towards fish and shrimp. Results are discussed in light of a possible valorization of marine invasive species for applications in aquaculture.

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PHYTOCHEMICAL VARIATION BETWEEN DIFFERENT ACCESSIONS OF *ARTEMISIA ABSINTHIUM* L.

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Great wormwood (*Artemisia absinthium* L.) is a well-known medicinal and aromatic plant, used notably for the production of several famous aperitif drinks. The chemical composition of the raw plant material, especially its contents in α-thujone and β-thujone, is important in order to assure high quality standard of the final product, as well as to respect legal requirements. Therefore, the agricultural production of selected lines is justified. Nevertheless, today, the species is still at a very early stage of its domestication process, and no real genetic improvement program has been carried out on it yet. As usual, selection and breeding of improved lines will rely on the genetic variation offered by the species.

If the existence of very distinct chemotypes in the species have been reported [1], the description of the genetic phytochemical variation, in compositions and proportions, offered by the species is still poorly documented. In order to precise the presence and the amplitude of this genetic variation, we gathered, and cultivated in parallel, a dozen of accessions, wild populations and cultivars, from very diverse origins. Plants were grown in the Botanical Garden of Neuchâtel (Switzerland) between 2021 and 2022. Along the regular monitoring of the development of the plants, samples of leaves and flowers were regularly collected at different stages during the season. Optimized extracts of dried plant material have been analyzed by GC-MS.

Large morphological and phenological (dates of flowering) variations were observed between the lines. And the chemical compositions of the different accessions were very variable too, especially in regard of their α-thujone and β-thujone contents, which varied between 0.6 and 5.8 mg/L, and 0.0 and 168 mg/L, respectively.

These results make it possible to consider the selection and breeding of lines optimized for their productivity and their organoleptic quality, and respecting the legislation relating to the thujone contents.

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PHENOLIC PROFILES AND BIOLOGICAL ACTIVITIES OF HYDRODISTILLATION WASTEWATERS

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Essential oils (EOs) from plant material are obtained mainly by distillation (either hydrodistillation or steam distillation). The biomass used in the process is returned as a solid reside together with variable amounts of wastewaters rich in water-soluble compounds, which are not addressed to any further application. The scope of our work is to evaluate the phenolic composition of hydrodistillation wastewaters (DWWs) of several aromatic plants (*Origanum vulgare*, *Thymus vulgaris*, *Salvia officinalis*, *Rosmarinus officinalis* and *Origanum majorana*) and asses their biological activities for future application in the cosmetic, pharmaceutical, nutraceutical and food fields. Phenolic profiles of the DWWs were determined by HPLC-DAD-ESI/MS[1]. A method of phenolic enrichment using absorbent resins has been developed. Free radical scavenging activity, oxygen radical antioxidant capacity and superoxide dismutase mimetic activity of the extracts were measured. To evaluate the potential use of these extracts in pharmaceutical and nutraceutical fields, an *in-vitro* experimental model of intestinal inflammation was used to evaluate their anti-inflammatory activity. Moreover, an *in-vitro* α-glucosidase and α-amylase inhibition test has been used to evaluate the potential hypoglycaemic activity. Finally, the interfering effect exerted by *Lactobacillus helveticus* (LH) and *O. vulgare* DWWs in the modulation of Candida albicans (CA) (an opportunistic pathogen, which in pathophysiological conditions can lead to invasive infections) has been investigated. Our results show that DWWs have antioxidant and significant antinflammatory activity, an higher hypoglycaemic activity than that of acarbose, the commercial drug and show that DWW stimulates the growth of LH which, in turn, significantly inhibits the growth of CA by both contact inhibition and secretome action. Our study contributes to the knowledge of the polyphenolic composition of the DWWs, confirming the presence of compounds with proven biological activity, which make this by-product of the essential oil production industry of potential application interest in the pharmacological, cosmetic and nutraceutical fields.

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ULVA SEAWEEDS AS A SUSTAINABLE ALTERNATIVE FOR NON-ANIMAL PROTEINS

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Given the recent interest in exploring alternative non-animal protein sources, seaweeds have emerged as a viable and sustainable option. Seaweeds are rich in proteins and dietary fibres, which are of great interest in the food industry; however, their intricate cell wall structure hinders the extraction of proteins, resulting in low yields. This study aimed to optimize the protein extraction process from the green seaweed *Ulva spp*. The influence of the seaweeds physical state (frozen vs dry) was examined, along with the application of an ultrasound (US) pre-treatment to disrupt the cell walls and facilitate protein extraction. The crude seaweed samples were characterized in terms of gross composition (protein content, lipids, mineral content and carbohydrates). Protein-rich extracts were successfully obtained by a pH shifting process, involving an alkaline extraction followed by a precipitation step at acid pH. The resulting extracts were characterized to assess their composition and antioxidant capacity. The results evidenced the potential of the US pre-treatment to disrupt the cell walls and facilitate protein release, as the extraction yields increased up to 2-fold, achieving a maximum protein extraction yield of 47%. In the subsequent pH shifting process, the optimal solubilization pH was 12, causing a significant swelling of the cell walls, as evidenced by optical and confocal microscopy. During the precipitation stage, the pH had a significant impact on the yield and protein purity, with pH=3 yielding the highest protein extraction. Interestingly, freeze-drying of the seaweeds caused a collapse in their cell wall structure, hindering significantly the protein extraction process and producing much lower yields.

The application of a short ultrasound pre-treatment proved to be a promising technique for enhancing protein yield in *Ulva*, offering a means to produce novel protein-rich ingredients without excessive energy consumption which will have a great potential for their use in the food industry.

POLYSACCHARIDE-PROTEIN GEL-LIKE STRUCTURES TO MODULATE FOOD PROTEINS DIGESTION

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The digestibility of proteins is defined by their intrinsic properties and by gastrointestinal conditions like pH, temperature, enzymes and physiological surfactants. Some peptides produced upon gastrointestinal digestion have been reported to have the potential to regulate hormonal responses involved in appetite and satiety modulation. Based on this, the development of polysaccharide-protein hybrid gel-like structures is proposed in this work as a promising strategy to modulate protein digestion and induce the release of larger peptide fragments in the intestine.

A sulphated polysaccharide (agar) and a labile food protein (casein) were used to develop hydrogels, which were then subjected to *in vitro* gastrointestinal digestions using the harmonized Infogest protocol. Subsequently, the obtained digestion products were characterized in terms of peptide profile, microstructure and molecular weight distribution and their behaviour was compared to micellar casein and to agar-casein blend solutions. Furthermore, the effect of the bile salts concentration was evaluated on the digestion mechanism of the different formulations.

The degree of gastric proteolysis of casein has been proven to be reduced by hybrid gel-like structures, while promoting the release of larger peptide fragments at the intestinal digestion phase. Even though certain protective effect was shown in the presence of agar in the blend solutions, this was enhanced when hybrid hydrogels were developed. Moreover, the effect of bile salts on the digestion process differed depending on the presence of the polysaccharide. This is expected to have strong implications on the type of structures formed by the assembly of the digestion products and their intestinal transport.

Polysaccharide-protein gel-like structures exhibit a great potential to limit the degree of hydrolysis undergone by dietary proteins at the gastric phase while simultaneously enhancing the release of larger peptides during the intestinal phase which can reach the far intestine to exert their bioactive functionalities. Therefore, they could have a beneficial impact on appetite modulation and satiety.

CCK AND GLP-1 SECRETION IN ENTEROENDOCRINE CELLS IN RESPONSE TO MILK PEPTIDES IDENTIFIED IN HUMAN JEJUNUM

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One of the functions of the enteroendocrine cells located at intestinal epithelium is to sense the luminal contents and, in response, release gut hormones that modulate a variety of physiological gastrointestinal and metabolic processes. Our previous results have shown that food peptides are able to trigger the secretion of cholecystokinin (CCK) and glucagon like peptide-1 (GLP-1), being the main inducers of GLP-1 (Santos-Hernandez et al., 2020; Santos-Hernández et al., 2023). The objective of this work was to characterize the CCK and GLP-1 secretagogue effect, in STC-1 cells, of peptides resistant to gastrointestinal digestion identified in human jejunum after casein and whey protein ingestion. After the peptidomic fingerprint of jejunal contents from 5 subjects, 17 consensus sequences derived from β-casein, κ-casein, and β-lactoglobulin found at least two individuals were chemically synthesized. Cell viability assays, intracellular calcium increase, and hormonal secretion were performed in STC-1 cells.
The intracellular calcium concentration was used as a screening prior to the hormonal secretion experiments. The exposure of STC-1 cells to all peptides produced an increase in intracellular calcium compared to the control. Nevertheless, we found some peptides that produced an outstanding activation, such as, β-casein peptides ^{172}PVPQ^{175} and ^{86}PPFLQPEV^{92}, although this was not translated into higher CCK or GLP-1 levels in the extracellular medium. A sequence-specific behaviour was observed in both, GLP-1 and CCK secretion. The loss of proline at the N-terminal end of peptide ^{81}PVVVPPFLQPE^{91} derived from β-casein, notably decreases GLP-1 secretion. Similarly, our results identified β-casomorphin-7 ^{60}YPFPGPI^{66} as a potent CCK and GLP-1 secretagogue, and interestingly, the loss of the last C-terminal two amino acids, proline-isoleucine (^{60}YPFPG^{64}), reduced CCK secretion but induced GLP-1 at the same level. Also, the progressive loss of amino acids at the N-terminal end of the ^{6}LNVPGEIVE^{14} peptide resulted in a stepwise decrease in CCK secretion. These results show the GLP-1 and CCK secretion in enteroendocrine cells induced by milk-derived peptides with proved *in vivo* resistance to gastrointestinal digestion and the importance of the sequence in the secretion of these hormones involved in satiety.

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METABOLOMIC PROFILE OF COMMERCIAL *PHASEOLUS VULGARIS* L. VARIETIES BY NMR SPECTROSCOPY AND MASS SPECTROMETRY

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Common bean (*Phaseolus vulgaris* L.) is considered as the most consumed legume around the world. Seeds possess nutritional properties due to the presence of protein, carbohydrates and lipids but they also contain several secondary metabolites that provide health benefits [1]. This project aims to the study of the metabolic profile of some Italian commercial varieties of *Phaseolus vulgaris* with the optimization of the method for the phytochemical analysis. Furthermore, the outcome of metabolomics analysis is a 'fingerprint' that provides information on the primary and secondary metabolites contained in legumes when they are consumed as food. Seeds of commercial beans have been extracted following a specific procedure: the extraction was performed in CH_2Cl_2/H_2O/MeOH 2:1:1 obtaining a hydrophilic extract and a lipophilic extract [2]. Both extracts, once separated, centrifuged, dried and lyophilized, have been analyzed through 1D and 2D-NMR experiments carrying out a qualitative analysis. 1D ^{1}H-NMR spectra of hydrophilic extract have been compared through an untargeted analysis with the PCA technique which allows to compare the varieties of the plant. A targeted approach has been applied on the 1D ^{1}H-NMR spectra of hydrophilic extract performing a quantitative analysis by qNMR with NMRProcFlow software. Lipophilic extract has been also analyzed with Mass Spectrometry by LC-MS/MS with the goal to identify fatty acids that cannot be well discriminated by NMR due to the overlapping of many similar signals.

Fig.1: workflow of the project

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POSTER SESSION 2

ST1 BIOACTIVE NATURAL PRODUCTS AND DRUG DISCOVERY

PO61 – PO97

TWO UNREPORTED ONNAMIDE ANALOGS FROM THE MARINE SPONGE *THEONELLA CONICA*

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Since the first report of the marine natural product spongothymidine in 1950,[1] there has been a dedicated effort by researchers to uncover new compounds from marine invertebrates, and as the results, more than 34,000 new metabolites have been reported to date.[2] Among them, onnamides or theopederins form a group of compounds that share the core skeleton derived from polyketide and nonribosomal peptide biosynthetic pathways. This class of compounds exhibit unique structures and demonstrate remarkable cytotoxicity against cancer cell lines. In this study, the isolation, structure elucidation and biological activity of two previously unreported onnamide analogs, 2*Z*- and 6*Z*-onnamides A (Figure 1) will be introduced.[3] Effects on histone modifications in relating to the cytotoxicity by analogs will be also discussed.

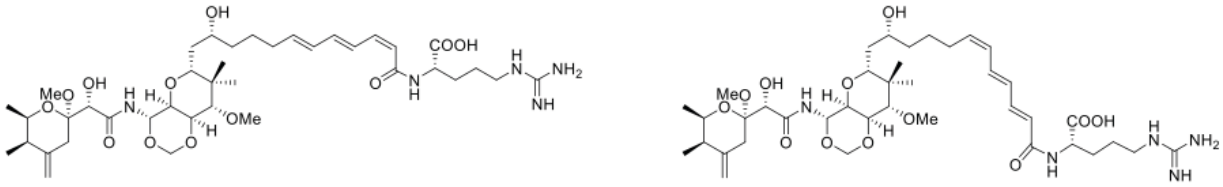

Figure 1. Structures of 2*Z*- and 6*Z*-onnamides A.

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SYNTHETIC STUDY OF HEDOAMIDE, A NEW LINEAR PEPTIDE FROM MARINE CYANOBACTERIA

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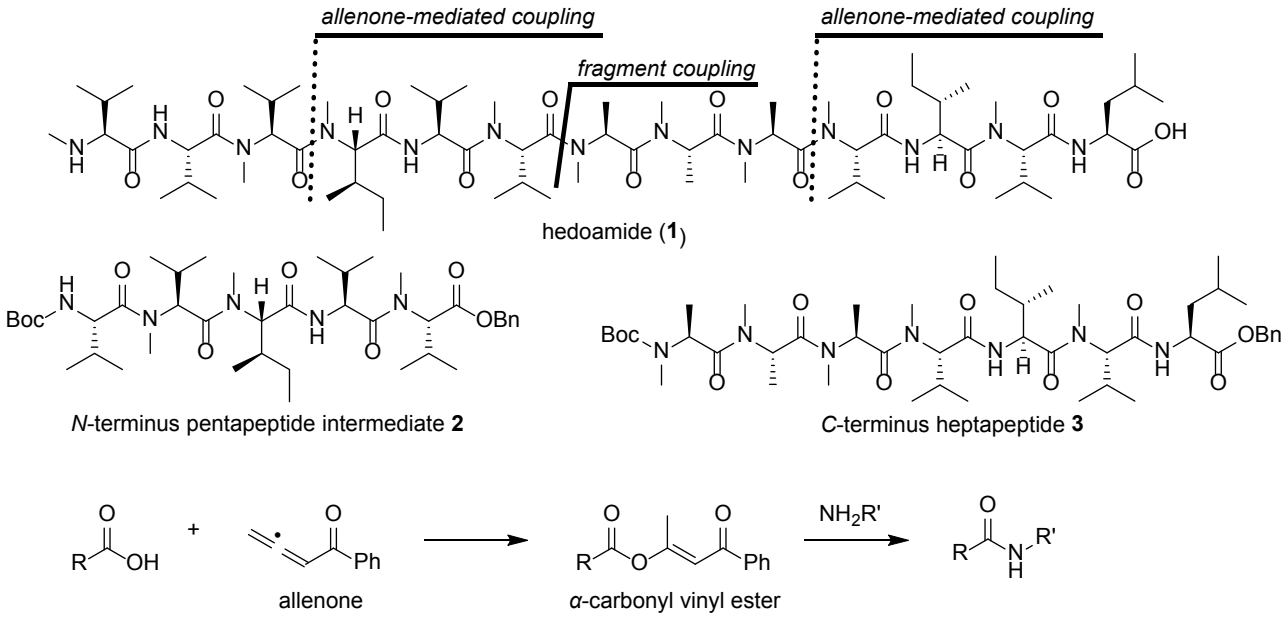
A novel antiparasitic drug is strongly desired due to the continuous appealed compounds with a novel pharmacophore and mode of action, we have investigated antiparasitic natural products from marine cyanobacteria collected in Okinawa. Hedoamide (**1**), a novel antiparasitic compound isolated from an unidentified marine cyanobacterium in our group. Hedoamide (**1**) was an extremely *N*-methylated tridecapeptide composed entirely of aliphatic amino acids including *N*-Me-Val, *N*-Me-Ile, and *N*-Me-Ala. To confirm the proposed structure and evaluate further biological activity, we have carried out the synthetic study of hedoamide (**1**).

Toward a total synthesis of **1**, we planned a fragment coupling reaction between *C*-terminus heptapeptide and *N*-terminus hexapeptide. Each fragment was elongated with HATU. However, because of the poor reactivity of *N*-methylated amine, we could access only *N*-terminus pentapeptide intermediate **2** and *C*-terminus heptapeptide **3**. The yields were low, only 1% each, thus requiring a highly convergent strategy. Hedoamide (**1**) is divided into four fragments, and they can be connected by allenone-mediated fragment coupling[1]. We reached every tri- and tetrapeptide fragment and coupling conditions will be investigated.



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IDENTIFICATION AND CHARACTERIZATION OF MOLECULAR TARGET OF LABDANE DITERPEN MANOOL

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Antimicrobial resistance is a global public health threat that creates the need for new sources of active compounds for the treatment of pathogenic microorganisms. Plants have developed numerous defense mechanisms such as barriers and antimicrobial specialized metabolites. This powerful defense mechanism suggests focusing on specialize metabolites, to search for antimicrobial molecules[1]. Extracts, essential oils and bioactive molecules from *Salvia officinalis* L. (Lamiacee) were reported to exert several biological activities including antibacterial, antifungal, anti-inflammatory, anticancer and antioxidant[2]. Recent studies[3] by our research group confirmed the antibacterial properties of *S. officinalis* extracts and components. In particular, the labdane diterpene manool was tested against various bacterial strains, both Gram+ and Gram-, and the most promising activity was obtained on *Streptococcus mutans*, a Gram+ bacterium responsible for dental caries in humans. However, the mechanisms underlying this antibacterial effect have still to be elucidated. The aim of this project was the identification of molecular target of manool in *S. mutans* by proteomic approaches and the target(s) validation. Firstly, expression proteomic studies in *S. mutans* treated or not with manool were carried out to define the pathways involved its bioactivity. Drug Affinity Responsive Target Stability (DARTS) coupled with mass spectrometry was used as a quick and reliable approach to identify potential cellular targets of manool. This approach allowed the identification of manool interacting proteins belonging to the ATP-binding cassette (ABC) superfamily, phosphotransferase system (PTS) and ATP synthase family. These proteins are mainly responsible for sugar transporting across the inner bacterial membrane, allowing biofilm formation. This is accomplished in parallel with phosphorylation of the sugar, which prevents efflux of the sugar back across the membrane. This process is a key part of an extensive signaling network that allows bacteria to efficiently utilize preferred carbohydrate sources. Preliminary study on the effect of manool on biofilm formation, in presence of different sugars, by *S. mutans* was investigated using the microplate crystal violet stain retention method. Our results confirmed the ability of manool to interact with *S. mutants* proteins playing different roles in proliferation and survival, suggesting a possible use of this compound to develop new antibacterial drugs.

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INTERACTIONS BETWEEN OLIGONUCLEOTIDE SECONDARY STRUCTURES AND PORPHYRINS

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Oligonucleotide analogues (ODNs) are biomolecules with great scientific potential due to their remarkable properties (higher bioavailability, target affinity, stability and resistance to nuclease degradation). They can therefore be used as nanoprobes and biosensors, but also for the development of new materials in the field of nanotechnology1. ODNs and their analogues can adopt several secondary structures that play an important role in biology, biotechnology and nanotechnology2. In particular, ODNs rich in guanine can adopt secondary structures called G- quadruplexes, which are also very interesting from a diagnostic and therapeutic point of view3. In the human genome, G-quadruplexes are found in regions of genes of high regulatory importance (such as enhancers, promoters and oncogenes). Stabilisation and destabilisation of these secondary structures have been shown to influence the onset of disease. There is a field of research investigating the interactions between small molecules and DNA secondary structures, including G-quadruplexes. In this paper we focus on the interactions between G-quadruplexes and modified porphyrins, a class of macrocyclic compounds that play a very important role in the metabolism of living organisms4.

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THE ANTICANCER POTENTIAL OF SPHINGOID LIPIDS ISOLATED FROM THE MARINE SPONGE *HALICLONA VANSOESTI*

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The discovery of novel anticancer compounds from marine organisms is continuously growing over the years [1]. Marine sponges represent one of the most promising sources of bioactive molecules [2].
In the framework of the project "Antitumor drugs and vaccines of the sea (ADViSE)", we analyzed the anticancer potential of the marine Demospongiae *Haliclona vansoesti*, an alien species for the Mediterranean Sea, previously collected in the Faro lake (Strait of Messina, Sicily). Applying a bioassay-guided screening platform [3], we demonstrated that an enriched solid phase extraction (SPE) fraction of this sponge induced cell death in human melanoma cells. Through a second step of orthogonal SPE fractionation, we identified two active subfraction mainly enriched of sphingoid based lipids that were characterized by Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS). In detail, we described and characterized the antitumor activity of glycosphingolipids and sphingosines, which are the main component of the two active subfractions, providing the first evaluation of the anticancer potential of polar lipids isolated from the marine sponge *H. vansoesti*.

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JA-ILE-LACTONE ENHANCED ALKALOID BIOSYNTHESIS IN TOMATO

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Plants use carbon dioxide to produce valuable secondary metabolites such as pharmaceuticals and materials. For example, paclitaxel from *Taxus cuspidata* is used as an anti-cancer drug, and isoprene from the rubber tree *Hevea brasiliensis* is used as a resource for natural rubber. These valuable secondary metabolite productions are enhanced by the plant hormone (+)-7-iso-Jasmonoyl-L-isoleucine (JA-Ile) that functions as a protein-protein interaction (PPI) inducer between F-box protein COI1 and transcription repressor JAZ[1]. However, JA-Ile-induced upregulation of secondary metabolite production also causes significant growth inhibition and thus is impractical. A new insight into JA-Ile-induced secondary metabolite production is keenly desired to overcome the current issue.

Figure. JA-Ile causes secondary metabolites production and growth inhibition.
JILa causes alkaloid production wituout growth inhibition.

We found that a cyclized JA-Ile (JA-Ile-lactone, JILa)[2,3] induces accumulations of tomatine, a steroidal glycoalkaloid, without causing growth inhibition in *Solanum lycopersicum*. On the other hand, JILa-induced accumulation of tomatine was not observed in COI1-impaired mutants. This result suggests that JILa activates tomatine biosynthesis in a COI1-dependent manner. We also found that JILa enhanced the expression of tomatine biosynthetic genes without affecting the expression of JA signaling marker genes, suggesting that JILa selectively activated tomatine biosynthesis without affecting canonical JA signaling.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF C-30 MODIFIED LUPANE TRITERPENOIDS

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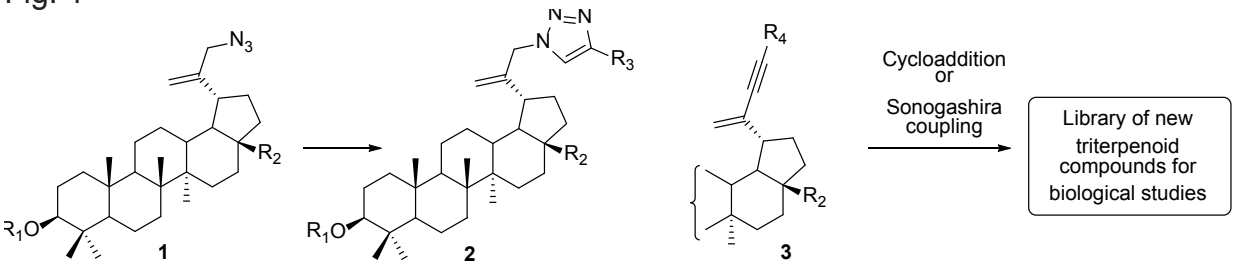
Pentacyclic triterpenoids are natural compounds obtainable from numerous plants. Many of these compounds have significant biological activities.[1] In our research group we focus on synthesis of selectively cytotoxic[1] or neuroprotective[2] compounds.

In the past an article was published on the transformations of azide **1** to selectively cytotoxic triterpenoids containing a substituted triazole **2** with heterocycle moiety at the position C-30.[3,4] However, the synthetic approach towards series of compounds **2** was rather complicated and provided very low yields. The main goal of the presented work was to explore new synthetic approach towards analogous C-30 modified triterpenoids and to discover new options for the synthesis of various conjugates. Precursor alkyne **3** was synthesized in high yields over 5 steps, which allowed preparation of large library of 27 new triterpenoid conjugates via cycloaddition reaction or Sonogashira coupling. Some of these final derivatives showed moderate cytotoxic or neuroprotective activity. Detailed synthetic procedures and IC_{50} values will be presented.

Fig. 1



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ACTIVE FRACTIONS ISOLATED OF *PHYSALIS* GENUS WITH PROTECTOR EFFECT AGAINST OXIDATIVE STRESS INDUCED IN MACROPHAGES

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In Colombian folk medicine, *Physalis* genus (Solanaceae) is recognized as a food supplier and a prolific source of new bioactive compounds that confer numerous medicinal properties. Some genus plants exhibit the presence of a calyx, an organ that protects the fruit. This particular organ is used to prepare teas to treat different diseases because the content of chemical compounds such as tropine alkaloids, withanolides, and physalins are the major groups of chemical compounds recognized by the anti-inflammatory, anti-tumor, cytotoxic, anti-microbial, hepatoprotective and immunomodulatory activities. All these biological activities are related to noncommunicable diseases and oxidative stress. Our research group obtained two active fractions, enriched in sucrose esters, from calyces of two physalis species (*P. peruviana* and *P. angulata*) with potent anti-inflammatory activity. The objective of this work was to evaluate the in vitro activity of two active fractions isolated of the calyces of two physalis species against oxidative stress induced in macrophages RAW 264.7 using a quantitative method for the evaluation of oxidative stress in cells, known as dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay. P. peruviana fraction (PPF) was extracted with methanol, and *P. angulata* fraction was extracted with dichloromethane (PAF). The results showed the potent activity of PAF to inhibit the ROS produced by LPS stimulation. This effect supports the traditional use of these plants and indicates the potential of calyces as an alternative treatment for a variety of pathologies that course with inflammation. PAF can be considered a source of substances that can neutralize the ROS generated in pathological states such as NCDs. Therefore, this important finding would drive the identification of the bioactive components present in this fraction.

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THE EFFICACY AND SAFETY OF SIPJEONDAEBO-TANG FOR CANCER PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Objective: This study was conducted to systematically analyze the therapeutic efficacy and safety of Shipjeondaebo-tang in cancer patients.

Methods: By August 29, 2022, a systematic review and meta-analysis were conducted through a screening process after searching for randomized controlled clinical studies in which Sipjeondaebo-tang was applied to adult cancer patients in 9 domestic and foreign databases. All the results reported in each individual study were summarized and meta-analysis was performed for studies with the same evaluation index, and qualitative analysis was performed for studies with different evaluation indexes. To evaluate the quality of individual studies included in this study, the risk assessment tool of bias in the Cochrane library was used.

Results: 430 studies were retrieved from 9 databases. A total of 37 studies were finally included according to the selection and exclusion criteria. Among them, 30 studies were included in the meta-analysis. After surgery, Sipjeondaebo-tang significantly improved ALB, PALB, TP as a nutritional index, RBC, Hb, TRF as a hematological index, and immune index in cancer patients after surgery. Sipjeondaebo-tang did not show any significant improvement effect on AMC, TSF, and wound drainage as nutritional index of cancer patients after surgery. Sipjeondaebo-tang showed significant improvement in WBC, Hb reduction, vomiting, KPS, CD4+, CD8+, CD4+/CD8+ during chemotherapy in cancer patients. Sipjeondaebo-tang did not show any significant improvement effect on RECIST, PLT, abdominal pain, TFG-beta1, and CD3+ during chemotherapy for cancer patients. Sipjeondaebo-tang significantly improved CD3+, CD4+/CD8+ in cancer patients who were unable to undergo surgery, chemotherapy, and radiation therapy. Sipjeondaebo-tang did not show any significant improvement effect on CD4+ and CD8+ in cancer patients who were unable to undergo surgery, chemotherapy, and radiation therapy. Sipjeondaebo-tang significantly improved the fatigue of cancer patients, and there were no serious side effects from Sipjeondaebo-tang.

Conclusion: Sipjeondaebo-tang significantly improved the nutritional and immune status of cancer patients. In addition, Sipjeondaebo-tang relieved cancer symptoms or side effects caused by chemotherapy, and there was no difference in safety compared to the control group. However, the quality of the individual studies included in this study is relatively low, and a well-designed large-scale RCT study is needed in the future.

DISCOVERY OF NEW ANTIPARASITIC DRUG CANDIDATES FROM MICROBIAL NATURAL PRODUCTS

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Leishmaniasis and American trypanosomiasis (Chagas disease) are neglected tropical diseases (NTDs), caused by the parasites *Leishmania* spp. and *Trypanosoma cruzi*, respectively, that lead on a yearly basis to thousands of deaths worldwide and lately are also emerging as a health concern in developed countries. New therapeutic solutions are required due to increasing resistance and adverse effects of existing treatments[1,2]. Natural products (NPs) are unique sources of chemical diversity and historically they have proved to be a rich reservoir of new bioactive compounds, with many drugs originated from NPs used today in clinical practice[3].

With the aim of discovering new NP scaffolds with novel mechanism of action (MoA) against *Leishmania* and *T. cruzi* and the development of a new parasite painting approach to characterize the MoA of candidate hits, a high content imaging screen (HCS) against intracellular *L. donovani* and *T. cruzi* has been run with 40K representative microbial extracts from MEDINA's NP collections. Active hits were submitted to LC/MS dereplication and the most promising ones prioritized to perform medium scale cultivations and activity confirmation, followed by selection of the best candidates for bioassay-guided isolation of the active molecules and structural elucidation using HPLC/MS and NMR. Non-cytotoxic compounds have been profiled in an innovative HCS parasite painting assay, to be developed in parallel, to propose potential MoA for the selected compounds.

From the 40K microbial extracts screened on HCS intracellular parasite models of *T. cruzi* and *Leishmania*, we have identified 983 (2.3%) and 270 (0.6%) active hits against *T. cruzi* and *Leishmania* respectively. A total of 16 and 12 pure bioactive compounds against *T. cruzi* and *L. donovani*, respectively, have been isolated so far. Among them, 9 and 7 active compounds against *T. cruzi* and *L. donovani,* respectively, were selected for further MoA and ADME studies.

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ANALYSIS OF VOLATILES OF *STEVIA OVATA*, *STEVIA SUAVEOLENS* AND *STEVIA TRIFLORA* BY HS-SPME-GC-MS

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Stevia is a genus of herbs and shrubs from the American continent that belongs to the Asteraceae family. The phytochemical studies include the presence of sesquiterpenoids and compounds from the guaiane, longipinene and germacrene groups [1]. The essential oil of *Stevia serrata* from Guatemala has shown antinociceptive and anti-inflammatory activities [2,3]. Thus, the objective of the research was to locate populations of other *Stevia* species in Guatemala to characterize the volatile composition and establish the presence of metabolites with possible biological activity.

Populations of *Stevia ovata* Wild, *Stevia suaveolens* Lag. and *Stevia triflora* DC. were located in departments of western Guatemala, at altitudes above 2000 m, associated with pine-oak forests. Aerial parts of the three species were collected between October and November 2022, then dried and extracted by headspace solid phase microextraction (HS-SPME) using a 75 μm carboxen/polydimethylsiloxane fiber (SUPELCO) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS) using a HP5 (5% phenylmethylsilicone) column.

Thus, *S. ovata* presented (*E*)-caryophyllene (27.9%), (*E*)-nerolidol (10.7%), β-humulene (8.2%), germacrene D (5.8%) while *S. triflora* presented (*E*)-caryophyllene (27.8%), linalool (9.1%), (*E*)-nerolidol (8.1%) and β-humulene (8.1%), as their main volatiles. On the other hand, *S. suaveolens* presented chamazulene (19.8%), (*E*)-caryophyllene (9.1%) and (2Z, 6Z)-farnesol (7.1%) as main volatiles. Chamazulene is considered as possible responsible of the antinociceptive activity of *S. serrata* [2], thus the composition and pharmacological activity of the essential oil of *S. suaveolens* should be investigated. Further studies regarding biological activity of the essential oils of the three species are suggested to evaluate uses as potential raw material for therapeutic products.

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HIGHLY SELECTIVE BUTYRYLCHOLINESTERASE INHIBITORS STRUCTURALLY INSPIRED BY AMARYLLIDACEAE ALKALOIDS - DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION

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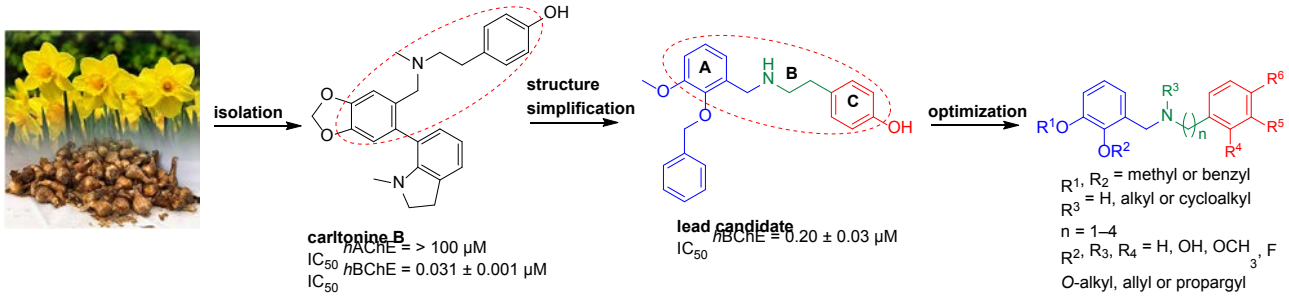
Butyrylcholinesterase (BChE) is one of the enzymes involved in the pathophysiology and progression of a devastating neurodegenerative disease affecting millions of people worldwide, namely Alzheimer's disease (AD). In the later stages of AD, the dominant levels of acetylcholinesterase are significantly reduced by more than 90%, while the level of BChE gradually increases up to 165% of the normal level, indicating a compensatory role [1]. In our efforts to develop novel drug candidates for AD, we focused on natural template structures, specifically the Amaryllidaceae alkaloids carltonine A and B, with high BChE selectivity. In this study, we designed, synthesized, and *in vitro* evaluated compounds with *h*BChE inhibitory potential ranging from micromolar to low nanomolar scale. For compounds with inhibition below 100 nM, CNS penetration was theoretically verified by calculating the BBB score algorithm and confirmed with the *in vitro* use of the PAMPA-assay. The safety profile was verified in human neuroblastoma and hepatocellular carcinoma cell lines. The study spotlight compounds **87** (*h*BChE IC_{50} = 3.8 ± 0.2 nM) and **88** (*h*BChE IC_{50} = 5.7 ± 1.5 nM) as the top-level BChE inhibitors. A crystallographic study was carried out to reveal the binding mode of the most potent inhibitor **87**, revealing essential interactions between **87** and the active site of *h*BChE [2].



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NEW PYRROLO[2,3-c]PYRIDINES: DESIGN, SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY EVALUATION

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Fused pyridine and pyrimidine derivatives constitute an interesting medicinal chemistry scaffold, since their structural resemble to purines results in their involvement in crucial biological processes. Numerous purine or purine-like compounds have already reported as cytotoxic agents, through a variety of investigated mechanisms. As part of a program aiming to discover novel derivatives with potential cytotoxic activity, our research group has previously synthesized a number of nitrogen containing heterocyclic compounds, that exhibited promising in vitro and/or in vivo anticancer activity. In this work, we have designed and synthesized a number of new, suitably substituted pyrrolo[2,3-c]pyridines, using as lead compound a hit, recently identified by our group. For the synthesis of the target derivatives 2-amino-3-nitro-4-methylpyridine was used as the starting material, which was initially converted to the key intermediate 7-chloropyrrolo[2,3-c] pyridine, followed by the introduction of appropriate substituents to this scaffold. The new derivatives were subsequently evaluated for their potential to inhibit the proliferation of human origin cancer cell lines. The evaluation of the cytotoxicity results revealed interesting SARs since certain compounds possessed strong antiproliferative activity, that could assist to the design of the next generation of derivatives. Interestingly, the new compounds proved to be more effective against the A431 cancer cell line, which expresses abnormally high levels of the epidermal growth factor receptor (EGFR) and contains no functional p53, a potent tumor suppressor gene.

THE BIOSYNTHESIS OF *DAPHNIPHYLLUM* ALKALOIDS

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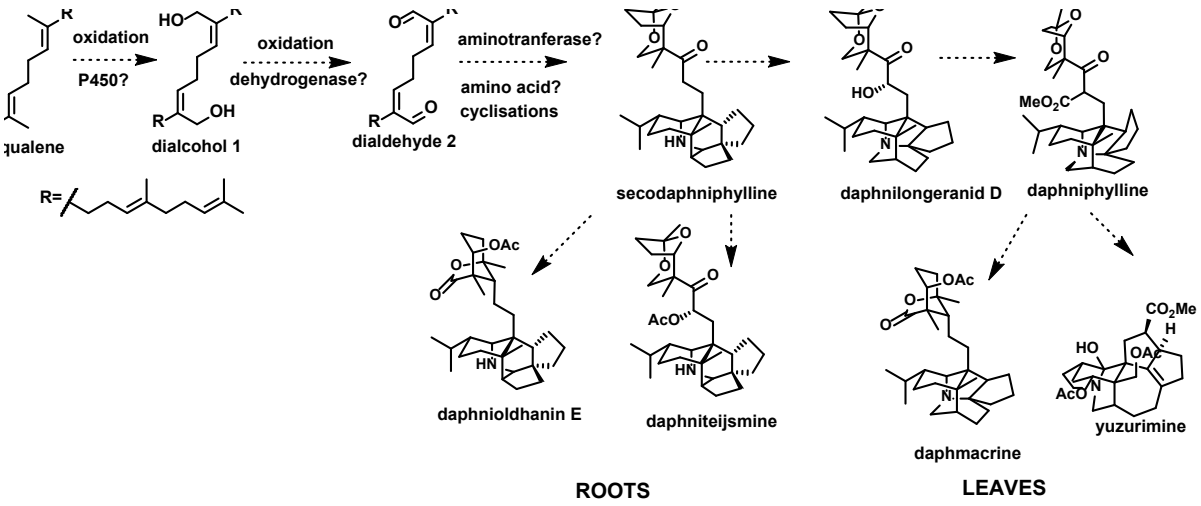
Plants from the genus *Daphniphyllum* have been used in Traditional Chinese Medicines to treat a range of ailments. The medicinal properties of this plant are largely due to its variety of complex polycyclic alkaloids that when isolated have exhibited a range of bioactivities that include anticancer, insecticidal and vasorelaxant properties[1]. Currently, the biosynthesis of *Daphniphyllum* alkaloids is unknown. The elucidation of the biosynthetic pathway will aid our understanding of the bioactivities of these compounds and allow for the development of novel enzymatic routes towards complex bioactive compounds. Prior studies have shown that *Daphniphyllum* alkaloids are likely derived from the isoprenoid squalene[2] that undergoes series of oxidations, introduction of a nitrogen and cyclisation to form secodaphniphylline that is then diversified into other *Daphniphyllum* alkaloids. We have used a variety of methods to investigate the biosynthetic pathway, including synthetic chemistry, metabolomics, LC/GCMS and alkaloid isolation. Results indicate that the early steps of the biosynthetic pathway occur in the roots and leaves starting with secodaphniphylline which is diversified differently in each tissue. This progress represents key first steps towards understanding and harnessing this highly complex biochemical system.



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MODE OF ACTION AND STRUCTURE-ACTIVITY RELATIONSHIPS OF THE ONCOLOGY DRUG TIGILANOL TIGLATE AND RELATED ANALOGUES

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Background: Tigilanol Tiglate (TT) is a naturally occuring epoxytigliane under development for the local treatment of solid tumours via intratumoural injection. Isolated from an Australian rainforest plant (*Fontainea picrosperma*), TT is a protein kinase C (PKC)/C1 domain activator that disrupts tumour vasculature and causes direct oncolysis of tumour cells. Together, these activities lead to haemorrhagic necrosis of injected tumours with rapid and enduring ablation of >70% of target tumours in both preclinical syngeneic mouse models and cutaneous tumours presenting in the veterinary clinic. TT is registered as a veterinary oncology pharmaceutical in Europe, the UK, the US and Australia. The drug has also completed safety trials in humans with strong evidence of local efficacy and signs of abscopal effects in some patients and is currently undergoing Phase II trials in head and neck cancer and soft tissue sarcoma. However, the underlying mode of action (MOA) of TT and its immunotherapeutic potential is not fully understood.

Results: At therapeutic concentrations, TT induces oncosis/pyroptosis in cancer and endothelial cells via a pathway involving caspase activation and cleavage of pore forming protein gasdermin E. TT enacts this by binding to endoplasmic reticulum (ER) membranes, causing an ER stress response that results in loss of mitochondrial membrane potential, ATP depletion, organelle swelling and oncosis/pryoptosis. This TT-directed cell death results in release of damage associated molecular patterns (DAMPs), indicative of an immunogenic cell death (ICD) pathway and, in the murine CT-26 tumour model, TT promotes the development of tumour-specific T cells. Whilst the induction of ICD is largely PKC-independent *in vitro*, PKC/C1 domain signaling appears necessary for efficacious tumour ablation *in vivo*. Consequently, we are assessing analogues of TT to identify and unravel the structural components necessary for the induction of oncosis/pyroptosis, the promotion of ICD and how these changes affect PKC/C1 domain activation and *in vivo* anti-tumour efficacy.

Conclusions: These data demonstrate that TT acts as an oncolytic small molecule with potentially broader immunotherapeutic effects in the treatment of cancer.

(3*R*,7*S*)-12-HYDROXY-JASMONOYL-L-ISOLEUCINE IS THE GENUINE BIOACTIVE STEREOISOMER OF A JASMONATE METABOLITE IN *ARABIDOPSIS THALIANA*

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The plant hormone (3*R*,7*S*)-jasmonoyl-L-isoleucine (JA-Ile) is widely known as a plant defense hormone against pathogens and chewing insects. The metabolism of JA-Ile into 12-OH-JA-Ile and 12-COOH-JA-Ile is the central mechanism for inactivating JA signaling. Recently, 12-OH-JA-Ile was reported to function as a ligand for the JA-Ile co-receptor COI1-JAZ. However, in previous studies, the "12-OH-JA-Ile" was a mixture of stereoisomers.[1, 2] Thus, the genuine bioactive form of 12-OH-JA-Ile has not yet been identified. In this study, we prepared pure stereoisomers of 12-OH-JA-Ile and identified (3*R*,7*S*)-12-OH-JA-Ile as the naturally occurring bioactive form.[3] We also revealed that the unnatural trans-isomer (3*S*,7*S*)-12-OH-JA-Ile functions as another bioactive isomer. Furthermore, the pure (3*R*,7*S*)-12-OH-JA-Ile causes partial activation of JA-signaling without affecting the negative feedback regulation of JA-signaling. Thus, (3*R*,7*S*)-12-OH-JA-Ile could cause weak and sustainable expression of certain JA-induced gene expressions until the catabolism of (3*R*,7*S*)-12-OH-JA-Ile into (3*R*,7*S*)-12-COOH-JA-Ile occurs. Our current results demonstrated that (3*R*,7*S*)-12-OH-JA-Ile is the genuine bioactive form of 12-OH-JA-Ile, and a metabolite of plant hormone plays a unique role *in planta*.

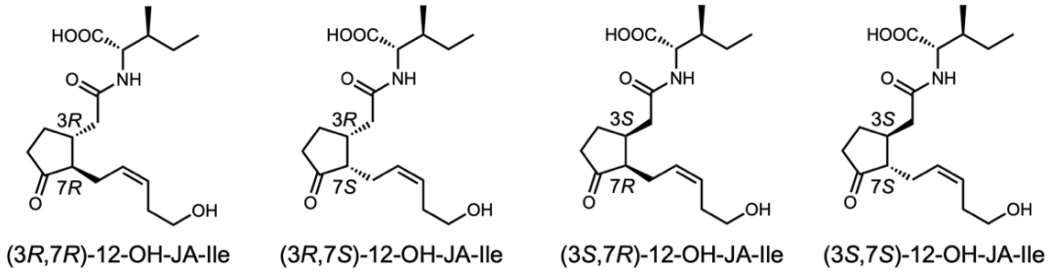

Figure. Chemical structure of stereoisomer of 12-OH-JA-Ile.

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TOTAL SYNTHESIS OF BISELYNGBYASIDE ANALOG

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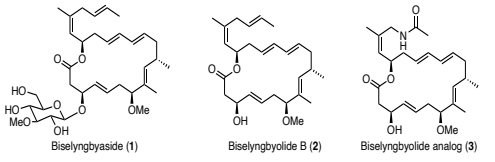
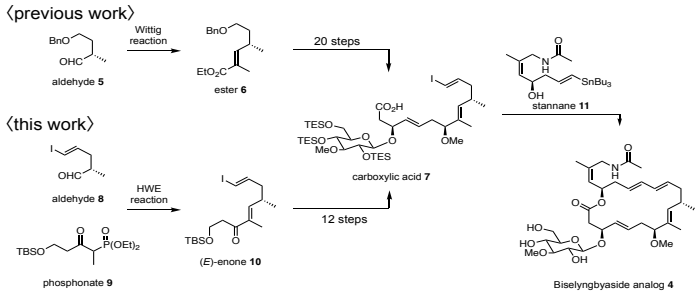
Biselyngbyaside (BLS, **1** an 18-membered macrolide glycoside) and its aglycone, biselyngbyolide B (**2**) were isolated from marine cyanobacteria collected at Okinawa, Japan by our laboratory [1]. We achieved the total synthesis of **1**[2], **2**[3], and artificial analog (**3**)[4] and reported the antimalarial activity and cytotoxicity of these compounds (Figure 1). Based on the above results, the methyl glucose and hydrophilicity of the side chain might affect the bioactivity. Thus, to exert selective antimalarial activity, we designed a new artificial analog **4** and worked on the total synthesis improving the synthetic route to the BLS scaffold.



compound	Growth-inhibitory activities IC_{50} (μM)			
	Human cells		Antimalarial activity	
	HeLa	MRC-5	K-1	FCR-3
Biselyngbyaside (**1**)	2.5	0.4	3.5	4.4
Biselyngbyolide B (**2**)	0.0028	0.23	24	23.5
Biselyngbyolide analog (**3**)	4.9	3	18	14



As shown in scheme 1, the synthetic route to the key intermediate carboxylic acid **7** was improved. The aldehyde **8** was synthesized from (*R*)-Roche ester in 8 steps and tried the Horner-Wadsworth-Emmons (HWE) reaction with phosphonate **9**. The desired product of the HWE reaction was initially obtained in low yield, so we investigated the conditions of this reaction. As a result, Hight selectivity (*E*/*Z*=7.3:1) and better yields (66%) for the *E*-enone **10** could be obtained using LiHMDS and toluene. The enone was then converted to **7**, reducing eight steps compared to the previous synthetic route. Next, an 18-membered ring was constructed by esterification of **7** and known vinyl stannane **11**[4] followed by an intramolecular Stille coupling reaction. Finally, three TES groups were removed to give biselyngbyaside analog **4**.

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ENDEMIC PLANT *RUMEX BALCANICUS*: PROMISING SOURCE OF POLYPHENOLS WITH ANTIOXIDANT, HYPOGLYCEMIC AND DEPIGMENTATION ACTIVITIES

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The species belonging to the *Rumex* L. genus (Polygonaceae), distributed throughout the world, have exhibited numerous health-promoting activities, such as antioxidant, anti-inflammatory, antidiabetic, neuroprotective, antimicrobial, and due to these attributes are used in traditional medicine for the treatment of diarrhoea, constipation, mild diabetes, as well as neurological, skin and liver disorders. However, there is almost no data on the chemical composition and pharmacological activities of *Rumex balcanicus* Rech. fil., an endemic species of the Balkan peninsula. In this context, dry hydromethanolic extracts of *R. balcanicus* fruit (RBF), root (RBR) and leaf (RBL), collected in Serbia, were analyzed regarding the polyphenolic profile, and tested for their *in vitro* antioxidant, α-amylase, α-glucosidase, acetyl-cholinesterase and tyrosinase inhibitory activities. The maximum total phenolic content, determined by the Folin-Ciocalteu method, was found in RBF (386.57 mg GAE/g). The conducted HPLC analysis of the investigated extracts revealed the presence of flavanols, flavonols, procyanidins, phenolic acids, anthranoides and naphtalene derivatives. The RBF was characterized by high amounts of miquelianin (28.84 mg/g) and procyanidin B_1 (28.11 mg/g), along with catechin (20.27 mg/g), rutin (8.15 mg/g) and epicatechin gallate (5.95 mg/g). The RBR was the richest in nepodin (54.06 mg/g), procyanidin B_2 (40,59 mg/g) and epicatechin gallate (24.46 mg/g), while quercitrine (18.38 mg/g), miquelianin (15.04 mg/g) and rutin (8.07 mg/g) were major compounds in RBL. The RBF exhibited significant antioxidant activity, evaluated by DPPH (IC_{50}: 4.86 μg/mL), ABTS (IC_{50}: 0.75 μg/mL) and FRAP (5.89 mmol Fe2+/g) assays. All extracts, particularly RBF, showed stronger α-glucosidase inhibitory activity (IC_{50}: 1.82-5.51 μg/mL) compared to acarbose (IC_{50}: 156.64 μg/mL). Moreover, RBF showed notable anti-tyrosinase activity (IC_{50}: 14.57 μg/mL), and moderate potency for inhibiting α-amylase and acetyl-cholinesterase. These results suggest that RBF could be the promising active substance of herbal products with antioxidant, hypoglycemic and depigmentation activities intended for treatment of the mild form of diabetes or skin pigmentation diseases.

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ENVIROMENTAL-FRIENDLY PROTOCOLS APPLIED TO THE DEVELOPMENT OF DUAL INHIBITORS AS NOVEL ANTICANCER AGENTS

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Until now, drug discovery focused on designing highly selective ligands for single biological targets. However, the “one molecule, one target” strategy is evolving, and the identification of novel molecules that can modulate multiple targets simultaneously, such as dual inhibitors and also natural products, holds promise as a therapeutic approach.[1] Thus, our objective is to synthesize new computationally designed chemical entities that target key epigenetic markers like Bromodomain-containing protein 9 (BRD9), a component of the SWI/SNF chromatin-remodeling complex, and c-Myc, a transcription factor. In fact, recent evidence highlighted that c-Myc expression is influenced by BRD9 activity, as transcript levels of c-Myc decrease after treatment with I-BRD9, a potent and selective BRD9 inhibitor. In this work, we present the rational design and synthesis of potential dual inhibitors using the molecular hybridization approach.[2]
These selected binders have been synthesized via effective and modern synthetic strategies that allow rapid access to the desired compound, prioritizing a green approach and minimizing safety and environmental impact.[3] Particular attention was given to the choice of solvents for the reaction steps because they represent the principal source of waste, carbon footprint, and energy consumption. Therefore, we preferred solvent-free processes or less hazardous and lower toxic solvents. When possible, we also switched from the classic thermal heating method to the microwave-assisted one, which is more efficient and cleaner.[4, 5]
Through environmental-friendly protocols we developed rapidly and sustainably new chemical entities as potential dual inhibitors for cancer treatment.

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TARGETING LIF/LIFR AXIS FOR THE TREATMENT OF PANCREATIC ADENOCARCINOMA

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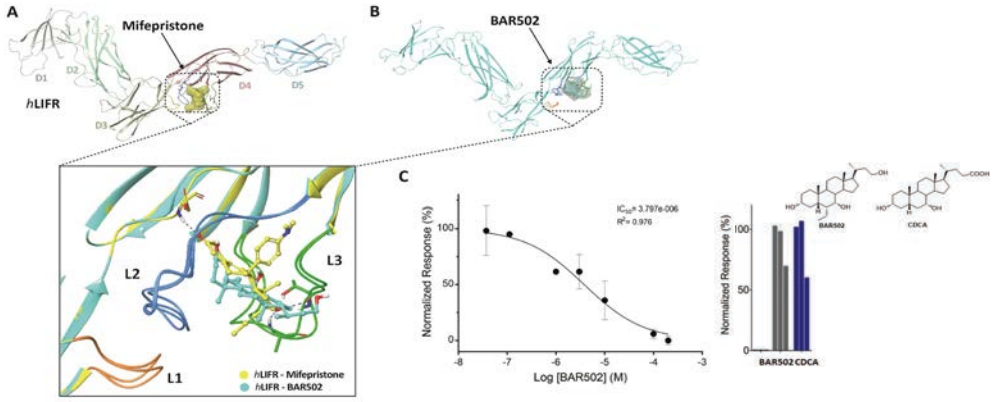
Pancreatic cancer is a growing cause of cancer mortality, with a projection of becoming the second-most common cause of cancer mortality in the next decade.[1]
A potential therapeutic candidate is the leukemia inhibitory factor (LIF), a cytokine belonging to IL-6 family, upregulated in pancreatic ductal adenocarcinomas (PDAC). LIF mediates its signalling using membrane receptor complex that is comprised of its receptor (LIFR) and glycoprotein 130 (gp130), leading to oncogenic signalling pathways activation including JAK/STAT3.[2]
LIF/LIFR axis is implicated in tumour growth and progression by acting on multiple aspects of cancer biology. We developed an *in silico* strategy and drug repositioning to identify potential LIFR antagonists. In this communication, we report the preliminary studies, allowed us the identification of steroidal-like agents such as mifepristone [3] and in-house library based on both natural and synthetic bile acids, such as BAR502,[4] regulating the LIF/LIFR pathway in relevant clinical settings such as LIF overexpressing-PDAC.

Figure 1. In-house steroidal compounds screened against LIFR



A-B) Best IFD docking poses in the *h*LIFR putative binding pocket. In the zoom-view, the superposition of both *h*LIFR-Mifepristone (yellow) and *h*LIFR-BAR502 (cyan) complexes. The three loops L1, L2 and L3 are highlighted in orange, blue, and green, respectively. **C**) Alphascreen analysis of BAR502 and CDCA and the inhibition curve of the interaction between LIF and LIFR due to increasing concentrations of BAR502

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NMR-GUIDED STUDY OF *LYSIMACHIA ATROPURPUREA*

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Lysimachia atropurpurea L. (Primulaceae) is an understudied species of Greek flora in terms of phytochemistry. Particularly, there is only one study in which five flavonoids were isolated from this species[1]. In our present work, the ethanol (95%) extract of *L. atropurpurea* was studied by 1D- and 2D-NMR techniques, which guided the whole analytical process. Up to now, eight chemical compounds were isolated and categorized mainly into triterpenoid saponins, and flavonoids. Among them, myrsinoside B and rutin were major chemical constituents of the extract. The ^{1}H-NMR spectrum of the crude extract unveiled the characteristic proton NMR pattern derived from myrsinoside B. In order to show the identification of this compound from the crude extract and the obtained fraction, we compared, herein, their ^{1}H-NMR spectra (Figure 1). Regarding triterpenoid saponins, previously undescribed chemical compounds that belong to the oleane-type triterpene saponin were isolated and identified for the first time.

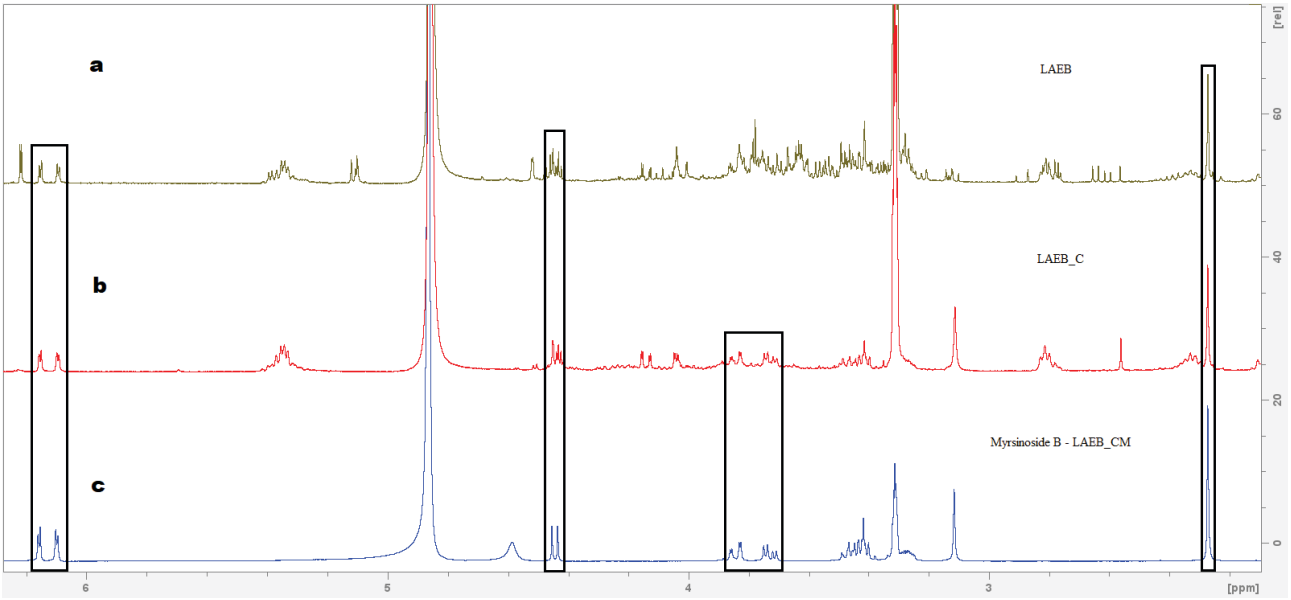

Figure 1: Overlaid ^{1}H-NMR spectra of the *L. atropurpurea* crude extract (a), fraction (b), and the isolated myrsinoside B (c).

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PHYTOCHEMICAL ANALYSIS OF *SIDERITIS RAESERI* SUBSP. *RAESERI* INFUSIONS

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Sideritis raeseri Boiss. & Heldr. subsp. *raeseri* (Lamiaceae) is a Balkan endemic that belongs to section *Empedoclia* (Rafin.) Bentham. of subgenus *Sideritis*[1]. Previous studies mentioned the presence of flavonoids, phenylethanoid glycosides, and phenolic acids in its extracts[2-5]. In continuation of our studies on *Sideritis* infusions[6,7], the main aim of the present study was to investigate the chemical composition of *S. raeseri* subsp. *raeseri* infusions from cultivated and wild populations by means of NMR, LC-DAD, LC-ESIHRMS, and GC-MS techniques, aiming to reveal potential differences. Preliminary ^{1}H-NMR analysis of infusions showed almost similar chemical profiles, including mainly carbohydrates (middle region: 5.50 and 3.10 ppm), flavonoids, and phenylethanoid glycosides (downfield region 8.00-6.20 ppm). The identification has been performed by the analysis of the spectra in comparison with data of reference compounds isolated in our previous studies. The polar samples were further analysed by LC-ESIHRMS in order to get a better insight into their chemical compounds, revealing the same constituents with slight differences in the amounts. For GC-MS analysis of the infusions, silylated derivatives were produced by using derivatisation reagent. Carbohydrates were found to be main constituents in both infusions. Caffeic acid was found in traces only in the cultivated sample, while quinic and chlorogenic acids were detected in both samples. Although the *Sideritis* samples originated from wild and cultivated populations, similar metabolite profiling was observed in the infusions.

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OPTIMAL CONDITIONS FOR ANTHOCYANINS DETERMINATION IN NATURAL PRODUCTS

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Anthocyanins are natural pigments found in fruits, vegetables and flowers and are responsible for their characteristic bright red, blue or purple color. They have been studied extensively and have been proven to possess beneficial effects for the plant, humans, and animals. However, their determination has many difficulties as there are more than 550 different anthocyanins in nature. Hydrolysis of Anthocyanins and the quantification of their aglycon forms (Cyanidin, Delphinidin, Malvidin, Peonidin, Petunidin and Pelargonidin) is a fast and efficient methodology for their determination in natural products. Literature describes different conditions for the acidic hydrolysis of anthocyanins extracted from several food matrices. In most of them the hydrolysis is performed in extract with hydrochloric acid.[1] The aim of this work is to investigate the stability of Anthocyanins of selected fruits and vegetables in different drying conditions and to find a rapid method for the hydrolysis of Anthocyanins to Anthocyanidins. For this purpose, ten different fruits and vegetables that are rich in Anthocyanins were collected and dried: naturally in a well-ventilated dark place, with the freeze-drying technique and in a laboratory oven at 45 oC. Subsequently, the dried raw materials were extracted and then the optimal hydrolysis conditions of Anthocyanins in the extracts were investigated. In particular, the effect of the nature of the acid, the temperature, and the time of the hydrolysis reaction on the Anthocyanidins content in the extracts was studied. Afterwards, the optimal hydrolysis conditions were applied directly to the dried raw materials and the levels of Anthocyanins were compared with those obtained from the hydrolysis of their extracts. Results of this study showed that the direct hydrolysis of Anthocyanins in freeze-dried fruits and vegetables with sulfuric acid, assisted by heating at 90 oC for 1 hour, and then HPLC-DAD quantification of their aglycon forms is an appropriate methodology for the determination of Anthocyanins in natural products. Overall, this is the first time that an analytical methodology for determination of Anthocyanins in natural products is described, where extraction and hydrolysis procedures are performed in one step.

Acknowledgments
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EXTRACTION AND EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF TWO ALGERIAN SPECIES: *ROSMARINUS OFFICINALIS L.* AND *THYMUS VULGARIS L.*

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Introduction: The exploration and development of essential oils in response to the increase in healthcare-associated infections (HAIs), which are known to be multi-resistant to the usual anti-infectives, appears to be a promising solution. Hence the interest of this study, which involved extracting EOs from two species of Lamiaceae: *Rosmarinus officinalis L*. and *Thymus vulgaris L*., and assessing their antimicrobial activities in vitro.

Materiel and medthods : The species *Rosmarinus officinalis* L. was harvested in the region of Aïn El Hammam at Tizi ouzou and the species *Thymus vulgaris L.* was harvested in the region of Ouled Chiha at Guelma, both located in Algeria. The antimicrobial activity was tested on the microbial strains incriminated in HAIs. This included the aromatogram, determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC).

Results and discussion: The average yield obtained by extracting EO from the dried aerial parts of *Rosmarinus officinalis L.* and *Thymus vulgaris L*. was 1,2% and 2% respectively. The aromatogram results show that the antimicrobial power of these EOs on the strains tested is significant, with inhibition zone diameters ranging from 22 mm to over 60 mm. Determination of MICs showed a strong inhibitory power of *Rosmarinus* officinalis L. EO on Enterococcus *faecalis, Acinetobacter baumanii*, *Staphylococcus aureus* 25, *Staphylococcus aureus* 43 and *Klebsiella pneumoniae* (MIC = 1.42 mg/ml for the first three strains and MIC = 2.84 mg/ml for the last two), bactericidal on *Pseudomonas aeruginosa* and bacteriostatic on the other strains. Strong inhibitory power of *Thymus vulgaris* L. EO on all strains (MoIC = 1.61 mg/ml).

Conclusion: The EO of *Thymus vulgaris* has a bactericidal activity against gram-negative and gram-positive bacteria at low concentrations. *Rosmarinus officinalis* showed a strong bactericidal potential on *Pseudomonas aeruginosa* and bacteriostatic on the IAS tested.

Key words: *Rosmarinus officinalis L., Thymus vu*lgaris L., essential oil, antimicrobial activity.

A HOLISTIC IN VITRO APPROACH OF ANTIOXIDANT AND GENO-PROTECTIVE ACTIVITY OF AN EXTRACT ORIGINATED FROM DIRECT HYDROLYSIS OF OLIVE LEAVES

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Nowadays, olive leaf polyphenols have been at the center of scientific interest due to their beneficial effects on human health. The most abundant polyphenol in olive leaves is oleuropein (OLE). It is comprised of three different components, namely hydroxytyrosol (HT), Elenolic acid and Glucose. The biological properties of OLE are mainly due to HT moiety, a drastic catechol group whose biological activity has been mentioned many times in the literature. In 2012, the European Food Safety Authority (EFSA), recognizing the beneficial effect of HT on human health, issued an opinion in favour of a specific health claim, described in EU Regulation 432/2012. So, in recent years many nutritional supplements, food products and cosmetics enriched in HT, have been developed and marketed by pharmaceutical, food and cosmetic industries, with unexpected positive results. However, the concentration of HT in olive leaves depends on several factors, such as olive tree variety, cultivation practices, and is also corelated with the activity of endogenous enzymes (polyphenol oxidase, peroxidase, β-glucosidase, and esterase). Since HT derived from OLE, the most abundant polyphenol in olive leaves, it is obvious that this by-product could be an ideal raw material for the production of HT rich extracts.

The main goal of the present study was to investigate, holistically, the antioxidant and geno-protective effects of an HT-enriched extract originated from olive leaves of Greek Olea europaea cultivars. Even though, there are studies which have mentioned the *in vitro* antioxidant activity of olive leaves extracts, less are known for hydrolysed extracts, as well as their bioactivity. In this study, we explore the antioxidant activity of a Hydrolysed Olive Leave Extract (HOLE) using cell-free and cell-based methods, as well as its ability to protect from H_2O_2-induced DNA damage. Moreover, in this study a rapid and easy methodology for HT-enriched extracts production from olive leaves, was also described. The proposed method is based on the direct acidic hydrolysis of olive leaves, where the extraction procedure and the hydrolysis of OLE is carried out in one step. To our knowledge, this is the first time that a one-step procedure is applied in olive leaves to produce HT-enriched extracts.

Acknowledgments
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ISOLATION, STRUCTURE DETERMINATION, BIOLOGICAL ACTIVITY AND SYNTHETIC STUDY ON TERUKUFAZOLINES FROM AN UNDESCRIBED MARINE CYANOBACTERIUM

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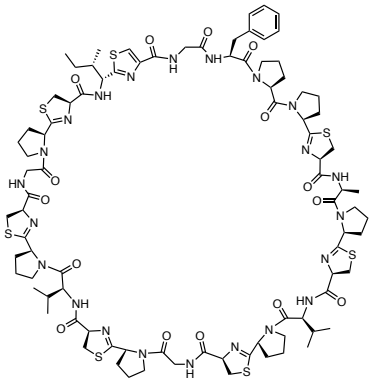
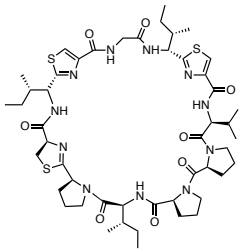
Marine cyanobacteria possess various biosynthetic genes encoding a variety of secondary metabolites, making them a valuable source for natural product discovery. We have previously reported that rare marine cyanobacteria inhabiting isolated islands tend to produce novel natural products at high rates[1,2]. In this study, we isolated novel cyclic peptides Terukufazolines A (**1**) and B (**2**) from undescribed marine cyanobacterium collected on an isolated island, Aguni Island, Okinawa Prefecture, Japan.



Terukufazoline A (**1**)



Terukufazoline B (**2**)

From ^{1}H NMR spectra, **1** was found to have many rotamers. 2D NMR spectra revealed the constituent amino acids, but determining their sequence was difficult due to overlapping signals. Next, we attempted tandem MS analysis and partial hydrolysis of **1**, but the amino acid sequence could not be unambiguously determined by these methods. Comparing the structure of terukufazolines and known natural products, it was inferred that terukufazolines are classified as cyanobactin biosynthesized by the RiPPs pathway. Therefore, we carried out a metagenomic analysis of the cryopreserved cyanobacterial sample and successfully revealed a biosynthetic gene cluster of terukufazolines along with their amino acid sequences. The positions of cysteine-derived thiazolines and thiazoles were clarified based on analysis of NMR spectra and tandem mass spectra.
Next, we determined the absolute configuration of terukufazolines. Epimerization of amino acids adjacent to the *N*-terminal of thiazoline or thiazole was suppressed by ozone degradation before acid hydrolysis. To determine the absolute configuration of six thiazolines of **1**, we hydrolyzed the thiazolines with deuterium chloride to distinguish the epimers formed during hydrolysis by deuterium labeling. Finally, the resulting amino acids were derivatized with a highly sensitive MS labeling reagent[3] and subjected to LC-MS analysis, which revealed that the cysteines constituting thiazoline are all L-forms. Terukufazolines showed selective toxicity against *Trypanosoma brucei rhodesiense*. We are currently working on the total synthesis of **1** to confirm its structure.

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SELECTIVE FUNCTIONALIZATION OF METHYLENE CARBONS BY *ASPERGILLUS NIGER* FURNISHES STEREOSELECTIVE HYDROXYLATED DERIVATIVES RELATED TO ANTI-TUMOR METABOLITES

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Stevia rebaudiana is a plant used as a sweetener, and reported to possess antitumor activity[1]. Isosteviol, a *S. rebaudiana* metabolite, is a talented compound with cardioprotective effect and antiproliferative activity against MCF-7 breast and human gastrointestinal cancer cell lines[2]. Antiproliferative activity of diterpenoids increase with the introduction of hydroxyl groups in their chemical structures. However, regio- and stereoselective hydroxylation of C-H bond functionalization reactions is challenging. In this work, isosteviol was obtained from hydrolyses followed by a Wagner-Meerwein rearrangement of Stevia power and fed to wild strains of the filamentous fungi *Aspergillus niger*, *Beauveria bassiana*, and *Syncephalastrum racemosum* aiming at introducing hydroxyl groups in strategic positions of isosteviol skeleton. Small scale experiments were followed by ^{1}H NMR showing that only *A. niger* produced hydroxylated derivatives. Biomass of this species was accumulated in 30 flasks containing potato dextrose broth (PDB) (200 mL/flask, 130 rpm; 25 °C, 4 days) prior to feeding isosteviol (50 μg mL^{-1}). After further ten days, broth was extracted with ethyl acetate. Chromatographic separation in silica gel allowed the isolation of three products. ^{1}H and ^{13}C NMR as well as HSQC, HSQC-TOCSY, HMBC, and NOESY NMR experiments allowed their identification as 1*α*-hydroxy, 16-oxobeyeran-18-oic acid, 7*β*-hydroxy,16-oxobeyeran-18-oic acid, and 1*α*,7*β*-dihydroxy,16-oxobeyeran-18-oic acid. Three-dimensional models suggested that the stereoselective hydroxylation of positions 1 and 7 may result from the alternately interaction of isosteviol molecule with the alpha and the beta faces with the enzymatic site responsible for the introduction of hydroxyl groups at C1*α* and C7*β*. The compounds obtained have structural similarities with cytotoxic isosteviol derivatives, and were assayed using WI-26 VA4 (non-tumor human lung fibroblasts; control) and RKO-AS45-1 (colon carcinoma) cell lines. None of the compounds were cytotoxic against WI-26 VA4 cell lines. The 1*α*-hydroxy derivative was the most cytotoxic compound against RKO-AS45-1 cells (IC_{50} 205.5 μg mL^{-1}) being a readily available and selective promising antitumor agent[3] (FAPEMIG, CNPq).

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MONOMERIC AND DIMERIC GERMACRANOLIDES FROM *ARTEMISIA ATROVIRENS*

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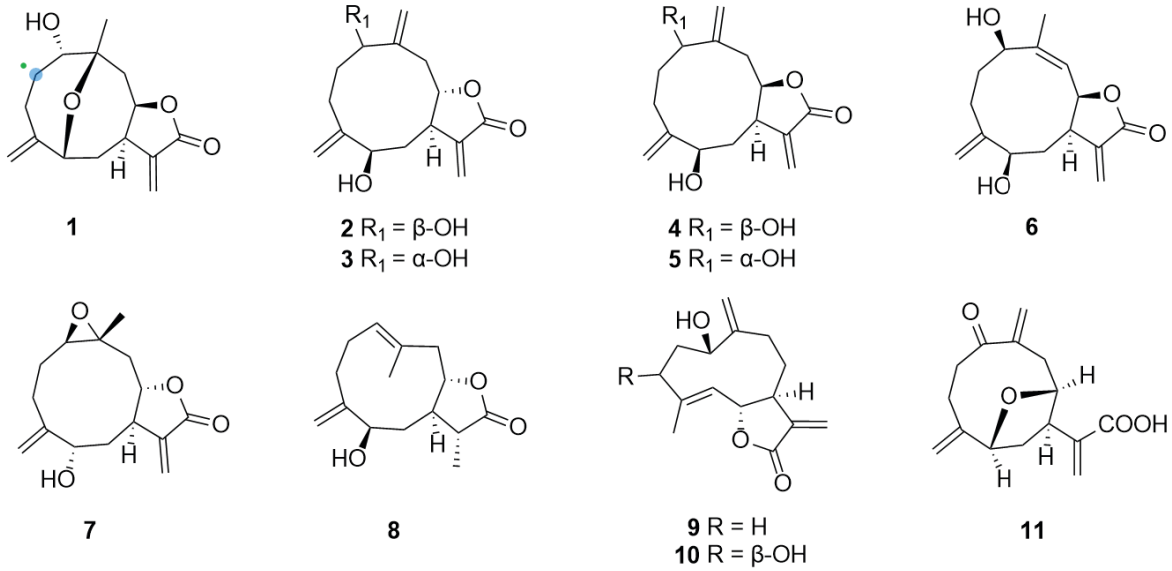
Sesquiterpenoids are a significant class of secondary metabolites present in various plants belonging to the Asteraceae family. This group encompasses several types of sesquiterpenoids, comprising germacranolides, eudesmanolides, and guaianolides, among others.

Artemisia atrovirens Hand.-Mazz., a perennial herbaceous plant, primarily grows on slopes, grasslands, and roadsides in southern China. In recent studies, researchers have continuously reported the presence of monomeric, dimeric, and trimeric guaianolides in this plant.[1-3] Our previous investigations reported the discovery of guaianolides, *seco*-guaianolides, and their dimers.[4,5] In addition to these compounds, germacranolides and the seldom-reported dimeric forms of germacranolides were identified from *A. atrovirens* in this study. The structures of these compounds were fully established through the comprehensive analysis of MS, IR, 1D- and 2D-NMR spectroscopic data, together with X-ray crystal diffraction, density functional theory (DFT) NMR calculation, and time-dependent DFT electronic circular dichroism (TDDFT ECD) calculation. Some compounds showed moderate inhibitory activity against HL-60 and A549 cell lines.



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COMPUTATIONAL STUDY OF THE INHIBITION OF PCSK9 FOR THE REDUCTION OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

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Over the years, cholesterol has earned a reputation as a high-risk factor for cardiovascular diseases. This is because the high concentrations of cholesterol in the blood may result in a build-up of plaque in the arteries that supply oxygen to the heart. Cholesterol build up in the arteries interferes with blood flow resulting in atherosclerotic cardiovascular diseases or a heart attack. Cholesterol is obtained by the body from dietary constituents, and many cells can synthesize their own endogenous cholesterol. Cholesterol in the blood is transported by lipoproteins (an assembly of proteins and lipids). Lipoproteins are classified based on their density, lipoproteins that consists of more lipids and less proteins are classified as low density lipoprotein (LDL), and lipoproteins that consists of less lipids and more proteins are classified as high density lipoproteins (HDL)[1]. This is because lipids have low density than proteins. LDL transport cholesterol to tissue cells, whereas HDL transports cholesterol from the tissue to the liver. Cholesterol in the liver may be used for a number of processes such as production of bile. The entry of cholesterol into the liver cells is facilitated by the LDL receptors (LDLR) on the liver cell membrane. PCSK9 protein binds to LDLR. When LDL binds to the PCSK9 bound LDLR, the complex is internalized into the lysosome where it is destroyed and LDLR is not recycled to the liver cell membrane and this will result in excess LDL cholesterol in the blood leading to atherosclerosis[2]. Two PCSK9 inhibitors (alirocumab and evolocumab) were approved by the US Food and Drug Administration and European Medicines Agency. This study uses computational methods to elucidate the inhibitory pathways of alirocumab and evolocumab and search for cheaper drugs with better activity than evolocumab and alirocumab that target PCSK9. This study involves protein-protein docking and protein-ligand docking and subsequent molecular dynamics of the complexes.

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ENGINEERING NATURAL PRODUCTS BY CHEM-BIO HYBRID SYNTHESIS FOR DRUG DISCOVERY OF DYSENTERY AMOEBIASIS

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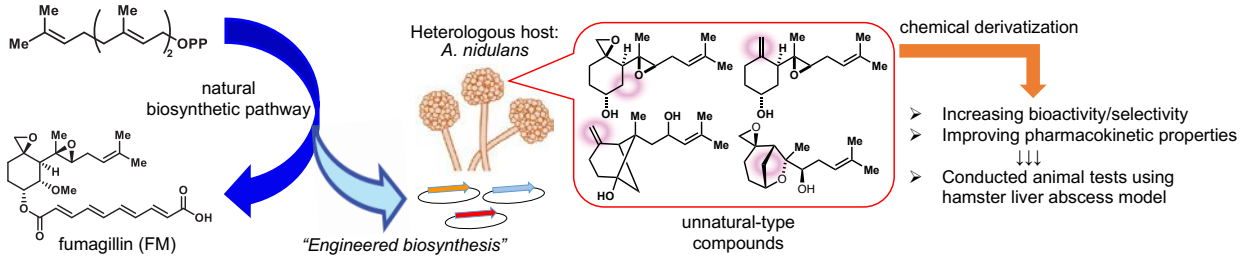
Dysentery amebiasis, caused by *Entamoeba histolytica*, affects 1% of the global population. The current treatment, metronidazole, has concerns such as potential genotoxicity and the emergence of drug-resistant mutants. Therefore, developing a new drug with a novel mode-of-action remains imperative. Our primary screening rediscovered that fumagillin (FM), a fungal secondary metabolite from *Aspergillus fumigatus*, suppressed the proliferation of amoeba in vitro and rescued amebic liver abscesses in hamsters. In the past decades, efforts to develop FM-based drugs through semi-synthesis have been conducted to treat various diseases, including cancer, diabetes, and protozoan infections. However, FMs have yet received approval due to their potential to cause adverse effects, such as neurotoxicity and thrombosis.



To address the issue, we conceived a strategy incorporating engineered biosynthesis into chemical diversification. We expressed the biosynthetic genes for FM and its analog ovalicin in a heterologous host, *Aspergillus nidulans*. This genes-to-molecules system allowed us to design and create unnatural-type natural products, one of which showed potent activity (compound **1**, ED_{50} 6.7 nM). Of note, the production titer of **1** increased to 40 mg/L through optimizing the fermentation condition, enabling us to obtain **1** with a gram scale. However, orally administered **1** was not sufficient for curing the hamster model. We disclosed that the drug-metabolizing enzymes in the hamster liver degraded the compound, making it inadequate in vivo. Thus, the structure of **1** was further modified through synthetic derivatization to improve its pharmacokinetic property. We developed a promising compound, YOK24, which exhibited metabolic resistance and demonstrated efficacy against amoebiasis in animal tests.

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ISOLATION AND STRUCTURE DETERMINATION OF AKUNOLIDES, NEW MACROLIDE GLYCOSIDES FROM A MARINE CYANOBACTERIUM

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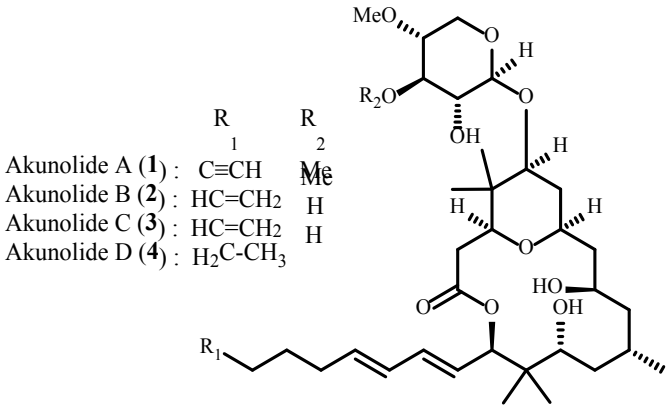
As a result of a series of investigations on secondary metabolites of marine cyanobacteria collected in Okinawa, new macrolide glycosides akunolides A-D (**1**-**4**) were isolated from a marine *Okeania* sp. cyanobacterium.

These planar structures were elucidated by spectroscopic analyses. The relative configuration of akunolide A (**1**) was revealed based on a detailed analysis of ^{1}H coupling constants and NOESY correlations. In addition, the relative configuration of akunolide B (**2**) was determined to compare the ^{1}H chemical shifts of the reduced derivatives to reduced **1**. The absolute configuration of akunolide B (**2**) was determined by modified Mosher's method[1]. The absolute configuration of akunolide A (**1**) was subsequently determined by comparing the specific rotation of the reduced derivative. Akunolide A (**1**) showed an antitrypanosomal activity against *Trypanosoma brucei rhodesiense* with 10-fold selectivity compared to normal fibroblast WI-38 cells. On that day, we are going to explain the details of the structure determinations of akunolide C (**3**) and D (**4**).



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ACCESS TO CHEMICAL DIVERSITY AND ANTIFUNGAL COMPOUNDS USING CO-CULTIVATION APPROACH

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The co-cultivation of phytopathogenic fungi of edible fruits in rice medium has shown to be a promising strategy in the search for compounds with chemical diversity and antifungal properties. This approach allows studying the interaction between fungi and fruits, as well as the production of secondary metabolites by fungi under specific cultivation conditions.[1,2]

Fruits such as papaya and pineapple have a high level of proteases that participate in the plant's defence system against pathogens. However, some fungi are capable of infecting them, suggesting that they produce protease inhibitors. Thus, in this work, microorganisms that infect these fruits were investigated through their isolation and cultivation in rice medium and co-cultivation to access different biosynthetic routes and obtain new bioactive metabolites from these interactions.

By combining the co-cultivation of phytopathogenic fungi of edible fruits in rice medium with ^{1}H NMR analysis, it was possible to identify compounds with antifungal properties and potential application in the protection of fruits against diseases caused by fungi. In this context, the co-cultivation of *Fusarium guttiforme* and *Fusarium proliferatum*, responsible for causing fusariosis in pineapples, was carried out in rice medium and also the co-cultivation of *F. guttiforme* with *Phytophthora palmivora*, a papaya infecting fungus.

So far, we have found that the axenic culture extract of *F. guttiforme* showed antifungal activity against the phytopathogenic fungus *Colletotrichum horii*. When cultivating this fungus with *P. palmivora*, the extract showed an higher antifungal activity against *C. horii*. A fraction the ethyl acetate extract of the axenic culture of *F. proliferatum* showed an antifungal activity against the fungi *F. guttiforme*, *Pestalotiopsis diospyri* and *C. horii*, respectively. When co-cultivating with *F. guttiforme* there was an increase in the antifungal activity against *P. diospyri* and *C. horii* fungi. We have also found that *F. proliferatum* produces high amounts of beauvericin in both axenic and in cocultivation conditions. These results are an evidence of potential of co-cultivation to access compounds that are not produced in the axenic culture.

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COMPARATIVE CHEMICAL ANALYSIS OF PEEL, PULP AND CALLUS EXTRACTS OF TWO APPLE (*MALUS DOMESTICA*) VARIETIES

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Malus domestica Borkh. (Rosaceae) comprises different varieties of commercially widespread apple around the world and available on the market all year round [1]. Apples are a rich source of bioactive compounds such as polyphenols and triterpenes present both in pulp and peel. The daily consumption of apples has been linked to the prevention of several diseases such as cancer, cardiovascular diseases, asthma, and type-2 diabetes [2]. Today, cell plant culture technology may be considered a source of valuable compounds [3] as, in some cases, bioactive compounds are higher in plant cell culture extracts than *in vivo* [4]. In the course of our continuing studies on apple callus culture, a comparative phytochemical analysis between peel, pulp and callus extracts of two apple varieties, “Annurca” and “Mela Rosa Marchigiana del Montefeltro”, two examples of local apple varieties from central-northern and southern Italy, respectively, was carried out. The *in vitro* callus culture was obtained starting from explants of the ripe pulp of the two fruits considered. To recover the metabolites, 500 mg of each sample were subjected to ultrasound-assisted extraction with 10 mL of EtOH-H_2O (80% v/v). The metabolomic analysis of the obtained extracts was carried out by ultra-high performance liquid chromatography coupled to high resolution electrospray ionization source-Orbitrap/mass spectrometry (UHPLC-HR-ESI-Orbitrap/MS). The findings of this study revealed slight differences in the chemical composition of the two apple varieties. Furthermore, the qualitative profile of peels and pulps was almost superimposable, while differences were observed in the callus extracts. In particular, pulps were rich in phenols including phlorizin, catechin and procyanidins; peels contained both phenols and triterpenic acids while, according to previous studies [5], callus extracts were characterized only by highly produced triterpenic acids, some of which were not found in the fruits (such as pomolic and tormentic acids). In conclusion, this study sheds light on how cell plant culture can be considered as an alternative system for producing secondary metabolites.

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THE NON-ALKALOID EXTRACTS OF *EPHEDRA SINICA* INHIBIT MUC5AC EXPRESSION AND CELL MIGRATION IN LUNG CANCER A549 CELLS

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Lung cancer is one of the leading causes of death globally, and metastasis is responsible for most lung cancer mortality. MUC5AC is a heavily glycosylated, secreted polymeric mucin protein produced by respiratory epithelial cells in the airways. Studies have demonstrated that MUC5AC is overexpressed in lung cancer tissues and plays a significant role in tumor metastasis and chemoresistance, indicating its potential as a therapeutic target for lung cancer treatment. *Ephedra sinica* is a traditional Chinese medicine that has been used to treat pulmonary diseases. The bronchodilator activity of *E. sinica* comes from ephedra alkaloids, but they also have adverse cardiovascular effects and dietary supplements containing Ephedra have been banned by FDA. This study aims to investigate the effects of *E. sinica* on MUC5AC expression and cell migration in lung cancer A549 cells, and to identify the active fractions and compounds. Our results show the ethanolic extract of *E. sinica* at non-cytotoxic concentrations (10 and 20 μg/ml) inhibited cell migration and MUC5AC expression in A549 cells, while ephedra alkaloids were ineffective. We thus used acid-base extraction to obtain a non-alkaloid fraction, which preserved the inhibitory effects on MUC5AC and cell migration. By using bioassay-guided fractionation, the active sub-fraction and the active compounds within it were isolated and identified. These results suggest a potential use of non-alkaloid extracts of *E. sinica* for prevention of MUC5AC-realated cancer metastasis.

NOVEL NAPHTHYLISOQUINOLINE ALKALOIDS FROM *ANCISTROCLADUS TECTORIUS* AND THEIR POTENTIAL INHIBITION ON NAV1.7 SODIUM CHANNEL

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Naphthylisoquinoline alkaloids (NIQs) are a class of structurally, biosynthetically, and pharmacologically remarkable natural products only derived from two phylogenetically related paleotropical families [11, 12]. *Ancistrocladus tectorius*, the only native species of the *Ancistrocladus* genus in China, has been widely used as a traditional folk medicine of the Li nationality for treating malaria, dysentery, and other infectious diseases [13-15]. Systematic investigation resulted in the isolation and identification of 31 NIQs by a comprehensive analysis of HRESIMS, 1D, and 2D NMR spectra as well as ECD spectra and single-crystal X-ray diffraction analysis with Cu *K*α radiation, which includes 12 new compounds bearing 6 different linkage types between the naphthyl and isoquinoline half. By summarizing the proton chemical shifts and coupling constants of the isolated NIQs with different linkage types, a easy and quick way to determine the linkage types and the relative configuration of C-1 and C-3 was achieved. In addition, all the NIQs showed inhibitory activities against the Nav1.7 channel stably expressed in HEK293 cells, and compound **2** was the most potent with an IC50 of 0.73 ± 0.03 μM. In acutely isolated dorsal root ganglion (DRG) neurons, compound 2 at 10 μM dramatically suppressed native sodium currents and action potential firing. In the formalin-induced mouse inflammatory pain model, local intraplantar administration of compound **2** (2, 20, 200 nmol) dose-dependently attenuated the nociceptive behaviors. In summary, NIQs represent a new type of Nav1.7 channel inhibitors and may act as structural templates for the following analgesic drug development.

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MACROLIDES WITH POTENT ACTIN-MICROFILAMENT DISRUPTION ACTIVITIES FROM RED SEA SPONGES

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Actin is a tubular and filamentous protein that composes versatile dynamic polymers, which can define cell polarity, control cell shape, organize cytoplasmic organelles, promote stable adhesions of cell–cell and cell–matrix, and produce protrusive forces needed for migration [1, 2, 3]. In cancer cells, these functions usually fail and become abnormal [1, 2, 3]. Latrunculin A was the first marine macrolide known to contain a 16-membered ring and the unique 2-thiazolidinone moiety linked together via a tetrahydropyran (THP) ring [4, 5, 6]. Latrunculins A and B and their derivatives possess important biological properties including antiproliferative, antiangiogenic, antimicrobial, and antimetastatic effects [7, 8]. In a continuation of our interest to explore bioactive marine-derived chemical entities from Red Sea sponges with biomedical importance, bioassay-guided fractionations of the organic extracts of the Red Sea sponges *Negombata magnifica* and *N. coticata* were performed. Chromatographic partition of the sponges' extracts and final HPLC purifications of active fractions led to the isolation of three new macrolides, latrunculins U-W, along with latrunculin A, B and 16-*epi*-latrunculin B. Structural determinations the compounds were confirmed by interpretation of their 1D and 2D NMR spectra and high-resolution MS spectra. The compounds displayed vairable actin-microfilament disruption activities. The structure-Activity-Relationship (SAR) of the compounds will be discussed and presented.

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ANTIOXIDANT CAPACITY OF WILD THYME EXTRACT-LOADED LIPOSOMES

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Wild thyme (*Thymus serpyllum* L., Lamiaceae) contains bioactive compounds that show antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, analgesic, and spasmolytic effects. However, the application of its bioactive components is limited due to their low stability, solubility, and consequently bioavailability. The encapsulation, as a process of entrapping bioactive compounds into particles, represents an appropriate way to overcome the mentioned disadvantages. Furthermore, the addition of sterols in a phospholipid mixture during liposomal preparation can result in liposomal particles with satisfied physicochemical characteristics. Hence, wild thyme extract-loaded liposomes were developed in the present study, and their antioxidant capacity was investigated. Liposomes were prepared using proliposome method and pure phospholipids (Ph) or the mixture of phospholipids and two different sterols (20 mol % of cholesterol or β-sitosterol). According to the results of ABTS and DPPH assays, pure wild thyme extract neutralized 74.0±1.3% of ABTS radicals and 57.7±0.1% of DPPH radicals. Additionally, the antioxidant activity of plain liposomes (without extract), which probably originates from an antioxidant compound added to the raw phospholipid mixture was 11.3-15.9% of ABTS neutralization and 9.89-12.7% of DPPH neutralization. All liposomal populations with extract have shown similar antioxidant potential towards free ABTS radicals. Namely, Ph, Ph+cholesterol, and Ph+β-sitosterol liposomes with extract neutralized 64.8±1.2, 64.7±1.0, and 65.9±0.6% of ABTS radicals, respectively. However, DPPH radical scavenging potential was statistically significantly lower (56.5±2.3, 48.1±2.8, and 47.8±2.2%). The addition of sterols significantly decreased the DPPH antioxidant capacity of the extract-loaded liposomes. Considering that the two used antioxidant tests are based on different principles and reactions, the obtained results may be different and provide good insight into the overall antioxidant activity of wild thyme extract-loaded liposomes. Due to shown antioxidant capacity of the liposomes with encapsulated bioactive compounds from wild thyme extract, they can be used in various cosmetic and pharmaceutical products for skin application.

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POSTER SESSION 2

ST4 INNOVATIVE AND ECO-FRIENDLY MATERIALS

PO98 – PO104

NANOCELLULOSE-BASED GAS BARRIER FILMS FOR SUSTAINABLE FOOD PACKAGING

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Food packaging protects food from biochemical and mechanical damage. Transparent films are commonly used as outmost sealing materials due to their clear visibility of the contents. However, these films require oxygen and moisture barrier properties to prevent oxidation and decay. Currently, aluminium foil and halogenated polymers are the most commonly used materials for oxygen and moisture protection, but they have drawbacks such as opacity and hazardous gas generation after use.
This work presents the development of gas barrier films made from eco-friendly and biodegradable materials for sustainable food packaging. The films are composed of a poly(lactic acid) (PLA) substrate coated with nanocellulose and poly(vinyl alcohol) (PVA) for gas barrier protection. The surface of the nanocellulose was modified to enhance the gas barrier properties by crosslinking with PVA. The presentation will cover the gas barrier properties, biodegradability, cytotoxicity, and biomass content of the developed sustainable food packaging film.

OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION FROM WALNUT HARD SHELLS: EVALUATION OF ANTIOXIDANT ACTIVITY AND PREPARATION OF ECOVIO® FILMS LOADED WITH NANOHYBRIDS CONSISTING OF HALLOYSITE NANOTUBES

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Agri-food wastes with low economic value are rich in bioactive compounds, which can be recovered and used for several potential industrial and nutraceutical applications[1].
A Box–Behnken design has been used to optimize the microwave extraction of compounds from walnut (*Juglans regia* L.) shells with high antioxidant activity. The effect of three independent factors, extraction time, ethanol/water (% v/v) concentration, and microwave power and their mutual interaction was investigated through response surface methodology (RSM), obtaining 3D-response surface plots for each dependent variable: total phenolic content (TPC) and antioxidant activity (TEAC, DPPH, and ORAC).
The optimized extract (EWS) was wholly characterized by HPLC-MS, retrieving qualitative and quantitative data with the identification of flavonoids, hydrolyzable tannins, and anacardic acids as the components responsible for antioxidant activity.
The extract was melt-mixed with the bio-based Ecovio® at different concentrations (0.5 and 1.5 w/w) by forming film specimens. A set of films was prepared to enhance the thermal material properties by adding halloysite nanotubes (0.5% and 1.5% w/w) to the blend. All formulations showed increased antioxidant features. Remarkably, adding 1.5 % boosted the bioplastic UV light resistance, improving mechanical properties.
Although the presence of the nanofiller fairly much increase thermal properties maintaining a high antioxidant degree, its addition implies photodegradation phenomena that impact the final application of the material.

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PILOT-SCALE APPLICATION OF THE *PHANEROCHAETE CHRYSOSPORIUM* SPORE SUSPENSION FOR THE BIODETERIORATION OF LOW DENSITY POLYETHYLENE

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In polymer biodeterioration studies, it is crucial to prepare a viable inoculum to initiate biological deterioration. The objectives of the current study were to develop a spore suspension from endolichenic fungi (ELF) and application of the spore suspension, as an enhancer for low density polyethylene (LDPE) biodeterioration. It was hypothesized that the application of the spore suspension of the best polyethylene deteriorating ELF isolate (out of the tested ELF species), can enhance the LDPE biodeterioration. Based on LDPE biodeterioration capabilities and results of quantitative enzymatic assays, *Phanerochaete chrysosporium* was selected as the potential candidate for the preparation of the spore suspension [1]. Pilot-scale testing of the spore suspension on LDPE strips dipped in an aqueous environment and strips buried in a soil environment, was carried out. Four set-ups were maintained for water and soil separately, considering the sterilized and non-sterilized samples, with and without spore suspension. Deterioration of the polymer properties was analysed after 105 days, based on percent reductions in weights and tensile properties, increments in degree of water absorption, changes in peaks of Infrared (IR) spectra and carbonyl index. Scanning Electron Microscopy (SEM) was used to detect the changes in surface morphology and changes in surface wettability were measured by contact angle measurements. Gas Chromatography-Mass Spectrometry (GC-MS) was used to detect the products liberated, during biodeterioration. Subsequent search in the degree of deterioration of LDPE strips, dipped in water revealed the efficacy of the spore suspension in deteriorating LDPE, amidst of normal aquatic microorganisms. After 3.5 months (105 days) of exposure, LDPE strips in the set-ups added with the spore suspension, showed well-satisfactory results in all the key parameters to analyse the polymer deterioration. Further, in the soil environment exposure studies also, it was concluded that the deterioration by the environmental soil micro-flora was enhanced due to the application of the spore suspension. Hence, the ELF species can be used to develop eco-friendly alternative treatment methods to deteriorate LDPE. The present study confirms that application of such fungal spores as inoculants can enhance deterioration of LDPE in the environment, mitigating the problems posed by their use.

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MEMBRANE-PROCESSED DEWATERING FOR CONCENTRATION OF LIQUID FOODS

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The imperative to establish environmentally friendly and sustainable food processing techniques has compelled the food industry to explore alternative approaches that uphold food quality, ensure nutritional integrity, and minimize energy consumption. Extensive research conducted in the past decade has substantiated the superiority of membrane-based dewatering technology over conventional methods, owing to its ability to retain nutrients effectively while minimizing energy requirements. Notably, forward osmosis (FO) and membrane distillation (MD) have emerged as viable membrane technologies for food processing in the industry. However, recent reviews have underscored the prominence of FO in the enrichment of liquid food, positioning it as a preferred choice among other membrane-based processes. This paper aims to elucidate the advancements and contributions of FO and MD in the realm of food processing while evaluating their maturity and technology readiness level for food concentration. Moreover, it endeavors to delineate specific parameters, including pretreatment techniques, membrane cleaning strategies, and membrane configurations/modules tailored to liquid food sources' distinct dewatering requirements. Although most FO and MD studies have focused on lab-scale fruit juice and whey concentration, future investigations should encompass pilot-scale process development alongside comprehensive techno-economic analyses to facilitate the smooth transition of these technologies to an industrial scale.

PREPARATION, STRUCTURAL CHARACTERISTICS AND PROPERTIES OF SERICIN COATED WOOL NONWOVEN FABRIC

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Recently, natural silk nonwoven fabric has attracted researchers' attention in biomedical and cosmetic applications because of its good mechanical properties and cytocompatibility. Although it can be easily fabricated using binding character of sericin, high cost of silk material may restrict its industrial use in a certain area. In this study, sericin as a binder was added to a cheaper ma-terial (wool) to prepare wool based nonwoven fabric and the effect of amount of sericin addition on the structural characteristics and properties of wool nonwoven fabric was investigated. It was found from SEM observation that the sericin coated the surface of wool fibers and filled the space between the wool fibers. With an increase of sericin addition, porosity, moisture regain, and contact angle of the sericin coated wool nonwoven fabrics decreased. Maximum stress and Young's modulus of nonwoven fabric increased with increasing sericin amount to 32.5%, and then decreased after that. Elongation decreased constantly with an increase of sericin addition. All nonwoven fabrics showed a good cytocompatibility which could be enhanced by adding sericin to wool. These results indicate that the wool based nonwoven fabrics can be successfully fabricated by adding sericin and its properties can be diversely controlled by altering the amount of sericin addition promising a good candidate for biomedical and cosmetic applications.

OPTIMISATION OF ULTRASONIC-ASSISTED EXTRACTION OF CANTHAXANTHIN FROM *CHROMOCHLORIS ZOFINGIENSIS* USING EUTECTIC SOLVENTS

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Carotenoids are well-known bioactive compounds and high-value nutritional molecules. *Chromochloris zofingiensis*, a unicellular green alga, is a promising source of commercial carotenoids owing to its robust growth, easy cultivation, and facile scale-up. However, this microalga has a very rigid three-layered cell wall that hinders extraction. Meanwhile, traditional extraction methods are complicated and cause serious pollution. To address these issues, a eutectic solvent (ES) was identified from 15 candidates for the ultrasonic-assisted extraction of canthaxanthin from *C. zofingiensis* for the first time. Remarkably, this hydrophobic ES comprising octanoic and decanoic acid displayed novel dual functions of breaking the cell wall and carotenoid extraction. Under the optimised extraction conditions obtained using a response surface methodology (ultrasound at 50 ℃ for 49 min, octanoic acid-to-decanoic acid molar ratio of 2.3:1, and solid-to-solvent ratio of 66.2 mg/mL), the maximum yield of canthaxanthin (60.5 μg/mL) was equal or superior to that using traditional organic extractant after grinding to break the cell wall. Furthermore, only ES as extraction solvent was considered, the canthaxanthin content obtained by pre-grinding under the same condition was much higher (70.4 μg/mL) than that of grinding and extraction with ethanol (62.2 μg/mL). Possible mechanisms of cell wall breakage and component extraction were investigated based on extraction kinetic analysis, molecular dynamics simulation, and scanning electron microscopy observation. This ES is non-volatile and environmentally friendly, making it a simple, sustainable, and effective alternative extractant for carotenoids in microalga. There is considerable potential for its industrial-scale development and applications in various fields such as food, medicine, and cosmetics.

NATURAL DEEP EUTECTIC SOLVENTS EXTRACTION OF ANTHOCYANINS FROM BLACK RASPBERRY (*RUBUS OCCIDENTALIS* L.) POMACE

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At industrial levels, a large amounts of fruits by-products are made. For overcoming challenges related to consequential environmental and economical elements, the re-utilization of redundant fruit biomass, as a source of active compounds with therapeutic properties, represent an idea that gained a lot of scientific community interest. Moreover, green extraction methods and selection of an appropriate solvent should be an integral part of achieving this goal. Thus, this study aimed to investigate the effect of natural deep eutectic solvents (NaDES) in comparison with conventional solvents on the ultrasound assisted extraction of cyanidin-3-O-rutinoside and total anthocyanins from black raspberry fruit pomace (*Rubus occidentalis* L., Rosaceae), their stability at 4, 25 and 40 ◦C, as well as to evaluate the impact of hydroxypropyl-β-cyclodextrin (HPβCD) on extraction capacity of NaDES and water. A range of NaDESs, composed of hydrogen bond donors (choline chloride or betaine) and hydrogen bond acceptors (organic acids, sugars, polyols, amide), were screened. Based on total anthocyanins content (TAC) and antioxidant activity, citric acid-choline chloride NaDES was selected for extraction conditions optimization by Box-Behnken design coupled with response surface methodology. The optimal conditions were found to be an extraction time of 52.93 min, a temperature of 65 ◦C, and 15.60% (*w/w*) water in NaDES, resulting in maximized extraction yields of target compounds, cyanidin-3-O-rutinoside (7.60 mg/g DW) and TAC (6.88 mg CGE/g DW). A 30-day stability study revealed that NaDES provided the best anthocyanins preservation at defined temperature range compared to extracts prepared with water, 70% ethanol and 70% methanol. By incorporating different concentrations of HPβCD (1.5, 3 and 6%, *w/w*) in citric acid-choline chloride NaDES, downward trend was observed, while in the case of water anthocyanins extraction was improved. Black raspberry fruit pomace is a promising herbal drug rich in anthocyanins that can be used in green technology oriented pharmaceutical and cosmetic industries.

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POSTER SESSION 2

ST5 BIODIVERSITY AND CHEMICAL ECOLOGY

PO105 – PO108

CONOCEPHALUM CONICUM L. LIVERWORT: AN INTEGRATED BIOLOGIC AND METABOLOMIC STUDY

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Bioaccumulation, ultrastructural and DNA damage, oxidative stress, and metabolomic analyses were carried out in vitro on the liverwort Conocephalum conicum L., in response to the heavy metal stress.[1] For this purpose, Conocephalum conicum L. samples were grown in vitro under heavy metal contamination environmental conditions, as measured in 2 sites of Sarno River: the upstream (C1 sample) and downstream river (C2 sample), characterized by very different environmental conditions, representative of a preserved environment (C1) and of a high anthropogenic pressure (C2).
The biological responses considered, ROS production and localization, antioxidant enzymes, ultrastructural damage responded consistently with the expected environmental stress.
For a fast and reliable dereplication of the secondary metabolites in C. conicum we used advanced mass spectrometry-based metabolomics and molecular networking tools.[2] This approach allowed to evaluate the metabolome induced by metal stress in C. conicum permitting a fast-tentative annotation of several known and unknown metabolites. The enhanced production of oxidized lipids, of members of betain lipid class and of C-glycosides, matches all well with previous findings on the role of these metabolites, as both stress chemical markers and protective agents and damage markers. The multidisciplinary approach proposed in the present study pave the way to future investigations aimed to further mining the secondary metabolome of plants, including those of medicinal and alimentary interest, when exposed to abiotic stress.

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LC-HRMS METABOLOMICS FOR THE DETERMINATION OF BIOMARKERS FROM *EX SITU* GENOTYPES OF *HELICHRYSUM AMORGINUM*

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The genus *Helichrysum* Mill. (Asteraceae) comprises approximately 600 species, mainly of African and Eurasian origin[1]. They are widely used in traditional and modern phytomedicine to tackle several problems and primarily infections. *Helichrysum amorginum* is a Greek endemic species that has never been studied for its polar (non-volatile) constituents. It is interesting to note that plant collection period is of primary importance since it affects *Helichrysum* properties and quality, through metabolites profile alternation[2]. Thus, the objective of this study was to identify and propose biomarkers responsible for the seasonal variation of *H. amorginum,* using metabolomic approaches.
Specifically, during the years 2016-2020, 62 genotype samples of *H. amorginum* were collected in the period from March to June. The samples were extracted with methanol and subjected to LC-HRMS-based metabolite profiling. Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) for clustering purposes and identification of statistically significant variables (VIP scores). Based on the results, *H. amorginum* genotypes do not differ significantly between collection years. However, show a significant variation trend between collection months. Flavonoids, phenolic acids and pyrone-phloroglucinols were identified as marker molecules responsible for the observed classification and correlated with the seasonality of *H. amorginum*.

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PHENYLPROPANOIDS FROM ESSENTIAL OILS OF *PIPER*: A NATURAL ALTERNATIVE FOR THE CONTROL OF *SITOPHILUS ZEAMAIS*

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The genus *Piper* stands out for its high extraction yields of EOs. Additionally, within its biological properties, it has been shown to be efficient in controlling insects and storage pests[1]. Such is the case with *Sitophilus zeamais*, which causes economic losses and damage to cereals such as maize[2]. Synthetic insecticides commonly used against *S. zeamais* often have negative effects on the environment and promote the development of resistance mechanisms[3]. Therefore, our objective is to find substances for controlling *S. zeamais* using EOs from the *Piper* genus. To achieve this, EOs were obtained by steam distillation from *P. aduncum*, *P. asperisculum*, *P. holtonii*, and *P. auritum*. The median lethal dose (LD_{50}) was determined by contact toxicity, and the EOs were characterized by GC-MS. The compounds were identified by analysing the mass spectra and comparing with literature data[4]. The major compounds were isolated using flash chromatography (FC), and they were characterized by NMR. The 24h LD_{50} values for the EOs of *P. aduncum*, *P. asperisculum*, *P. holtonii*, and *P. auritum* were to 121, 73, 144, and 67 µg/insect, respectively; and their major constituents were the phenylpropanes dilapiol 27.48%, myristicin 29.85%, apiole 46.63%, and safrole 64.29%, respectively. Dilapiol was isolated from *P. aduncum*, myristicin from *P. asperisculum*, and apiol from *P. holtonii*, while safrole was obtained commercially. Dilapiol, at the maximum evaluated dose, does not exhibit toxic activity upon contact. However, for myristicin, apiole, and safrole, a 24h LD_{50} of 51, 70, and 36 µg/Insect, respectively, was determined. Apparently, dilapiol is not responsible for the activity of *P. aduncum*, which suggests that the toxic effect of the EO may be due to other components or to a synergistic effect. Nevertheless, the phenylpropanes present in the EOs of *P. asperisculum*, *P. holtonii*, and *P. auritum* may be responsible for the insecticidal activity. Safrole and myristicin could be molecules of interest for the development of natural insecticides. This work was developed with resources from the project BPIN 2020000100342 (Colombia) and covered by the Contract for Access to Genetic Resources and Derivative Products No. 121 addendum No. 21.

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EMERGING PHARMACEUTICAL POLLUTANTS IN SURFACE AND WASTEWATER OF THE DEPARTMENT OF ATLÁNTICO-COLOMBIA

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Water pollution represents an environmental concern due to the risk it poses to health and ecosystems. Emerging pollutants (EP) are micropollutants, generally of an organic type, with toxicological capacity for aquatic and terrestrial organisms. Wastewater discharged to surface water directly or after inefficient treatment processes are the main access routes to the environment. Pharmaceutical products represent a group of EP that, even in small amounts in the environment by the extensive use in human and veterinary medicine. Includes compounds such as antibiotics, analgesics, antidepressants, anti-diabetics, among others[1]. The scientific community now recognizes that continuous exposure at low doses can produce long-term effects on the environment and human health. Due to their high polarity and low volatility, pharmaceuticals tend to be easily transported and discharged into bodies of water. In this work, six medications from various frequently used therapeutic categories (Acetaminophen, Ibuprofen, Naproxen, Acetylsalicylic Acid, Carbamazepine, and Metronidazole) were studied in wastewater (Hospital and Domestic) and surface waters of eight municipalities of the department of Atlántico-Colombia. Extraction of analytes was performed by SPE using 500 mg/6 mL Bond Elut PPL cartridges, followed by derivatization with BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide trimethylchlorosilane (TMCS)). For the detection, gas chromatography and mass spectrometry were used, and the identification of each compound was made by comparing the retention time and the mass spectrum with its respective derivatized standard. The quantification was carried out by selective ion monitoring (SIM). The results indicated that no sample analyzed was free of EP, regardless of their origin. It was found that wastewater of hospital origin has a higher percentage of emissions of these substances at 75% compared to domestic wastewater samples with 24%. The pharmaceutical products that prevailed in all the samples and presented the highest concentration levels were Acetaminophen (78.42 µg/L), Ibuprofen (4.18 µg/L), and Naproxen (2.74 µg/L), indirect indicators of the increased consumption of analgesics and anti-inflammatories by the target population.

Keywords: Pharmaceutical products, Wastewater, Surface water, Emerging pollulants.

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POSTER SESSION 2

ST6 NEW METHODOLOGIES IN NATURAL PRODUCTS RESEARCH

PO109 – PO118

STABILIZATION OF BEETROOT PIGMENTS WITHIN SILICA GELS

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The water-soluble pigments of beetroots are widely used as color additives in food, cosmetics, and pharmaceuticals, and serve as antioxidants.[1] Betanin, the primary pigment in commercial beetroot extract, has remarkable antiradical properties and has shown potential to inhibit tumor growth in humans and prevent lipid and hemoglobin oxidation in patients with β-thalassemia. It also exhibits low toxicity and stability within the pH range of 3 to 7, rendering it suitable for use in dairy products. However, the broad application of betalains and their derivatives is somewhat restricted due to their instability towards hydrolysis and oxidation. Improvements have been made to the persistence of betanin in aerated solutions through encapsulation with maltodextrin or via emulsification,[2] yet these methods only enhance its stability to a certain degree. We present a method to stabilize betanin through encapsulation within silica aerogels and cryogels. This involves the use of a mixture of betanin, its epimer isobetanin, and other betacyanins, or alternatively, high-purity betanin, and the sol-gel method.[3] Depending on the specific conditions applied, betanin is either covalently attached to the silica matrix or merely adsorbed. Regardless of the procedure, only a portion of the initial betanin is encapsulated. After solvent removal using supercritical carbon dioxide or controlled freeze-drying, we obtain magenta aerogels and cryogel, respectively. The color of these materials is far more persistent compared to solid betanin. Moreover, the use of silica not only provides an atoxic environment but also enables the application of betanin in aqueous or organic media, as well as in complex matrices. We anticipate that these materials will offer potential applications as non-toxic pigments and for the development of bioinspired chemical sensors.

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DETERMINATION OF SULFITE IONS IN FOOD SAMPLES USING HEADSPACE LIQUID-PHASE MICROEXTRACTION WITH AN OPTICAL PROBE (HS-LPME-OP)

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Sulfite is a well-known enzymatic and non-enzymatic reactions inhibitor, that is used during food and beverages preparation and storage. It is also added to the wines because of antimicrobial and antioxidant properties. From the other side, sulfite concentration must be carefully controlled in food and beverages due to its possible allergic effect[1,2]. Because of complex sample matrix there is still a need for a simple method with sufficient sensitivity and selectivity for the determination of sulfite in food and beverages.
Sulfite ions were determined in alcoholic beverages, like wine, cider, juices and jams with the help of HS-LPME-OP. Determination is based on sulfur oxide liberation from sample in acidic medium with its next absorption with 0.1 mM 5,5`-dithiobis-(2-nitrobenzoic) acid. This procedure requires almost no additional sample preparation, except dilution. Juices do not require extra preparation step, and jams were previously dissolved and filtered. Free sulfite was determined in wines without previous pretreatment. But for total sulfite determination, bounded sulfite should be liberated first with the help of sodium hydroxide. HS-LPME-OP combines advantages of headspace single-drop microextraction and optical probe, eliminating their disadvantages, like problems with drop stability and difficult compatibility with UV-Vis spectrophotometry. It also does not require any organic solvents. Method LOD was calculated as 14.4 μg L^{-1}. Method also showed high enough selectivity toward compounds present in food and beverage samples.

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UREA SYNTHESIS USING RENEWABLE RESOURCES: CARBON DIOXIDE, AIR AND WATER

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Urea [$CO(NH_2)_2$] is the first organic product that was successfully synthesized in the laboratory, opening the field of organic chemistry. Today, over 190 million tons per year of urea is produced as a fertilizer[1]. This is accomplished by adding liquified carbon dioxide (CO_2) to liquid ammonia (NH_3) under harsh conditions (400-500 K, 150-250 bar). High temperatures have the drawback of quickly introducing degradation and unwanted by-products such as biuret, cyanuric acid, ammelide, ammeline, and melamine[2]. Ammonia is synthesized from nitrogen (N_2) and hydrogen (H_2) by the Haber-Bosch process under high pressure and high-temperature conditions (100-200 psi, 400-500 °C), which is sufficient to break the N≡N triple bond (941 kJ mol-1)[3,4]. This process accounts for more than 2% of the global energy consumption[5]. The hydrogen comes from a petroleum source, adding to the negative environmental impact. Viable urea synthetic approaches under milder conditions are widely sought to reduce environmental pollution from the present industrial process.

Water (H_2O) microdroplets are sprayed onto a graphite mesh with a bismuth oxide copper oxide (Bi_2O_3/CuO) coating using a 1:1 mixture of N_2 and CO_2 as the nebulizing gas. The resulting splash of microdroplets contains urea [$CO(NH_2)_2$], which is detected by mass spectrometry and 1H and ^{13}C nuclear magnetic resonance. This gas-liquid-solid heterogeneous catalytic system synthesizes urea on the 0.1 millisecond timescale. The conversion rate reaches 6.3 mmol g^{-1} h^{-1} at 25 °C and 25.2 mmol g^{-1} h^{-1} at 65 °C, with no applied external voltage. Water microdroplets serve as the hydrogen source and the electron transfer medium for N_2 and CO_2 in contact with Bi_2O_3/CuO. Water-gas and water-solid contact electrification are speculated to drive the reaction process. This strategy couples N_2 fixation and CO_2 utilization in a one-step, eco-friendly, low-cost approach to produce urea, converting a greenhouse gas into a value-added product.

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CHANGES OVER TIME OF THE METABOLITE PROFILE IN THE DECAY PROCESS OF A MARINE SPONGE

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A number of secondary metabolites with unique structures and biological activities have been isolated from marine sponges[1], especially the marine sponge of genus *Theonella* is known to be a rich source of natural compounds such as onnamides[2] and theonellamides[3-4]. Recently, it has become revealed that the symbiotic microorganisms are the real producers of the sponge derived secondary metabolites[5]. In spite of the recent progress in understanding the biosynthetic process of marine natural products, little is known about the degradation process of secondary metabolites within the host organisms in an environment where symbiotic relationships are lost after the cessation of life activity.
In this study, to gain knowledge on the degradation process of secondary metabolites in the sponge after the end of the symbiotic relationship, we used LC-MS to analyze changes over time of the secondary metabolite profiles in the marine sponge *Theonella* sp. As a result, we found metabolites thought to be degradation products of theonellamides.

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REVISITING THE EXTRACTION OF BETANIN AND OTHER BETACYANINS FROM RED BEETROOTS

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Betalains are natural products that emerged as biocompatible antioxidants, redox mediators, pharmaceuticals, and safe dyes for food and cosmetics. Chemically, all betalains contain the same chromophore derived from L-tyrosine, but magenta betacyanins are distinguished by the presence of a cyclo-DOPA portion. Betanin (2*S*/15*S*, CI Natural Red 33, betanidin 5-*O*-β-glucoside) is the main component of a food color additive approved by the EFSA and FDA.[1] It is also an important source of betalamic acid, the starting material for the partial synthesis of bioinspired betalains and quasibetalains.[2] The extraction of betanin and its 2*S*/15*R* epimer isobetanin from beetroots have been extensively described.[3, 4] However, although most coloring applications do not require pure betanin, methods for the obtaining of high purity betanin are scarce. Here, we outline a method to extract betacyanins from beetroots and to obtain betanin in high purity by subsequent chromatographic separation. Commercially available red beetroots contain 300–600 mg of betacyanins/kg fresh weight (FW), mostly betanin/isobetanin.[2] We used a solid-liquid extraction method to extract betacyanins from raw beetroots, successfully preventing betanin decomposition by inhibiting the action of peroxidases, polyphenoloxidases, β-glucosidase, and betalain oxidase. Betacyanins (~200 mg/kg_{FW} of red beetroots) were precipitated by using an environmental benign organic antissolvent. This mixture is suitable for coloring applications requiring magenta natural products. Purification of betanin from beetroot juice using column chromatography is costly because browning substances tend to strongly adsorb in C18 silica gel. Nevertheless, the purification of betacyanins was successful, producing a mixture of betanin/isobetanin pure enough for most applications. For applications that require high purity betanin, the betanin/isobetanin mixture was further purified using HPLC under reversed-phase conditions.

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UNRAVELING THE ENIGMATIC WORLD OF NATURAL SCALEMATES: CHALLENGES AND INSIGHTS THROUGH CHIRAL SEPARATIONS AND THE INVERTED CHIRALITY COLUMNS APPROACH

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It is not entirely clear whether natural scalemates, which which are enantiomerically enriched mixtures, exist in nature. Chiral natural products are typically produced by enzymes, making them enantiomerically pure, or they may self-assemble from enzyme-generated reactive species, in which case they would be racemic (a mixture of diastereomers if stereogenic elements exist). The concept of scalemates deviates from the binary logic of enantiomeric purity or racemic state. Racemic refers to an equal mix of two enantiomers, while scalemic refers to an uneven ratio of two enantiomers, which can vary widely. Different enantiomeric forms of natural products have been known since the 19th century. Racemic and scalemic natural products are increasingly documented. Some compounds thought to be racemic were later found to be racemized, but there are indeed racemic natural products. It is now understood that biogenetic pathways are responsible for generating racemic natural products[1]. On the other hand, the occurrences of scalemic natural products are more enigmatic and challenging to detect and study, even through chromatography. Chromatography is a comparative technique, and one of the main problems encountered in the stereoselective analysis of complex molecules with one or more stereogenic elements is that the minor enantiomer, or racemate, is often not available as a reference sample and is often very difficult to synthesize. The lack of reference samples for the minor enantiomer or the racemate poses challenges in the stereoselective analysis of complex molecules. Identifying enantiomers in natural products that are produced as single enantiomers or with extreme enantiomeric excess (ee) can be challenging. However, the Inverted Chirality Columns Approach (ICCA) offers a solution by utilizing oppositely bonded Pirkle-type chiral stationary phases (CSP). By using Whelk-O1 columns, this technique helps identify enantiomeric pairs in complex mixtures and enables analysis of enantiomeric traces. Thus, by switching between two CSP-packed columns having the same bound selector but opposite configurations, under the same experimental conditions, it is possible to observe the reversal of the elution order of a given enantiomeric pair. Especially when the aim of the chromatographic analysis is to find extreme enantiomeric excesses and the minor enantiomer elutes in the tail of the major one, this simple feature allows identifying an enantiomeric pair (even in complex matrices) and controlling the order of elution (eluting the minor enantiomer before the major one), simply by carefully choosing the column with the “right” selector configuration[2]. In this contribution, several examples of ICCA used to elucidate the stereochemical composition of natural products are reported.

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UNLOCKING NATURE'S POTENTIAL: CHOLESTEROL-POLYMER FUSION IN DRUG DELIVERY

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Cholesterol, a steroid lipid, plays a pivotal role as a fundamental organic molecule in mammalian cell membranes, governing their fluidity, integration, and permeability.[1,2] Beyond its structural significance, cholesterol also serves as a vital precursor in the biosynthesis of essential biological substances, such as bile acids, vitamin D, and sex hormones.[3] Due to its multifaceted significance, cholesterol is a prevalent selection for guiding molecule incorporation into cell membranes within Smart Drug Delivery Systems (SDDS).[4] Studies have shown that even a single cholesterol group at the end of a polymer chain is sufficient for effective interaction with the cell membrane.[5] Introducing cholesterol groups to the side chains using the modern method of controlled polymerization with Reversible Addition-Fragmentation Chain Transfer (RAFT) will be presented. A poly(N-isopropylacrylamide) (PNIPAAm) block was added to ensure solubility and thermosensitivity.

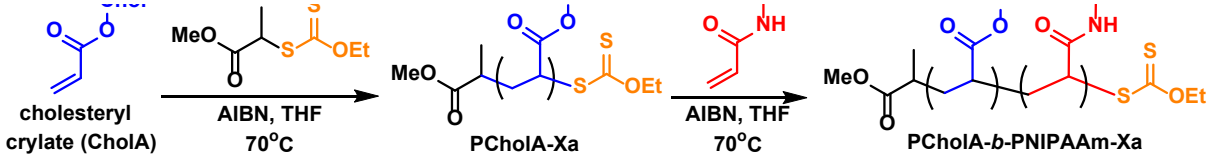

Figure 1. RAFT polymerization of cholesteryl acrylate and block copolymerization of *N*-isopropylacrylamide.

Acknowledgments

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2. Analyses were performed in the Centre of Synthesis and Analyses BioNanoTechno of the University of Bialystok. The equipment in the Centre of Synthesis and Analysis BioNanoTechno of the University of Bialystok was funded by the EU as a part of the Operational Program Development of Eastern Poland 2007-2013, project: POPW.01.03.00-20-034/09-00 and POPW.01.03.00-20-004/11.

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MACE - MASS SPECTRA FOR CHEMICAL ECOLOGY

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Mass spectral libraries of EI-mass spectra are an important tool in the identification and structure elucidation of natural products by GC/MS, a typical task in Chemical Ecology. Unfortunately, spectra of new compounds are usually only available as figures even in recent publications, making it tedious to keep track of them and difficult to integrate them into user libraries. We therefore implemented an open access data repository for EI mass spectra, called MACE[1]. In this database high quality spectra not found in common databases can be downloaded as a collection in a simple format, readily integrable into local spectral databases. The spectra are from original publications that have been synthesized or isolated. Compounds found widespread commercial databases such as NIST 17 or Wiley 7 are not included. MACE is therefore an add-on database of high quality EI-mass spectra.

MACE is designed as an community effort with open access, needing input from research groups worldwide. The list of compounds will be continuously extended, hopefully with spectra submitted by other groups. We have started with compounds from our own group, but further additions are very welcome. Submitted spectra will be checked for quality and added to MACE. The complete MACE library text file can be downloaded from the research data repository of TU Braunschweig, thus ensuring long-term data storage[2]. The data can be freely distributed for non-commercial use according to the creative commons license CC-BY-SA. There is a twitter account for news on MACE: @MACE4GCMS.

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QUICK AND SIMPLE TOOL FOR TESTING THE QUALITY OF FRUIT DISTILLATES

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Food fingerprinting has become one of the most promising analytical tools to solve the problem of food quality and authentication, especially in complex cases such as distinguishing originality of fruit alcohol distillates[1,2]. This work describes possibilities for the simple screening of the originality and quality of selected fruit alcohol distillates using gas chromatography with flame ionization detector (GC-FID). We used qualitative and quantitative analysis methods such as normalization of the area, the external or internal standard method and the standard addition method. The external standard method with single-point calibration was chosen for semi-quantitative analysis. The proposed method was used to quantify methanol, n-propanol, and ethyl acetate in selected samples of fruit alcohol distillates. A high concentration of these compounds significantly affects the quality and taste of the distillate alcohols (aroma, taste, quality of raw material, etc.). The aim of this work was to select and design a simple and functional analytical tool for the identification and quantification of some significant components of the distillates for the rapid assessment of the quality and taste of analyzed samples.

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BUILDING BLOCK EXTRACTOR: AN MS/MS DATA MINING TOOL FOR TARGETED DISCOVERY OF NATURAL PRODUCTS WITH SPECIFIED FEATURES

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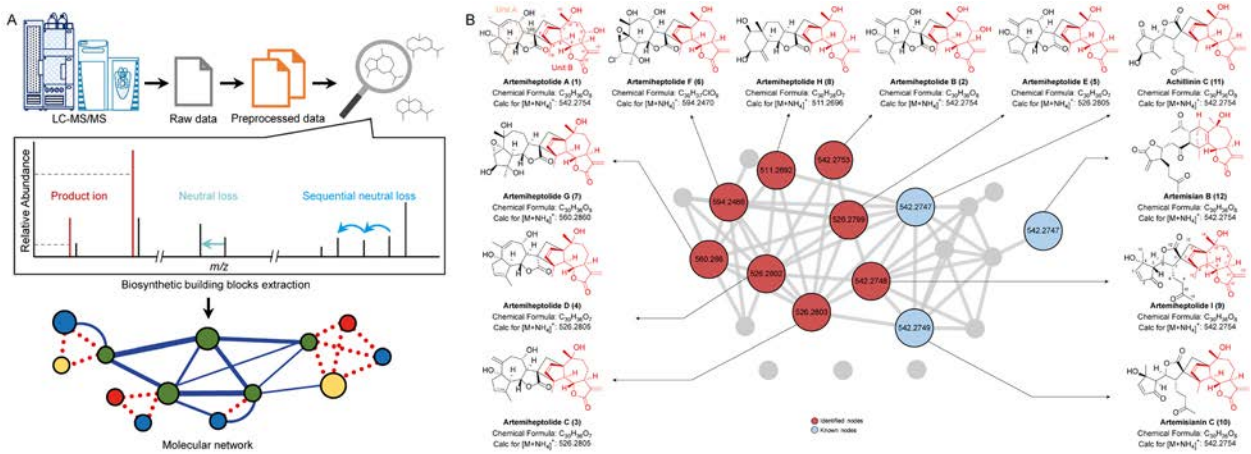
The building blocks-based molecular network is an efficient strategy to explore the unknown chemical space of natural products.[1] However, structure-based automated MS/MS data mining remains challenging. In this work, we propose Building Block Extractor, an MS/MS data mining program with a graphical user interface that automatically extracts user-defined specified features. In addition to the characteristic product ions, their relative abundance and the sequential neutral loss features were integrated as building blocks for the first time. Nine undescribed sesquiterpenoid dimers were discovered and isolated from *Artemisia heptapotamica*, demonstrating the power of this tool. Artemiheptolide I (**9**) suppresses the infection of Influenza A/Hongkong/8/68 (H3N2) in vitro with IC_{50} of 8.01±6.19 μM. In addition, two known guaianolide derivatives (**16** and **17**) possessed remarkable antiviral activity, including Influenza A/Puerto Rico/8/1934 H1N1, H3N2, and influenza B/Lee/40 with IC_{50} values ranging from 3.46 to 11.77 μM. In addition to the efficient discovery of novel natural products, this strategy can be generally applied to grab derivatization with particular fragments and expand the annotation power of LC-MS/MS analysis.



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POSTER SESSION 2

ST1 BIOACTIVE NATURAL PRODUCTS AND DRUG DISCOVERY

PO119 – PO121

TOTAL SYNTHESIS OF (4*Z*)-LACHNOPHYLLUM LACTONE, A SPECIALIZED BIOACTIVE ACETYLENIC FURANONE PRODUCED BY *CONYZA BONARIENSIS*

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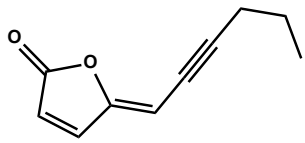
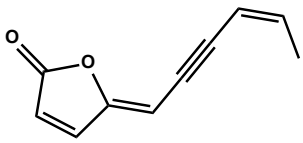
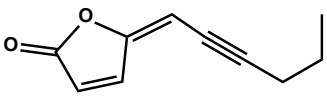
Conyza bonariensis, an invasive plant native to America, severely affecting many crops worldwide that use allelopathy as part of a successful strategy to outcompete neighboring plants. (4*Z*)-lachnophyllum lactone is the main acetylenic furanone (constituted by a 2-furanone ring bonded to an unsaturated chain) produced by this plant that induced a rapid cessation of broomrapes (*Orobanche* and *Phelipanche* spp.), holoparasitic weeds that severely infesting a large number of important crops and causing severe yield losses in agriculture.[1,2] Thus, (4*Z*)-lachnophyllum lactone was selected as a model for the synthesis of new environmentally friendly herbicides. A versatile synthetic strategy was developed and also used to obtain two of its natural analogues, namely (4*E*)-lachnophyllum lactone and (4*Z*,8*Z*)-matricaria lactone (Figure 1). These compounds were evaluated against four types of pests affecting Mediterranean agriculture, i.e. the stem parasitic weed *Cuscuta campestris*, the root parasitic weeds *Orobanche minor* and *Phelipanche ramosa,* the autotrophic weed *Conyza bonariensis* and the fungal pathogen *Verticilium dahlia* (isolated from olive trees in Spain). In this comminication, the methodology for total synthesis of these acetylenic furanones on gram-scale, will be reported as well as the results of the biological activity. The developed methodology is an effective strategy to afford these acetylenic furanones and could be easily modified to obtain several structural analogues for SAR studies. Finally, the design and implementation of its effective formulation in suitable matrix will allow us to obtain an easy-to-use bioinspired pesticide for the agrochemical industry.



(4*Z*)-Lachnophyllum lactone



(4*Z*,8*Z*)-Matricaria lactone



(4*E*)-Lachnophyllum lactone

Figure 1. Bioactive acetylenic furanones

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HR-LCMS AND EVALUATION OF ANTI-DIABETIC ACTIVITY OF *HEMIDESMUS INDICUS* (ANANTMOOL): KINETIC STUDY, AND MOLECULAR MODELLING APPROACH

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This study delved into the exploration of novel antidiabetic medications acquired from natural resources, utilizing the Ayurvedic Rasayana herb *Hemidesmus indicus* through cutting-edge chemoprofiling and molecular modelling techniques. The methanolic extract of *Hemidesmus indicus* root exhibited the highest extractive yield (24.70 ± 0.08 %) and contained substantial levels of total phenolic and flavonoid content as 154.15±1.24 mg Gallic Acid Equivalent/g extract and 70.61±0.35 Quercetin Equivalent/g extract respectively. Invitro study revealed the potent inhibitory potential of methanolic extract of the herb against essential carbohydrate hydrolytic enzymes α-amylase (IC50= 4.19±0.04 mg/ml) and α-glucosidase (IC50= 5.78±0.10 mg/ml). Further, the enzyme kinetic study demonstrated the competitive mode of inhibition of both enzymes. HR-LCMS analysis identified the major phytoconstituents present in the extracts, including Solanocapsine, Cyclovirobuxine C, Lucidine B, Zygadenine, Aspidospermidine, silychristin, 3beta-3-Hydroxy-18-lupen-21-one, Manglupenone, and 19-Noretiocholanolone. Molecular docking, molecular dynamic simulation, and MM/GBSA analysis have proved stable, rigid, compact, and folded form of complexes during the entire 100ns simulation, illustrating Zygadenine, Solanocapsine, and Cyclovirobuxine C as the superior inhibitors of α-A protein, while Zygadenine, Plumieride, and Phlegmarine exhibited greater inhibitory behaviour towards α-G protein than the FDA-approved drug acarbose. Collectively, our findings indicate that the *Hemidesmus indicus* could be a promising source of α-A and α-G inhibitors, potentially serving as a lead in order to develop medications for type-2 diabetes.

Keywords: Type-2 diabetes mellitus; Hemidesmus indicus; Enzyme inhibition; Computational study.

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PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON SELECTED SOUTH AFRICAN MEDICINAL PLANTS

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Traditional medicine is a cultural practice with a long history in South Africa, the African continent and the rest of the world. However, there is still a fair amount of scepticism about its use, mostly due to lack of scientific information to support this practice. Presently nearly every "upmarket" mall in RSA's has several, successful herbal shops which are thriving over the idea that "natural is better than synthetic". Approximately 3000 plants, out of a national biodiversity represented by about 30 000 higher plant species, are used as traditional medicine. Some of these medicinal plants have been investigated in our lab for their phytochemical composition, and this paper will describe some of the contributions that have been made in this regard over the few years. Selected plant species, which included *Sutherlandia (Lessertia) frutescens*[1,2], *Ecklonia maxima*[3], *Artemisia afra*[4], some *Searsia* species[5], and *Protea cynaroides*[6] among others, were subjected to extraction with organic solvents followed by chromatographic fractionation and spectroscopic identification of the isolated compounds. Flavonoids, terpenoids and their glycosides were among representative compounds isolated, while water extracts were shown to be rich in pectic polysaccharides. Some of the crude extracts and purified compounds were also studied for antimicrobial and enzyme inhibition properties. Most of the results showed that there is justification for the use of these plants for medicinal purposes.

Sutherlandia frutescens

Protea cynaroides

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SATELLITE WORKSHOP AT
ISCNP31 & ICOB11 BY EU-OPENSCREEN
ACCELERATING NATURAL PRODUCT DISCOVERY BY ACADEMIC AND INDUSTRIAL COLLABORATIVE INITIATIVES

MARINE NATURAL PRODUCTS BIOPROSPECTING AND COLLABORATION OPPORTUNITIES AT EU-OPENSCREEN

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EU-OPENSCREEN is the European Research Infrastructure Consortium (ERIC) for chemical biology and early drug discovery. EU-OPENSCREEN supports all stages of a small molecule discovery project, including assay adaptation, high-throughput screening, and chemical optimisation of the hit compounds, and operates an open-access database and a unique, common compound collection (ECBL). EU-OPENSCREEN has over 20 affiliated high-throughput screening and chemistry facilities at partner sites in 10 European countries operating as a distributed infrastructure and offers researchers from Europe and around the world open access to a uniquely broad range of cutting-edge technologies and tools for the screening of chemical substances for their biological effects. EU-OPENSCEEN provides open access to the most advamced screening and medicinal chemistry technologies and expertise, and invites chemists to ensure long term sustainabilty of their compounds as part of ECBL.
Among the core capacities of a relevant number of EU-OPENSCREEN partner sites is the bioprospection of natural products, involving access to terrestrial and marine biological resources, natural products libraries of extracts and expertise in the screening and medchem development of compounds from natural products scaffolds. These capacities have enabled to propose an alternative offer of the discovery tools to researchers involved in natural product bioprospecting.
The presentation will briefly illustrate the different types of nodes involved, and the collaborations established by EU-OPENSCREEN with natural product bioprospecting researchers, research centers and consortia, as well as the new discovery platform EUREMAP engaging three infrastutures to respond to the needs of academia and industrial marine natural products biodiscovery.

EU-OPENSCREEN: A MULTINATIONAL INITIATIVE TO SUPPORT COLLABORATIVE PROJECTS IN CHEMICAL BIOLOGY

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The quality of a screening collection is one of the most critical factors of success in drug discovery. Academic compound collections often predominantly consist of compounds that are commercially available, but proprietary compounds synthesized by academic chemists as well as natural products represent a rich, untapped source for novel chemical diversity. In order to make the invaluable chemistry accessible to a broader scientific community and to allow chemists to uncover novel bioactivities of their compounds, EU-OPENSCREEN offers chemists the opportunity to make their compounds available, in a regulated and transparent framework, to a wider community of biologists, who screen these compounds in suitable bioassays. By doing so, chemists can expose their compounds to a broad range of different biological/drug targets to screen for unknown bioactivities of their compounds, which would otherwise not be feasible in individual one-to-one-collaborations. Once a compound has been identified as an active hit compound, a research collaboration between the chemist (who submitted the compound) and the biologist (who developed the bioassay) can be initiated.
The European research infrastructure EU-OPENSCREEN (www.eu-openscreen.eu) was founded in 2018 with the aim to support projects in early drug discovery. Through its ca.30 academic partner institutes in 10 European countries, it offers complementary expertise and instrumentation for the development of novel chemical research tools for the Life Sciences community. EU-OPENSCREEN partner sites jointly use a unique diversity compound collection containing commercial as well as proprietary compounds submitted by chemists from all over Europe. The primary screening data will be made available to the scientific community through its open-access European Chemical Biology Database (www.ecbd.eu).

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NATURAL PRODUCTS RESEARCH AT LATVIAN INSTITUTE OF ORGANIC SYNTHESIS AS A DRIVER FOR EXCELLENCE IN INNOVATION (NATALION)

Aigars Jirgensons,[1] Dace Karkle,[1] Stefano Donadio[1,2]

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NATALION is a Horizon Europe ERA Chair project of HORIZON-WIDERA awarded to a consortium comprising of Latvian Institute of Organic Synthesis (LIOS) and an Italian company NAICONS. The aim of NATALION is to foster innovation excellence of LIOS by the establishment the Natural Products Research group and implementation of the LIOS Innovation hub as a complementary structural change. The total budget of NATALION is 2.5 M Eur and duration is 5 years. The introduction of new research strands and the structural changes will be achieved under the leadership of experienced R&I professional ERA Chair holder, Dr. Stefano Donadio.

NATALION

Natural products (NPs) as drug leads have revitalized the interest of pharma industry as a result of revealing untapped biological resources, advances in genome mining and engineering, and the availability of powerful analytical tools.[1] Sourcing of NPs with high molecular diversity and wide spectrum of biological activity would strongly synergize with the existing LIOS competencies in drug discovery and will enable to sustain the competitivness of LIOS. Moreover, this would constitute an efficient engine to develop new technologies and products with high innovation potential not only for the pharma industry but also for agricultural and animal health industries.
https://natalion.osi.lv/

Acknowledgment: The Natalion project has received funding from the European Union's Horizon Europe Programme Widening Participation and Spreading Excellence under grant agreement No 101087181. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

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MARINE BIOPROSPECTING WITH AN ARCTIC PERSPECTIVE: THE MARBIO SCREENING PLATFORM AND ITS INVOLVEMENT IN LARGER EUROPEAN PROJECTS

Espen Holst Hansen,[1] Kine Østnes Hansen, [1] Teppo Rämä [1] and Jeanette Hammer Andersen[1]

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Marbio is a screening platform for marine natural products hosted by UiT the Arctic University of Norway. The main focus of Marbio is to identify and characterize bioactive secondary metabolites from Arctic marine plants, animals and microorganisms, and to evaluate their potential as starting points for drug development. In addition, we also run projects focusing on identifying new ingredients for food, feed and cosmeceutical applications. The marine biodiscovery pipeline at Marbio will be presented, and a couple of collaborative projects will be highlighted to illustrate how we work together with European partners from both industry and academia to increase the impact of our research.

MICRO4ALL: A MAJOR CHANGE IN DRUG DISCOVERY

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The world needs sustainable solutions to cope with an increasing and aging human population. Drug R&D programs are time-consuming, costly, and with no guarantee of success. Given the world population increase, fast and efficient development of new drugs is essential. Natural products have found numerous applications to treat and prevent diseases in humans, animals, and plants, and are widely used as research reagents to probe biological processes. They are a proven and sustainable answer to make the R&D process faster and more successful. *micro4all* is a platform that provides access to an extensive online Catalog of Molecules, present in ready-to-screen samples produced by NAICONS proprietary microbes. NAICONS has a collection of 45,000 microorganisms, among the most diversified in the world, and of 49,000 ready-to-screen extracts, a number that is being expanded continuously. Each microorganism produces hundreds of molecules that are present in the extracts and that are identified using a proprietary automated informatic process. The molecules, the extracts and the producing microorganisms are listed in a growing database called Catalog of Molecules. *micro4all* provides a search-and-order engine where Users can access information, molecules, extracts and the producing microorganisms. We will provide an overview of how the Catalog was created, what it contains and demos on the type of searches users can perform.

EMBRC-ESFRI ROLE IN THE DEVELOPMENT OF CUTTING EDGE RESEARCH SERVICES, A FOCUS ON MARINE BIOPROSPECTING

Donatella de Pascale

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The **European Marine Biological Resource Centre (EMBRC) is** a 'distributed research infrastructure' focused on research on marine organisms and ecosystems, promoting the sustainable characterization of their biodiversity and their biotechnological application. Its Headquarter is in Paris at the Sorbonne University, and its nine member countries are: Belgium, France, Greece, Israel, Italy, Norway, Portugal, Spain and Sweden. One of the strategic drivers of EMBRC is the development of its bioprospecting potential, creating a stronger intersection between the technological platforms of its operator and a tighter connection between the marine environments and their biotechnological value. EMBRC has, now, a service catalogue with more 430 services, often scarcely used, redundant between each other and difficult to navigate for the user. A more objective-driven and optimized service offer should draw together complimentary facilities and platforms to organising them into full service capable to provide solution to complex scientific issues or **connect services into advanced "pipelines"** managed by multiple operators. For this reason, the creation of an enhanced and sustainable **biodiscovery pipeline** from poorly studied marine ecosystems is considered pivotal of the successful positioning of EMBRC in the bioprospecting field and in 2020 an *ad hoc* Working Group has been established to reach this goal: the WG has already mapped the capabilities of EMBRC members to identify the scope of activities possible and to identify the technological platforms (equipment, pipelines, expertise) within EMBRC, identifying where opportunities for development exist and establish the basis for strategy implementation through funding and application to competitive projects. In 2021 this brought to a strengthened connection with the RI EU-OPENSCREEN and to support the starting grants in the H2020 RI VIS project, promoting interaction between the Institute for Microbial Biotechnology and Metagenomics (IMBM) at the University of Western Cape in South Africa and EMBRC. Moreover, SZN, Tel-Aviv University, University of Gent, CCMAR and Sorbonne University joined forces with other institutes for the preparation of the EUREMAP H2020 INFRA-DEV Project, aimed to fill the gaps and increase the interactions among EU-OPENSCREEN, ELIXIR and EMBRC RIs for a more efficient and innovative bioprospecting pipeline. This funded project will create a wider impact of marine bioprospecting towards PMIs dedicated to aquaculture, drug discovery, agronomy and, overall, societal changes.
Lastly, in September 2023 the SZN BluBio Department hosted the first official meeting of the EMBRC Bioprospecting WG, setting-up the starting point of new activities and defining the state-of-the-art of marine Bioprospecting in Europe, with a critical view of the gaps and potential for improvement of the actual pipelines.

ARE THERE BIODISCOVERY PROGRAMS IN LATIN AMERICA? THE IMPORTANCE OF ESTABLISHING INTERNATIONAL COLLABORATIVE INITIATIVES

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Biodiscovery programs established in USA and Europe proved as extremely powerful venues for the development of natural products chemistry associated with the unveiling of new biologically active metabolites, new biosynthetic pathways, biotechnology processes, sustaining global biodiversity and promoting knowledge to the society on the value of biodiversity conservation. However, very few of such programs have been developed locally between megabiodiversity countries in Latin America. The Merck-Inbio program was first idealized and settled in 1989, between the Costa Rican and American governments, to explore Costa Rica biodiversity for the discovery of biologically active compounds.[1] The International Cooperative Biodiversity Groups program was established by the US government led by the Fogarty International Center (FIC) of the NIH, and provided opportunities for collaborations between US and megabiodiverse countries around the world, including Latin America countries.[2] The National Cooperative Natural Products Drug Development Groups NCI/NIH program was similarly established by the US funding agencies, and also provided opportunities for collaborations between US and abroad scientists.[3] These were great initiatives to promote science and disseminate knowledge on the value of biodiversity and its conservation.

However, to the best of our knowledge, Brazil is the Latin American country that established two in-house biodiscovery programs: the FAPESP-BIOTA program, between the period of 2003 and 2020, and the CNPq-SISBIOTA program, between 2011 and 2016. Both Brazilian programs led to the numerous discoveries of bioactive compounds, biotechnological products and to the promotion of biodiversity knowledge, that are of extremely importance in the nowadays scenario of biodiversity loss and Earth degradation due to human activities and its consequences. A summary of data of FAPESP-BIOTA and CNPq-SISBIOTA programs will be presented and discussed, aiming to promote new venues between Latin American researchers towards biodiscovery and biodiversity conservation.

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INTEGRATION OF MASSIVE METABOLITE PROFILING DATA OF NATURAL EXTRACTS IN BIOACTIVITY SCREENING CAMPAIGNS FOR BIOACTIVE HITS PREDICTION AND EFFICIENT PRIORITIZATION

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Recent advances in high resolution mass spectrometry data dependent MS/MS analyses (HRMS/MS) have enabled the acquisition of increasingly precise data on plant and microorganism metabolomes. This allows mapping the composition of natural extracts at an unprecedented precision level [1]. This particularly permits the construction of virtual chemical libraries based on all putative annotation obtained. By combining annotation data sets generated from raw extracts, researchers can efficiently prioritize valuable natural products (NPs) for drug discovery. In this context, to improve the annotation confidence of our molecular networking (MN) approaches using ultra-high performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS/MS), we have integrated automated natural product (NP) class annotations and taxonomically informed scoring [2]. To further support this effort, we recently established an online resource for tracking NP structures and their occurrences in their respective source organisms [3]. We have applied this integrated approach to the investigation of a chemodiverse collection of 1,600 plant extracts from Pierre Fabre Laboratories which holds one of the largest plant samples library worldwide with over 17,000 samples. For chemical space exploration of such a massive metabolite profile dataset, we developed novel computational tools to efficiently prioritize and target the isolation of high-value bioactive NPs [4,5]. We have also started to use knowledge graph database that contains interconnected, spectral, structural, taxonomical and bioactivity data to interrogate such multi-informative datasets. The proof of concept and the exploration of such data in combination with results of various bioassays (anti-infective, anticancer, antiparasitic activities) will be exemplified. The potential benefits and challenges of using these approaches to transform the field of pharmacognosy in the current era of omics and digital science will be discussed.

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