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ABSTRACTS

Session I: Natural Products in Drug Discovery -1

Climate Change and Medicinal Plants

Thomas Efferth

Department of Pharmaceutical Biology, Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Mainz, Germany

Abstract:

Climate change and global warming reached utmost attention in recent years. Negative effects are not only expected for humanity but for all creatures on earth. Climate change will severely affect ecosystems or even destroy them. Plants are not only used in agriculture for food production but also for medicinal purposes. According to the World Health Organization (WHO), up to 80% of the populations in low-and-middle-income countries depend on medicinal plants from traditional medicine systems for primary health care. Increased environmental stress by increasing temperatures do not only affect the biomass production of medicinal plants but also alter their contents of pharmacologically active phytochemicals. We introduce several aspects of quantitative and qualitative changes in medicinal plants and discuss actions for plant conservation as well as sustainable cultivation and commercialization of medicinal plants.

Biomolecules, Medicinal Properties of Dessert Plants and their Nano Crystals for Better Therapeutic Effects

Sivakumar S. Moni

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Abstract:

The pharmaceutical importance of dessert plants is still yet to be explored for the significance of human society. However, traditionally desert plants have been used for therapeutic purposes to treat many ailments. The study investigated the biomolecules and their medicinal values of *Caralluma retrospiciens* and *Aloe fleurentiniorum*. Furthermore, injectable nanocrystals from the dessert plant, a novel method for creating a superior delivery system that increases the effectiveness of biomolecules and can serve as a better therapeutic agent.

In Silico Natural Products Optimization to Reverse Multidrug Resistance

Daniel J. V. A. dos Santos[1,2]*, Patrícia Rijo[1], Maria-José U. Ferreira[2], Vera M.S. Isca[1], David S. P. Cardoso[2], Ricardo J. Ferreira[3]

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Abstract:

Multidrug resistance (MDR) to anticancer drugs is one of the major impairments in current chemotherapeutic regimens. Aiming at developing new and selective modulators towards the most relevant ABC efflux pumps linked to MDR (P-glycoprotein, P-gp; Multidrug-resistance Protein 1, MRP1; Breast Cancer Resistance Protein, BCRP), small libraries of natural products with different scaffolds like macrocyclic diterpenes, flavanones, monoterpene Indole alkaloids or royleanones were studied using different *in silico* techniques (molecular dynamics, molecular docking, QSAR, pharmacophores) aiming at characterizing the ligand-protein interactions that increases the MDR reversers potency of the molecules. *In silico* techniques like molecular docking were used to assess the accuracy of structure-based drug discovery techniques in the prediction of the experimental MDR-reversal activities along with ligand-based drug discovery techniques to characterize the relationship between chemical modifications and the respective modulation capabilities. Our results show that, while selective efflux modulation can be achieved for each pump, some compounds additionally modulate drug efflux in more than one ABC transporter. New structure-activity relationships from ligand- and structure-based approaches allowed the identification of structural features related with the selective inhibition of more than one efflux pump.

Acknowledgments:

Project ILIND Seed Funding, CoSysCan: Combining Synergistic Approaches to Fight Cancer, (COFAC/ ILIND/ CBIOS/1/2021), is acknowledged for funding.

Tryptophan as a Source of p53 Activators with Anticancer Potential

Maria M. M. Santos

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Abstract:

Tryptophan is an essential amino acid present in the structure of several natural peptides, proteins, and indole alkaloids with a wide range of biological activities. Tryptophanol, obtained by reduction of tryptophan, is also present in the structure of several molecules with antimalarial and anticancer activities. In this talk, we will present our most recent results on the development of novel tryptophanol-derived p53 activators. Recently, we disclosed tryptophanol-derived oxazoloisoindolinones as new p53- activating agents with *in vitro* and *in vivo* antitumor activity against hepatocellular carcinoma. The pharmacokinetic profile of these tryptophanol-derived isoindolinones was investigated. From the metabolites' identification, performed by liquid chromatography coupled to high resolution tandem mass spectrometry, followed by their preparation and structural elucidation, it was possible to identify that the indole C2 and C3 are the main target of the cytochrome P450 (CYP)-promoted oxidative metabolism in the tryptophanol-derived isoindolinone scaffold. Based on these findings, to search for novel p53 activators a series of enantiopure tryptophanol-derived isoindolinones substituted with a bromine in indole C2 was prepared and their antiproliferative activity evaluated in human colon adenocarcinoma HCT116 cell lines with and without p53. Structural optimization led to the identification of two (*S*)- tryptophanol-derived isoindolinones 1.9 to 3.9-fold more active than the hit previously identified. Through differential scanning fluorimetry (DSF) experiments, the most active compound of the series in cell assays led to an increase in the protein melting temperature (T_m) of 10.39 °C, suggesting an effective binding to wild type p53 core domain.

Treatment with Herbs or Drugs

Emília p. T. Leitão

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Abstract:

The oldest written evidence of the use of medicinal plants to prepare drugs was found on a Sumerian clay slab from Nagpur, approximately 5,000 years old. The clay tablet contained 12 recipes with more than 250 different plants, some alkaloids like poppy, henbane and mandrake. People may feel more comfortable using a "natural" remedy prepared with a medicinal plant, which does not require a medical prescription, than using a synthetic drug (medicine) prepared through a synthesis process. Natural products are not subject to the same testing, manufacturing, and labeling standards and regulations as drugs. This means no universal regulatory system is in place to ensure plant remedies' quality, efficacy, and safety. In preparing an active pharmaceutical ingredient, the control is very tight by the competent authorities (FDA and EMEA). The FDA considers a product made from plants and used solely for internal use as an herbal supplement, not a drug. The use of natural products should be prescribed by those who know the area. However, it is essential to highlight that plant and animal products were the primary sources of drugs. This work shows the differences between "natural" remedies and medicines.

Discovery of Drug Leads and Chemical Probes from Australian Flora and Fauna

Rohan A. Davis

Griffith Institute for Drug Discovery (GRIDD), Griffith University, Brisbane, Australia

Abstract:

Australia is one of 17 countries described as megadiverse. While these 17 megadiverse nations cover <10% of the global surface, they collectively support >70% of the Earth's biodiversity. Biodiversity typically equates to chemical diversity, an important element for those undertaking biodiscovery, thus the use of Australian-sourced biota for natural products drug discovery and chemical biology research has a strategic advantage, since this unique resource has only been superficially explored for new pharmaceutical agents and chemical probes. This presentation will: (**i**) give a brief overview of NatureBank - a unique biodiscovery platform based on natural product extracts and fractions derived from Australian plants, fungi and marine invertebrates; (**ii**) describe the identification and biology associated with several new natural products that have recently been discovered by the Davis group and their potential impact to the field of biodiscovery; and (**iii**) detail the design and synthesis of several drug-like screening libraries that were created using isolated natural product scaffolds and semi-synthetic methodology.

Discovery of Anti-MRSA Protein from *Streptomyces Pluripotens* with Wound-healing Properties in MRSA-infection Mice Model

Learn-Han Lee[1]*, Yi-He Kuai[1], Jodi Woan-Fei Law[1], Vengadesh Letchumanan[1], Kar-Wai Hong[1], Kok-Gan Chan[2,3], Bey-Hing Goh[4,5], Tan Loh Teng-Hern[1,6]

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Bandar Sunway 47500, Malaysia; [2]Division of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia; [3]International Genome Centre, Jiangsu University, Zhenjiang 212013, China; [4]Biofunctional Molecule Exploratory Research Group (BMEX), School of Pharmacy, Monash University Malaysia, Bandar Sunway 47500, Malaysia; [5]College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China; [6]Clinical School Johor Bahru, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Johor Bahru 80100, Malaysia

Abstract:

This study demonstrates the promising result of a potentially novel protein discovered from the Streptomyces pluripotens strain A genome isolated from Malaysia mangrove soil. Biosynthetic gene clusters prediction using bioinformatic tools, such as antiSMASH, PRISM, DeepBGC, GECCO and SeMPI, and a hierarchical cluster analysis were performed to identify protein of interest. The gene coding for Protein A was selected and expressed in a heterologous expression system. Protein A exhibits anti-MRSA activity at sub-MIC level to which the standard drug for MRSA infection, vancomyin loses its efficacy. In the full-thickness wound mouse model of MRSA infection, topical application of Protein A confers wound healing activity. The delayed wound closure rate in the MRSA-infected mice was ameliorated by Protein A treatment. At day 6 post-treatment, the recovery for the infected wound treated with Protein A was the same as the non-infected wound (60% wound closure) while the infected wound with no treatment achieved 30% wound closure only. The skin histopathological analysis shows that mid-dose Protein A significantly improves the wound healing process, achieving the highest score in all the histological parameters (re-epithelialization, granulation tissue formation and collagen deposition). Furthermore, in silico characterization and protein structure prediction were also performed to better understand the functions of Protein A and the ongoing investigation of its interactions with relevant protein targets. In conclusion, Protein A could be developed into a new treatment for MRSA infections and improve wound healing in diabetic patients, contributing to the overall health and well-being of the population.

Session II: Natural Products in Drug Discovery -2

Alkaloids as Lead Compounds to Overcome Drug Resistance in Cancer

Maria-José U. Ferreira

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal

Abstract:

Medicinal plants have been playing a crucial role as source of drug lead compounds. The discovery of plant-derived bioactive compounds, based on their use in traditional medicine, has been considered an encouraging approach. Characterised for bearing diverse and complex scaffolds, alkaloids, found particularly in plants, are nitrogen-containing compounds commonly with strong biological properties. Aiming at obtaining compounds with anticancer activity to overcome drug resistance, in our research group, we have been focused on the phytochemical study of plants able to produce large amounts of alkaloids, allowing further molecular derivatization. The ability of natural alkaloids and derivatives as ABC transporter inhibitors, has been assessed in different resistant cancer cells. Several indole and Amaryllidaceae-type alkaloid derivatives were found to be strong P-gp inhibitors. Some of them displayed selective antiproliferative activity to P-gp/MRP1 overexpressing cells. To find new active compounds against breast and ovarian cancer, the anti-proliferative activity of libraries of alkaloid derivatives was also evaluated in an array of human breast and ovarian cancer cells. We have identified an indole alkaloid derivative that targeted homologous recombination

DNA repair defects, by disrupting BRCA1-BARD1 heterodimer complex, in triple-negative breast and ovarian cancers. Furthermore, it showed high in *vitro* and *in vivo* antitumor activity and synergised conventional anticancer drugs.

Acknowledgements:

Fundação para a Ciência e a Tecnologia (FCT), Portugal (project PTDC/MED-QUI/30591/2017).

Metabolomic Profiling of Mixed Endosymbiotic Fungal Culture in the Enhancement of Anti-biofilm Activity

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Abstract:

Biofilm formation by pathogens has played a vital role in multi-drug resistance bacteria like those of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. An increasing number of novel bioactive compounds are recently being isolated from endosymbiotic fungi. In this study, metabolomic approaches assisted media selection for optimum fungal growth and increase the yield of antibacterial secondary metabolites. In parallel, metabolomics tools were also used to pinpoint and efficiently improve the further isolation of pure antibiofilm agents from fungal-bacterial and fungal-fungal co-cultures by using statistical tools like multivariate analysis to correlate with the bioassay result. High resolution mass spectrometry and NMR were used to dereplicate and elucidate the biologically active agents recovered from the different co-cultures, which may be either new or have been previously discovered. The goal of this project was to investigate the metabolomic changes of anti-biofilm active metabolites in mixed endophytic cultures. The integration of orthogonal biological approaches was used to assess a set of chemical compositions and environmental variables, and to better understand the effects of metabolites with anti-biofilm activity. Different strategies have been utilised to activate such silent genes, including co-culture or mixed fermentation, that is a cultivation-based approach. This study highlights the potential of co-culture of endophytic microorganisms to induce the production of new metabolites as well as to increase the yields of respective target metabolites with pharmacological potential, and indirectly improved the biological activity of a crude extract.

Exploration of the Argentinian Flora Chemodiversity in the Search of Metabolites as New Leads in Drug Discovery

Maria Cecilia Carpinella

Instituto de Investigaciones en Recursos Humanos y Sustentabilidad José Sánchez Labrador S.J., IR-NASUS-CONICET. Universidad Católica de Córdoba. Córdoba, Argentina.

Abstract:

Plants are an excellent reservoir of biologically active compounds with a significant contribution to cancer and microbial chemotherapy. The great biodiversity of existing plants results in a wide variety of secondary metabolites which enjoy the presence of varied pharmacophores and a high degree of stereochemistry that give them high possibilities of emerging as prominent therapeutic agents. The need of alternative drugs, particularly for the treatment of pathologies resistant to

clinical therapies, encourages the search for new substances in the plant world. This bioprospection becomes even more relevant and novel when it involves species from the low explored Argentinian flora. As part of our continuing search for agents to treat cancer and microbial infections, a panel of 170 extracts from mostly native plants from Argentina and the bioactive metabolites obtained from these, were screened in order to determine their cytotoxic and multidrug-resistance (MDR) reversing potential against sensitive and P-gp-overexpressed cell lines. In addition, their inhibitory effect against efflux transporters expressed in *Candida* species was evaluated, as well as their inhibitory activity on growth of sensitive and resistant pathogenic bacteria. The most effective principles were further studied in view of determining their modes of action. Derivatives of these metabolites were designed and synthetized showing improved activity in comparison to the structural leads. These findings provide important evidence that the identified compounds may be a promising entities to be further investigated to develop agents able to overcome MDR and to treat resistant tumors and emerging infections.

Session III: Marine Natural Products-1

Design of Antimalarial Compounds Inspired by Natural Products

Rui Moreira

Department of Pharmaceutical Sciences and Medicines, Portugal

Abstract:

Natural products continue to play an important role in identifying novel and structurally unique chemotypes endowed with antimalarial activity. Typically, phenotypic high throughput screening campaigns performed in industry and academia, implies prioritization of hit compounds based on stringent requirements including chemical diversity, physicochemical properties, and potency against intraerythrocytic and liver stage malaria parasites. We will disclose the optimization of one hit selected from a publicly available data set into dual-stage antiplasmodials capable of killing intraerythocytic and liver-infecting parasites. The structure-activity relationship (SAR) investigation of the novel chemotype enabled the generation derivatives active against intraerythrocytic and liver stage malaria parasites, and endowed with oral efficacy. In addition, we provide details on the studies performed to explore the mechanism of action, showing that the novel class compounds can hit different targets in the respiratory chain of the parasite.

Valorization of Brown Seaweeds as a Natural Source of Valuable Bioactive Compounds

Susana M. Cardoso*, Marcelo D. Catarino, Ana R. Circuncisão, Sónia Silva, Artur M.S. Silva

LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Abstract:

Seaweeds have been part of the human nutrition for centuries, particularly among the Far East populations to which seaweed cultivation, processing and consumption represent a great source of economic income. Indeed, seaweeds are a well-known source of bioactive compounds, with more than 15,000 novel compounds being reported over the last years and taking place in countless industrial applications. Following different extraction strategies, we were able to obtain economic-affordable extracts rich in specific compounds, including phlorotannins, fucoxanthin, fucoidans, laminarans and alginates from important European commercially important brown macroalgae, namely F.

vesiculosus, L. digitata and H. elongata. Naturally, different compositions have different biological properties, and we have demonstrated that some of them can exert interesting anti-inflammatory activities through inhibition of the NF-kB signaling cascade, while others may activate apoptosis in tumor cells, contribute interfere with important enzymes associated to metabolic disorders (e.g. α-amylase, α-glucosidase and pancreatic lipase) or modulate gut microbiota growth and activity. Moreover, and considering the challenges of the modern agrifood sector to move towards eco-friendly practices, we have found that these extracts can also be used as crop bio-stimulants, with great protective effects against abiotic stress, particularly drought. The overall results ultimately demonstrate that seaweeds offer a plausible strategy to tackle several SDGs simultaneously and contribute for a world where we can live better together.

Effect of Prior Frozen Storage and Octopus Waste Addition to the Packing Medium on the Rancidity Stability of Canned Fish

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[1]Department of Food Technology, Marine Research Institute (CSIC), Vigo, Spain; [2]Department of Food Technologies, CIFP Coroso, Ribeira, Spain.

Abstract:

To increase the rancidity stability of canned seafood, natural antioxidants from different sources have been tested as packing systems. This study focused on the antioxidant behaviour of octopus cooking liquor (OCL) resulting from common octopus (*Octopus vulgaris*) processing. In it, the effects on lipid damage in canned horse mackerel (*Trachurus trachurus*) of a prior frozen storage (−18 ^{0}C) period and the presence of an OCL in the packing medium were investigated. An increase of the frozen storage time favoured an increase of free fatty acid (FFA), peroxide, and thiobarbituric acid reactive substance contents and a decrease of the phospholipid (PL) value and polyene index. Furthermore, an increased presence of OCL in the packing medium led to an inhibitory effect on fluorescent compound formation as well as to retention of the PL and FFA compounds. Colour determination showed a substantial increase of *L** and *b** values in canned fish with previous frozen storage time. Nevertheless, this increase was partly reduced by the OCL presence in the packing medium. It is concluded that previous holding time has led to an increased lipid oxidation development and loss of beneficial lipid constituents (i.e., PLs and polyunsaturated fatty acids). Remarkably, the presence in the packing medium of preservative compounds (i.e., antioxidants) included in waste juice obtained from octopus processing provided an effective tool for lipid preservation and quality enhancement in canned fish.

Structural Diversity and Antimicrobial Activity of South African *Laurencia* Natural Products

Jameel Fakee,[1] Marilize le Roes-Hill,[2] John J. Bolton,[3] Edith M. Antunes[4] and Denzil R. Beukes[5*]

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Abstract:

Natural products remain the leading source of new antibiotics against pathogenic microorganisms. However, the rapid development of resistance against common antibiotics combined with the slow pace of new antimicrobial drug discovery present a significant risk to human health. Coastal marine organisms, such as seaweeds, are exposed to significant levels of microbial pathogens (including human pathogens) but suffer remarkably low levels of microbial infection. This may be due the presence of natural products with antimicrobial activity. In the current study, we screened a library of more than 200 marine algal extracts for activity against *Acinetobacter baumannii*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus subsp. aureus* and *Candida albicans*. The natural products with the most promising antimicrobial activities were isolated and their structures determined using standard spectroscopic methods. Halogenated sesquiterpenes, C15 acetogenins and indole alkaloids from *Laurencia* spp showed potent antimicrobial activities (MIC < 5 µg/mL).

Session IV: Bioactivity of Natural Products, Functional Food and Food Supplements and Natural Products in Drug Discovery

Application of Essential Oil in Veterinary Sciences

Douglas Siqueira de Almeida Chaves

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Abstract:

Brazil has always played an important role in the export of essential oils. Historically, the export of Rosewood EO (1930 – 1980), sassafras EO (1940 – 1980), cornmint EO (1960 – 1990), orange EO (1970 – current) and eucalyptus (EO 1950 – current) have generated a lot of wealth in Brazil. The essential oils market demands a minimum of quality and reproducibility for the final product. Due to the success of oil-based products, the high biological activity in veterinary models and the increase in the number of works in the area, the pet market is aligned with the One Health (alternative to pest control products that do not cause negative impacts on human and animal health, the environment, and natural resources it is promising. The world pet market is currently the fastest growing in the world. The Brazilian Pet Industry Association estimates that the world pet market had turnover of US $ 124.6 billion in 2018, representing growth of 4.3% in relation to 2017, when the turnover was US $119.5 billion. The United States is the country with the highest sales, accounting for approximately 40.2% of all world sales, the use of medicines with less severe side effects, including for the prevention and treatment of infestations by parasites such as fleas and ticks, as well as with public health regarding the transmission of pathogens from animals to humans. In addition to human exposure, medicines administered to pets to control fleas and ticks can be introduced into the wastewater treatment system during the routine bathing of animals, since they are often bathed in residential bathrooms or by professional services where the waste is sent directly to the sewer system. Fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus*) transmit diseases to domestic animals, requiring their followed by Brazil. China now has a share of 3.1% of the world market, while until 2016, it was not among the top 10 worldwide. We are present several potent essential from native an exotic plant to treat fleas, and possible compounds for the development of new products based in the One Health.

Prebiotic Proanthocyanidins Inhibit Bile Reflux-induced Esophageal Adenocarcinoma through Reshaping the Microbiome-metabolome Axis

Laura A. Kresty[1,2]*, Katherine M. Weh[1,2], Connor L. Howard[1,2], Yun Zhang[1,2], Jennifer L. Clarke[3], and Amy B. Howell[4]

[1]Department of Surgery, Section of Thoracic Surgery, University of Michigan, Ann Arbor, Michigan, USA; [2]Rogel Comprehensive Cancer Center, University of Michigan, Ann Arbor, Michigan, USA; [3]Department of Statistics, Department of Food Science Technology, Quantitative Life Sciences Initiative, University of Nebraska-Lincoln, Lincoln, Nebraska, USA; [4]Department of Plant Pathology and Biology, Marucci Center for Blueberry and Cranberry Research, Rutgers University, Chatsworth, New Jersey, USA

Abstract:

The gut and local esophageal microbiome progressively shift from healthy commensal bacteria to inflammatory-linked pathogenic bacteria in patients with gastroesophageal reflux disease, Barrett's esophagus and esophageal adenocarcinoma (EAC). However, mechanisms by which microbial communities and metabolites contribute to reflux driven EAC remain incompletely understood and in turn challenging to target. Herein, we utilized a rat reflux-induced EAC model to investigate targeting the gut microbiome-esophageal metabolome axis with cranberry proanthocyanidins (C-PAC) to inhibit EAC progression. Sprague Dawley rats, with or without reflux-induction received water or C-PAC *ad libitum* (700 µg/rat/day) for 25 or 40 weeks. C-PAC exerted prebiotic activity abrogating reflux-induced dysbiosis, and mitigating bile acid metabolism and transport, culminating in significant inhibition of EAC through TLR/NF-κB/P53 signaling cascades. At the species level, C-PAC mitigated reflux-induced pathogenic bacteria including *Clostridium perfringens, Escherichia coli,* and *Proteus mirabilis*. In addition, C-PAC specifically reversed reflux-induced bacterial, inflammatory, and immune-implicated proteins and genes (i.e., *Ccl4, Cd14, Crp, Cxcl1, Il6, Il1β, Lbp, Lcn2, Myd88, Nfkb1, Tlr2 and Tlr4)* which aligns with changes documented in human EAC progression, as confirmed through public databases. C-PAC is a safe promising dietary constituent which may be utilized alone or potentially as an adjuvant to current therapies to prevent EAC progression through ameliorating reflux-induced dysbiosis, inflammation and cellular damage. Importantly, C-PAC is well tolerated, exerting cancer inhibitory effects at behaviorally relevant dietary concentrations.

Antineoplastic Effects on Pancreatic Ductal Adenocarcinoma Cell Line using Cyclic Terpenophenolic Varinoid, Olivetoids and their Derivatives

Maite Docampo-Palacios[1]*, Giovanni Ramirez[1], Arianna Collins[1], Yousef Mzannar[2], Husain Khan[2], Omar Aboukameel[2], Asfar Azmi[2], Prakash G. Jagtap[1], Kyle P. Ray[1], Westley Cruces[1]

[1]Colorado Chromatography Labs LLC., United State; Karmanos Cancer Institute, Wayne State University, United States

Abstract:

Tetrahydrocannabivarin (THCV), a rare cannabinoid, is a homologue of THC, with three atoms in alkyl chain. THCV has garnered attention in clinical trials as an anti-obesity drug treating glucose issues. Hexahydrocannabinol (HHC), and Hexahydrocannabivarin (HHCV) are hydrogenated analogues of THC and THCV respectively (Figure 1). Increased popularity of these rare cannabinoids has led to proposed experimentation leading to assessing the cytotoxicity of these cannabinoids toward cancer cells of the pancreas (MIA-PaCa2, HPAF-II, and PANC1). Synthesizing analogs with similar pharmacophore motifs, CCL-104 and CCL-105 were produced, and testing for cytotoxicity towards pancreatic cancer cells. The data evaluated through this studies led to the statement that these cyclic cannabinoids can be used towards the treatment of pancreatic cancer due to the efficacy as single agent antineoplastics compared to common single agent antineoplastics, with evidence being

strongly presented when compared to commercially available anticancer agents poly (ADP-ribose) polymerase (PARP) inhibitors. (Table 1)

Table 1. IC50 values of cannabinoid compounds and commercially available anticancer agents.

Compound	PANC-1 (μM)	HPAF-II (μM)	AsPC-1 (μM)	Mia-PaCa2 (μM)
THCV	14.7	8.8	8.9	9.6
(R)-HHC	10.3	10.8	19.6	12.6
(S)-HHC	18.9	11.6	13.9	27.2
HHCV	5.5	*nd*[1]	*nd*[1]	21.2
CCL-104	1.06	*nd*[1]	*nd*[1]	1.3
CCL-105	2.55	*nd*[1]	*nd*[1]	6.1
Olaparib	*nd*[1]	21.4	*nd*[1]	9.1
Veliparib	*nd*[1]	35.3	*nd*[1]	25.2

nd[1]: not determined.

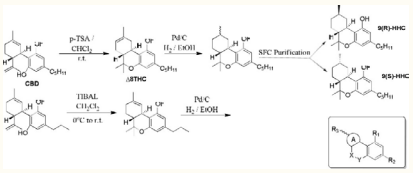

Figure 1: Synthesis pathways of hydrogenated cannabinoids and structure scaffold of CCL compound

Studies of Natural Products against Metabolic Disorders

Fatima Rivas [1]*, Taotao Ling[1], and Benedict Barras[1]

Louisiana State University A &M[1], 431 Chemistry & Materials Building, Chemistry Department, USA, *Baton Rouge, LA 70803*

Abstract:

Adipocytes, specialized lipid storage cells play a critical role in energy homeostasis. However, adipose tissue dysfunction occurs with aging and has systemic effects, including peripheral insulin resistance, ectopic lipid deposition, and inflammation. Glucocorticoid signaling plays an integral role in the progression of dysfunctional adipose tissue. We and others have shown that selective inhibition of 11β- hydroxysteroid dehydrogenase type 1 (11β-HSD1) can directly regulate glucocorticoid action at the tissue level. However, there are other factors that may play a role in preadipocyte differentiation, proliferation, and ectopic lipid deposition in adipose tissue. Our laboratory has shown that the natural product colletoic acid (CA) significantly reducesadipogenesis of pre-adipocytes via selective inhibition of 11β-HSD1, dialing down glucocorticoid action without inducing cytotoxicity. We solved the first x-ray structure of CA-11β-HSD1 complex to demonstrate the exact mode of action of CA. However, the parameters that regulate adipogenesis and overall cellular homeostasis are broader than glucocorticoid signaling, and we hypothesize that in combination with reactive oxygen species modulation dysfunctional adipose could be prevented and treated. Recently, we have identified other natural products that can modulate adipogenesis in combination with CA to modulate lipid droplet size. We will discuss these findings and propose the mode of action of these compounds. This investigation provides new chemical tools based on natural products and valuable knowledge on selectively targeting an important signaling pathway in adipose biology, which will serve for the development of future therapeutic strategies to support clinical endeavors.

Informed High Throughput Platform to Identify Selective Modulators of Cell Death

Taotao Ling[1*], Jack Menard[1] and Fatima Rivas[1]

Louisiana State University A &M[1], 431 Chemistry & Materials Building, Chemistry Department, USA, Baton Rouge, LA 70803

Abstract:

Cancer is the second leading cause of death worldwide and the identification of targeted and selective new anticancer agents would offer alternative treatments to minimize the detrimental side effects of non- selective chemotherapeutic agents. Approximately 65% of approved anti-cancer drugs and antibiotics originated from molecular structures isolated from natural sources. However, screening and deconvoluting natural product extracts can be time and financially constrained. To circumvent this obstacle, an informed-phenotypic cell-based high throughput platform was designed to screen a library of natural product fractions from American terrestrial plants with the objective to rapidly identify novel hit compounds against cancer cell models, occupying a diverse chemical space from current candidate leads. We used isogenic cancer cell models with pro-apoptotic Bcl2 members (Bax and Bak) double knockout and anti-apoptotic (Bcl2 members, Mcl-1, Bcl-XL, Bcl-W, and Bfl-1) knockin for the primary screen, enabling the identification of genotype selective natural product fractions. Our study identified several natural product fractions that display cytotoxicity against cancer cell lines with little or no cytotoxicity against normal cells. Herein, specific natural products will be highlighted to demonstrate thepromise of these academic endeavors with natural products.

Flash Talks

Research of Natural Antiviral Substances against Mosquito-borne Flaviviruses in the Flora of the Reunion Island, using Bio-guided Fractionation and LC-MS/MS-based Molecular Networking

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Abstract:

Reunion Island, a french overseas region, is classified as a biodiversity hotspot due to its exceptional flora and endemic plants that could be sources of new medicines. Tropical and subtropical countries are areas that are prone to viral infectious diseases. Dengue virus (DENV) and Zika virus (ZIKV) belong to the genus Flavivirus of the Flaviviridae family and are transmitted to humans by mosquitoes of the *Aedes* genus. The epidemic of DENV is currently taking place in Reunion Island. Epidemics of emerging ZIKV have been recently recorded in the Americas, Asia, and the Pacific. Infection with DENV or ZIKV has been linked to serious clinical outcomes in humans. To date, there is still no specific vaccine or treatment for ZIKV or DENV. In order to meet this need, we performed an *in vitro* antiviral screening on 526 plant extracts prepared from endemic or indigenous plant species from Reunion Island. Our screening has allowed the identification of 10 endemic plants that are able to inhibit ZIKV and DENV infection of Vero cells at non cytotoxic concentrations. Based on an exhaustive review of the literature, 2 species *Bertiera borbonica* and *Turraea ovata* are retained. The bio-guided fractionation combined with the molecular network analysis using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS2) are applied to investigate the extracts

from *B.borbonica* and *T.ovata*. Thanks to this double approach, chemical families responsible for the antiviral activity are identified and the new active phytocompounds can be isolated. Together, our data provide novel insights for the potential of Reunion Island plants as promising source of natural antiviral compounds in the fight against medically important flaviviruses.

Diversity-oriented Synthesis of Halimane Derivatives for Improved Biological Activity

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Abstract:

Plectranthus spp. (Lamiaceae) have been used in traditional medicine across Africa, Asia, and Brazil, to alleviate digestive disorders, skin and respiratory conditions, infections, fever, pain, and musculoskeletal conditions. Their main phytochemical constituents, diterpenes and phenolic compounds, could play a role in drug discovery and development. *P. ornatus* Codd. has been used traditionally for alleviating a wide range of ailments, including infections. The halimane compound 11*R**-acetoxyhalima-5,13*E*-dien-15-oic acid (**HAL**) is the main constituent of the *P. ornatus'* acetonic ultrasound-assisted extract, and was found to have interesting biological activities, such as moderate anti-inflammatory effects, antimycobacterial activity and cytotoxicity. In a prior study, we found promising results on the antimycobacterial activity of a derivative of **HAL**, which inhibited bacterial growth to a similar extent as a clinically used antitubercular drug. In the herein work we present the physicochemical characterization of the starting molecule of **HAL** that was characterized through SCXRD, ^{1}H- and ^{13}C-NMR, FTIR, HSM (170 °C), DSC and TG. Namely, SCXRD results showed that carboxylic groups of **HAL** which is involved in hydrogen bonds giving rise to $R_2^2(8)$ homosynthons. Moreover, some new **HAL** derivatives were synthesized, functionalized using amines, to potentially improve the biological activity. **HAL** derivatives **1, 2** and **3** were successfully prepared and their structural characterization was confirmed by ^{1}H-,^{13}C-NMR, and FTIR. Further physicochemical and biological activity characterization of the analogues are on-going.

The Investigation of the Anticonvulsant and Anxiolytic Effects of some *Stachys* L. and *Teucrium* L. Taxa and Bioassay-guided Isolation of Active Substances

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Abstract:

Stachys and *Teucrium* taxa (Lamiaceae) have been traditionally used for the treatment of various disorders, including epilepsy and anxiety (1,2). The aim of the study was to rationalise its traditional usage on the basis of scientific results by using bio-guided fraction. The anticonvulsant and anxiolytic activities of the extracts of the *S. byzantina*, *S. officinalis*, *S. cretica* subsp. *anatolica*, *T. chamaedrys* subsp. *chamaedrys* and *T. montanum* was investigated in the PTZ model of seizures and elevated plus maze test in BALB-C albino mice, respectively. The aqueous fraction of *S. byzantina*, which was exhibited the highest potential, was fractionated to isolate the effective compounds. Three compounds were isolated and their structure were elucidated using spectroscopic methods (MS, 1D-2D NMR). The most effective total extract in terms of both activities was found to be *S. byzantina* extract. The results demonstrated a significant increase in latency to PTZ-induced myoclonic seizures provided by *S. byzantina* total extract (60.17±2.66 s), aqueous fraction (68.83±4,11 s), active fraction separated by the column chromatography (68.83±3.52 s), isolated compound-I (60.33± 2.95 s) and compound-II (Chlorogenic acid) (78,4±4,01 s) compared to the control (42.17±3.32 s) ($p < 0.05$*). These results showed that the total extract and aqueous fraction of *S. byzantina*, and the compound isolated from aqueous fraction exhibited significant anticonvulsant properties ($p < 0.05$*). This is the first study on the anticonvulsant and anxiolytic effects of these taxa which are growing in Turkey.

Acknowledgements:

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Valorization of the Neglected Species *Verbascum pulverulentum* Vill. from Côa Valley for Possible Cosmetic Applications

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Abstract:

Verbascum pulverulentum Vill. is a common plant species, in the landscapes of the Northeast of Portugal, namely in Côa Valley. Interestingly, this territory is classified as UNESCO World Heritage Site since 1998, once it is recognized as "the most important open-air Paleolithic rock art site". In traditional medicine, *Verbascum* spp. are used to treat several skin ailments, including eczema, insect bites, nail infections, and wounds. Despite its abundance, *V. pulverulentum* could be considered a neglected plant species once it lacks proper scientific validation for its popular uses, such as the poultices made with the leaves that are used for wounds' healing. Bearing in mind the several skin-related popular uses of this plant species, we decided to screen its cytotoxic effects and antioxidant activity to potentially, in the future, incorporate it into scientific-validated plant-based cosmetic formulations of the cosmetic brand that we will create for Côa Valley. Therefore, two hydroalcoholic extracts (EtOH 80%) were independently prepared from flowers and leaves of this plant, and total phenolic compounds (TPC) and total flavonoids content (TFC) were determined. Non-cytotoxic concentrations for potential topical application were determined *in vitro* using the Normal Human Dermal Fibroblasts (NHDF) cell line, through the Alamar blue® and sulforhodamine B® (SRB) assays. Accordingly, flowers and leaves' extracts were non-cytotoxic for concentrations ≤ 0.8 mg/mL and ≤ 0.4 mg/mL, respectively. Afterwards, non-cellular techniques were used to screen the antioxidant activity, namely through the DPPH, ABTS, CUPRAC and FRAP assays, showing that leaves' hydroalcoholic extract has higher antioxidant potential over the flowers' extract.

Obtaining Carvacrol from *Origanum onites* L. Essential Oil and Developing Carvacrol-loaded Nanoformulation for Use in Cosmetics

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Abstract:

Carvacrol is an essential natural component and is found prominently in aromatic plants as an essential oil and is well known for its anti-hyaluronidase, anti-collagenase and anti-elastase activity. The aim of this study is to obtain carvacrol from the *Origanum onites* L . essential oil, and to prepare carvacrol-loaded polycaprolactone (PCL) nanoparticles (NPs) as an approach in cosmetic industry with anti-aging activity. The components of the obtained carvacrol were analyzed using GC-MS. The carvacrol-NPs were synthesized by the single-emulsion method, and characterized by using UV-Vis spectrometry, Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM). It was determined that the carvacrol-NPs had a 199.5 nm average particle size, 0.070 PdI, and -8.51 mV zeta potential. The encapsulation efficiency and loading capacity were calculated as 98.55% and 20%, respectively, and the *in vitro* drug release study showed that carvacrol was released 95.55% over the 48 h period.

Plectranthus ecklonii Benth.: A Potential Plant with Clinical Use?

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**Presenting author. †Both authors shared first authorship.*

Abstract:

Plectranthus species (Lamiaceae family) have widespread ethnobotanical and traditional uses around the world. The extensive use of these plants suggests that this genus may be highly promising for the discovery of medicinal compounds.This work provides insight into the current knowledge of phytochemistry and studied bioactivities of one species of the *Plectranthus* genus, the *P. ecklonii* Benth. The results showed that 29 compounds have been isolated and structurally elucidated to date, being 5 of them the diterpenes Parviflorone D (ParvD), Parviflorone E, Parviflorone F, Parviflorone G, and Sugiol. Moreover, several bioactivity assays have been performed with the extracts, fractions, and isolated compounds being the most promising ones the antitumoral, dermatological, and antidiabetic. ParvD isolated from acetonic extract showed a more pronounced activity in glioblastoma (GB) cell lines and at lower doses than the TMZ (current drug of choice) (13 and 175 times lower); the mechanism involved in this effect was proven to be the intrinsic apoptosis in these tumour cells. In another study, it was also evidenced that the isolated compound ParvD inhibits tyrosinase (72.7±5.6 % enzyme inhibition), collagenase (84.6±5.9 % enzyme inhibition), and to a lesser extent, elastase (52.8±3.8 % enzyme inhibition). On the other hand, a recent paper published on this specie evidenced its antidiabetic potential since some of their components possess alpha-amylase and alpha-glucosidase activities. In summary, all this evidence corroborates that *P. ecklonii* and its isolated components are natural products of special interest for pharmaceutical drug development.

TransfersomILs: A Biobased Strategy for the Skin Delivery of Hydroxycinnamic Acids

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Abstract:

Hydroxycinnamic acids, such as caffeic and *p*-coumaric acids, are commonly present in natural sources, such as plants (e.g., bamboo shoots), cereals (e.g., corn, rice), vegetables (e.g., beans), and fruits

(e.g., orange, apples). These compounds are the largest group of phenolic acids, displaying several interesting properties (antioxidant, anti-inflammatory, and antimicrobial) for the pharmaceutical or cosmetics industries. However, these compounds present low aqueous solubility, which impairs their incorporation into drug delivery systems. TransfersomILs, an innovative combination of biobased ionic liquids (ILs) with transfersomes, may be a valuable tool for the skin delivery of hydroxycinnamic acids. These new nanocarriers combine the elasticity and deformability of transfersomes with the multifunctionality of biobased ILs. Our goal was to formulate TransfersomILs to load caffeic or *p*-coumaric acid, in the presence of (2-hydroxyethyl) trimethylammonium phenylalaninate [Cho][Phe] or (2-hydroxyethyl) trimethylammonium glycinate [Cho][Gly]. These nanosystems were characterised in terms of physicochemical properties, storage stability, and *in vitro* bioactive release. TransfersomILs loading hydroxycinnamic acids presented a smaller hydrodynamic diameter and improved association efficiency than transfersomes without ILs. ILs also contributed to the increment of the colloidal stability of the nanocarriers, upgrading their storage stability. Additionally, TransfersomILs improved the amount of active released over time. Therefore, TransfersomILs seem to be valuable nanocarriers for the skin delivery of hydroxycinnamic acids. Studies on the safety and efficacy of these nanosystems are ongoing.

Acknowledgements:

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Enhancing Breast Cancer Therapy via Protein Kinase α Activation: Derivatization of Natural Cytotoxic Diterpenoid

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Abstract:

Breast cancer represents a pressing global health concern, demanding the exploration of novel and effective therapeutic strategies. Protein kinase C-α (PKC-α), a serine/threonine kinase of the PKC family has been implicated as a crucial protein involved in the pathogenesis of breast cancer. *Plectranthus* spp. (Lamiaceae) is rich in bioactive compounds, particularly diterpenes. Among these compounds, the cytotoxic abietane diterpenoid 7α-acetoxy-6β-hydroxyroyleanone (**1**, Figure 1) was identified as the major constituent of *P. grandidentatus* acetonic extract. Furthermore, compound **1** has shown potential as a lead molecule for interacting with PKC isoforms and has exhibited promising activity in several breast cancer cell lines. The main goal of this work was to functionalize lead **1**, through esterification, to enhance its cytotoxic effect. Previous studies have demonstrated that ester derivatives have improved stability and bioactivity compared to the original royleanone. A collection of new royleanone analogs were prepared by hemi-synthesis of **1** and were evaluated on breast cancer cell lines (MCF-7, MDA-MB-231, and MDA-MB-468) and non-tumorigenic fibroblasts (HFF-1). Some of the derivatives exhibited selectivity towards cancer cells. Among them, the derivatives **2** to **5** (Figure 1), were selected for further investigation on a yeast-based assay screening assay, as PKC-α activators. Derivative **2** emerged as the most promising candidate, exhibiting significant potency as a PKC-α activator. To further validate its efficacy, derivative **2** was evaluated in a PKC kinase activity assay. Encouragingly, derivative **2** demonstrated a higher degree of PKC-α activation potential compared to the positive control (phorbol 12-myristate 13-acetate, PMA), indicating its

ability to modulate this important signaling protein. These findings provide a strong basis for further exploration of derivative **2**, including studying the mechanisms of PKC modulation and evaluating its therapeutic efficacy in preclinical models of breast cancer.

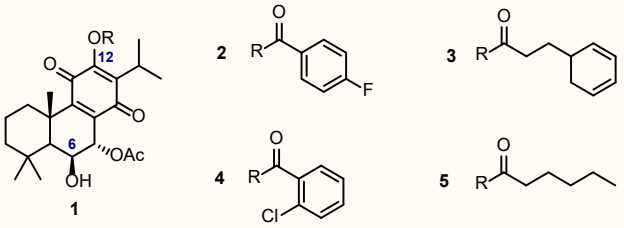

Figure 1 - Abietane diterpenoids: natural 7α-acetoxy-6β-hydroxyroyleanone (**1**) obtained from *P. grandidentatus* and semi-synthetic derivatives evaluated as PKC-α activators (**2** to **5**)

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Centauri Honey: A Promising Medicinal Ingredient?

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Abstract:

Honey is a natural product that has been used over the centuries as a medicine due to its biological activities. Centauri Cave Nymph Honey is a Cave honey extracted from 2500 meters high altitude above sea level from a deep cave by professional speleologists and is located at Caucasus Mountains of Turkey. The *Apis mellifera* Caucasica bee colony is located 50 kilometers away from human residences, ensuring its isolation from other colonies and maintaining a varroa mite-free status. The aim of this work is to analyze the physicochemical parameters and the bioactivity of Centauri honey. The physiochemical parameters that have been examined include color, moisture content, conductivity, pH, acidity, HMF (5-hydroxymethylfurfural), diastase index, and proline. In addition, it was also evaluated the ash, protein, sugars, carbohydrates, and energy. The biological activity was evaluated through the antioxidant (TBARS), antimicrobial activities and cytotoxicity in different cell lines (AGS, CaCo-2, MCF-7, NCI-H460, PLP2, HFF-2, and HaCat), and anti-inflammatory activity (RAW 264.7 macrophages). Ongoing research is focusing on the potential protetive effects of consuming

Centauri Cave Honey against lung and prostate cancers. In vivo studies are expected to shed more ligt on the additional health benefits that this honey may offer.

Acknowledgements:

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Molecular Docking Studies and In Vitro Analysis of P-gp Inhibitors from *Plectranthus* Diterpenoids

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Abstract:

As cancer cases worldwide continue to increase, there is an urgent need to discover new treatments to combat the disease. Unfortunately, in the case of multi-drug resistant (MDR) cancers, effective therapy is hindered by the overexpression of membrane transport proteins such as P-glycoprotein (P-gp). Plectranthus species, well-known for their medicinal properties, are a valuable source of diterpenes, such as the 7α-acetoxy-6β-hydroxyroyleanone (Roy) and Coleon U, which have demonstrated cytotoxicity against various cancer cell lines[1]. Based on molecular docking, SwissADME and ADMET simulations[2], semi-synthetic derivatives of Roy and Coleon U, that displayed strong P-gp interactions in silico, were prepared. To evaluate the potential antitumor activity of the compounds, resistant human cancer cell lines NCI-H460/R and DLD1-TxR were tested. The MTT assay was used to assess cell viability, while Annexin V/PI was used to determine cell death induction. The findings revealed that Roy derivatives 2, 3, and 4 exhibited significant selectivity (2.7, 2.3, and 2.6 times, respectively) for cancer cells compared to normal lung fibroblasts (MRC5). Additionally, Roy derivatives 2, 3, and 4 demonstrated a reduction in P-gp activity in the Rho123 accumulation assay and showed P-gp inhibition in the DOX accumulation assay for resistant cell lines NCI-H460/R and DLD1-TxR. These results demonstrate that abietane diterpenoid derivatives are capable of inducing P-gp inhibition in MDR cancer cell lines, presenting a novel set of selective compounds for the treatment of lung and colon cancer. Further investigations are ongoing to ascertain the anticancer activity of the Coleon U derivatives to obtain hit P-gp modulators.

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C. elegans: An Alternative *In vivo* Model for Discovering Biological Activities of Natural Compounds

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Abstract:

Caenorhabditis elegans (*C. elegans*) has served as an *in vivo* model organism for screening the biological activity of natural compounds for several years and has become a valuable tool for the discovery of novel therapeutic agents due to its simple anatomy, short lifecycle, and significant genetic similarity to humans. Its approximately three-days of lifecycle and three weeks of lifespan -that becomes even shorter at high temperatures- enables rapid and efficient screening of large number of compounds and extracts. Additionally, *C. elegans* has a transparent body, which makes internal organs and cellular processes easy to observe. Its simple nervous system and transparent body make it an excellent tool for investigating the effects of natural compounds on a wide variety of physiological processes. Furthermore, *C. elegans* possesses a well-characterized genome and a variety of genetic manipulation tools, allowing researchers to investigate the mechanisms underlying the effects of natural compounds. As a model organism, *C. elegans* enables researchers to investigate various physiological processes, including memory, aging, cytotoxicity, reproduction, neurodegenerative diseases, antimicrobial activity, and drug screening. Using *C. elegans* as a model organism for the screening of natural compounds can help researchers comprehend the potential therapeutic applications of these compounds across a broad spectrum of physiological processes, thereby facilitating the discovery of new therapeutic agents.

Synthesis and Cytotoxic Activity Evaluation of a Small Library of Resveratrol-related Stilbenoids

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Abstract:

Within the huge class of plant secondary metabolites, resveratrol-derived stilbenoids present a wide structural diversity and mediate a great number of biological responses relevant for human health. In particular, resveratrol is known to modulate several pathways directly linked to cancer progression, as well as its analogue pterostilbene, characterized by increased metabolic stability and significant pharmacological activities. In order to study the potential cytotoxicity of other natural stilbenoids, a small collection of simplified analogues has been synthesized and tested on melanoma A375, non-small cell lung cancer H460 and prostate PC3 tumour cell lines and human normal skin WS1 fibroblasts. The structural determinants responsible for the activity have been highlighted, indicating that both lipophilicity and geometry of the most potent molecules might play a role on their antiproliferative activity. Moreover, to evaluate the ability of the selected molecules to produce DNA damage, the expression of the γ-H2AX after compound exposure was evaluated in WS1, H460 and A375 cell lines. Low levels of DNA damage were evidenced in normal WS1 fibroblasts exposed to 200 μM resveratrol, whereas in H460 and A375 lines the compound showed a significant damaging activity, confirming its selective behaviour towards cancer cells. In the same way, stilbenoid analogues showing a conjugated benzofuran moiety revealed a very interesting profile compared to other already known derivatives. Further studies are still ongoing in order to identify more precise structure-activity relationships on resveratrol congeners.

Nematicidal Activity of Oxygen-containing Compounds against the Root Lesion Nematode

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Abstract:

The root lesion nematode, *Pratylenchus penetrans*, is a serious threat to agricultural crops worldwide, being extremely difficult to control with common pest management practices. New EU commission policies on the development of plant protection products restrict the application of synthetic nematicides. Aiming at the development of a sustainable and environment friendly approach for nematode control, 20 oxygen-containing compounds were evaluated, at 2 mg / mL, for their: (1) nematicidal activity by direct contact assays for 24 h, (2) minimum time period required to reach > 99% mortality, and (3) nematicidal activity by indirect contact (fumigant) for 24 h. All bioassays were performed using acetone as negative control and Oxamyl (a systemic nematicide) as positive control. Overall, *P. penetrans* was remarkably resistant to the tested compounds, with the exception of benzaldehyde, carvacrol, 3-octanol and thymol, which were able to achieve > 99% mortality, surpassing the activity of the positive control Oxamyl. For these compounds, after ca. 60 min a 50% mortality was recorded, while at 18 h mortality was already > 99%. Using the indirect contact method, mortality was < 65% for the tested compounds (benzaldehyde > 3-octanol > thymol > carvacrol). Ongoing research is testing the effectiveness of these compounds against *P. penetrans* parasitizing potato, one of its main hosts. Simultaneously, the mode of action is being studied using a transcriptomic approach.

Acknowledgements:

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Ocean Bioprospecting: Preliminary Investigation of Metabolites Profile and Cytotoxic Activity of Soft Coral *Sarcophyton sp.* Extracts

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Abstract:

Secondary metabolites extracted from soft corals have been extensively studied for their potential pharmacological properties of interest to human health especially in relation to their possible

cytotoxic effect against different cancer cell lines. However, the comparison between the viability study on the cancer cell lines and on the healthy cells of the same tissue remains to be further investigated. In this work, the soft coral *Sarcophyton sp.* was extracted by using ethyl acetate and dichloromethane solvents. Moreover, further purification by column chromatography of the ethyl acetate extract led to three fractions and the metabolites profile was determined by applying a multi-analytical approach based on mass spectrometry and by comparison with reference data from the current literature. By MTT assay was found that ethyl acetate and dichloromethane crude extracts caused a reduction in viability of human colorectal cancer (CRC) cell lines SW480 and E705, while were only weakly active against human healthy mucosa cell line CCD841. Furthermore, fraction 2 and fraction 3 displayed cytotoxicity against CRC cell lines while showed a lower effect towards CCD841. This study provides a better understanding of the chemical nature of *Sarcophyton sp.* and indicates a promising fraction which represents an excellent starting point for further work on the isolation, structural characterization, and biochemical investigation of new compounds with a potential anticancer effect. In addition, the future aim will be to extend the study to other soft corals genera as well as other marine organisms.

Chemical and Biological Analysis of *Thymbra capitata* and *Thymus sipyleus* Species: In Vitro and Ex Vivo Approaches

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Abstract:

The two species of Lamiaceae, *Thymbra capitata* and *Thymus sipyleus* subsp. Rosulans methanolic and infusion extracts were used to find out their phytochemical composition and biological activities. The phytochemical investigation revealed that rosmarinic acid was the most significant component of the studied extracts (15.85–26.43%). The extract's total phenolic and flavonoid contents range from 83.43–127.52 mg GAE/g and 9.41–46.34 mg RE/g, respectively. The methanolic extract of T. capitata showed the highest ABTS radical scavenging capacity (379.11 mg TE/g), followed by T. sipylus (360.93 mg TE/g). Similarly, the methanolic extract of T. capitata had the highest reducing ability in the CUPRAC assay, with 802.22 mg TE/g. The phosphomolybdenum ability ranged from 2.39 to 3.61 mmol TE/g. Methanol extracts were found active on the enzyme, inhibited tyrosinase (83.18–89.66 mg KAE/g) and BChE (3.79–4.36 mg KAE/g) more actively and while the water extracts showed less (18.74–19.11 mg KAE/g) or no inhibitory effect on the respective enzyme. Additionally, the tested extracts were found effective in preventing the LPS-induced upregulation of COX-2 and IL-6 gene expression in isolated colon, thus indicating promising anti-inflammatory effects. Overall, these findings suggest that these species showed good antioxidant, enzyme inhibitory, and anti-inflammatory activity, suggesting their capability in the management of different applications related to health promotion and disease prevention.

Phytochemical and Biological Investigations on Pollen Extracts from Industrial Hemp Male Inflorescences

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Abstract:

The topic of hemp pollen is almost completely unexplored in the literature. The aim of this multidirectional study was to investigate phytochemical and biological properties of pollen extracts from male inflorescences of industrial hemp, obtained with biocompatible solvents suitable for food use, as water and hydroalcoholic solution. Pollen extracts have been assayed to evaluate the qualitative and quantitative composition of phenolic compounds through HPLC-DAD-MS analyses. The intrinsic antiradical and enzyme inhibition properties were assessed, as well. Ecotoxicological investigations were conducted for defining the biocompatibility limits in eukaryotic organisms. The biocompatibility limits of the extracts were also evaluated through MTT test in prostate PC3 and myocyte C2C12 cell lines. Finally, antimicrobial effects were investigated towards bacterial, fungal and dermatophyte species. The extracts from hemp pollen were found particularly rich in phenolic compounds, such as hydroxytyrosol, coumaric acid, and hesperetin. The *brine shrimp* lethality test revealed a higher toxicity of the hydroalcoholic extracts. In analogy, in the *daphnia magna* test, no toxicity attributable to the extracts was highlighted. Additionally, in prostate PC3 and myocyte C2C12 cells no relevant changes in vitality induced by the extracts were detected. The extracts were found effective as inhibitors of different bacterial and fungal strains. The experimental data obtained confirms the innovativeness of a product obtained directly from bees, suggesting the hemp pollen extracts as innovative sources of antimicrobial agents. These could become new high-quality products to be studied and improved for applications in the field of self-care and well-being.

Neuroprotective, Anti-diabetic and Anti-tumoral Activity of Portuguese Endemic *Thymus* spp. Hydroethanolic Extracts

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Abstract:

Thymus capitellatus Hoffmanns & Link and *Thymus carnosus* Boiss. are near-threatened species endemic to Portugal, whose phytochemical characterization was recently reported. Among various glycoside derivatives of flavonoids, their hydroethanolic extracts (HE) contain various salvianolic acids and high concentrations of oleanolic and ursolic acids. *Thymus* spp. are widely used in traditional medicine and pharmaceutical formulations for their bioactivities, and thus, due to their unique phytochemical profile, *T. capitellatus* and *T. carnosus* should also be screened for health-promoting effects. This research evaluated the neuroprotective, anti-diabetic and anti-tumoral activities of *T. capitellatus* and *T. carnosus* HE. Neuroprotection was assessed as anti-acetylcholinesterase and anti-tyrosinase activity, anti-diabetic activity as anti-α-glucosidase activity, and anti-tumoral potential as cell viability by Alamar Blue assay, using a hepatocarcinoma cell line (HepG2). Both extracts induced significant inhibition of acetylcholinesterase, and mild tyrosinase inhibition. Comparing both extracts, *T. capitellatus* extract produced the highest acetylcholinesterase inhibition (IC50= 360±4 µg/mL), while *T. carnosus* extract induced higher anti-tyrosinase inhibition than *T. capitellatus*. Regarding anti-diabetic activity, both extracts presented anti-α-glucosidase activity, being *T. capitellatus* extract the best inhibitor (23% inhibition; 1 mg/mL). Nevertheless, *T. carnosus* extract produced higher anti-proliferative activity in HepG2 cells (IC50= 103±2 µg/mL), than *T. capitellatus* extract (IC50= 447±7 µg/mL), revealing potential as an anti-tumoral agent. In summary, both extracts present potential as novel functional foods, presenting neuroprotective and anti-diabetic activity, with emphasis to

T. capitellatus. Regarding the potential use as chemopreventive, *T. carnosus* presented higher anti-proliferative activity, which should be further addressed in future studies.

Acknowledgements:

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The Effect of Hydroxylation on Flavonoid Structure in Antioxidant Activity and Ultraviolet Protection

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Abstract:

Oxidative stress is a significant factor in disease development and aging, resulting from the formation of reactive oxygen species (ROS) that cause changes at the cellular level, such as lipid oxidation, DNA mutations, and alterations in metabolism and cell division. Ultraviolet (UV) radiation is the primary environmental factor triggering ROS formation in the skin, leading to skin cancer. Flavonoids are a group of natural compounds that exhibit various physiological bioactivities, including the ability to inhibit free radicals and protect cells from metabolic, protein, and lipid changes. Hydroxylation, a structural alteration of flavonoids, leads to differences in their bioactivities. In this study, we aimed to investigate the effect of hydroxylation of flavonols on their antioxidant activity and their protective action against sun UV radiation. We selected three compounds, kaempferol, quercetin, and myrcitin, and evaluated their antioxidant capacities using different in vitro assays. Results showed that hydroxylation of flavonols led to different activities, and each compound exhibited varying abilities to inhibit/scavenge different free radicals. Additionally, the same compound showed a scavenging/inhibitory effect dependent on the radicals in study. We found that hydroxylation affects the ability of flavonoids to absorb UV radiation. Therefore, hydroxylation of flavonoids alters their bioactivity, which depends on the number and position of hydroxyl group. In conclusion, this class of flavonoids demonstrated high antioxidant capacity and sun protection potential, and hydroxylation was a significant factor in their activity. However, the compounds exhibited different potential for radical scavenging and UV radiation protection.

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Anti-inflammatory Effects Induced by an Aqueous Aged Black Garlic Extract Supplemented with Vitamins D, C and B12 on Cardiovascular System

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Abstract:

Multiple studies demonstrated biological activities of aged black garlic, including anti-inflammatory, antioxidant, and cardioprotective effects. We aimed to investigate the protective effects of an aged black garlic water extract (ABGE) alone or in association with multivitamins consisting of combined Vitamins D, C and B12, on mouse heart specimens exposed to E. coli lipopolysaccharide (LPS). ABGE, vitamins D and C, as well as the Formulation suppressed LPS-induced gene expression of cyclooxygenase (COX)-2, tumor necrosis factor (TNF)-α, interleukin (IL)-6, nuclear factor-kB (NF-kB) and inducible nitric oxide synthase (iNOS), on mouse heart specimens. The beneficial effects induced by the extract could be related to the pattern of polyphenolic composition, with particular regard to gallic acid and catechin. Concluding, the present findings showed that ABGE, alone and in association with multivitamins, exhibited protective effects on mouse heart. Moreover, further suggesting its potential use on cardiovascular disease.

Bioprospecting Marine Microbiota from Volcanic Environments

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Abstract:

Marine ecosystems are unique and rich reservoirs of biodiversity with high potential towards improving the quality of human life. The ocean's extreme physico–chemical conditions have propitiated marine organisms to produce a great variety of new molecules as a mechanism to ensure their survival. Such compounds possess great biopharmaceutical interest. In particular, marine microbiota represents a promising and near-inexhaustible source for the development of new drugs. These special characteristics have encouraged us to bioprospect the singular volcanic marine ecosystem in the Canary Islands. Our research team has participated in monitoring oceanographic cruises carried out around volcanic and submarine volcanic eruptions, with the aim to explore the microbial biodiversity associated with both rock samples and deep-sea invertebrates to probe their pharmaceutical potential. Besides the biodiversity these environments represent, we have evaluated different culture conditions in order to explore their metabolic potential. Our results represent an excellent starting point to accessing novel secondary metabolites and enzymes with potential for biotechnological applications.

Session I: Bioactivity of Natural Products-1

Looking at Underexplored Species as Natural Resources for Application in Medicinal or Nutritional Formulations

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Abstract:

Plant medicinal uses are well documented as well as their potential to deliver natural products that can or have become a commercial drug. Actually, medicinal plants used in folk medicine are being increasingly studied and used in pharmaceutical and nutraceutical fields. In fact, several molecules approved for drug administration are pure natural compounds. Moreover, natural products have garnered increasing attention in therapy because they are viewed as more biologically friendly and consequently less toxic, although this is not always the case. The main objectives of our studies on natural products are the valorisation of underexploited species,1 the confirmation of the species medicinal and/or nutritional value, and also to understand the extension of the climate changes' impacts on species metabolome profile. To accomplish these goals, GC- and UHPLC-MS techniques were employed to obtain the species metabolome profile. However, in some cases, phytochemical studies to isolate and characterize the major secondary metabolites were also performed. Among recognised medicinal plants are Calendula species,2 Artemisia campestris L.3 and Genista tridentata L.,4 which could become a value resource of bioactive compounds. In this communication, we will also present results that confirm the nutritional or medicinal value of several species,5 highlighting the salt tolerant species Spartina maritima, Puccinellia maritima and Limonium vulgare,6 as well as the climate changes' impact on Olea europaea7 metabolome profile.

Acknowledgements:

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Selective Targeting of Cancer-related G-Quadruplexes by Natural Compounds

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Abstract:

In the search of selective G-quadruplex (G4)-targeting chemotypes, natural compounds have been thus far poorly explored, though representing appealing candidates due to the high structural diversity of their scaffolds. A high diversity in-house library composed of ca. one thousand individual natural products was investigated through a combination of molecular modelling and experimental assays. Five hit binders of telomeric and oncogenic G4s, i.e., Bulbocapnine, Chelidonine, Ibogaine, Rotenone and Vomicine were identified. Biophysical studies unambiguously demonstrated the

selective interaction of these compounds with G4s compared to duplex DNA. The rationale behind the G4 selective recognition was suggested by molecular dynamics simulations. From biological assays, Chelidonine and Rotenone emerged as the most active compounds of the series against cancer cells, also showing good selectivity over normal cells. In a follow-up optimization study, some analogues of bioactive G4 binders were tested. Among them, Dicentrine was found to thermally stabilize telomeric and oncogenic G-quadruplexes without affecting the control duplex. Molecular dynamics simulations indicated that Dicentrine preferentially binds the G-quadruplex groove or the outer G-tetrad for the telomeric and oncogenic G-quadruplexes, respectively. Finally, biological assays proved that Dicentrine is highly effective in promoting potent and selective anticancer activity by inducing cell cycle arrest through apoptosis, preferentially targeting G-quadruplex structures localized at telomeres. Taken together, these data validate a few natural products as putative anticancer candidates that target selectively cancer-related G-quadruplex structures.

Composition and Biological Effects of *Pelargonium quercetorum* Agnew Extracts

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Abstract:

Pelargonium quercetorum Agnew is a medicinal plant traditionally used for treating intestinal worms. In the present study, the chemical composition and bio-pharmacological properties of *P. quercetorum* extracts were investigated. Enzyme inhibition and scavenging/reducing properties of water, methanol and ethyl acetate extracts were assayed. The extracts were also studied in an ex vivo experimental model of colon inflammation, and in this context the gene expression of cyclooxygenase-2 (COX-2) and tumor necrosis factor α (TNFα) were assayed. Additionally, in colon cancer HCT116 cells, the gene expression of transient receptor potential cation channel subfamily M (melastatin) member 8 (TRPM8), possible involved in colon carcinogenesis, was conducted as well. The extracts showed a different qualitative and quantitative content of phytochemicals, with water and methanol extracts being richer in total phenols and flavonoids. This could explain, at least in part, the higher antioxidant effects showed by methanol and water extracts, compared with ethyl acetate extract. By contrast, ethyl acetate was more effective as cytotoxic agent against colon cancer cells, and this could be related, albeit partially, to the content of thymol and to its putative ability to downregulate TRPM8 gene expression. Additionally, the ethyl acetate ex-tract was effective in inhibiting the gene expression of COX-2 and TNFα in isolated colon tissue exposed to LPS. Overall, the present study supports the use of bioactive extracts of the plant as potential anti-inflammatory agents, in the colon.

Sesquiterpene Lactones Extraction: The Case Study of Cynaropicrin and its Anti-inflammatory and Anti-cancer Potential

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Abstract:

Sesquiterpene lactones (SL) group, comprises more than 6000 compounds, with high biological potential. Access to SL biological properties could be achieved by solid-liquid extraction, with solvent and extraction process as key factors for successful extraction. With new SL discovered in the last years, new biological activities have been tested, different action mechanisms (synergistic and/or antagonistic effects), as well as molecular structure–activity relationships described. We have previously described Cynara cardunculus, commonly named cardoon, as a rich source of SL (94,5 g/kg DW), being cynaropicrin the most abundant SL presented in cardoon leaves (87,4 g/kg dw). Those results lead us to improve cynaropicrin extraction, using ultrasound assisted extraction. The extraction methodology was further optimized by response surface methodology. Optimal conditions were found for a solid/liquid ration of 1/27, amplitude of 67% and temperature of 44ºC. Those extracts were further used to develop chitosan-based films with different cardoon leaves extracts, and the anti-inflammatory properties evaluated. Chitosan-based films with 5% (w/w) leaves cardoon extracts presented an interesting anti-inflammatory activity. Bj5-ta cells stimulated with liposaccharides (LPS), presented a reduction of 86% on IL-6 cytokine levels, after exposure to chitosan with 5% film extract. The expression of tumor protein markers were also assessed in human breast cancer cells (MDA-MV-231) in the presence of cardoon leaves extracts, or cynaropicrin. The results underlie high potential biological properties, namely anti-inflammatory and anti-cancer for cardoon leaves extracts rich in sesquiterpene lactones, particularly cynaropicrin.

Pharmacological Assessment of *Adiantum incisum Forsk*. Affirms Ethnobotanical Use in Diabetes

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Abstract:

Diabetes mellitus (DM) is a lifelong clinicopathological metabolic disease affecting the major population in the world. Plants are a source of at least 25% of all modern medications. The current study evaluated the phytochemical and antidiabetic potential of the fern *Adiantum incisum* (*A. incisum*) Forssk. The fern was extracted with various solvents of increasing polarity. The single-dose study in alloxan-treated diabetic mice showed maximum antihyperglycemic effect with ethyl acetate fraction (E.A.F; 250 mg/kg) which was further subjected to 21 days evaluation in alloxan diabetic animal model (n=5). The results showed a highly significant ($p < 0.001$) decrease in blood glucose levels on 7th, 14th and 21st days of treatment. At the end of treatment, a significant increase in the insulin (↑216.4%) and a significant ($p< 0.05$) decrease in L-lactate, pyruvate, plasma free fatty acids (11.1%), glycated hemoglobin ($p<0.001$) and malondialdehyde ($p<0.001$) levels were observed. The fraction also significantly increased the glycogen content in the liver and skeletal muscle and decreased in the heart. The *A. incisum* E.A.F fraction significantly ($p < 0.001$) improved liver, kidney, and lipid profile. Histopathological analysis of pancreas, liver and kidney in all treated groups showed the presence of intact cells when compared with the control diabetic group. The phytochemical analysis showed the presence of gallic acid, ferulic acid and kaempferol. The study concluded that the ethyl acetate fraction of *A. incisum* increases insulin levels and possess potential antidiabetic and antioxidant activity and can be potentially explored further as a therapeutic agent in diabetes.

Session II: Bioactivity of Natural Products-2

Natural Food Ingredients Applications: From Plants and Mushrooms

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Abstract:

Over the years, the study of natural ingredients of edible matrices has increased exponentially, driven by the discovery of scientific evidence that validates the wide variety of applications and benefits arising from their use. Accordingly, the use of plants and mushrooms to improve health epitomizes a significant cultural legacy, these been traditionally used as a source of highly nutritional foods and medicinal preparations. Besides their high nutritional richness, mushrooms and plants are known to perform various pharmacological functions, holding a wide range of high-value natural ingredients for various industries, acting as colorants, preservatives, and conferring bioactive properties when added to other products. Polyphenolic extracts of *Melissa officinalis* L. (lemon balm) and *Castanea sativa* Mill. flowers (sweet chestnut) showed to exert a good antioxidant and antimicrobial activities, while *Foeniculum vulgare* Mill. (fennel), and *Matricaria chamomilla* L. (german chamomile) were used for preservative purposes in loaf bread, cupcakes, yogurt, cheese, wine, and cottage cheese. Also, bioactive colouring molecules like betalains (gomphrenins, isogomphrenins) from *Gomphrena globosa* (purple globe amaranth) and anthocyanins (cyanidin, delphinidin, and malvidin derivatives) from *Rose canina* (rose), were applied in yogurt, waffles, and donut topping. As for mushrooms, ergosterol and vitamin D2 have been successfully extracted and used for functionalized dairy beverages, cheese, and flours. Together, these results highpoint the effectiveness of natural ingredients of different mushrooms and plant species, promoting the valorisation of these products as a source of naturally based ingredients able to be incorporated into widely consumed and appreciated food products at an industrial/commercial level.

CoaMedPlants: From Ethnobiology to Plant-based Health Solutions

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Abstract:

A UNESCO World Heritage Site since 1998, the Foz Côa Valley is considered "the most important open air Palaeolithic rock art site". However, the natural heritage of the Côa Valley should also be preserved and valorized. Bearing in mind this exceptional natural heritage, our interdisciplinary research project aims at the preservation of the cultural heritage related to the practices with medicinal plants in Côa Valley, and the valorization through the scientific validation of their properties based on biochemical characterization, study of biological activities, and mechanisms of action of their extracts. This project brings to light the traditional knowledge of medicinal practices in Côa Valley, with information from the ethnobotanical survey and understand that it summarizes the results of

experiments made by trial and error over centuries, and create an interface of science, medicine, and humanities, which bridges past and present, and inspire fresh investigations and innovative research strategies for tomorrow's health care. It addresses a fundamental challenge of the 21st century, the need for new drugs and new strategies for the discovery of such medicines. By linking medical tradition with contemporary medical needs in a creative association, it will suggest new avenues for fresh pharmacological investigations and innovative research strategies. In this presentation, it will be summarized the path of this project. From the ethnobotanic survey and herborization of the around 500 species catalogued, passing to the scientific validation of the uses through chemical characterization of the extracts and assessment in various disease models.

Anti-inflammatory Potential of Digested *Brassica* Sprout Extracts in Human Macrophage-like HL-60 Cells

Bruno Ramos-Molina[1]*, Paula García-Ibañez[2], María A. Núñez-Sánchez[1], Alba Oliva-Bolarín[1], María A. Martínez-Sánchez[1,3], Antonio J. Ruiz-Alcaraz[3], Diego A. Moreno[2]

[1]Biomedical Research Institute of Murcia (IMIB), Spain; [2]Centro de Edafología y Biología aplicada del Segura (CEBAS-CSIC), Spain; [3]University of Murcia, Spain.

Abstract:

Cruciferous vegetables have been reported to be a great source of anti-inflammatory compounds. Specifically, sprouts from the Brassicaceae family stand out for their high content of glucosinolates (and their bioactive derivatives, isothiocyanates), phenolic acids, and anthocyanins. Despite the evident anti-inflammatory activity of certain Brassica phytochemicals such as sulforaphane or phenolic acids, the effect of digested Brassica vegetables on inflammation remains understudied. In this work, we aimed to evaluate the anti-inflammatory potential of the bioaccessible forms of cruciferous bioactives (from red cabbage sprouts (RCS) and red radish sprouts (RRS)) obtained upon *in vitro* gastrointestinal digestion in the HL-60 macrophage-like differentiated human cell line. The study was performed under basal conditions or stimulated with a low dose of LPS for 24 hours as a validated *in vitro* model of chronic inflammation. The cell viability was determined by MTT assay. The gene expression and production of pro-inflammatory cytokines were determined by RT-qPCR and ELISA respectively. Our results revealed no cytotoxicity with any of the treatments in LPS-stimulated macrophage-like HL60 cells. Regarding cytokine production, digestates significantly decreased the production of pro-inflammatory cytokines at concentrations of 50 and 100 μg mL^{-1}. Furthermore, the RT-qPCR analysis showed a decrease in the relative expression of pro-inflammatory cytokines in LPS-stimulated cells treated with RRS digestates at 100 μg mL^{-1} but not with red cabbage digestates. In conclusion, RRS bioaccessible compounds in the extracts could be used as dietary coadjuvants given their potential anti-inflammatory effect on this *in vitro* model of chronic inflammation.

Protective Effects on Selected Pro-inflammatory and Pro-oxidant Markers Induced by *Allium sativum* L. Extracts on an Ex Vivo Experimental Model of Ulcerative Colitis

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Abstract:

Inflammatory bowel diseases (IBDs) are chronic and multifactorial inflammatory conditions of the colonic mucosa (ulcerative colitis), characterized by increased and unbalanced immune

response to external stimuli. In this context, antioxidant/anti-inflammatory herbal extracts were found to contrast IBD-related symptoms by reducing various pro-inflammatory and oxidative biomarkers, such as reactive oxygen/nitrogen (ROS/RNS) species, prostaglandins, and cytokines. Garlic and its bioactive constituents were reported to exert various biological effects, including anti-inflammatory, antioxidant and immunomodulatory activities ant to exercise protective effects against ulcerative colitis. We aimed to evaluate the protective effects of a hydroalcoholic (GHE) and a water (GWE) extract from a Sicilian variety of garlic, known as Nubia red garlic, on an ex vivo experimental model of ulcerative colitis, involving isolated LPS-treated mouse colon specimens. Both extracts were able to counteract LPS-induced cyclooxygenase (COX)-2, tumor necrosis factor (TNF)-α, nuclear factor-kB (NF-kB), and interleukin (IL)-6 gene expression in mouse colon. Moreover, the same extracts inhibited prostaglandin (PG)E2, 8-iso-PGF2 , and increased the 5-hydroxyindoleacetic acid/serotonin ratio following treatment with LPS. In particular, GHE showed a better anti-inflammatory profile. The anti-inflammatory and antioxidant effects induced by both extracts could be related, at least partially, to their polyphenolic composition, with particular regards to catechin. Concluding, our results showed that GHE and GWE showed protective effects, as confirmed by the inhibitory effects on selected pro-inflammatory and pro-oxidant markers, in LPS-stimulated colon, suggesting a potential role in the prevention and management of ulcerative colitis.

Neuroprotective Potential of Medicinal Plant Extracts

Clara Grosso[1]*, Cristina Delerue-Matos[1]

[1]REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

Abstract:

According to the World Health Organization, about 80% of the world population (c.a. four billion people) rely on herbal medicinal products for their primary healthcare. Since ancient times, medicinal plants have been recognized for their therapeutic effects which results from a rich variety of bioactive compounds. This presentation will focus on our recent studies on the neuroprotective effect of medicinal plants extracts, namely those prepared from *Scutellaria baicalensis* Georgi, *Ginkgo biloba* L., *Hypericum perforatum* L., *Curcuma longa* L., *Lavandula angustifolia* Mill., *Trigonella foenum-graecum* L., *Rosmarinus officinalis* L., *Coffea arabica* L. and *Annona muricata* L.. Since there are several enzymes involved in the etiology of neurodegenerative and neuropsychiatric disorders, extracts have been tested as enzyme inhibitors against acetylcholinesterase, butyrylcholinesterase, monoamine oxidase A/B, tyrosinase and glycogen synthase kinase-3β. Moreover, emphasis will also be given to oxidative stress, by discussing the extracts with strong potential as scavengers of oxygen and nitrogen reactive species. Finally, the effect of these extracts on different cell lines will also be presented.

Acknowledgements:

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Session III: Marine Natural Products-2

Classical and Metabolomics-guided Discovery of Bioactive Natural Products from Brown Algae

Deniz Tasdemir[1]

[1]GEOMAR Centre for Marine Biotechnology, Research Unit Marine Natural Products Chemistry, GEOMAR Helmholtz Centre for Ocean Research Kiel, [2]Faculty of Mathematics and Natural Sciences, Kiel University, Kiel, Germany

Abstract:

Seaweeds (marine macroalgae) are a diverse group of photosynthetic organisms that play key roles in maintenance of healthy marine ecosystems in coastal areas. Brown algae (Phaeophytes) are prolific producers of various classes of natural products, e.g., diterpenes, phlorotannins and carotenoids. These metabolites are mostly produced as an evolutionary response to environmental pressures, and their ecological relevance underlies their pharmaceutical potential for treatment of human diseases. Most of the work on these organisms have employed classical natural product chemistry approaches, and the full stereochemical assignments of the isolated compounds have been difficult. Seasonal variations in the metabolite and bioactivity profile of brown algae are critical for timing of sampling campaigns, but this has often been neglected. Furthermore, untargeted metabolomics has been rarely used for initial chemical profiling of algal extracts to monitor downstream chemical and bioactivity-guided isolation studies. Hence, this presentation will highlight our efforts in seasonal variation studies, automated extraction methods, computational tandem mass spectrometry-based metabolomics studies allowing targeted purification and detailed structure elucidation of bioactive natural products from edible European brown algae.

Innovative Approaches for the Extraction of Ulvans

Herminia Domínguez* and Mª Dolores Torres, Noelia Flórez-Fernández

**CINBIO, Faculty of Science, University of Vigo, Spain*

Abstract:

Ulvan is the major polysaccharide in green macroalga of Ulva genus. These sulfated polysaccharides, mainly composed of rhamnose, uronic acids and xylose, are biocompatible, biodegradable and non-toxic. They exhibit a variety of biological activities and can be used for the preparation of novel biomaterials for pharmaceutical applications. The polysaccharide chemical composition and structure are determined by the seaweed species, the collection area, geographical and seasonal conditions. These characteristics define the functional and biological properties of ulvan. In addition, the extraction and purification strategies are strongly influencing the polysaccharide characteristics. The adequate definition of these processes is needed to obtain more active products. Ulvan extraction is usually carried out with hot water under stirred conditions. The incorporation of intensification strategies to shorten extraction time and to increase the yields have been suggested. Subcritical water extraction and assistance by enzymes, microwaves or ultrasound proved suitable to enhance the process performance and the effect of some of these strategies on the composition, molecular weight and properties is discussed.

Botryllus schlosseri as a Unique Chordate Model for the Study and Modulation of Innate Immunity in Hematopoietic Stem Cell Transplantation

Shelly Oisher, Oron Goldstein, Edna Ayerim Mandujano-Tinoco, Shani Talice, Orly Gershony-Yahalom, and Benyamin Rosental*

**The Shraga Segal Department of Microbiology, Immunology, and Genetics. Faculty of Health Sciences. Regenerative Medicine and Stem Cell Research Center. Ben Gurion University of the Negev. Beer Sheva, 8410501, Israel.*

Abstract:

The mechanisms that sustain immunological non-reactivity are the basis for understanding the maintenance of tissue in syngeneic and allogeneic settings. While most transplantation rejection occurs due to the adaptive immune response, the pro-inflammatory response of innate immunity is necessary for the activation of adaptive immunity - both in syngeneic and allogeneic settings. We study a unique chordate model, *Botryllus schlosseri*, that lacks a classic adaptive immune system, yet has the ability to reject allogeneic individuals or form chimeras with compatible animals. This organism demonstrates three major innate immunity responses: non-inflammatory program cell removal, acute rejection (between non-compatible animals) and allogeneic resorption (between compatible colonies that formed chimeras). Using flow cytometry, whole-transcriptome sequencing of defined cell populations and tissues, and diverse functional assays, we isolated 34 *B. schlosseri* cell populations, identified hematopoietic stem cell (HSC), progenitors, immune-effector cells, and the HSC niche. Completing a full model for HSC transplantation. Furthermore, we identified a *B. schlosseri* cytotoxic cell population originating from large granular lymphocyte-like cells and demonstrated their function in acute and chronic rejection processes. Studying the molecular and cellular framework underlying loss of tolerance to allogeneic tissues within the *B. schlosseri* chimera, we found that developmental cell death programs license cytotoxic cells to eliminate histocompatible partners. This study demonstrates that interactions between pro-inflammatory and damaged tissue removal lead to robust cytotoxic and phagocytic clearance programs within the allogeneic microenvironment.

Acknowledgements:

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Session IV: Other fields related to Natural Products, Natural Products Chemistry, Natural Products in Drug Discovery, and Bioactivity of Natural Products

A New Approach to the Production of *Schisandra* Lignans using Plant Biotechnology Methods

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**Chair and Department of Pharmaceutical Botany, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland*

Abstract:

Schisandra lignans, namely dibenzocyclooctadiene lignans, are the main secondary metabolites specific to the *Schisandra* genus of confirmed high medicinal activities, e.g., hepatoprotective, anticancer, antioxidant, anti-inflammatory and adaptogenic. Our studies aimed to investigate the lignan production in *S. chinensis*, *S. chinensis* cv. Sadova, *S. henryi*, and *S. rubriflora* microshoot cultures using different comprehensive and innovative biotechnological methods (e.g., elicitation, cultivation in plant bioreactors). The high production of lignans in agar, agitated, and PlantForm bioreactor cultures of *S. chinensis* and *S. chininsis* cv. Sadova (238, 195, 547 and 574, 375, 313 mg/100 g DW, respectively) were confirmed. For the first time the production of dibenzocyclooctadiene, aryltetralin, dibenzylbutane, tetrahydrofuran lignans and neolignans has been confirmed in *S. henryi* (874 mg/100 g DW) and *S. rubriflora* male and female lines (251 and 221 mg/100 g DW). The high antioxidant, anti-inflammatory and antimicrobial potential of obtained tissue extracts were confirmed. The results show the new possibilities of bioactive *Schisandra* lignans production using biotechnological methods.

Acknowledgements:

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Discrimination of *Quercus pyrenaica* Honeydew Honey through the Volatile Profile

Soraia I. Falcão[1,2]*, Kheira M. Mouffok[1,2], Miguel Vilas-Boas[1,2]

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Abstract:

Honey is a natural product produced by *Apis mellifera* bees from the nectar of flowers, and called nectar honey, or secretions of plants or excretions produced by plant-sucking insects and called honeydew honey. The production of these honey can be observed in the north of the Iberian Peninsula, where forests of black oak (*Quercus pyrenaica*) exist, from the honeydew secretions produced in the plant, which result of the insect's injuries or through phloem sap exudate in its acorns. The aim of this work is the discrimination of the black oak honeydew honey through its volatile profile. For that, forty-two samples, obtained in September of 2021, from four apiaries located in black oak forests within Montesinho Natural Park, Portugal, where characterized by the volatile profile. Also, acorn secretions were collected in *Q. pyrenaica* trees located near the apiaries. Volatiles were sampled by headspace solid phase microextraction (HS-SPME) and the chemical identification was performed by

gas chromatography-mass spectrometry (GC-MS). A complex total ion chromatogram was obtained. The alcohols, aldehydes and terpenic derivatives were the most likely to relate the honeydew honey to its botanical origin, being 1-nonanol, α-terpineol, nonanal, hotrienol and phenylethyl alcohol the most abundant volatiles. Compounds such as 2,3-butanediol and cis-linalool oxide were presented and previously described in honeydew honey with forest origin. The above methodology was suitable for the isolation of low-molecular-weight aroma compounds that are important for authentication of *Q. pyrenaica* honeydew honey.

Natural Hydrophobic Deep Eutectic Solvents for the Extraction of Bioactive Compounds from Complex Samples

Cecilia Cagliero[1]*, Giulia Mastellone[1], Gaia Bechis[1], Arianna Marengo[1], Barbara Sgorbini[1] and Patrizia Rubiolo[1]

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Abstract:

The valorization of plants and their products is fundamental in terms of sustainable development. In recent years, public attention to environmental sustainability has increased dramatically, and regulatory agencies are developing strategies and designing roadmaps to promote the efficient use of resources the restoration of biodiversity, and the reduction of waste. In this sense, natural products can be used as a valuable source of phytochemicals to produce more environmentally friendly solvents for various applications, including more sustainable extraction methods, according to the principle of Green (Analytical) Chemistry. In particular, deep eutectic solvents (DESs) represent a more environmentally friendly alternative to conventional solvents, thanks to their easy preparation and low costs of raw material. They consist of two or more components that form a hydrogen bonding network, which is the key to the formation of DESs. Natural compounds, such as those isolated from essential oils, can be used as hydrogen bond donors or acceptors to form hydrophobic (H)DESs. In this communication, the potential of natural HDESs for the extraction of bioactive compounds from complex samples is presented. The possibility of isolating non-volatile compounds from natural resources and volatile potential allergens from cosmetic products using the described HDESs is reported, as well as the possibility of combining (micro)extraction with analytical characterization by various chromatographic techniques.

Thioesterase-mediated Macrocyclization of Non-ribosomal Peptides and Polyketide Natural Products

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Abstract:

Macrocyclic bacterial polyketides and non-ribosomal peptides are macrocyclized as one of the last steps in their biosynthesis by thioesterases (TEs). These enzymes catalyze the offloading of the covalently bound linear natural product from the biosynthetic enzyme complexes. In addition to macrocyclization, some TEs catalyze hydrolysis of the intermediate to generate the free acid. Efforts to elucidate the underlying mechanism that controls TE selectivity for hydrolysis versus

macrocyclization has proven to be highly challenging. Herein we develop two biochemical models for macrocyclization, a conformational sampling model and a preorganization model, and show how TEs can be used synthetically for the chemoenzymatic synthesis of complex macrocyclic natural products.

Further Studies on a New Family of Antibiotics Produced by Cryptic Biosynthesis in Extremophilic Fungi

Andrea Stierle1*, Donald Stierle1, Nigel Priestley2

1Department of Biomedical and Pharmaceutical Sciences, University of Montana, USA; 2Department of Chemistry and Biochemistry, University of Montana, USA

Abstract:

According to the CDC, at least two million people are infected with antibiotic-resistant bacteria every year, and over 35,000 die annually as a direct result of these infections. The *Infectious Disease Society of America* published a policy report that outlined the consequences of the alarming rates of antibiotic resistance and the dire need for reinvestment in the search for new antibiotics to overcome the lean development pipeline. In 2017 the Stierle research lab reported a new class of fungal macrolide antibiotics produced in co-culture by two extremophilic fungi. These fungi were isolated from the Berkeley Pit, an acid mine waste lake in Butte, Montana, USA. Unlike canonical bacterial macrolide antibiotics, the berkeleylactones lack the sugar moieties responsible for both the activity and induced resistance associated with other classes of macrolide antibiotics. The berkeleylactones also have a unique mode of action. The lead compound, Berkeleylactone A, targets multi-drug resistant strains of *Staphylococcus aureus* and *Bacillus anthracis* with MIC values near 1 µg/mL. The berkeleylactones are undetectable in axenic cultures of the contributing fungi, which suggests that co-culture can elicit cryptic biosynthesis in participating organisms. Efforts to determine the mode and mechanism of action of the berkeleylactones, to develop more potent analogues of this new class of antibiotics, and to assess their *in vivo* efficacy will be discussed.

High-value Compounds Produced by the Landfill-isolate *Coelastrella cogersae* sp. nov.: Scaling-Up, Stress Induction and Lipid Extraction

[1,2*]David Suárez-Montes, [2]Sara Gutiérrez-Valderas, [1]Victor Casado, [2]Jose Manuel Rico.

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Abstract:

During the last century, technological and cultural development of the society has modified different aspects related to nutrition. There is a relationship between the decrease on the consumption of fruits and vegetables and the increase in the mortality of people with coronary heart disease and cancer, probably related with a lack of antioxidant compounds in the diet (Li *et al.*, 2007). Lipids and carotenoids produced by microalgae and cyanobacteria have been established as one of the most important sources of bioactive compounds. Carotenoids, such as astaxanthin from *Haematococcus pluvialis* (Machado et al., 2014) are well established in the nutraceutical market. However, the prospection of new strains in unexplored environments joined to industrial scale up are mandatory. *Coelastrella cogersae* was isolated and identified as a potential source of bioactive chemicals

(Suarez-Montes et al., 2022). Scaling-up protocols were developed from lab-scale to pilot scale (100L close photobioreactors), analyzing growth parameters parallelly. Then, nutrient deprivation and NaCl addition were chosen to increase lipid fraction and total carotenoids content. Stressed and no-stressed biomass were extracted using a mix of solvents. Specifically, fractions without any pre-treatment resulted in less lipid content comparing to those which were pre-treated. The best combination of stress was 25g/L of NaCl and NPK^- deprivation, obtaining 28,1% of lipids (w/w). On the other hand, carotenoids production was statistically significative between control cultures and the combination of NK^- and 25g/L of NaCl, obtaining around 1-1,2% of dry biomass. These results make *C. cogersae* a suitable source of high-value products.

Carbonic Anhydrase and Anticholinesterase Effect of Antarctic Algae Extracts

Belma Konuklugil[1]*, Ibrahim Seyda Uras[2], Bulent Gozcelioglu[3], Murat Senturk[2]

[1]Lokman Hekim University, Turkey; [2]Agri Ibrahim Cecen University, Turkey; [3]The Scientific and Technological Research Council Of Türkiye, Turkey

Abstract:

Marine organisms obtained from Antarctica are prominent sources for many important activities. Algae are known for their ability to adapt to various adverse environmental conditions and for producing secondary metabolites with various biological activities. Enzyme inhibitors have a significant impact on the treatment of various diseases. Inhibitors of specific forms of the enzyme Cas (e.g., CA I / II) have been used to create new drugs for conditions such as epilepsy, edema, and glaucoma. As a result, new inhibitors of CA isoenzymes need to be developed because of their potential to be used as therapeutic agents. Specific inhibitors can be utilized to treat motor neuron diseases like dementia, myasthenia gravis, and Alzheimer's by decreasing the activity of AChE/BChE. *Cystosphaera jacquinotii, Gigartina skottsbergii, Palmaria decipiens, Desmarestia menziesi, Monostroma hariotii and Desmarestia antarctica* collected from the marine environment of Antarctica's Robert Island, King George Island and Nansen Island. In this study, inhibitory properties of six different algal extracts on carbonic anhydrase (CA) I, II, acetylcholinesterase (AchE) and butyrylcholinesterase (BchE), glutathione reductase (GR), and α-glucosidase (AG) enzymes were investigated. *M. harioti* and *C. jacquinotii* extracts demonstrated highest AChE and CA I enzymes inhibiton while *C. jacquinotii* and *D. antarctica* extracts presented highest inhibitory activity against BChE and CA II enzymes. While *M. harioti* and *D. antarctica* extracts presented highest GR enzyme inhibiton, *C. jacquinotii* and *D. antarctica* extracts showed highest enzyme inhibition against *α*-Glucosidase. The results indicate that these extracts are potent cholinesterases and new potential drugs.

Poster Presentations

In-person Posters

Acacia pods: A Potential Source of Promising Antibacterial Agent against MRSA and Other Resistant Pathogens

Abdalla Mohamedsalih*

University of The West of Scotland, Paisley, UK

Abstract:

Herbal medicine has become the preferred treatment for a large fraction of the world population, to cure and prevent various common diseases. Medicinal plants are inexpensive and valuable source of unique phytochemicals with distinct therapeutic properties. In traditional medicine, among hundreds of plants, *Acacia* spp. has a wide range of therapeutic uses in a large number of countries. In this project, the antibacterial activity of the methanolic extract of the plant pods has been evaluated against Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis*. The antibacterial susceptibility test was carried out using Agar Diffusion Method (ADM). According to the inhibition zone, the Acacia extract has exhibited promising inhibitory activity against all the mentioned strains and can be a potential source of new antibacterial agents, particularly against MRSA. LC-HRMS analysis followed by bio-guided isolation of the antibacterial metabolite(s) is under way.

Preparation of β-carboline Derivatives for Circumventing Drug Resistance in Cancer

Filipa Barbosa[1]*, Bianca Montsch[2], Petra Heffeter[2] and Maria-José U. Ferreira[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Portugal; [2]Center for Cancer Research, Medical University of Vienna, Austria

Abstract:

Cancer is one the major causes of death globally, accounting for approximately 10 million deaths in 2020 (WHO). Drug resistance is one of the major impairments for the efficacy of chemotherapy in cancer treatment. Aiming at finding effective compounds for reversing drug resistance in cancer, a natural β-carboline indole alkaloid was selected for derivatization. A set of new derivatives was prepared, by reaction with different aliphatic and aromatic isocyanates, whose structures were determined mainly by NMR, including two-dimensional NMR experiments. The ability of compounds for overcoming drug resistance was evaluated, using several models of sensitive and resistant human cancer cells. Firstly, the antiproliferative activity of the compounds was evaluated through 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenylthetrazolium bromide (MTT) viability assay. The most significative results were found for a derivative, against most of the cancer entities tested, selectively killing resistant cancer cells, thus having collateral sensitivity effect. The ability of the compounds to act as inhibitors of the ABC transporters was also investigated.

Acknowledgements:

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Indole Derivatives and Protoflavones Hybrid Molecules to Tackle Triple-negative Breast Cancer

Ricardo Ferreira 1*, Elizabeth A. Lopes 1, Gábor Girst 2, Lídia M. Gonçalves 1, Hui-Chun Wang 3, Attila Hunyadi 2, Maria M. M. Santos 1

1Research Institute for Medicines, Universidade de Lisboa, Faculty of Pharmacy, Portugal; 2Institute of Pharmacognosy, Interdisciplinary Excellence Center, University of Szeged, Hungary; 3 Graduate Institute of Natural Products, Kaohsiung Medicinal University, Taiwan

Abstract:

Triple-negative breast cancer (TNBC) is an aggressive tumor with poor prognosis, representing 10-15% cases of breast cancers. For that reason, the development of new drugs able to act on TNBC is highly needed. Two important therapeutic targets to tackle TNBC are the tumor suppressor protein p53 and the ataxia telangiectasia and Rad3 related protein (ATR). p53 because it is inactivated in breast cancers and ATR because it plays a central role in DNA damage response. The indole moiety is a privileged structure widely found in different natural products with several biological activities, such as anticancer. Our research group has been involved in development of both wild-type and mutant p53 reactivators based on the indole moiety, in order to reactivate the p53 tumor suppressor functions. ATR is considered a selective antitumor target, and promising clinical studies are ongoing to develop the first ATR inhibitor .4 Interestingly, ATR inhibitors were also shown to kill p53-deficient cancer cells, which also makes this class potential drugs to complement p53-targeted therapies. In order to develop new drugs to tackle TNBC, we decided to combine two distinct pharmacophores (indole p53 activators and protoflavone ATR inhibitors). In this communication we will present the synthesis of the hybrid molecules, as well as the results obtained in breast cancer cells.

Acknowledgments:

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Overcoming Multidrug Resistance: Search for Human P-glycoprotein Modulators and Insights on Drug Efflux Mechanism

Cátia A. Bonito1*, Ricardo J. Ferreira2, Maria-José U. Ferreira3, Fernando Durães4, Emília Sousa4,

Jean-Pierre Gillet5, Natália D. S. Cordeiro1, Daniel J. V. A. dos Santos6,1,3

1LAQV@REQUIMTE, University of Porto, Portugal; 2Red Glead Discovery AB, Sweden;

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5URPhyM, NARILIS, University of Namur, Belgium; 6CBIOS, Universidade Lusófona de

Humanidades e Tecnologias, Portugal

Abstract:

Over-expression of efflux pumps as P-gp, is one of the most significant mechanisms in Multi Drug Resistance (MDR) in cancer cells. The poly-specificity of the drug-binding pocket (DBP) is considered the major disadvantage for the development of more potent and selective P-gp modulators. The intracellular coupling helices (ICHs) located at the interface between the transmembrane domains (TMDs) and nucleotide-binding domains (NBDs) have been described as interesting hotspots for the development of non-competitive modulators. In this work, and through *in silico* methodologies, a

refined human P-gp model was built and used to study the impact of four MDR-related mutations on P-gp's architecture and efflux mechanism. The results demonstrated that P-gp is sensitive to the presence of these mutations and/or ligands within the DBP, having both a direct impact in the helices repacking, and in the drug-binding-sites (DBSs) properties, mostly affecting the binding affinity and binding mode of the ligands. Interestingly, the helical repacking also affect the ICH-NBDs residues interactions, thus indicating a TMD-NBD communication pathway through ICHs. Two allosteric DBSs (aDBSs) were identified, located in-between the ICHs and specific NBDs motifs, able to bind thioxanthone and flavanone derivatives with favorable binding energies and reducing the P-gp verapamil-stimulated ATPase activity, thus suggesting that these putative aDBSs are druggable. An IC50 of 81 µM is reported for a narigenin derivative, thus demonstrating the "proof-of-concept" that P-gp can be modulated by an allosteric mechanism.

Acknowledgments:

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Commiphora Myrrha Essential Oil Antimicrobial Agent against Microorganisms Tested *In Vitro* Condition

Miroslava Kačániová[1]*, Natália Čmiková[1], Lucia Galovičová[1], Marianna Schwartzová[1], Simona Kunová[2], Milena Vukic[1,3]

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[2]*Institute of Food Science, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia*

[3]*Department of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Serbia*

Abstract:

Myrrh is an aromatic oleogum resin that can be extracted from several plants of the Burseraceae family and from the stalk of *Commiphora myrrha*. It is a potent antimicrobial drug used to treat brucellosis, glandular fever, sinusitis, gingivitis, mouth ulcers, and parasitic infections. Additionally, the volatile oils from myrrh and their unrefined preparations showed a variety of biological effects, including cytotoxic, anesthetic, anti-inflammatory, and antimicrobial effects. In our study it was tested against three Gram-positive (*Staphylococcus aureus* subsp. *aureus* CCM 2461, *Enterococcus faecalis* CCM 4224, and *Streptococcus pneumoniae* CCM 4501), three Gram-negative bacteria (*Escherichia coli* CCM 3988, *Salmonella enterica* subsp. enetrica CCM 3807, and *Shigella sonei* CCM 1373) and four yeast (*Candida albicans* CCM 8186, *Candida glabrata* CCM 8270, *Candida krusei* CCM 8271, and *Candida tropicalis* CCM 8223). This study used two techniques to track antimicrobial action against pathogenic microorganisms. Both the disk diffusion technique and the broth dilution method were used to assess the antimicrobial activity of essential oil. With an inhibition zone measuring 16.67 mm in size, we discovered that myrrh essential oil was the most efficient against the yeast *Candida albicans* and least effective against *Shigella sonei*. Using the broth dilution technique, myrrh essential oil had the strongest antimicrobial activity against all of the pathogenic microorganisms that were tested in the range of concentrations from 125 L/mL to 250 L/mL.

Acknowledgements:

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Styrax tonkinensis Essential Oil Tested against Different Microorganisms *In Vitro*

Natália Čmiková[1]*, Lucia Galovičová[1], Marianna Schwartzová[1], Simona Kunová[2], Milena Vukic[1,3], Miroslava Kačániová[1]

[1]Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture in Nitra, Nitra, Slovakia
[2]Institute of Food Science, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia
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Abstract:

Fast-growing, oil-producing woody shrub Styrax tonkinensis is also used for medicine and as a source of wood. Particularly in the last 20 years, natural products and their semi-synthetic analogs have played a significant role in the formulation and development of antimicrobial drugs. This research concerned the antimicrobial and antifungal properties of Styrax tonkinensis essential oil. Using inhibition zones in agar media and minimum inhibitory concentration (MIC) bioassays, the antimicrobial activity was examined in comparison to bacteria, including both Gram-positive and Gram-negative bacteria. Using a solid medium assay, the antifungal action was tested on Aspergillus flavus, Botrytis cinerea, and Candida albicans. According to the results, Styrax tonkinensis essential oil was highly effective at inhibiting both Gram-positive (Listeria monocytogenes, Micrococcus luteus, and Staphylococcus aures) and Gram-negative (Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa) bacteria. The maximum inhibition zones and MIC values were found to range from 12.67 to 19.33 mm and 3.9 and 62.5 µL/mL. In addition, the Styrax tonkinensis essential oil exhibited antifungal action against A. flavus, B. cinerea, and C. albicans. Research results have shown that Styrax tonkinensis essential oil can be a useful source of natural compounds that can be used as novel antimicrobial agents against microorganisms.

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Flavonoid Derivative FMC, as a Potent Cytotoxic and Apoptosis Inducer in Several Human Cancer Cell Lines

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Abstract:

Synthetic flavonoids with new substitution patterns have attracted attention as potential anticancer drugs. Here, fourteen flavonoids were synthesized and their antiproliferative activities against five human tumour cells were evaluated. These flavonoids derivatives include two cyclic compounds either with or without a furoyl radical. The structure-activity relationship (SAR) revealed that (i) the presence of a 2′ amino group in 4-methoxychalcone generated a more cytotoxic compound than the corresponding 2′-hydroxy against leukemic cells, and (ii) the introduction of a furoyl radical in position 2′ as an ester or an amide group enhanced the cytotoxicity against leukaemia and melanoma cells; and (iii) the substitution of 2′-hydroxy for a 2′-amino group in 3,4,5-trimethoxychalcones enhanced the cytotoxicity but the corresponding furoyl derivatives did not enhance it as in the case of 4-methoxychalcones. The 4-methoxychalcone containing a furoyloxy radical at 2′ on the A ring (FMC) displayed less cytotoxicity against human peripheral blood mononuclear cells and fibroblast-like Vero cells. Treatment of U-937 and HL-60 cells with FMC inhibited colony formation, induced cell cycle arrest at the G2-M phase, an increase in the percentage of sub-G1 and annexin-V positive cells, the release of mitochondrial cytochrome *c*, activation of caspase and poly (ADP-ribose) polymerase cleavage. In addition, it inhibited tubulin polymerization *in vitro* in a concentration dependent manner and induced changes in BCL-2 family proteins expression and MAPK activation. Cell death triggered by this chalcone was decreased by a pan-caspase inhibitor and was dependent of the generation of reactive oxygen species.

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Lignan Profiling and Antioxidant, Anti-inflammatory and Anticancer Activities of Extracts from *Schisandra henryi* Leaves and Microshoot Cultures Maintained in Plant-Form Bioreactors

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Abstract:

Schisandra henryi is a rare vine-plant species, known in traditional Chinese medicine, but of scientifically unproven biological activities. The aim of our studies was estimation of phytochemical profile and biological activities of leaves as well as established for the first time in vitro cultures of this species. The microshoot cultures were cultivated in PlantForm bioreactors on Murashige-Skoog medium with 2 mg/l indolyl-3-butyric acid and 0.5 mg/l 6-benzyladenine over 30-days (3 series). The antioxidant potential was assessed using: CUPRAC, FRAP, DPPH tests and the total content of polyphenols was assessed with Folin-Ciocalteu assay. The anti-inflammatory activity was measured with *in vitro* inhibition tests of $sPLA_2$, 15-LOX, COX-1 and COX-2. The antitumor activities (antiproliferative and cytotoxic) were estimated against Jurkat, MCF-7, HT-29 and HEK-293 HeLa lines. The lignan profiles of leaf and microshoot extracts were done with UHPLC-MS/MS method. The antioxidant activity of the microshoot cultures assessed by CUPRAC, FRAP and DPPH tests was 3.8-, 5.6- and 3.3-times

higher than leaves, respectively. The total polyphenol content was 4.1 times higher. Microshoot extracts showed the highest activity of COX-1 and COX-2 inhibition (76% and 66%, respectively). For the leaf extracts that was 70% and 36%, respectively. Tested extracts showed antiproliferative activity against tested tumor cells. The highest activity was shown by the leaf extract against HT-29 cells. As the dominant lignans in the phytochemical estimations were indicated: schisantherin A and B, licarin A and deoxyschisandrin. This is the first report confirming the high pharmacological potential of extracts from *S. henryi* leaves as well as from biomass of microshoot cultures grown in PlantForm bioreactors.

In Vitro Biological Activity and Phenolic Profile of Selected Portuguese Monofloral Honeys

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Abstract:

Portuguese monofloral honeys from carob tree, chestnut, bell heather, eucalyptus, incense, orange, and strawberry tree, were evaluated in vitro for antimicrobial, antioxidant, wound healing, and cell viability effect, compared to manuka honey 850+. Antimicrobial activity was determined against Gram+-and Gram- bacteria and yeast. Antioxidants, wound healing, and cell viability effects were studied in the Human Keratinocyte (HaCaT) cell line. Chestnut, bell heather, eucalyptus, manuka and strawberry tree honeys were most effective against S. aureus with a minimum inhibitory concentration (MIC) of 12.5%-25.0% (w/v), and greater ability to decrease reactive oxygen species (ROS) production (> 75%), than manuka honey (68%). Incense and orange honeys exhibited high wound healing rates, 89% and 86%, respectively, higher than manuka honey, 53%. Honeys showed cell viability > 76%. Bell heather and strawberry tree honeys exhibited the highest total phenolic content, 38 and 137 mg/100 g honey respectively, being more effective against the microorganisms tested and showing greater antioxidant activity. Opposite, incense, and orange honeys with lower phenolic amounts, 11 and 15 mg/100g honey, respectively, achieved higher wound healing ability. Flavonoid aglycones were the

most abundant flavonoids in all honeys. This knowledge can be further explored in formulations that take the best out of each honey type composition and biological activity capacity.

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Extraction of Phospholipids, Alpha-tocopherol and Omega-3 Fatty Acids from Squid Waste by Low-toxic Solvent Employment

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Abstract:

Processing marine species generates a great amount of by-products, which constitute an important source of high-added value molecules but also led to environmental contamination. This study focused on the recovery of bioactive compounds from Patagonian squid (*Doriteuthis gahi*) by-products. Extraction conditions of phospholipids (PLs), α-tocopherol, and ω3 fatty acids (FAs) were analysed by employing different concentrations of three low-toxic solvents (ethanol, acetone and ethyl acetate). A remarkable attention was also accorded to FA ratios (polyunsaturated FAs/saturated FAs and ω3 FAs/ω6 FAs). Results were compared to yields obtained by traditional (i.e. chloroform/methanol) extraction. As a result, ethanol-including extracting systems led to higher PL values than the traditional procedure and any other eco-friendly system tested. Contrary, acetone- and ethyl acetate-containing systems led to a higher recovery of α-tocopherol, eicosapentaenoic acid, docosahexaenoic acid compounds and greater polyunsaturated FA/saturated FA ratio; in such cases, levels were higher than those obtained by the traditional extraction procedure. Finally, ethanol-containing systems provided higher ω3 FA/ω6 FA values than any other eco-friendly system and were similar to those detected in the lipid extract obtained by the traditional procedure. Differences are discussed and explained based on the different polarities and extracting capacities of the different solvents. The suitability of low-toxicity solvents tested was concluded, matching present international interests in the search for alternatives for extracting systems that provide valuable constituents on healthy and nutritional properties from a waste marine substrate.

Iris xiphium L. Flowers Extract Induces Selective Antiproliferative Activity and G1 Cell Cycle Arrest in A549 Lung Cancer Cells

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Abstract:

Currently, lung cancer is the leading cancer-causing death worldwide (18% of total cancer deaths). Non-small cell lung cancer (NSCLC) represents approximately 80% of cases [1]. High mortality rates in patients with NSCLC are caused by resistance and lack of selectivity of the available therapies. The importance of finding new selective anticancer drugs is the key to fighting cancer. Some useful clinical anticancer drugs are derived from plants [2]; for this reason, we focused on a random screening of plants collected in Andalusia for cytotoxic activity on A549 lung cancer cells versus HaCaT non-malignant cells. Cell viability was measured with the Resazurin assay. An extract of the naturalised *Iris germanica* L. rhizome showed cytotoxicity and selectivity against A549 cells (IC50 35,4 ± 3,97 µg/mL and selectivity index 7,46). Our next aim was to collect and prepare extracts of Iris plants in several areas of Andalusia. The anticancer activity of the extracts was evaluated by the Resazurin assay on A549 and HaCaT cells, as well as on two cell lines of other common types of cancer, melanoma MeWo cells and bladder cancer T24 cells. A549 cells were the most sensitive cells to all Iris extracts, highlighting the extract of the flowers of *Iris xiphium* L. (IC50 8,66 ± 2,18 µg/mL and selectivity index 38,92). Cell cycle analysis indicated that this extract caused the arrest of A549 cells in the G1 phase. These results suggest that Iris species may have potential as source of compounds to treat lung cancer.

Cloning of Strawberry's Malonyltransferase Genes and Characterisation of their Enzymes

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Abstract:

Malonyltransferases (MATs) are enzymes that play a key role in the biosynthesis of secondary metabolites in plants, such as flavonoids and anthocyanins. As a kind of flavonoid-rich fruit, strawberries are an ideal model to study MATs. From Goodberry metabolome data, in the hybrid generation of 2 strawberries various, *Fragaria* × *ananassa* cv. 'Senga Sengana' and 'Candonga', we found the malonylated flavonoid concentration is significantly higher in 'Senga Sengana' compared with 'Candonga'. Therefore, we want to study the malonyltransferases in strawberries that take responsibility for this difference. In this study, we have cloned, expressed and characterized 5 MATs from strawberries catalyzed the malonylation of flavonoid substrates: quercetin-3-glucoside, kaempferol-3-glucoside, pelargonidin-3-glucoside, and cyanidin-3-glucoside. All four compounds reacted with FaMATs to varying degrees. These MATs have important implication into strawberries' flavonoid biosynthesis, and also provide insights into the regulation of secondary metabolism in plants and may have practical applications for improving the nutritional quality and flavour of crops.

Phytochemical and Bioactive Screening of Natural Plant Matrices from the Cumbira Forest Reserve (Cuanza Sul, Angola): *Lablab purpureus* (L) Sweet, *Dombeya rotundifolia* (Hochst.) Planch. and *Commelina africana* L.

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Abstract:

The exploitation of natural matrices as rich sources of bioactive compounds is a topic of great scientific relevance since the chemical and bioactive characterization of plants can benefit both the rural and global communities. *Lablab purpureus* (L) Sweet, *Dombeya rotundifolia* (Hochst.) Planch. and *Commelina africana* L. are plants native to Africa, commonly used by rural Angolan communities, particularly those living in the surrounding area of Cumbira Forest Reserve, in Cuanza Sul province. The leaves of these species are consumed as a vegetable, besides being used as fodder in animal feed. This work aims to determine the nutritional and chemical profiles, as well as the bioactive potential of hydroethanolic extracts of the leaves of these species, promoting their consumption while ensuring greater sustainability in their exploitation and conservation. Their nutritional profile is mainly rich in proteins, ashes, and carbohydrates, while several organic acids, sugars, fatty acids, and tocopherols were also identified. The extracts showed good antioxidant activity, with EC_{50} values ranging between 215 and 219 µg/mL for the TBARS assay. Additionally, all extracts showed excellent antimicrobial activity, with the minimum inhibition concentration (MIC) ranging between 0.6 and 10 mg/mL. Overall, the *D. rotundifolia* hydroethanolic extract showed to be the most efficient in inhibiting both food and clinical bacteria. Together, the attained results clearly demonstrated that these species are valuable sources of nutrients and bioactive compounds able to perform different biological activities, validating their use not only as food but also as a source of compounds with different medicinal assets.

Rutin-loaded Cerosomes: Enhancing the Delivery of a Bioactive Natural Compound for the Management of UV-induced Skin Diseases

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Abstract:

Many dermatological conditions, such as xeroderma pigmentosum, are associated with UV induced damages. Currently, these diseases can be managed by therapeutic strategies, but the incidence of adverse effects and lack of efficacy raise the need for adjuvant treatments based on photoprotective cosmetic formulations. In the last years, researchers have been exploring the possibilities offered by natural compounds, mainly those extracted from plants. In this context, Rutin, a well-known flavonoid, may be a promising active compound, due to its valuable antioxidant and photoprotective features. Nevertheless, the skin delivery of rutin is impaired by its high molecular weight and low water solubility. Accordingly, the aim of this work was to develop a nanovesicular system made of ceramides and lecithin – so-called cerosomes - for the topical delivery of Rutin, using a Box-Behnken factorial design (BBD). The BBD approach was settled considering the independent variables: lipid

concentration, ceramide concentration, and lipid/edge activator ratio. To evaluate the effect of the variables on cerosomes' physicochemical properties, the formulations were characterized in terms of size, polydispersity index (PDI), zeta potential (ZP), and encapsulation efficiency (EE). The optimized cerosomes showed promising properties for the skin delivery of rutin: size < 300 nm, PDI < 0.25, ZP < –35 mV (ZP), and EE > 50%. Hence, BBD was a useful tool to optimize rutin-loaded cerosomes and further studies are ongoing to ascertain the stability, safety, and efficacy of these innovative nanovesicular systems.

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Selective Antitumoral Activity of *Thymelaea lanuginosa* (*Lam.*) *Ceballos & C. Vicioso.* Extract

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Abstract:

In a previous work it was presented the preliminary results for the selective cytotoxic activity of *Thymelaea hirsuta* (L.) Endl. extracted with ethanol/ethyl acetate/water (1:1:1), in a panel of cancer cell lines. Since then, we have focused on active search for species from the *Thymelaea* genus and found that *T. lanuginosa* (Lam.) Ceballos & C. Vicioso. (Extracted with the same solvents' mixture previously mentioned) showed IC_{50} values 10000-fold lower in lung cancer cells (A549) comparing with normal cells (HaCaT). This activity may be due to the presence of daphnane diterpenes previously described in the Thymelaeaceae family. To know which of the solvents was responsible for extracting the active compounds, two different extracts were prepared: 1) Water and ethanol (1:1) and 2) Ethyl acetate 100%. However, both showing similar IC_{50} values like those of the original extract in the A549 cell line. In fact, thin layer chromatography patterns were very similar for all the extracts. Therefore, it was carried out preparative thin layer chromatography (PTLC) to recover the five separate different fractions from ethanol/ethyl acetate/water (1:1:1) extract and perform a preliminary evaluation of their phytochemistry and bioactivity. Considering the fact that the antitumoral activity *in vitro* evaluation of the fractions was similar to the one of the initial extract, this leads to think that the activity shown could be due to synergetic effects between different compounds that need to be clearly identified and structural characterized on ongoing studies.

SOMAÍ Approach: Non-cannabinoids Study to Setting New Standards in Medical Cannabis

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Abstract:

Cannabis, renowned for its versatility in textiles and food, [1] has emerged as a prominent therapeutic resource. Extensive research focuses on unravelling the molecular composition of over 100 cannabinoids found in cannabis, exploring their interactions with the endocannabinoid system to develop innovative therapeutic approaches. The current era marks the golden age of cannabinoid research, as cannabis is considered a chemical "treasure trove" of novel compounds that can be studied and brought to the medical market [2]. In addition to cannabinoids, the unique constituents of cannabis are increasingly acknowledged for their potential impact on its medicinal properties. These components possess inherent medicinal qualities and can synergistically interact with cannabinoids. At the forefront of research, SOMAÍ Pharmaceuticals is dedicated to establishing groundbreaking standards in therapeutic innovation. Our mission is to alleviate the burden of debilitating chronic conditions. We take pride in our commitment to utilizing the finest raw materials to formulate novel medications. By prioritizing high bioavailability and specifically targeting the endocannabinoid system, we strive to deliver effective relief for specific conditions. This study aimed to quantify well-known components such phenolic content, flavonoids, waxes, and chlorophylls in both the raw and purified extracts of SOMAÍ. The goals were two-fold: to gain insights into the preservation of these components during the extraction and purification process; and to lay the foundation for future characterization studies. These characterizations will facilitate the examination of how these components impact the formulations developed by SOMAÍ.

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Biological Activity Screening of Plant Oils for Skincare Applications

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Abstract:

Essential and vegetables oils are both types of oils derived from plants, but they have distinct characteristics and uses. Essential oils are a precious source of secondary metabolites (mainly polyphenols, alkaloids and terpenes) potentially endowed with a plethora of biological activities. Vegetable oils, also known as plant oils, are lipidic extracts derived from plants that are commonly used for cooking, cosmetic formulations, and various industrial applications. The development of high-added-value products from the essential and vegetable oils industries can be considered a priority due to their broad range of bioactive compounds. Those bioactive compounds could be used for human consumption, medicinal uses and other health products applications, including cosmetics. The aim of this work was to explore the biological potential of commercially available oils. We studied plants that have been traditionally used due to their antimicrobial, anti-inflammatory and antioxidant activities. *Moringa oleifera* Lam. (*Moringaceae* family), *Lavandula angustifolia* and *Ocimum kilimandscharicum* Gürke (Lamiaceae family), *Spirostachys africana* Sond. (Euphorbiaceae family), *Chrysopogon zizanioides* and *Cymbopogon citratus* (DC.) Stapf (Poaceae family), *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson and *Eucalyptus globulus* (Myrtaceae family) were the species studied. The antiproliferative potential was evaluated against HaCaT and HFF-1 cells using the sulphorodamine B colourimetric assay. The antioxidant activity was assessed throught DPPH and TBARS assays. The antimicrobial activity was tested against several bacterial and fungal strains using the broth microdilution method. Additionally, the anti-inflammatory capacity through the measurement of oils capacity to inhibit the formation of the nitric oxide in a murine macrophage cell line. Ongoing research is being conducted to further explore the therapeutic properties and potential health benefits of the eight oils extracted from plant materials, emphasizing their use and applications in promoting well-being.

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Exploring the Inhibitory Effects of Côa Valley Plant Extracts on Osteoclast Differentiation: Chemical Characterization and Cytotoxicity

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Abstract:

Osteoclasts are specialized cells with active mitochondria that play a crucial role in bone resorption. RANKL is essential for osteoclast differentiation and activation, promoting the production of reactive oxygen species (ROS) involved in osteoclast formation and function. The decline in estrogen levels during menopause increases the susceptibility to osteoporosis. Current therapies for osteoporosis have limitations, requiring the development of safer and more accessible alternatives. In this study, we obtained decoction and ethanolic maceration extracts from plants indigenous to the Côa Valley (*Equisetum ramosissimum* Desf. (ER), *Urtica dioica* L. (UD), *Urtica urens* L. (UU)) and explored their potential for treating osteoporosis. Through LC-MS analysis, flavonoid and phenolic compounds were identified in the extracts. Our goals included perform plant extractions, analyse their chemical composition, and evaluate their antioxidant activity. Subsequently, we aimed to evaluate the extracts cytotoxicity to gain insights into their inhibitory effects on osteoclast differentiation. Using RAW 264.7 cells exposed to aqueous and ethanolic extracts at concentrations ranging from 1 mg/mL to 0.063 mg/mL over a 7-day period, we observed a decrease in cell viability when cells were exposed to decoction extracts from all plant species. Conversely, the ethanolic extracts demonstrated no cytotoxicity until 1 mg/mL for ER and UD, and for UU, no toxicity was observed with concentrations up to 0.125 mg/mL. Preliminary data showed that these extracts inhibit osteoclast differentiation. Ongoing research aims to further explore the inhibitory effects of these extracts and elucidate their mechanisms of action, to identify the chemical compounds responsible for the bioactivity.

Extraction and Purification of Algae Extracts (*Asparagopsis armata*) and Evaluation of its Antimicrobial Activity

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Abstract:

Natural products have revealed to be the major source of compounds for drug development, and consequently, a large number of plants, animals, bacteria, and fungi have been examined for drug discovery. The excessive and inadequate use of drugs has caused an increase in resistant bacteria and, consequently, in infectious diseases. As a way of overcoming this limitation, several species of macroalgae have been studied, since they present bioactive compounds with pharmacological activity, in addition to presenting a large number of compounds with antioxidant activity. The red macroalgae *Asparagopsis armata* (Rodophyta) was used in this study with aim to extract and characterize bioactive secondary metabolites in terms of antimicrobial activity. The organic and aqueous extracts were evaluated in terms of the antioxidative activity, phenol concentration and antimicrobial activity. The extract obtained using acetone showed higher antioxidant value (25,79%), based on scavenging activity method, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, while the phenol content in the extracts, obtained using acetone, presented higher values (0.077 mg/ml Gallic Acid Equivalent). Regarding the aqueous extract, it was previously purified using chromatographic techniques (ion exchange and molecular exclusion) in order to isolate a wide range of aqueous extracts. Based on the antimicrobial activity analysis, the organic and purified extracts were tested in *Escherichia coli* gram (-) and in *Bacillus subtilis* gram (+) bacteria. Based on the assays, some extracts showed a slight antimicrobial activity, but, in the next future, it will be necessary to optimize the extraction and purification conditions to confirm those preliminary results.

Is Supplementary Chromium (III) Beneficial for Treating Diabetes? A Reference to Own Study

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Abstract:

Many clinical studies have demonstrated a significant improvement of glucose tolerance after Cr (III) supplementation in type 2 diabetics. However, other trials failed to confirm beneficial effects in diabetics. Irrespective of controversial scientific opinions, various Cr (III) compounds have been advertised worldwide, as popular dietary supplements to improve glycemic control, or reduce appetite. Besides, Cr itself has been included into the list of nutrients in many national dietary guidelines. The aim of this presentation is to review of the current state-of-the art about Cr (III) that has been considered for over 60 years as an essential micronutrient for animals and humans. Chromium (III) has a documented effect on carbohydrate, lipid, and protein metabolism; however, the mechanism(s) of its action on the molecular level have not been fully understood. The recent studies performed on experimental animals raised some doubts on the essentiality of Cr for mammals, including humans, and made researchers re-evaluate dietary recommendations on Cr. If Cr (III) is not an essential element for mammals, but at certain dosages improves impaired glucose and lipid homeostasis, its action could be called "pharmacological" at best. Recent own study performed in this project* demonstrated that supplementary Cr (III) at pharmacologically relevant doses increased the antioxidant protection in type 2 diabetic rats. The mode of action of Cr (III) at a molecular level is still an area of active debate, however, it suggests it acts as a second messenger, amplifying insulin signalling.

Acknowledgements:

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Steviol Glycosides and Glucose Metabolism-related Protein Levels in Diabetic Rats

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Abstract:

Stevia rebaudiana Bertoni and its glycosides are popular mainly for its sweet taste. However, steviol glycosides exert health-promoting effects, most importantly antidiabetic properties. The mechanisms of these effects have not been fully understood. The aim of this in vivo experiment was to determine the effect of the addition of stevioside and rebaudioside A to a high-fat diet (HFD) on glucose metabolism related proteins (GLUT-4, IRS, IR, AMPK, AKT) levels in tissues of type 2 diabetic rats, hypothesizing that the tested compounds can affect the expression of the key signaling proteins in the course of diabetes. The study was performed on 70 male Wistar rats with induced type 2 diabetes (HFD feeding + streptozotocin injection). Experimental diets in groups (n = 10) were as follows: AIN-93M (healthy C group), HFD, HFD + metformin and HFD enriched with the test substances (stevioside and rebaudioside A (0.5 or 2.5%). For protein examination, properly truncated tissues

(adipose tissue, muscle tissue) were analysed using Western blot technique. The results of statistical analysis showed that supplementary compounds have no effect on the level of selected proteins. However, a number of statistically significant correlations were found between certain biochemical parameters (liver enzymes, glycaemia, insulinaemia) and several glucose metabolism related proteins (IR, IRS, GLUT-4) levels in tissues of diabetic rats. This study provides indirect evidence that stevia-derived compounds can modulate glucose metabolism in type 2 diabetes. The outcome allows to conclude that these compounds may be regarded as supportive therapy for type 2 diabetes.

Bioaccumulation of Health-promoting Elements in the Biomass of Verbena officinalis In Vitro Cultures

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Abstract:

Scientists constantly are looking for innovative sources of substances aimed at meeting the needs of the body and supplementing the deficiency of nutritional elements. A promising solution is biofortification, which involves enriching plants with the right nutrients at the source, i.e. at their growth stage [1]. This process can be carried out using *in vitro* cultures, that is, plant material grown under controlled sterile conditions. In the work, *in vitro* cultures of *Verbena officinalis* L. were used due to the rich array of pharmacological properties (e.g. anti-inflammatory, analgesic, antioxidant, antibacterial, antifungal, gastroprotective) [2] of this plant. Shoot *in vitro* cultures of *V. officinalis* were enriched with health-promoting elements (Ca, Mg, Fe, Cr, Li, Zn) at specific concentrations (Ca^{2+} i Mg^{2+} - 1, 5, 10, 25, 50 mg/l and Fe^{3+}, Cr^{6+}, Li^{+}, Zn^{2+} - 10, 25, 50 mg/l). After the culture were completed, mineralization was carried out, and then the elements were quantified using the Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) method. The highest bioaccumulation was observed in cultures enriched with lithium and chromium. For the results of biofortification of lithium at a concentration of 50 mg/l, an increase of 1,446,693% was obtained, and for the results of biofortification of chromium, at a concentration of 10 mg/l, an increase of 1,179,521% was reached. Zinc, iron and magnesium are also elements well absorbed by this plant species. The smallest bioaccumulation in relation to the control sample was confirmed in the case of culture enriched with calcium.

Effect of *Fucus vesiculosus* Aqueous Extract on Cholesterol Transport and Permeation: A Potential Functional Food

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Abstract:

In recent years, the search for a healthier lifestyle has increased around the world, associated with the growing consumption of natural products such as algae. The brown seaweed, Fucus vesiculosus, has traditionally been used to prevent hypercholesterolemia, among other uses. However, its molecular mechanism of action is still not fully understood. This work aims to study the in vitro effect of the aqueous extract of F. vesiculosus, rich in phlorotannins and peptides, on the expression of different proteins involved in the synthesis and transport of cholesterol using HepG2 cells and caco-2 cells differentiated into enterocyte-like cells though western blot, qRT-PCR and gel-based proteomic analysis. This work also intends to study the effect of F. vesiculosus on cholesterol absorption simultaneously with the drug Ezetimibe. In enterocyte-like cells, the proteomic results showed that the extract increased the expression of NPC1, an important protein in cholesterol transport. Higher levels of inhibition of cholesterol absorption were also observed in the presence of the extract and the drug ezetimibe when compared to the action of the drug alone. In liver cells, the extract decreases the expression of two important cholesterol transporter proteins, NPC1L1 and ABCG5, as well as decreases NPC1L1 mRNA levels. These effects may be beneficial in increasing biliary excretion of cholesterol and, consequently, decreasing cholesterol accumulation in the blood and tissues. Our study demonstrates some possible mechanisms of action of the aqueous extract of F. vesiculosus which may explain its hypocholesterolemic effect, opening doors for its use as a functional food.

Carbonic Anhydrase and Anticholinesterase Effect of Turkish Plant Extracts

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Abstract:

Turkey is home to a wide variety of plants. Many plants growing in different parts of the country have been the subject of study and studies on plant extracts showing various bioactivities have been brought to the literature. Enzyme inhibitors have a significant impact on the treatment of various diseases. Inhibitors of specific forms of the enzyme Cas (e.g., CA I / II) have been used to create new drugs for conditions such as epilepsy, edema, and glaucoma. As a result, new inhibitors of CA isoenzymes need to be developed because of their potential to be used as therapeutic agents. Specific inhibitors can be utilized to treat motor neuron diseases like dementia, myasthenia gravis, and Alzheimer's by decreasing the activity of AChE/BChE. *Diplotenia cachrydifolia, Anarrhinum orientale, Fumaria asepola, Rhabdosciadium microcalycium, Zosima absinthifolia, Salvia pseudeuphratica* and *Ferulago stellat*a collected from Bitlis, Turkey. In this study, inhibitory properties of seven different plant extracts on carbonic anhydrase (CA) I, II, acetylcholinesterase (AchE) and butyrylcholinesterase (BchE) enzymes were investigated. The extract of *Zosima absinthifolia* both AchE and BchE showed a very active inhibition profile (IC50 1.26 ± 0.01 µg/mL for AchE and 1.32 ± 0.02 µg/mL for BchE). The results indicate that these extracts are potent cholinesterases and new potential drugs.

Development and Sensory Analysis of *Adansonia Digitata L.* Fruit-based Beverages

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Abstract:

Múcua is the fruit of the *Adansonia digitata L.* tree, native to Africa, known for its high content of ascorbic acid, dietary fiber, and antioxidant properties. In June 2023, a study was conducted to develop múcua beverages and evaluate their sensory properties and purchasing intentions. The beverages were prepared using a handmade process, one with water, múcua, and honey (MB), and another with these ingredients along with mango and ginger (MMB). Participants (n=117) tasted the beverages and completed a questionnaire covering socioeconomic characteristics, acceptability tests (9-point Likert Type Scale) for MB and MMB, and willingness to pay and frequency of consumption. MB received a median (P25; P75) score of 6 (4; 8) for attributes such as appearance, color, aroma, texture, and flavor. It scored 5 (3; 7.5) for sweetness, (3; 7) for acidity, and 7 (4; 8) for overall appreciation. MMB had a median (P25; P75) score of 7 (4; 9) for the same attributes, with sweetness scoring 6 (3.5; 8.5), acidity scoring 5 (3; 8), and overall appreciation scoring 7 (4; 9). Around 30% and 40% of the participants expressed willingness to consume the MB and MMB, respectively, 1 to 6 times a week if they were available at an affordable price. The median (P25; P75) prices they would be willing to pay for the MB and MMB were €2 (€1.55; €3) and €3 (€2; €4), respectively. The panelists showed good acceptance of the beverages under study. However, there is room for improvement in future formulations to enhance certain attributes.

Virtual Posters

Evaluation of the Effect of Polymeric Micelles-based Hydrogel Loaded with Oregano Essential Oil on Skin Tags

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Abstract:

Origanum vulgare var. *vulgare* essential oil gained attention in the dermato-cosmetic industry due to an impressive series of therapeutic applications (i.e., acne, aging, wound healing) revealed in recent years [1]. Withal, skin tags occur with a prevalence of 50–60% in the adult population and can become disturbing for the patient [2]. The study was designed for the evaluation of the effect of polymeric micelles-based hydrogel loaded with oregano essential oil on skin tags. Twenty volunteers were diagnosed with skin tags by means of dermoscopy and Woof lamp examination and were further enrolled in the study. The evaluation was approved by the research ethics committee of UMFT, Romania (Nr. 04 a/17.06.2022). Non-invasive measurements, determined with Multiprobe Adapter System (MPA5) from Courage-Khazaka Electronics, Germany, equipped with Mexameter® MX18, Tewameter® TM300 and Corneometer® CM 825 probes, were used in order to appreciate modifications in physiological skin parameters. A histological examination of the unfallen skin tags was also conducted. Increases in trans-epidermal water loss and erythema index were recorded, while skin hydration decreased during treatment with OEO-PbH. Clinically, the volunteers presented a lowering of the number of skin tags with 50% after two months of treatment. Hydrogel application did not influence skin tags larger than 5 mm. In the same time, 70% of the patients confronted with mild xerosis of the skin around the treated zone, which subsided promptly after treatment with emollients. The results of the histological examination were consistent with those observed during clinical assessment.

Acknowledgement:

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Acylglucose and Acilinositols Present in Natural Extracts Inhibitors of Cdr1 and Mdr1 Efflux Transporters in Resistant Candida Strains

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Abstract:

Candida species represent more than 80% of all nosocomial infections. Failures in the antifungal therapies due to the development of resistance lead to an urgent need of agents able to overcome this phenomenon. The azoles represent the drugs of choice for the treatment of fungal infections, being fluconazole (FCZ) the main representative. Resistance not only affects the effectiveness of azoles but also of multiple drugs (MDRs). One of the main mechanisms of MDR is the overexpression of genes encoding the transporter proteins such as Mdr1 and Cdr1, which expel antifungal drugs out of the cells therefore decreasing their intracellular concentration. To determine the ability of plant-

derived extracts to reverse MDR by inhibiting FCZ efflux, resistant Candida albicans and C. glabrata clinical isolates overexpressing Mdr1 and Cdr1, as well as Saccharomyces cerevisiae strains that selectively express Mdr1 (AD/CaMDR1) or Cdr1 (AD/CaCDR1) were tested by agar chemosensitization assays. Of the 137 extracts tested at a maximum concentration of 200 µg/ml, those obtained from Acalypha communis and Solanum atriplicifolium showed to be the most active by decreasing the minimum inhibitory concentration (MIC) of FCZ 4 to 32 times with minimum effective concentration (MEC) values of 25 µg/mL. S. atriplicifolium was submitted to bioguided isolation yielding different acylglucose and acylinositols, which show promising resistance reversing effects. These compounds arise as promising candidates to sensitize Candida resistant strains to fluconazole.

New Guanidine-chalcone Hybrid Agents are Potent Antiproliferative Compounds against Human Leukemia Cells

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Abstract:

Secondary metabolites of plants exhibit a broad spectrum of pharmacological properties, including anticancer activities. One of these metabolites are chalcones, the major precursors of flavonoid biosynthesis that are found in a wide variety of foods in the diet. They can interact with various anticancer drug targets, exhibiting promising activities *in vitro* and *in vivo*. On the other hand, guanidines, whose derivatives are widely distributed in nature, are versatile organosuperbases with important biological properties and chemical and pharmaceutical implications. In this communication, I wish to disclose the evaluation antiproliferative against cancer cells of 11 guanidine-chalcone hybrids containing different substituents on the guanidine functional group. The structure-activity relationships revealed that the most potent inhibitors of cancer cells viability were a *p*-toluenesulfonylguanidine containing an *N*-methylpiperazine and a *N*-methyl-*N*-phenylguanidine containing a piperidine ring. These compounds induced apoptotic cell death in the leukemic cell line U-937, which was associated with cytochrome *c* release, caspase activation, and PARP cleavage, while overexpression of the antiapoptotic protein Bcl-2 did not block the inhibition of cell viability by these compounds. Both compounds showed less cytotoxicity and less increase in the percentage of annexin V-positive cells against peripheral blood mononuclear cells of healthy donors. All this suggests that these compounds may have a promising future as possible drugs with significant therapeutic value.

Evaluation of Essential Oils from the Brazilian Species *Baccharis trimera* and *Mimosa verrucosa* against *Ctenocephalides felis felis*

Nayana de Figueiredo Pereira[1]*, Bianca Augusto de Souza[1], Diefrey Ribeiro Campos[1], Nathalia Camargo[1], Daniel Falcão Lopes Princisval Carlos[1], Cristiano Jorge Riger[1], Yara Peluso Cid[1], Douglas Siqueira de Almeida Chaves[1]

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Abstract:

Essential oils shown potential to control ectoparasites, being an alternative to the use of synthetic products. The aim was to evaluate the essential oil of *Baccharis trimera* and *Mimosa verrucosa*, against the ectoparasite *Ctenocephalides felis felis,* and to evaluate the toxicity from the model *Saccharomyces cerevisiae* eukaryote. Essential oil was obtained by hydrodistillation and characterized by GC/FID/MS. Mortality at different immature stages and among adult fleas was measured through *in vitro* filter paper tests at different concentrations of EOs. The major compounds for *B. trimera* were carquejila acetate (33.0%) and *β*-pinene (5.5%) and for *M. verrucosa* were *β*-pinene (14.2%) and *γ*-himachalene (8.1%). A low toxicity was record for both EO at 0.2 mg.mL^{-1}. The *in vitro* tests showed the essential oil of *B. trimera* ideal against adult fleas (800 µg.cm-$^{-2}$; LC_{50}= 369.22 µg.cm^{-2}) in 24 and 48h and *M. verrucosa* essential oil ideal against flea larvae (791.2 µg.cm^{-2}; LC_{50} = 266.29 µg.cm^{-2} and LC_{50} = 240.54 µg.cm^{-2}) in 24 and 48h, respectively. It is a study with results and with potential for the development of alternative products from the essential of *B. trimera* and *M. verrucosa* in the control against *C. felis felis*, which show low toxicity and great activity.

Quantification and Characterization of Phenolic Compounds present in *Foeniculum vulgare* by LC/MS/MS

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Abstract:

The interest in aromatic plants as *Foeniculum vulgare* is driven by their many applications, including food and pharmaceuticals. Phenolic are the main phytoconstituents found and have been linked to the prevention of diseases related to oxidative stress. Leaves of *F. vulgare* were extracted by decoction (10% w/v for 15 min) filtered and dried by lyophilization. The extract was processed in methanol and submitted to HPLC-DAD and an ion trap mass spectrometer in negative mode. The quantification of total phenolics and flavonoids were based on the spectrophotometric method using Folin-Ciocalteau reagent and aluminum chloride, respectively. A C_{18} column was used and gradient method of water (0.01% formic acid) and acetonitrile, flow 1 mL/min. To identify glycosylated flavonoids an HPLC/DAD/MSn analysis were performed comparing mass spectra with known flavonoid standards and literature data. The extract showed levels similar to those reported in the literature, both for phenolics (8.43mg of gallic/100 mg of extract) and flavonoids (1.15mg quercetin/100mg extract). Fourteen compounds were identified. By the successive losses were deduced aglycone fragment ions, such as m/z 301 (quercetin), m/z 285 (luteolin), m/z 315 (isorhamnetin) and m/z 285 (kaempferol) from [M − H]$^{-}$, possessing sugars (glucose, galactose and pyranose) and feruloylquinic acid derivatives. The monosaccharide units were determined by observing the fragment ions that are indicative of the loss of sugar residues and suggest the interglycosidic bond is broken, such as the rhamnosyl (146 u) and glucosyl residues (162 u). Given the rich phenolic composition and antioxidant properties, *Foeniculum* can be used as a functional food with potential health benefits.

In Vitro Analysis of the Activity of the Essential Oil of *Schinus molle* L. and its Terpinol Isolate against *Leishmania amazonensis* as well as its Effect on Vertebrate Host Cells

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Abstract:

Leishmaniasis belongs to a group of parasitic diseases caused by protozoa, and *Leishmania amazonensis* is one of the species that are epidemiologically important, because they cause the cutaneous and cutaneous-diffuse forms in America. First-choice drugs promote a high toxicity and cost. In the search for an alternative treatment less harmful and costly to patients are essential oils (EO). The popular use of them in treatments of leishmaniasis has already been reported. The species *Schinus molle L.* and its isolate terpinol were chosen because of medicinal properties are described. Dried leaves of *S. molle* were submitted to hydrodistillation, obtaining an yield of 3.2%. The results showed the EO has leishmanicidal activity IC_{50} of 0.03 µg/mL and its isolate 1.5mM for promastigote forms after 24h, demonstrating a dose-dependent effect. In peritoneal macrophages of murine BALB/c, the amastigote forms were affected by EO at concentrations of 0.06 and 0.1 µg/mL, decreasing from 45 to 47% after 24 h and by terpinol at 3.0 and 6.0 mM, with a decrease of 53 and 60%, respectively, the association between parasites and host cells. The EO reduced the number of promastigotes by 22, 30 and 44% in the treatments by 0.03; 0.06 and 0.1 µg/mL and terpinol 26, 56 and 80% for 0.75; 3.0 and 6.0mM. In the cytotoxicity assay (XTT), the EO showed no relevant changes, demonstrating a low toxicity, while terpinol showed toxicity at higher concentrations. The results indicate a promising possibility of the compounds for use as chemotherapy against *Leishmania amazonensis* and its infective forms.

Antimicrobial Activity of EOs from *Baccharis trimera*, *Eremanthus erythropappus*, *Illicium verum* and *Mimosa verrucosa* against Bacteria Associated with Periodontal Disease

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Abstract:

Periodontal disease is an important infectious-inflammatory oral pathology in veterinary medicine. Several species of bacteria accumulate in dental plaque, and the frequent pharmacotherapy promote bacterial resistance. Plants produce essential oils (EOs), described in the literature as natural antimicrobials, mostly consisting of phenylpropanoid and/or terpenoid derivatives. The objective of this work was to verify the antimicrobial activity of four EOs (*Baccharis trimera, Eremanthus erythropappus, Illicium verum* and *Mimosa verrucosa*) against bacteria associated with canine periodontitis. The elucidation of EOs by gas chromatography (GC) identified 30 compounds.

Qualitative and quantitative tests were carried out, the disc diffusion test demonstrated antimicrobial activity against Gram positive bacteria (*S. aureus* and *E. coli*). The MIC of *M. verrucosa* planktonic cells inhibited the growth of strains of MRSA, MSSA, sensitive and resistant *E. coli* at concentration of 43.2 mg/µl and the MBC was 8.6 mg/µl and 21.6 mg /µl, for sensitive *E. coli* and *MRSA* respectively. The MIC of 50.9 mg/µl of *I. verum* inhibited the strains: *MRSA*, sensitive and resistant *E. coli* and the CBM showed activity from 81.4 mg/µl for sensitive *E. coli*. MIC and MBC of sessile *M. verrucosa* and *I. verum* cells were the same, 69 mg/µl for *MSSA* and 50.9 mg/µl for *MRSA*, sensitive and resistant *E. coli*, relatively. While the other EOs (*B. trimera* and *E. erythropappus*) did not demonstrate antimicrobial activity at the concentrations tested in the quantitative tests.

Valorization of Agrifood By-products for the Extraction of Phenolic Compounds and Production of Zero-valent Iron Nanoparticles

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Abstract:

The agrifood sector is facing increasing scarcity due to diminishing resources. Up to one third of food is lost or discarded annually, and 14% of global food production is wasted before reaching the retail stage. The valorisation of waste produced by this agrifood industries is of the utmost importance for the sustainability of the sector. The phenolic content and antioxidant activity through TPC, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities, and FRAP assay of various agrifood wastes were assessed and the ones displaying the best results were used to produce nano zero-valent iron (nZVI), a low-cost, environmentally friendly nanoparticle, widely researched for remediation of groundwater and soils, and removal of metals due to its small particle size, large surface area and high reactivity. The post-distillation residue of *Cistus ladanifer* L. stems and leaves and spent coffee grounds were the samples presenting the highest TPC values, as well as those displaying the strongest $DPPH^{\bullet}$and $ABTS^{\bullet+}$ scavenging activities. For the FRAP assay, the highest values obtained were for the spent coffee ground and frozen coffee silverskins. The *Cistus ladanifer* L. stems and leaves and spent coffee grounds were then used as the extracts to produce the nZVI. nZVI characterization is currently being conducted.

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The Hexane Extract of *Ardisia crispa* Root Inhibits Angiogenesis and Metastasis in Colorectal Cancer, In Vitro

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Abstract:

Ardisia crispa (Thunb) A.DC from the family Primulaceae is a local medicinal plant, used in treating various inflammatory-related ailments. Over the past few decades, extensive research has been conducted on the root part of the plant. Previous studies on the hexane extract of the plant's root, denoted as ACRH, has been reported to exert promising inhibitory effects on pain and inflammation, arthritis, carcinogenesis, and angiogenesis. Hence, this study aims to further investigate the potential of ACRH in impeding angiogenesis and metastasis in human colorectal cancer cell lines, HCT116, and LoVo cells via various angiogenesis assays. The ACRH's IC_{50} for HCT116 and LoVo cells was found at 1.9±0.32 and 2.1±0.21 µg/mL, respectively via MTT assay. Therefore, for subsequent experiments, ACRH was tested at concentrations of 0.02, 0.2, and 2.0 µg/mL, respectively. ACRH also significantly induced early and late apoptosis in both cancer cells. Additionally, ACRH significantly suppressed wound healing, migration, adhesion, and invasion ($P<0.05$) of both cancer cells, respectively. Suppression of urokinase-type plasminogen (uPA) by ACRH occurred in LoVo cells only, but matrix metalloproteinase-2 (MMP-2) was significantly attenuated in both cancer cells, thus indicating ACRH's role in inhibiting the extracellular matrix (ECM) degradation. Molecular-wise, ACRH at different concentrations, was also shown to suppress AKT, BRAF, ERK, KRAS, VEGF-A, VEGF-C, and PI3K protein expressions via ELISA assays, in both cancer cells, respectively. These current results suggest the promising potential of ACRH acting as antiangiogenic and antimetastatic mediated via suppressing angiogenesis and metastatic pathways in colorectal cancer.

Antioxidant Activity of Essential Oils from Plants of the Lamiaceae Family and of their Main Phytochemicals: Relevance in Biological Processes

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Abstract:

Essential oils (EOs) are a natural extract derived from aromatic and medicinal plants, constituted by phytochemicals which are a product of their secondary metabolism. EOs consist of a complex mixture of bioactive compounds such as monoterpenes, sesquiterpenes, and phenylpropanoids, which frequently exhibit antioxidant activity [1]. The use of EOs as natural antioxidants is a field of growing interest in food and pharmaceutical industries, namely as an alternative to synthetic antioxidants that could be harmful for human health [2]. In this study, we evaluated the antioxidant activity of *Thymus vulgaris* L. and *Origanum vulgare* L., and of their main phytochemicals thymol and carvacrol, respectively. The activity was assessed through *in vitro* colorimetric methods for NO• (nitric oxide) and HO• (hydroxyl) radicals scavenging, as well as β-carotene bleaching, as model for lipid peroxidation [3]. Both EOs presented great antioxidant activity, namely scavenging HO• radical in concentrations ≥ 0.1 mg/mL (*T. vulgaris*: 68.4 % inhibition; *O. vulgare*: 68.7 % inhibition), which was correlated to the presence of thymol and carvacrol, as they also presented significant antioxidant activity. Both *T. vulgaris* and *O. vulgare* presented higher HO• than NO• radical scavenging activity. Regarding the individual components, overall thymol performed better than carvacrol. In β-carotene bleaching, both *O. vulgare* EO and thymol presented higher potential to prevent lipid peroxidation than the others. In conclusion, these natural occurring compounds show antioxidant activity relevant to biological processes and could be an alternative to synthetic antioxidants, however it is important to study their biocompatibility to guarantee their safety.

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Caffeic and Rosmarinic Acids Induce Cytotoxicity and Morphological Changes in Breast Cancer Cell Models

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Abstract:

In recent years, breast cancer has become one of the biggest health issues worldwide, mainly affecting women. Chemotherapy is one of the main therapeutic approaches used in breast cancer treatment, but it presents various drawbacks, like the reduced efficacy of chemotherapy drugs due to multidrug resistance. Phenolic compounds, commonly found in herbs, fruits and vegetables, present a wide array of bioactivities, among which the anti-tumoral activity is frequently addressed. In addition, various compounds have been screened as potential coadjuvants of chemotherapy drugs, aiming to enhance drug's efficacy. In the present work we have evaluated the anti-proliferative activity of caffeic (CA) and rosmarinic (RA) acids, in two human breast cancer cell lines (MCF-7: adenocarcinoma; BT-474: ductal carcinoma), as an initial screening for their potential as chemotherapy co-adjuvants. The anti-proliferative activity was evaluated through Alamar Blue assay and morphology analysis through bright-field microscopy. We observed that at the highest concentration tested (200 µM), CA caused a significant decrease in MCF-7 and BT-474 cells viability (for 24 h and 48 h exposure), while RA only presented cytotoxicity towards BT-474 cells. At 72 h exposure, both phenolic acids significantly reduced MCF-7 and BT-474 cells viability. Morphological changes were detected in both cell lines when exposed to CA and RA (48 h and 72 h exposure). In conclusion, the selected compounds can induce cytotoxicity in breast cancer cell line models on its own, and thus present potential for future studies aiming to assess phytochemical-drug interactions to enhance chemotherapy drugs' efficacy.

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A System of Pomegranate *(Punica granatum)* Freeze-dried Flower Extract and a Prebiotic Substance, as a Combination with Multidirectional Benefits

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Abstract:

The combination of a substance possessing prebiotic activity with a raw plant extract can have a dual benefit, as it has the ability to support the intestinal microbiota while also influencing the properties of the extract. This study aimed to evaluate the properties of lyophilized pomegranate *(Punica granatum)* flower extract and extract systems in a 1:1 *(m/m)* ratio with a prebiotic substance (PS), including α-cyclodextrin, methyl-β-cyclodextrin, (2-hydroxypropyl)-γ-cyclodextrin, inulin, croscarmellose, and arabic gum. The study demonstrated that the combination of the extract with PS exhibited growth-stimulating activity of probiotic bacteria in contrast to the freeze-dried extract alone. Furthermore, the combination with a prebiotic substance did not diminish the freeze-dried extract's antidiabetic and antioxidant activity potential. The antidiabetic activity was evaluated by inhibiting α-glucosidase, and additional efficacy was confirmed by using *in silico* molecular docking technique. Antioxidant activity was evaluated by CUPRAC and DPPH methods. The stability of

anthocyanins present in the raw material was evaluated to determine whether the combination with PS improved the stability of the extract. The results showed that the combination with PS had a favorable effect on the stability of anthocyanins in the extract under elevated temperature conditions for methyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin and arabic gum. Infrared spectral analysis (FT-IR) allowed the determination of interactions between the prebiotic substance and the active compounds present in the extract. In conclusion, the combination of pomegranate flower extract shows beneficial biological effects and can improve the physicochemical properties of the system.

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Neuroprotective Activity of Different Varieties *of Humulus lulupus* Leaves and Strobiles

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Abstract:

Humulus Lupulus studied varieties were Blisk, Lunga, Nugget, Bačka, and Saphir. Hop strobiles and leaves were extracted with ultrasound-assisted extraction with 70% methanol at 50°C. The contents of the xanthohumol and lupulone were determined with the use of the HPLC-DAD method. Total phenolic content was also studied. The antioxidant activity studies were performed by DPPH, CUPRAC, FRAP, and ABTS methods. Inhibition of acetylcholinesterase and butyrylcholinesterase were measured spectrophotometrically. The greatest content of xanthohumol was found in Lunga strobiles – 2638.88 ± 156.25 µg/g of plant material, and lupulon in Nugget strobiles – 9671.5 ± 79.66 µg/g. The leaves and strobiles differed in the amount of polyphenol content. The greatest antioxidant potential was found for Blisk strobiles in DPPH (83.19 ± 2.77 mg trolox/g plant material), CUPRAC (75.25 ± 0.72 mg trolox/g plant material), FRAP (56.39 ± 2.13 mg trolox/g plant material), and ABTS methods (76.78 ± 1.65 mg trolox/g plant material). Blisk strobiles also had the greatest potential in inhibiting acetylcholinesterase and butyrylcholinesterase (IC_{50} values 15.9 ± 3.12 mg plant material/ml and 17.98 ± 0.37 mg plant material/ml, respectively). Blisk strobiles showed the greatest potential in anti-neurodegenerative activity which should be further investigated *in vivo*.

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Influence of Extraction Method on the Properties of Portuguese Propolis Extracts

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Abstract:

Propolis is a product produced by bees essentially by collecting chemically complex resins of plant origin and mixing them with wax and salivary enzymes. Propolis seems to have several functions in the hive such as waterproofing and air flow control in addition to hive protection from invaders and from microorganisms. In addition to the benefits for bees, this natural product looks promising for humans due to the numerous properties that have been attributed to it, such as antioxidant, antibacterial, antifungal, anticancer and anti-inflammatory activities. Several studies indicate that the extraction method and the solvent used in this process influence the composition and consequently the biological activities of propolis extracts. In this work, a Portuguese sample of propolis was extracted by maceration with differentsolvents (ethanol and ethanol 70%), as well as different stirring, filtration, and drying conditions.The obtained extracts were then analyzed for total ortho-diphenols, phenolics and flavonoids contents and compared in terms of antioxidant activity and antimicrobial efficacy against a panel of bacteria. A higher yield of extraction as well as higher antioxidant and antimicrobial activities were observed for the ethanol extract of the propolis sample.

Phaeodactylum tricornutum as a Stable Platform to Produce Bioactive Compounds at Pilot Scale: Growth, Lipid content, Fucoxanthin Analysis and Antioxidant Capacity

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Abstract:

In recent years, the nutraceutical market has grown because of changes in consumer preferences in natural and organic products. Carotenoids produced by microalgae has been studied largely. Moreover, fucoxanthin extracts from the diatom *Phaeodactylum tricornutum* were investigated in different fields. However, pilot and industrial scale experiments to perform a continuous and stable production of *P. tricornutum* and fucoxanthin are limited. Growth parameters were analysed during scale-up and cultivation in 400L tubular photobioreactor. Two different light intensities were used to detect potential differences in terms of productivity, lipid content and fucoxanthin content. Then, biomass was extracted by a soft solvent and oil fraction was measured to determine lipid and fucoxanthin content. Antioxidant capacity of medium light intensity extracts was analysed by ABTS assay, measuring the relative ability of antioxidants to scavenge the $ABTS^{\bullet+}$ radical, as compared with a vitamin C standard. Low light intensity experiments yielded suitable results, reaching a stable lipid percentage throughout the different batches. Also, fucoxanthin content increased to between 3,1-3,5% (w/w dry biomass) and productivity was around 0,4-0,5g/L. d^{-1}. On the other hand, medium light intensity results were similar in terms of biomass productivity (around 0,4-0,6g/L. d^{-1}) and lipid content. However, fucoxanthin concentration was slightly higher than low light intensity experiment (between 3,8-3,9% of w/w dry biomass). Antioxidant capacity was decreasing during 0, 12 and 18 months of storage (366, 257, 190 CEAM, µM Vitamin C), but maintained over time. These results offered new insights of cultivation microalgae to obtain a continuous production of bioactive compounds.

Euphorbia Propolis from Morocco: Mineral Content, Volatile and Phenolic Profiles and Biological Activities

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Abstract:

This study aimed to characterize the mineral content, volatile and phenolic profiles, and the *in vitro* biological activities of three *Euphorbia* propolis samples collected in Morocco. *Euphorbia* propolis pollen analysis showed *E. resinifera* pollen to dominate in one sample (P1) (> 58%) and *E. officinarum* pollen in the two other samples (P2 and P3) (>44%). P1 mineral content was higher than in the remaining samples, Mg leading in this sample, whereas Fe dominated in P2 and K in P3. Monoterpene hydrocarbons (> 31%) and oxygen-containing monoterpenes (> 14%) were the main grouped fractions in *Euphorbia* propolis volatiles. Higher amounts of phenols, flavonoids, and dihydroflavonoids were found in P2 sample. This sample showed also better antioxidant activity determined by the ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) free radicals, superoxide anion radicals, and to prevent lipid peroxidation through the thiobarbituric acid reactive substances (TBARS) assay. P1 and P3 samples had the best ability for inhibiting the glucosidase activity, whereas P2 had best ability for inhibiting acetylcholinesterase, lipoxygenase, tyrosinase, and xanthine oxidase activities. The minimum inhibitory concentration (MIC) varied between 50-300 mL/mL against the tested *S. aureus* and MRSA strains, being the MIC value of P1 the lowest (50-120 mL/mL) and the P3 with the highest MIC values (250-300 mL/mL). The tested propolis samples showed antibiofilm capacity against MRSA strains and an *Escherichia coli* multi-resistant strain.

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Chemical Characterization and Antioxidant Potential of *Euphorbia resinifera* Floral Cyathia

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Abstract:

Euphorbia resinifera O. Berg is a plant endemic to the Northern and Central regions of Morocco, which has been used since the ancient Roman and Greek times for its medicinal properties. The plant is mainly known for secreting a poisonous latex containing resiniferatoxin. However, *E. resinifera* pseudo-inflorescences called cyathia are devoid of laticifers, therefore, do not secrete latex. Instead, they exudate nectar that local honeybees collect and craft into honey, nowadays labeled with the Protected Geographic Indication of Morocco. Honey and water extracts of floral cyathia find a broad range of applications in the traditional medicine of Northern Africa as ointments and water decoctions. Given the relevance of *E. resinifera* floral-derived products for bee nutrition and honey production and the health benefits of water decoctions and ointments, this study aimed to provide a comprehensive phytochemical screening of its cyathia. *E. resinifera* cyathia showed a plethora of different classes of specialized metabolites, including carotenoids, flavonoids, and polyamines which confer antioxidant properties to water decoctions, measured with *in vitro* antioxidant assays. We also measured abundant content of hexoses, amino acids and vitamins, along with high levels of benzaldehyde and nonanal, all of which are important in honeybees' attraction and honey production. In conclusion, our analyses revealed that *E. resinifera* cyathia are a great source of antioxidant molecules and a good food source for the local foraging honeybees.

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In vitro Anti-chlamydial Activity of Isolated Compound from *Urolepis hecatantha* (DC.) R. King and H. Robins (Asteraceae)

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Abstract:

Chlamydial infections in humans are widely distributed and are responsible for a variety of acute and chronic diseases, which sometimes lead to complications and sequelae. Currently, recommended

antibiotic treatment has shown failures and proven unsatisfactory efficacy in chronic infections. Species from the genus *Eupatorium* have reported ethnomedical uses related to antimicrobial activity. Aerial parts from *Urolepis hecatantha* (DC.) R. King and H. Robins (Asteraceae) (syn. *Eupatorium hecatanthum* (DC) Baker) are chewed as antitussive, while the infusion or decoction is used topically for gangrene and ulceration treatment. Isolated compounds such as flavonoids, terpenoids, and benzofuran derivatives from *U. hecatantha* belong to the phytochemical groups that have reported antichlamydial activity. This study aimed to test the *in vitro* antichlamydial activity of an isolated compound obtained from *U. hecatantha* against *C. trachomatis*. Euparina was isolated from dicloromethane extract of aerial parts of *U. hecatantha,* identified by MS, 1H, and 13C-NMR spectra, and heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC) and correlated spectroscopy (COSY) (Elso et al., 2021) and assessed in a non-cytotoxically concentration (50 µg/mL) on cell culture as previously described by our group of research (Entrocassi et al., 2021) using LLC-MK2 cell line and *C. trachomatis* ATCC strain L2/434/BU. Euparina showed high inhibitory activity (99% condition B, 90% condition C and 84% condition D) during the inoculation steps of the Chlamydial life cycle. Research continues into the pathways involved in Euparin's impairment in *Chlamydia* entry, with a view to developing a promising new anti-chlamydial agent.

Fisetin Amorphization as a Co-dispersion Effect of Dissolution in Supercritical Carbon Dioxide

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Abstract:

Amorphization represents a promising strategy to improve the solubility and bioavailability of poorly soluble pharmaceutical compounds. In this study, we investigated the amorphization of fisetin, a compound with limited solubility, using supercritical CO_2 as a solvent. X-ray powder diffraction (XRPD) analysis confirmed the successful conversion of crystalline fisetin to its amorphous form. We further evaluated the solubility of amorphous fisetin and compared it to the crystalline counterpart, revealing a substantial improvement in solubility for the amorphous fisetin. This enhanced solubility holds significant potential for enhancing therapeutic efficacy and achieving a faster onset of action. Moreover, we explored the antioxidant activity of amorphous fisetin using four *in vitro* models. The results demonstrated the robust antioxidant capacity of amorphous fisetin, suggesting its potential application in combating oxidative stress-related disorders. Furthermore, we investigated the inhibitory effects of amorphous fisetin on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), enzymes implicated in neurodegenerative diseases, including Alzheimer's. Our findings revealed significant inhibition of AChE and BuChE by amorphous fisetin, highlighting its potential as a neuroprotective agent for neurodegenerative disorders. Overall, this study presents a comprehensive exploration of the amorphization of fisetin using supercritical CO_2, leading to enhanced solubility and demonstrating its potent antioxidant activity. The significant inhibition of AChE and BuChE further supports the potential therapeutic application of amorphous fisetin in neurodegenerative diseases.

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