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I Bioactive Natural Products Research Meeting 2019

Auditório Agostinho da Silva (ULHT)

Book of Abstracts

27-28 September 2019

ULHT, Lisboa

Portugal

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Welcome Message

On behalf of the Organizing Committee for the **I Bio.Natural** Meeting, we are pleased to invite you to the **I Bio.Natural** – Bioactive Natural Products Research Meeting, that will be held in Lisbon, on **September 27-28** (Universidade Lusófona de Humanidades e Tecnologias; Auditório Agostinho da Silva).

Bio.Natural Meeting aims to be a forum for researchers that are developing projects exploring the multiple applications offered by natural products. These compounds are being screened as new 'drug lead' on drug discovery, or being modified by rational drug design and semi-synthesis, in order to offer innovative therapeutic alternatives for the treatment of cancer, infectious diseases or other health problems. Other studies are focused on the potential of marine, herbal, or mineral materials as bioactive agents for cosmetics or medical devices. Moreover, the proven benefits of nutrients from naturally sourced ingredients are demanding new approaches for research on food and food supplements for human nutrition.

Besides to the scientific content, there will be an exciting exhibition of health, functional ingredients and supplements with the partnership of **FISSIN** and **Naturales Medicinae** with companies in the global market in the Natural Products sector. This event will gather nutritionists, pharmacists, researchers, students and producers, companies and end users from Natural Products field, in a welcoming environment.

The **Bio.Natural Meeting** is propitious to promote products and services, extend the contact network for business, partnerships, research and clients and will provide a face-to-face contact with potential partners and professionals with researchers and companies.

Additionally, the **Bio.Natural Meeting** will have a Special issue "Selected papers from I Bio.Natural Meeting" on Journal Biomolecules: Impact Factor (2018): 4.694, ranking 58/298 (80.70%, Q1) in 'Biochemistry and Molecular Biology'; CiteScore (2018): 5.72, ranking 33/407 (Q1) in 'Biochemistry' and 44/375 (Q1) in 'Molecular Biology

https://www.mdpi.com/journal/biomolecules/special_issues/Bio_Natural_2019

*All abstracts accepted can further be submitted to the Special issue "Selected papers from Bio.Natural Meeting 2019" on Journal Biomolecules.

Honour Committee

Manuel de Almeida Damásio, President of the Lusófona Group

Mário Caneva Moutinho, Rector of ULHT

Luis Monteiro Rodrigues, General Director CBIOS

Patrícia Rijo, Chairman of the congress

Organization

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Practical information

I Bio.Natural – Bioactive Natural Products Research Meeting (September 27-28, 2019)

Conference venue

The conference venue will be held at the Auditório Agostinho da Silva, Universidade Lusófona de Ciências e Tecnologias.

Contact details

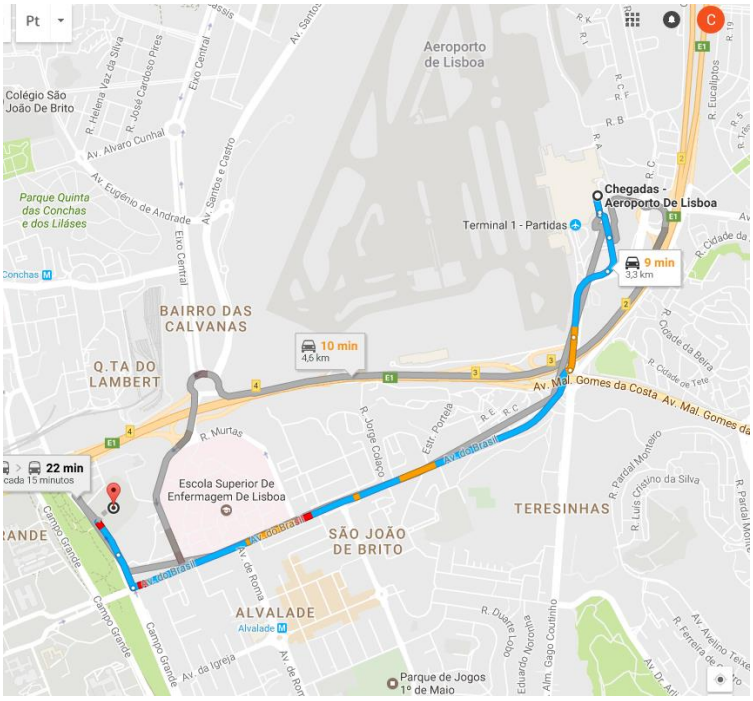
Escola de Ciências e Tecnologias da Saúde,
ULHT Campo Grande 376, 1749-024 Lisboa
(+351) 217515550
Email: patricia.rijo@ulusofona.pt

Language

English is the official language of the congress.

Location

The easiest way to get to the CBIOS - Universidade Lusófona de Humanidades e Tecnologias is either by bus, metro or taxi. All means of transportation are available at the aeroport.



How to arrive to the venue

If you decide to go on a bus, you need to catch 783 AMOREIRAS at the airport, and then catch another bus 717 or 767 at Júlio de Matos. Get off after 2 stops: 717 and 767 stops in front of the University. You can buy your ticket inside the bus. Each ticket costs 2 € for just one journey. If you decide to come by bus, you will have to buy two tickets.

If you decide to catch the metro at the airport, you must go to Alameda (Red Line) and change to the Green Line, heading to Telheiras. You must go off the metro at Campo Grande, and then walk to the University (~8 minutes).

The Taxi to the City Center is ~15 EUR.

Daily tickets for all transportations (bus and metro) are available at the price of 1.45€ per travel found inside the metro buying viva-lisboa card (0.5€).

Other option is to buy 24h bus+ metro pass for 6.40€ (called as *Bilhete diário Carris*).

The university is located at Campo Grande, 376.

Scientific information

Oral communications

The congress has a large number of oral presentations covering several topics of natural products compounds and medicinal chemistry.
The oral communications are divided in:

- Plenary sessions (30 minutes).
- Oral communications (10 minutes).
- Flash oral communications (3 minutes).

Some time for scientific discussion is included in all above sessions.
Speakers on the 27th September are kindly asked to contact the reception desk to provide their presentation data and verify the presentation preview.

Poster communications

Two posters sessions will be held in the congress, given the opportunity for exchange of ideas and networking between all the congress participants.
The maximum size for the posters is 120 x 90 cm.

Scientific detailed program

27th of September:

8:00-8:30	**Registration of participants / Poster setting**
8:30-9:00	**Opening** Gonçalo Calado (Vice-Rector ULHT); Luis Rodrigues (CBIOS Director); Patricia Rijo (CBIOS); Graça Mariano (DGAV)
9:00-9:30	**Session 1 Chairman: Graça Mariano (DGAV, Portugal) and Paula Bico (DGAV, Portugal)** **P1: M. J. Umbelino Ferreira (iMed.ULisboa, Portugal):** "Reversing multidrug resistance in cancer: plant-derived compounds as a promising approach"
9:30-9:45	**IL1: C arlos A. M. Afonso (iMed.ULisboa, Portugal):** "From Furan Based Biorenewable Resources to Highly Functionalized Molecules"
9:45-10:00	**IL2: Mariana Almeida (Medinfar, Portugal):** " New trends in the Pharmaceutical Industry: natural ingredients?"
10:00-10:15	**IL3: Tiago Rodrigues (IMM, Portugal):** " Machine intelligence to deconvolute the pharmacology of natural Products"
10:15-10:30	**IL4: Miguel González (CSIC, Spain):** "Advances in Natural Products Chemistry performed in Valencia: synthesis and biological activity of abietane diterpenoids"
10:30-11:00	***Coffee Break / Posters / Exhibition***
11:00-11:15	**Session 2 Chairman: Maria M. M. Santos (iMed.ULisboa, Portugal) and Daniel Santos (iMed.ULisboa, Portugal)** **IL5: Attila Hunyadi (University of Szeged, Hungary):** " ROS scavenging by hydroxycinnamates forms valuable antitumor leads"
11:15-11:30	**IL6: Emilia Leitão (HOVIONE, Portugal):** " Asthma - Traditional treatment with herbs and drugs"
11:30-11:45	**IL7: Mattia Mori (Università di Siena, Italy):** "Rational design of isoflavones as multitarget Hedgehog pathway inhibitors with anticancer effects"
11:45-12:00	**IL8: Ahmed A. Hussein (CPUT, South Africa):** "Chemical diversity of *Helichrysum* genus"
12:00-12:15	**IL9: Ricardo Ferreira (Uppsala University, Sweden):** "Computational Approaches in Natural Products: valuable tools in Cancer and Antibacterial Research"
12:15-14:00	*Lunch / Posters / Exhibition*
14:00-14:30	**Session 3 Chairman: L. M. Rodrigues (CBIOS, Portugal) and Marta Borges (DGAV, Portugal)** **P2: Isabel Ferreira (CIMO, Portugal)**: " Natural and bioactive? Plants and Mushrooms"
14:30-14:45	**IL10: Joana Mota (Lecifarma, Portugal):** " Product Development: From concept to market"
14:45-15:00	**IL11: Luisa Custódio (CCMAR, UALG, Portugal):** " The role of halophytes as sources of natural products in a changing world: current knowledge, challenges and future perspectives"
15:00-15:15	**IL12: Mariana Reis (CIIMAR, Portugal):** "Strategies to explore the chemical diversity of Cyanobacteria"
15:15-15:30	**Flash Oral Communications**
15:30-16:00	***Coffee Break / Posters / Exhibition***
16:00-16:15	**Session 4 Chairman: M. Céu Costa (CBIOS, Portugal) and Bruno Sousa (CBIOS, Portugal)** **IL13: Jose Prieto (UCL, UK):** "Artificial Intelligence and Statistic tools to predict, find and interpret the bioactivities of complex natural products"
16:15-16:30	**IL14: Nelson Tavares (Natiris, Portugal):** " Food Supplements and Elderly people"
16:30-16:45	**IL15: Paula Castilho (UMA, Portugal):** "Synthesis versus extraction of bioactive oligosaccharides"
16:45-16:55	**Oral Communication 1: Carmen S.R. Freire (CICECO, Portugal):** "Bacterial nanocellulose membranes loaded with bioactive compounds for skin treatment"
16:55-17:05	**Oral Communication 2: Mihayl Varbanov (CNRS, France):** "Advances on antimicrobial activity of *Morus* spp. plant extracts: viruses in the spotlight"
17:05-17:15	**Oral Communication 3: Ana T. Belo (INIAV, Portugal):** "Mediterranean Shrubs: Nutraceuticals for sustainable goat production"
17:15-17:45	**Awards and Closing session**

P: Plenary, **IL**: Invited Lecture, **OC**: Oral Communication

28th of September:

9:30-12:15	***Exhibition and network with Companies* / Posters (*with coffee-break)***

Plenary Lectures abstracts

PL 1: Reversing multidrug resistance in cancer: plant-derived compounds as a promising approach

Maria José Umbelino Ferreira

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal
E-mail: mjuferreira@ff.ulisboa.pt

The emergence of multidrug resistance (MDR) in cancer has been pointed as one of the major hurdles for a successful chemotherapy. It is characterized by the development of resistance by cancer cells to drugs that are structurally unrelated and with distinct mechanisms of action. The most significant mechanism of MDR is due to the overexpression of transmembrane transporter proteins of the ATP-binding cassette (ABC) superfamily, which act as efflux pumps for chemotherapeutic agents, decreasing their intracellular concentration. The three most implicated ABC transporter proteins are P-glycoprotein (P-gp), multidrug resistance protein (MRP1) and breast cancer resistance protein (BCRP), which play a key role on MDR.

Aiming at finding plant-derived compounds for reversing multidrug resistance in cancer, we have evaluated the ability of a great number of compounds, with different scaffolds, as ABC transporter modulators. Among the most relevant results are those obtained for some terpenoids and indole alkaloids, which were found to be strong P-gp inhibitors. Some compounds were also able to inhibit MRP1 and displayed collateral sensitivity activity to MRP1-overexpressing cancer cells [1-5].

Acknowledgements: Fundação para a Ciência e a Tecnologia (FCT), Portugal (projects PTDC/MED-QUI/30591/2017, SAICTPAC/0019/2015).

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PL 2: Natural and bioactive? Plants and Mushrooms.

Carla Pereira, Lillian Barros, **Isabel C.F.R. Ferreira**

Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
E-mail: iferreira@ipb.pt

Humans have been relying on Nature since the beginning of time. It provides us food and water, but also phytochemicals that play essential roles in biological systems. In this perspective, plants and mushrooms can be pointed out as excellent sources of bioactive molecules such as phenolic compounds. These compounds are present in mushroom caps and stipes and in plant leaves, seeds, barks, and flowers, and are widely known for their innumerable bioactive properties [1, 2]. Recent researches have been focusing on the extraction of these compounds from the referred matrices for further application in food industry, mainly to fortify and/or functionalize food products. Interestingly, some of these compounds can also confer preservative and colouring features to foodstuff, beyond beneficial health effects.

With that in mind, phenolic acids (rosmarinic acid), flavonoids (quercetin derivatives), and ellagitannins (sanguiin H-10, lambertianin) from mushrooms, wild strawberry, rosemary, mountain sandwort, and flowers of *silva brava* were incorporated in gelatin, yogurt, and cottage cheese [e.g. 3]. Given their antioxidant and antimicrobial properties, polyphenol extracts from strawberry-tree, basil, lemon balm, sweet chestnut flowers, fennel, and German chamomile were used for preservative purposes in loaf bread, cupcakes, yogurt, cheese, and cottage cheese, namely flavonoids (catechin, quercetin and luteolin derivatives), phenolic acids (rosmarinic, chicoric, lithospermic, caffeic, caffeoylquinic acids), and hydrolysable tannins (trigalloyl-HHDP-glucoside) [e.g. 4]. Also, bioactive colouring molecules like betalains (gomphrenins, isogomphrenins) from purple globe amaranth and anthocyanins (cyanidin, delphinidin, and malvidin derivatives) from rose, dahlia, centaurea, strawberry-tree, roselle, blueberry, sweet cherry, fig peel, blackthorn epicarp, etc., were applied in yogurt, waffles, and donut topping, among many others [e.g. 5].

These results reflect the applicability of natural extracts from plants and mushrooms in foodstuff, allowing the development of enhanced products.

Aknowlegments: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2019). To the European Regional Development Fund (ERDF) through the Regional Operational Program North 2020, within the scope of Project NORTE-01-0145-FEDER-023289: DeCodE and project *Mobilizador* Norte-01-0247-FEDER-024479: ValorNatural®. And to FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E.

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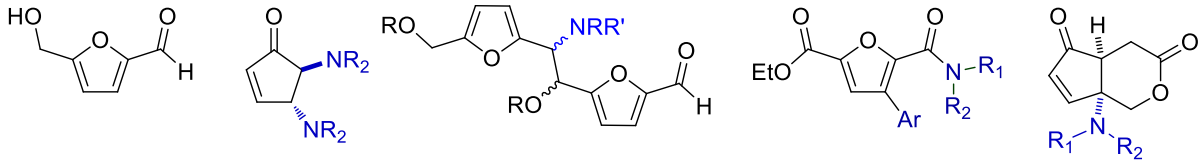
Invited Lectures abstracts

IL 1: From furan based biorenewable resources to highly functionalized molecules

Carlos A. M. Afonso[a]

[a] *Faculty of Pharmacy, University of Lisbon, Av. Professor Gama Pinto, 1649-003 Lisbon, Portugal*
E-mail: carlosafonso@ff.ulisboa.pt

Due to the reduction of fossil resources for energy consumption and platform chemicals for different purposes, several building blocks derived from renewable resources such as ethanol, glycerol, lactic acid, furfural, succinic acid, levulinic acid, are already in use or considered with potential importance in the near future.[1] Among them, 5-hydroxymethyl-furfural (HMF) has been considered a promising intermediate building block due to its potential rich chemistry that allows different transformations such as to biofuels, polymer monomers, levulinic acid, adipic acid, caprolactam and caprolactone and many other more specific molecules.[2] In line with our interest in the valorization of natural resources, will be described recent achievements from this laboratory on the transformation of HMF and furfural to several highly functionalized molecules by taking advantage of the reactivity of the furan core such as via amine condensation-rearrangement-cyclization **2**, homo Mannich reaction of trienamine/iminium-ion pair **3**,[3] chemoselective enzymatic desymmetrisation / Ru-catalysed C–H activation sequence **4**[4] and activation of HMF via Knoevenagel condensation with Meldrum's acid followed by reaction with a secondary amine to undergo a furan ring-opening/cyclization/lactonization cascade process **5**.[5]



HMF **2** **3** **4** **5**

Acknowledgements: Fundação para a Ciência e a Tecnologia (FCT) and European Research Area Network; ERANet LAC (ref. ELAC2014/BEE-0341) for financial support.

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[4] (2019) *ChemSusChem* 12: 420 (VIP).
[5] (2019) *ChemSusChem* ASAP, DOI: 10.1002/cssc.201902051 (VIP)

IL 2: New trends in the Pharmaceuticals Industry: natural ingredients?

Mariana Almeida

Grupo Medinfar, Lisbon, Portugal
E-mail: mariana.almeida@medinfar.com

Healthcare reforms, shifting market demands and increasing regulatory pressures are transforming the pharmaceutical industry and the health care systems. Within this frame, Innovation is a key word, since new pharmaceutical products can be crucial for maintaining the quality and longevity of human life. Pharmaceutical innovation is considered as a hardly and a predictable process, following a technology-push model dependent on a scientific breakthrough path with rough timings and hard outcomes.

Natural Ingredients have proved pharmacological and biological activities with therapeutic benefit being an important source of inspiration for the development of potential novel drugs. Besides, for the past few decades, it has been noticed an extensive study of natural ingredients for their promising prospects in several pharmaceutical applications. These trends provide new opportunities and constraints for the investigation of active principles for phytomedicines, nutritional additives and cosmetics.

IL 3: Machine intelligence to deconvolute the pharmacology of natural products

Tiago Rodrigues

[a]*Instituto de Medicina Molecular (iMM) João Lobon Antunes, Faculdade de Medicina, Universidade de Lisboa, Av Prof Egas Moniz, 1649-028 Lisboa, Portugal*
E-mail: tiago.rodrigues@medicina.ulisboa.pt

Machine intelligence is a growing concept in chemical biology and molecular medicine, which enables the discovery of latent data patterns that are otherwise imperceptible to human reasoning and intuition [1]. Resorting to a range of heuristics, machine intelligence has found particular applicability to the identification of drug targets for small molecules and natural products, with only a fraction of cost and time relative to chemical proteomics [2,3]. Herein, I discuss the application of (un)supervised machine learning to chart the natural product space and generate statistically motivated research hypotheses regarding both on- and off-targets that may correlate with therapeutic efficacy and liabilities. For example, (-)-englerin A was confirmed to modulate $Ca_v1.2$ channels [4], whereas beta-lapachone and piperlongumine were validated as allosteric 5-lipoxygenase and TRPV2 inhibitors, respectively [5,6]. Finally, I discuss the impact that the increasing amount of biological/chemical data will have in future natural product research.

IL 4: Advances in Natural Products Chemistry performed in Valencia: synthesis and biological activity of abietane diterpenoids

Miguel A. González-Cardenete[a], Alberto Yuste[b], Eva García-Martínez[b], Marisa Nicolai[c], Ramón J. Zaragozá[d], and Patricia Rijo[c],

[a] *Instituto de Tecnología Química (UPV-CSIC), Universitat Politècnica de Valencia-Consejo Superior de Investigaciones Científicas, Avda de los Naranjos s/n, 46022 Valencia, Spain*
[b] *Universitat Politècnica de València, Departamento de Tecnología de Alimentos, Grupo de Investigación e Innovación Alimentaria (CUINA), Camino de Vera s/n, 46022 Valencia, Spain.*
[c] *Research Center for Biosciences & Health Technologies (CBIOS), Universidade Lusofona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal*
[d] *Departamento de Química Orgánica, Universidad de Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain*
E-mail: migoncar@itq.upv.es

Nearly 200 years ago, the study of the chemistry of terpenoids started with the analysis of turpentine oil, investigating the first resin acid, abietic acid from pine oleoresin.[1] Abietic acid occurs in plants of the genus *Abies* and is the first member of a class of plant metabolites, the abietane-type diterpenoids. They are characterized by a tricyclic ring system and have shown a wide range of chemical diversity and biological activity.[2,3] Medicinal chemists have studied derivatives of two readily available materials such as dehydroabietic acid and dehydroabietylamine .[3] To date, there is only one commercial drug, Ecabet® [ecabet sodium], based on abietanes, which is used for the treatment of reflux esophagitis and peptic ulcer disease. The simplest phenolic abietane, ferruginol, exhibits anticancer effects in human ovarian cancer and inhibition of cancer cell migration.

It is now a decade since our first work on abietane chemistry, particularly abietic acid derivatives.[4] During the last 10 years, we have synthesized several naturally occurring abietanes and derivatives, including ferruginol and other analogues for further biological study. Recently, the simultaneous isolation (in 2014) by Hua and co-workers, of the new abietane liquiditerpenoic acid A, a sugiol analogue, from the resin of *Liquidambar formosana* [5] and from *Pinus massoniana*,[6] by Kuo and co-workers named independently as abietopinoic acid, and the lack of synthetic studies prompted us to synthesize it and study its antitumor, GABAA modulation and antileishmanial properties along with some analogues.[7]

Further investigation in collaboration with Prof. Rijo at Universidade Lusofona has led to a study on anti-acetylcholinesterase activity. In this communication, we will present an overview of synthetic and biological studies on abietane chemistry performed by our group in Valencia, Spain, which includes the most recent achievements as potential antioxidant agents.

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IL 5: ROS scavenging by hydroxycinnamates forms valuable antitumor leads

Laura Fási[a], Ahmed Dhahir Latif[a,b], István Zupkó[b], György Tibor Balogh[c], **Attila Hunyadi**[a,d,*]

[a]Institute of Pharmacognosy, Interdisciplinary Excellence Centre, University of Szeged Szeged, Hungary [b]Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary [c]Chemistry Department, Gedeon Richter Plc., Budapest, Hungary [d]Interdisciplinary Centre for Natural Products, University of Szeged, Szeged, Hungary
E-mail: hunyadi.a@pharm.u-szeged.hu

Hydroxycinnamic acids represent a versatile group of dietary plant antioxidants. Biomimetic oxidation of methyl-*p*-coumarate (pcm) and methyl caffeate (cm) was previously described to yield some potent antitumor metabolites [1,2]. In the course of an antioxidant-inspired drug discovery initiative, we aimed at investigating the potential of these two hydroxycinnamates to form chemically stable metabolites upon free radical scavenging, and to study these metabolites for their antitumor action.

By processing various oxidized mixtures obtained from pcm, we identified a new, potent and selective antitumor lead, graviquinone. By in vitro and in silico studies, the formation of graviquinone was found to take place when pcm scavenges free radicals [3]. Several further, potentially bioactive compounds were formed when pcm was oxidized by the biorelevant reactive nitrogen species (RNS) peroxynitrite. Bioactivity-guided isolation from the AAPH-mediated oxidation of cm led to the identification of a lignan derivative with strong antitumor activity.

Our results demonstrate the ROS/RNS dependent formation of several bioactive, chemically stable metabolites from dietary antioxidant hydroxycinnamic acids. The formation of potent antitumor leads upon ROS scavenging also suggests that research efforts to investigate such oxidative transformations of antioxidants can serve as the basis of a novel drug discovery strategy.

Acknowledgments:
This work was supported by the National Research, Development and Innovation Office, Hungary, (K119770), and the János Bolyai fellowship by the Hungarian Academy of Sciences. AH is grateful to the bilateral mobility grant 2018-2.1.15-TÉT-PT-2018-00016 for supporting the presentation.

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IL 6: Asthma - Traditional treatment with herbs and drugs

Emília P. T. Leitão

Hovione FarmaCiência SA, Process Chemistry Development,
Estrada do Paço do Lumiar, Campus do Lumiar Building S, 1649 – 038 Lisboa, Portugal
eleitao@hovione.com

Asthma is a chronic inflammatory disease of the airways that attacks the respiratory system and results in reduced or obstructed airflow. It has no cure, but the symptoms can be controlled through inhaled corticosteroid (ICS) therapy (such as Fluticasone propionate) and plant remedies. Unfortunately, there is no universal regulatory system in place that insures the quality, efficacy and safety for the plant remedies. However, for the preparation of an active pharmaceutical ingredient, such as Fluticasone propionate, the control is very tight by the competent authorities (FDA and EMEA). This work describes some medicinal plants used for centuries in asthma treatment. As well as the studies carried out to synthesize Fluticasone propionate, avoiding the use of an ozone depleting substances such as bromofluoromethane.

IL 7: Rational design of isoflavones as multitarget Hedgehog pathway inhibitors with anticancer effects

Mattia Mori[a], Simone Berardozzi[b], Ludovica Lospinoso Severini[c], Deborah Quaglio[b], Flavia Bernardi[c], Cinzia Ingallina[b], Elisa De Paolis[b], Francesca Ghirga[d], Paola Infante[d], Lucia Di Marcotullio[c], Bruno Botta[b]

[a]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, via Aldo Moro 2, 53100 Siena. Italy. [b]Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy. [c]Department of Molecular Medicine, Sapienza University of Rome, viale Regina Elena 291, 00161 Rome, Italy. [d] Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, viale Regina Elena 291, 00161 Rome, Italy.
E-mail: mattia.mori@unisi.it

Pharmacological inhibition of the Hedgehog (Hh) signaling pathway has emerged as a valuable anti-cancer strategy.[1] A number of small molecules able to block the Hh pathway at the upstream receptor Smoothened (Smo) or the downstream effector Gli1 have been designed and developed.[2] Recently, we took advantage by the highly versatile and privileged isoflavone scaffold to design, synthesize, and teste new Hh inhibitors.[3] The introduction of specific substitutions on the isoflavone's ring B allowed the identification of molecules targeting preferentially Smo or Gli1. Biological assays coupled with molecular modeling corroborated the design strategy,and provided new insights into the mechanism of action of these molecules. We also showed that the simultaneous administration of isoflavones targeting Smo and Gli1 provided synergistic Hh pathway inhibition stronger than single administration, which stands as a relevant to overcome the drug resistance particularly at the level of Smo.

By combining the most profitable pharmacophores targeting Smo and Gli1 in a novel non-natural isoflavone, i.e. compound 22, we inhibited the Hh pathway at both upstream and downstream level with a single molecule. We demonstrated that this multitarget agent suppresses medulloblastoma growth *in vitro* and *in vivo* through antagonism at both Smo and Gli1, which is a novel mechanism of action in Hh inhibition.

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IL 8: Chemical diversity of *Helichrysum* genus

Ahmed A. Hussein[a], Olugbenga K. Popoola[b], Jeaning L. Manewick[b]

[a]*Chemistry Department and* [b]*Oxidative Stress Research Centre, Cape peninsula university of Technolgy, Bellville 7535, Western Cape, South Africa*
E-mail: mohammedam@cput.ac.za

South Africa is one of the most biodiverse country on the world, it has a unique flora especially in the southern part which called Cape Floristic Region with more than 9.0k species, ~70% of them are endemic. *Helichrysum* genus represented by ~250 species widely distributed in the country with tremendous morphological diversity.

The chemistry of *Helichrysum* genus is quite diversified and including different classes of compounds. Diterpenes, flavonoids, phloroglucinols, and cannabinoids are among the most important secondary metabolites. Only 25% have been studied for their chemical constituents, and resulted in few hundreds of compounds. The majority of the species in SA were not explored for their biological potential and required great efforts discover more potential compounds. Few compounds have been isolated and belong to phyto-cannabinoids with certain modulation effects on cannabinoids receptors. On the other hand, phloroglucinols are phenolic secondary metabolites with unique polarity profile. The biological activity of the phloroglucinols-type natural compounds not fully understood. This quick review will highlights the recent scientific research and applications of *Helichrysum* different species.

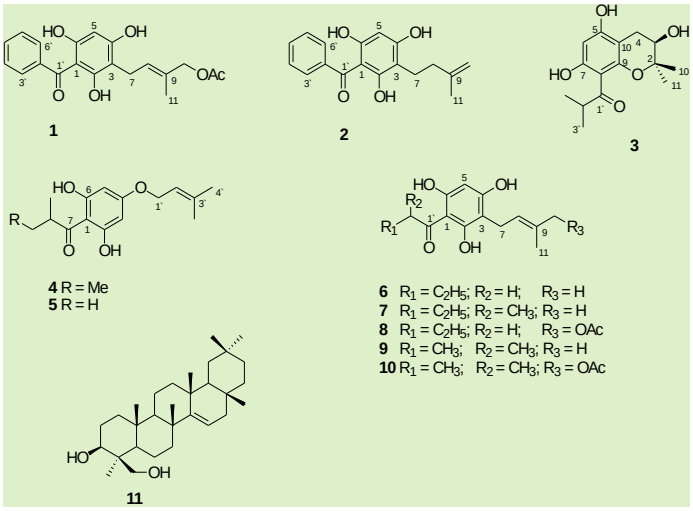

Figure 1. Compounds isolated from *H. niveum*

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IL 9: Computational Approaches in Natural Products: valuable tools in Cancer and Antibacterial Research

Ricardo J. Ferreira

Department of Cell and Molecular Biology - Molecular Biophysics, Uppsala University, Husargatan 3, 751 24 Uppsala, Sweden

E-mail: ricardo.ferreira@icm.uu.se

Nature is an unquestionable source for novel compounds, but quite often the specific mechanisms by which molecules isolated from natural sources may interact with cellular targets remain elusive, hampering the identification of which specific moieties contribute to the observed activity and/or to which targets they exert the most of its activity. Even if the target is known the molecular mechanism involved in the observed biological activity is often unknown and, more relevant, impairs further development of more potent analogues towards any given targets of interest.

Computational tools are, in that respect, one of the most common approaches to gain additional insights about the mechanisms by which natural compounds or its derivatives interact with cellular targets. Either through the development of structure-activity relationships by means of pharmacophoric hypothesis and regression models or, when a crystallographic structure is available, using molecular docking and dynamics such approaches are becoming widely used in the field of natural products in order to allow the design and optimization of novel derivatives from isolated scaffolds of interest.

During this presentation several examples of computational techniques applied to multidrug-resistance, in cancer or bacteria, will be presented. Regarding cancer, *in silico* techniques as molecular docking and molecular dynamics are the basis for studying the interaction with ABC transporters, allowing the discrimination between efflux substrates and modulators based on a previously published work-flow including relative free energies of binding and normal mode analysis (1,2). Oppositely, for the development of novel antibiotics towards ESKAPE Gram-negative pathogens the evaluation of permeabilities through the outer membrane (OM) remains a significant challenge and contributed for the “discovery void” registered since the 1990s [3]. Herein, permeability predictions using the inhomogeneous solubility-diffusion (ISD) model through the major OM porin, OmpF, are used to assess which scaffolds are more prone to accumulate in bacteria or, alternatively, which chemical modifications are expected to improve the permeability of a given scaffold. A new platform using outer membrane vesicles (OMVs) additionally allows the experimental validation of the predicted permeabilities, achieving a Pearson correlation coefficient of R = 0.97 with the predictions obtained using the ISD model [4].

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IL10: Product development: From concept to market

Joana Mota

LECIFARMA is a Pharmaceutical Company that works, since its foundation in 1985, for third parties, within contract manufacturing of medicines, medical devices, food supplements, cosmetics and personal care products.

The Product Development Department comprises a multidisciplinary team that studies every project from different perspectives such as formulation development and stability, manufacturing from laboratorial to industrial scale, manufacturing process validation, analytical method development and validation and regulatory affairs.

This presentation will show you the pipeline of a project, from the initial idea to the final product.

IL 11: The role of halophytes as sources of natural products in a changing world: current knowledge, challenges and future perspectives

Luísa Custódio
Centre of Marine Sciences, University of Algarve, Faculty of Sciences and Technology, Ed. 7, Campus of Gambelas, 8005-139 Faro, Portugal
E-mail: lcustodio@ualg.pt

Salt tolerant plants (halophytes) are abundant in coastal areas and can cope with adverse stressful conditions, such as high salinity and intense UV radiation, partially due to the synthesis of several bioactive secondary metabolites, as for example phenolic compounds and alkaloids. Besides their protective role for the plant, these molecules display important bioactivities, including anti-oxidant, anti-inflammatory, antidiabetic and neuroprotective, which may explain the several ethnomedicinal and-veterinary uses [1-3], and biological properties ascribed to a high number of halophytic species [4, 5]. For example *Salicornia* L. species (sea asparagus) are used in traditional medicine against obesity and diabetes while *Chrithmum maritimum* L. (sea fennel) is used as diuretic and antiscorbutic [4]. *Pistacia lentiscus* L. is used against jaundice and diabetes, as animal fodder and feed, and has ethnoveterinary uses as anthelminthic [1]. Halophyte's ethnomedicinal uses and chemical richness literally opens a cornucopia of naturally available bioactive products with a high added value in different commercial segments. Several species are edible and highly procured in the food industry due to their nutritional properties: this is the case of quinoa seeds (*Chenopodium quinoa* Willd.), sea asparagus and sea fennel with a high commercial value and highly valued in gourmet cuisine. Quinoa and sea buckthorn (*Hippophae rhamnoides* L.) are also widely used in food supplements. In addition active ingredients obtained by *in vitro* technologies and/or botanical extracts obtained from selected species, as for example sea fennel and *Armeria maritime* (Mill.) Willd. are used in cosmetic formulations. Halophytes have an enormous potential to supply important commercial products, and their high salt tolerance enables their use in sustainable saline agriculture and in production systems using a range of saline irrigation water resources and/or underutilized soils [5-7]. Moreover the levels of secondary metabolites, that are responsible for the medicinal properties of halophytes, may be manipulated by agrotechnical practices as for example irrigation water quantity and salinity, fertilization and harvest time and cycle [8]. However, research on the biotechnological applications of halophytes is still in its infancy. This talk will give a brief overview of the role of selected halophyte species as source of natural products targeting different commercial applications, in the context of climate change, especially soil and water salinization. This includes species with ethnomedicinal/veterinary uses, *in vitro* and *in vivo* relevant biological properties and possible cultivation methods.

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IL 12: Strategies to explore the chemical diversity of Cyanobacteria

Leonor Ferreira[a], João Morais[a], Marco Preto[a], Vitor Vasconcelos[a,b], Ralph Urbatzka[a], Pedro Leão[a], **Mariana Reis[a]**

[a] Interdisciplinary Center of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Terminal de Cruzeiros de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal, [b] FCUP, Faculty of Sciences, University of Porto, Rua do Campo Alegre 1021/1055, 4169-007 Porto

E-mail: mreis@ciimar.up.pt

Cyanobacteria represent an important group of photosynthetic bacteria capable of producing metabolites with pharmaceutical applications [1]. A successful example that reached the market is the anticancer drug Brentuximab vedotin 63 [2], reinforcing the role of cyanobacterial natural products in drug discovery.

The Culture Collection of cyanobacteria (LEGEcc), at Blue Biotechnology and Ecotoxicology group at CIIMAR, holds a valuable and underexplored natural resource that can underpin the discovery of promising bioactive compounds. LEGEcc comprises more than 350 different cyanobacterial strains, collected in different ecosystems and locations [3]. Hence, to search for potential bioactive compounds, the chemical diversity of cyanobacteria from the Nostocales order was assessed by two main strategies. The classical bioassay-guided approach involves the creation of a natural product library, and to find successful anticancer lead candidates the screening employs physiologically relevant cancer models (3D tumor spheroids). Additionally, the modern genome-guided strategy, through the genomisotopic approach [4], will be key to unveil cryptic compounds and give insights into their biosynthesis.

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IL 13: Artificial Intelligence and Statistic tools to predict, find and interpret the bioactivities of complex natural products

Jose Maria Prieto[a]

[a]Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University College London, 29-39 Brunswick Square, London (UK), WC1N 1AX.
E-mail: j.prieto@ucl.ac.uk

Complex Chemical Entities (CCE) include natural products such as Herbal crude extracts, herbal semi purified fractions and Essential oils (EOs). These are vastly used as active principles (APIs) of medicinal products in both Clinical and Complementary/Alternative Medicine. In the food industry, they add 'functionality' to many nutraceuticals. However, the intrinsic variability of their composition and synergisms/antagonisms between major and minor components make difficult to ensure consistent effects through different batches.

The use of Artificial Neural Networks (ANNs) for the modelling and/or prediction of the bioactivity of CCE as a substitute of laboratory tests has been actively explored during the last two decades. Similarly, advanced statistical methods are also gaining a preponderant role in extracting information from complex numerical matrices (Multiple regression and cluster analysis) [1].

Evaluation of antioxidant and antimicrobial properties of natural products have been a common target for researchers. We successfully achieved their prediction by modelling the chemistry of CCE in ANNs [2,3]. The accuracy of the predictions seems to be limited only by the inherent errors of the modelled tests and the lack of international agreements in terms of experimental protocols. With sufficient accumulation of suitable information, ANNs can become reliable, fast and cheap tools for the prediction of anti-inflammatory, antioxidant, antimicrobial and anti-inflammatory activities, thus improving their use in medicine and nutrition [1].

Finding active principles in CCE is a similarly big challenge. Multiple regression analysis could reveal 6 active compounds from 22 EOs with Acetylcholinesterase inhibitory activity [4].

Interpretation of the mode of use of CCE by other Medical Systems may lead to new medicines underpinned by molecular pharmacology. By applying cluster analysis, we could understand patterns between their Traditional Chinese Attributes and their differential inhibition of lipid peroxidation and/or eicosanoids release [5].

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IL 14: Food Supplements and Elderly people

Nelson Tavares[a]

[a]*Natiris S.A.*
E-mail: nelsontavares@natiris.pt

The current lifestyle imposes on the Central Nervous System a number of factors like stress, food and nutrition, smoking and certain diseases such as diabetes and hypertension that can contribute to further deterioration of higher mental functions such as memory. Thus, it is normal that with advancing age, small forgetfulness begins to appear which may remain stationary or worsen rapidly. The frequency and intensity of these complaints can be considered as simple physiological changes or reach variable pathological levels. Some foods and nutrients appear to have a protective effect on brain metabolism, including fish oils and essential fats from nuts, for example. Other nutrient-rich foods may promote an antioxidant effect by protecting the Central Nervous System from the deleterious effects of aging and from oxidative reactions that occur at metabolic level. On the other hand, ingested as dietary supplements they can have a protective effect on brain cells, decreasing early cell death and mental decline associated with various pathologies. Examples of these nutrients include phosphatidylserine, coenzyme Q10 and resveratrol, among others.

IL 15: Synthesis versus extraction of bioactive oligosaccharides

Paula C. Castilho[a], Gonçalo N. Martins[a], Onofre Figueira[a], Andreia Gomez-Zavaglia[b]

[a]Centro de Química da Madeira, Universidade da Madeira, campus Universitário da Penteada, 9020-105 Funchal, Portugal [b]CIDCA-CONICET La Plata), La Plata, Argentina
E-mail: pcastilho@staff.uma.pt

Oligosaccharides have important bioactive roles, either while covalently bound to proteins or lipids or in the free form. For instance, more than 130 unbound oligosaccharides have been identified in human milk (5-10 g/L) and their physiologic role is not limited to the enhancement of the growth of *L. bifidus* flora and indirectly to the protection against gastrointestinal infections. It is now known they have an important role in defenses against viruses, bacteria, and their toxins.

Fructooligosaccharides (FOS) and Galacto-oligosacharides (GOS) are interesting ingredients in functional foods due to their prebiotic capability, the later with special relevance on the infant formula production.

FOS can be obtained from plant materials by water extraction and enzymatic hydrolysis of inulin or by enzymatic transfructosylation reactions using sucrose as starting material. The two processes share identical market quota. In both cases, glucose, fructose and sucrose are byproducts.

GOS can also be obtained by enzymatic synthesis, using lactose as substract, and by water extraction from plant materials. However, significant differences in structure and in byproducts are observed: β-GOS obtained by synthesis have lactose (and glucose) as main contaminants, undermining their use by lactose intolerant individuals, while α-GOS obtained from plants present glucose, fructose and sucrose, plus other types of water soluble byproducts.

While production of FOS and GOS is technologically straightforward [1], the removal of contaminants is complex and leads to enormous decrease in yields, so it must be carefully evaluated according to the application.

This work is part of a project [2] aiming at the protection of lactic acid bacteria during cryopreservation processes and, for that, high purity FOS/GOS are needed. Several methods used for the production of FOS/GOS and those for the minimization of undesirable contaminant will be comparatively discussed.

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Oral Communications abstracts

OC 1: Bacterial nanocellulose membranes loaded with bioactive compounds for skin treatment

Daniela F.S. Fonseca, Eduarda S. Morais, Nuno H.C.S. Silva, Carla Vilela, Armando J.D. Silvestre, **Carmen S.R. Freire**[a]

[a]CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Portugal
E-mail: cfreire@ua.pt

Bacterial cellulose (BC), an extra-cellular form of cellulose produced by some non-pathogenic bacteria, is a promising source of new functional bio-based materials for myriad applications. The exploitation of this highly pure and crystalline form of cellulose, with peculiar properties, in the biomedical, pharmaceutical and cosmetic fields, has become an active area of research as a result of the potential of this nanostructured material as a bioactive molecules carrier, due to its 3D network of cellulose fibrils.[1]

An overview of the recent research efforts on BC-based membranes for skin treatment performed in our group at CICECO – Aveiro Institute of Materials will be presented with focus on the use of (i) neat BC membranes, (ii) BC-multilayered based systems and (iii) microneedle devices for the topical administration of different bioactive compounds.

Acknowledgments: This work was developed within the scope of the project CICECO – Aveiro Institute of Materials (FCT Ref. UID/CTM/50011/2019), financed by national funds through the FCT/MEC. The research contract of C.V. is funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19. The research contract of N.H.C.S.S. is funded by project NANOBIOINKS (CENTRO-01-0145-FEDER-031289). FCT is also acknowledge for the doctoral grants to D.F.S.F. (PD/BD/115621/2016) and E.S.M. (SFRH/BD/129341/2017) and the research contract under Stimulus of Scientific Employment 2017 to C.S.R.F. (CEECIND/00464/2017).

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OC 2: Advances on antimicrobial activity of *Morus spp.* plant extracts: viruses in the spotlight

Inès Thabti[a,b], Stephanie Philippot[a], Arnaud Risler[a], François Dupire[a], Brenda Westerhuis[c], Stéphane Fontanay[a], Thomas Kassab[a], Walid Elfalleh[b], Ali Aferchichi[b], Raphaël E. Duval[a], **Mihayl Varbanov**[a]

[a]*Université de Lorraine, CNRS, L2CM, F-54000 Nancy, France*
[b]*Laboratoire d'Aridoculture et Cultures Oasiennes, Institut des régions Arides de Médenine, Route el Djorf, Médenine 4119, Tunisia*
[c]*Academic Medical Center, Department of Medical Microbiology, Meibergdreef 15, Amsterdam, Netherlands*
E-mail: mihayl.varbanov@univ-lorraine.fr

Viral infections cause life-threatening diseases in millions of people worldwide every year and are responsible for worldwide epidemic outbreaks, thus representing a heavy burden to their hosts [1]. In the absence of specific treatments for human viral infections, natural products offer an alternative in terms of innovative drug therapies [2]. In search of effective bioactive molecules, we analyzed the antiviral properties of leaves and stem bark of the mulberry tree (*Morus spp.*), known for their multiple biological activities [3]. In our study we compared the antiviral activity of *Morus spp*. on enveloped and non-enveloped viral pathogens, such as human coronavirus (HCoV), human poliovirus (PV), human adenovirus (HAdV), human parechovirus (HPaV) and a norovirus surrogate (feline calicivirus-F9, FCV-F9). The antiviral activity of 12 water and water: alcohol plant extracts of leaves and stem bark of three different species of mulberry - *Morus alba* var. *alba*, *Morus alba* var. *rosa* and *Morus rubra* were evaluated. Our results showed that several extracts were able to reduce the viral titer and cytopathogenic effects (CPE). Curiously *Morus alba* var. *alba* leaves water: alcohol extracts exhibited maximum antiviral activity on enveloped viruses (HCoV), while two particular water: alcohol extracts, one of stem bark of *Morus rubra* and one of leaves of *Morus alba* var. *rosa* were the most effective on poliovirus and norovirus surrogate. On the other hand, the antiviral impact on HPaV was exclusively due to stem bark and leaves water extracts of the three mulberries species tested. Our HPLC analysis revealed the presence of different polyphenolic compounds in the extracts, with coumarins, tannins, triterpenes, and flavonoids were the major ones. We further identified specific compounds found in the majority and in the most effective antiviral extracts, such as alkaloids (1-deoxynojirimycin), prenylated flavonoids (kuwanon G) and stilbenoids (mulberroside A). Our current work is based on the analysis of the antiviral molecular mechanisms of selected compounds isolated from the mulberry extracts and the observed important virucidal effect offers promising applications in antiviral strategies.

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OC 3: Mediterranean Shrubs: Nutraceuticals for sustainable goat production

Costa, C[a,b], Almeida, S.M[c], Cavaco-Gonçalves, S[a], Barbas, J.P[a], Ribeiro, J.M.B.F[a], Belo, C.C[a], Padre, L[b], **Belo, A.T,**[a]

[a]*INIAV, Santarém – National Institute of Agrarian and Veterinarian Research, Quinta da Fonte Boa, 2005-048 Vale de Santarém*
[b]*ICAAM – Department of Veterinary Medicine, Évora University, Pólo da Mitra, 7006-554 Évora*
[c]*UTAD – Trás-os Montes and Alto Douro University, Quinta de Prados, 5000-801 Vila Real*
E-mail: anateresa.belo@iniav.pt

Bioactive natural products have become an important research topic and development area in different fields of study, owing to their numerous potentialities. In animal production, the anthelminthic, antioxidant and antimicrobial properties of natural vegetation can improve the animals' productive efficiency and wellbeing, and product quality (meat and milk). It is known that goats have a very selective feeding behavior and a higher sensitivity to gastrointestinal strongyles (GIS) infections compared to other ruminants, and that certain plants have beneficial effects in animal health through the synergy between their nutritive value and bioactive compounds. Thus, the project "VegMedCabras" intends to characterize and explore the nutraceutical value of mediterranean shrubs for sustainable goat production, by controlling parasitic infection as well as preventing environmental contamination and residues in the final product from the continuous use of synthetic anthelmintics [1,2,3]. Charnequeira breed goats were observed during browsing/grazing of Mediterranean vegetation, from February to July, in order to document their shrub species preferences and evaluate the nutraceutical value of the final diets. All selected plants were analyzed for nutritive value (protein and fiber content, digestibility), as well as bioactive compounds content such as total phenolic compounds (TPC), total tannins (TT), condensed tannins, flavonoids and saponins. Antioxidant activity was also evaluated. Each goat was individually evaluated for natural GIS infection, by egg elimination levels, and health status, by metabolic blood parameters. A significant drop in EPG (eggs per gram of feces) elimination (± 65%) was observed by the end of the study period. As soon as goats started browsing the Mediterranean vegetation, EPG counts started to decrease as the proportion of shrubs rich in TPC increased in the diet. Species with the highest contribution were *Pistacia lentiscus* and *Quercus coccifera*, which presented an average TPC and TT of 163,6 and 134,2 mg GAE (gallic acid equivalent)/g MS vs. 138,4 and 130,2 mg GAE/g MS, respectively. Nutritive value of the diets selected by goats was estimated in order to determine the adequacy to their physiological requirements. With the increased resistance of GIS to synthetic anthelmintics and social demand for quality products, it is important to establish multidisciplinary options such as the use of nutraceutical shrubs, a safer, ecological and inexpensive solution.

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Flash Oral communications

F 1: Nematicidal effect of terpene molecules in *Bursaphelenchus xylophilus* and *Pratylenchus penetrans*

Pedro Barbosa[a], Jordana Branco[a], Claudia Vicente[ab], A. Cristina Figueiredo[c], Manuel Mota[de]

[a]*ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Évora, Portugal.*
[b]*Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, Oeiras, Portugal.*
[c]*Centro de Estudos do Ambiente e do Mar (CESAM Lisboa), Faculdade de Ciências da Universidade de Lisboa, Centro de Biotecnologia Vegetal (CBV), DBV, C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal.*
[d]*Departamento de Biologia, Laboratório de Nematologia/ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Évora, Portugal.*
[e]*Departamento Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal.*
E-mail: pbarbosa@uevora.pt

Nematode control in forestry or in agricultural systems is extremely difficult, expensive and not cost-effective. Chemically-based nematicides offer systemic protection and all are potent acetylcholinesterase inhibitors [1]. Due to their short-term effects on human health and in the environment, they are extremely hazardous and forbidden or in phase of fading out. In the most recent years, natural compounds produced by plants, like the ones from secondary metabolism, gained increasingly importance as valid tools for disease control [2]. *Bursaphelenchus xylophilus* (pine wood nematode, PWN) and *Pratylenchus penetrans* (root lesion nematode, RLN) are two of the most important species responsible for worldwide productivity losses in a significant number of plant species. Nematicidal activity from 25 terpene molecules (13 oxygen-containing- and 12 monoterpene hydrocarbons molecules) was accessed separately in the two species. PWN was multiplied in *Botrytis cinerea* according to [3]; RLN was multiplied in carrot discs according to [4]. Bioassays were performed fallowing the methodology employed in [2], except in the number of nematodes per well in RLN. At 2 mg/mL after 24h-exposure, nine oxygen-containing- hydrocarbons achieved 100% mortality in *B. xylophilus*. For *P. penetrans* only two compounds (carvacrol and thymol) achieved the 100% mortality, followed by eugenol and methyl salicylate with around 70%. The mortality from the monoterpene hydrocarbons was low (<10%) in both nematodes. On-going research is evaluating the minimum inhibitory concentration from the compounds able to achieve full mortality.

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F 2: Anti-inflammatory and anticarcinogen potential of sesquiterpenes present in Chicory

Diogo Carregosa [a], Ana Matias [a], Regina Menezes [a], Cláudia Nunes dos Santos [a,b]

[a]*iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal*
[b]*CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Portugal*
E-mail: claudia.nunes.santos@nms.unl.pt

Sesquiterpenes have been described as potential antimicrobial, anti-inflammatory, antiparasitic and anticarcinogen, however the potential of many molecules is still unknown.[1]

Chicory (*Chicorium intybus L.*) is a plant commonly cultivated in the northern Europe. Its leaves have been used as a dietary product in salads and its root as substitute for coffee in many countries inside Europe. Commercially exploit of chicory root have been extended to the extraction of inulin in the last couple of years followed by the characterization of the compounds present in chicory leaves and roots that have been unveiled. [2] Sesquiterpenes in chicory, still uncharacterized in terms of bioactivity have been opening the doors to evaluate the potential anticarcinogen and anti-inflammatory of these molecules.

The capability of these molecules to passively cross the membranes and reach circulation was predicted using an *in silico* model of passive permeability to infer the physiological relevance of this molecules.

The anticarcinogen potential of these molecules was evaluated by accessing their ability to impair Ras/Raf interaction. Ras is a proto-oncogene and upregulation in cancer. Nearly 30% of tumors carry a Ras mutation. [3] Therefore finding new molecules that can inhibit will be advantageous.

To evaluate the capacity of a molecule to inhibit two key inflammatory pathways have been considered NFAT and NF-κB. In both cases, a yeast model as used in order to screen the molecules. In order to evaluate NFAT, the yeast homologue to the human NFAT, Crz1 have been used. In NF-κB pathway the expression of human NF-κB subunit p65 was used as a reporter.

The chicory sesquiterpene are predicted to passively cross the relevant barriers and reach circulation. Bioactivity assay reveal that these molecules however did not significatively impact the Ras/Raf interaction.

Regarding inflammation some of the sesquiterpenes evaluated showed a very significant impact on NFAT and NF-κB yeast models.

In conclusion, these novel sesquiterpene molecules present in Chicory seem to be physiologically relevant according to the *in silico* model and impact the inflammatory process through the NF-κB and NFAT and modulate the release of inflammatory cytokines, opening the doors to explore more deeply the mechanisms of action behind these molecules.

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F 3: *In silico* approach for design of new derivatives of 7α-acetoxy-6β-hydroxyroyleanone from *Plectranthus* spp. based on PKC-δ activation

Vera M. S. Isca[a,b], Epole Ntungwe[b], Joana Tavares[b], Filipa Siopa[a], Joana Almeida[c], Lucília Saraiva[c], Mattia Mori[d], Maurizio Botta[d], Carlos A.M. Afonso[a], Patrícia Rijo[a,b]

[a]*Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal;*
[b]*Center for Research in Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal;*
[c]*LAQV/REQUIMTE, Departamento de Ciências Biológicas, Faculdade de Fármacia da Universidade do Porto, Portugal;*
[d]*Dipartimento di Biotecnologie, Chimica e Farmacia, Università di Siena, Italy*
E-mail: patricia.rijo@ulusofona.pt

Cancer is one of the prevalent causes of death worldwide. Protein Kinases (PKCs) have important effects in processes of tumorigenesis and metastatic dissemination. PKC-δ works as tumor suppressor in colon cancer, one of the most dominant cancers and cause of cancer mortality worldwide [1].

Plectranthus genus is an important source of bioactive royleanones [1, 2]. 7α-acetoxy-6β-hydroxyroyleanone (Roy) is an example of a lead molecule isolated in high amounts from *P. grandidentatus*. This royleanone showed promising biological properties, including antitumoral activity [3]. Additionally, Roy has hydroxyl groups suitable for derivatization, essential for drug development.

In a previous study, the patented diterpene 6β-benzoyloxy-12-O-benzoylroyleanone (RoyBz), obtained by semi-synthesis of Roy [4], improved the cytotoxic properties, with selective activation of PKC-δ [5].

The aim of the present work is to preform several hemi-synthetic reactions in the lead compound, to prepare a small library of new compounds with ability to activate PKC-δ. Considering this, molecular docking was used as a tool to design the new royleanone derivatives. Theoretical derivatives of Roy were designed through modification of the C-12 and C-6 hydroxyl groups. *In silico* screening of these theoretical derivatives were carried out against the crystallographic structure of human PKC-δ regulatory domain.

Analysis of the docking poses suggests that small groups such as -OH, -OMe and -OAc, in position C-12, should favor PKC-δ activation, while position C-6 can bear a large diversity of structural groups. Based on the docking achievements further derivatives are currently been prepared by hemi-synthesis, using Roy as starting material for future structure-activity relationships.

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F 4: Spiropyrazoline oxidoles as promising inhibitors of p53-MDMs protein-protein interaction

Elizabeth A. Lopes[a], Margarida Espadinha[a], Mattia Mori[b], Maurizio Botta[b], Maria M. M. Santos[a]

[a] *iMed.ULisboa, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal*
[b] *Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy*
E-mail: mariasantos@ff.ulisboa.pt

Natural products play a highly important role in drug discovery for the treatment of human diseases. Specifically, oxindole alkaloids are known to possess a broad range of biological activities. Thus, there is a huge interest in industry and academia to develop novel spirooxindoles with interesting biological activities[1].

In all types of human cancers, the p53 tumor suppressor function is inactivated by mutation or gene deletion or by negative regulators such as MDM2 and MDMX. Currently, the most popular approach to activate the wild-type p53 is the inhibition of the protein-protein interaction (PPI) of p53 with these two regulators using small molecules. However, there aren't any small molecules acting as dual p53-MDM2/X PPI inhibitors in clinical trials[2]. For this reason, it is urgent the development of small molecules that inhibit the interaction of p53 with both negative regulators for a full reactivation of p53.

Our research team has been working on the development and optimization of spiropyrazoline oxindoles to obtain dual p53-MDM2/X PPI inhibitors (Figure). Hence, we have already developed derivatives with good antiproliferative activities in HCT-116 $p53^{(+/+)}$ human colon cancer cell line, which induces apoptosis and cell cycle arrest at G0/G1 phase, upregulate p53 steady-state levels, and lead to a decrease of MDM2 levels[3]. In this communication, we report the structure-based computational optimization of this chemical family for the development of novel p53-MDM2/X interaction inhibitors. Our studies will shed light on the possible binding mode of spirooxindole derivatives to MDM2 and MDMX and will drive the *hit-to-lead* optimization strategy. Furthermore, we report our most recent optimization of the synthesis of these new spiropyrazoline oxindoles derivatives.

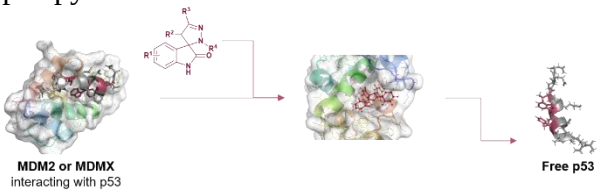

Figure – Spiropyrazoline oxindoles interacting with MDM2 and MDMX, releasing and activating p53

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Posters abstracts

P 1: Molecular biosystem analysis of the hypoxia inducible domain family member 2A: implications in cancer biology

Celia Salazar[a], Osvaldo Yañez[b], Alvaro A. Elorza[c,d], Natalie Cortes[e], Olimpo García-Beltrán[e], William Tiznado[b], Lina María Ruiz[a]

[a]*Instituto de Ciencias Biomédicas, Facultad Ciencias de la Salud, Universidad Autónoma de Chile, Santiago, 8910060, Chile*
.[b]*Computational and Theoretical Chemistry Group, Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andres Bello, 8370251, Chile.*
[c]*Institute of Biomedical Sciences, Faculty of Medicine and Faculty of Life Sciences, Universidad Andres Bello, Santiago, 8370146, Chile.*
[d]*Millennium Institute on Immunology and Immunotherapy, Santiago, 8331150, Chile.*
[e]*Facultad de Ciencias Naturales y Matemáticas, Universidad de Ibagué, Carrera 22 calle 67, Ibagué, 730002, Colombia.*
E-mail: lina.ruiz@uautonoma.cl

The expression of *HIGD2A* is dependent on oxygen levels and glucose concentration as well as on cell cycle progression [1]. This gene encodes for protein HIG2A, which is in mitochondria and nucleus, promoting cell survival in hypoxic conditions. The genomic location of *HIGD2A* is in chromosome 5q35.2, where several chromosomal abnormalities are related to numerous cancers. The analysis of high definition expression profiles of *HIGD2A* suggests a role for HIG2A in cancer biology [1]. Accordingly, our objective was to perform a molecular biosystem analysis of *HIGD2A* that allow knowing their implications in cancer biology. For these, public databases such as SWISS-MODEL[2], STRING[3], COSMIC[4], GEO[5], and MethHC[6] were accessed. We also evaluated by RT-qPCR the expression of *Higd2A* gene in healthy bone marrow-liver-spleen tissues of mice after quercetin (50 mg/kg) treatment[7]. Thus, among the structural features of HIG2A protein that may participate in their translocation to the nucleus are; an importin α-dependent nuclear localization signal (NLS), a motif of DNA binding residues and a probable SUMOylating residue. *HIGD2A* gene is not implicated via mutation in cancer. In addition, DNA methylation and mRNA expression of *HIGD2A* gene present significantly alterations in several cancers. Such as, *HIGD2A* gene showed significant higher expression in Diffuse large B-cell lymphoma (DLBCL). Hypoxic tissues characterize the "bone marrow-liver-spleen" DLBCL type. The relative quantification by RT-qPCR showed that *Higd2A* expression is higher in bone marrow than the liver and spleen. Besides, it was observed that quercetin modulated the expression of *Higd2A* gene in mice.

As an assembly factor of mitochondrial respirasomes, HIG2A might be involved in the change of cellular energetics that occurs in cancer. It is worth continuing to explore the role of *HIGD2A* in cancer biology.

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P 2: *In vitro* and *in vivo* exploratory physiological effects of Kefir on modulation of human microbiota applied to skin care: metabolic activities on skin aging related enzymes.

Yarabin De Icaza[a], Joana M. Andrade[b], Luis Rodrigues[b], António Pedro Louro Martins[c], Catarina Rosado[b], Patrícia Rijo[b], **Maria do Céu Costa[b]**

[a]Internship student CBIOS, Faculty of Bioscience Engineering, University of Gent. Coupure Links, 653 (9000), BELGIUM.
[b]CBIOS, the University Lusofona´s Research Center for Biosciences and Health Technologies, Av. Campo Grande 376 Lisboa (1749 024), PORTUGAL.
[c]Instituto Nacional de Investigacão Agrária e Veterinária (INIAV I.P.), Av. Da República, Quinta do Marquês 2780-157 Oeiras, PORTUGAL.
Email: ydicaza@gmail.com

The microbiome is defined as all microorganisms in the human body and their respective genetic material, in a particular location, such as the Gastrointestinal Tract (GI) or skin [1]. Human cutaneous and intestinal microbiota has a close relation with skin health, so manipulation of these microbiota may have health benefits for the skin and so, contributing with premature aging skin [2]. CIDCA Kefir is a well characterized product [3] whose potential to deliver actives for topical formulations and/or orally administration within dermatological disorders scenarios has never been explored.

To explore the potential of the Kefir probiotic processed milk in skin homeostasis.

We investigated the effects on bio-metrological measurements of skin hydration and trans-epidermal water loss along with *in vitro* assessment of acetylcholinesterase, collagenase and tyrosinase activities as important markers for skin health.

A significant inhibition of acetylcholinesterase, collagenase and tyrosinase activities ($p < 0.05$) was observed.

Kefir has significant potential to be used as skin hydrating and anti-aging agent.

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P 3: Cytotoxicity evaluation of *Annona muricata* L. seeds on human cancer cell lines.

Behzad Mohaddesi[a], Ashvin V. Dudhrejiya[b], Navin. R. Sheth[c],

[a]*Department Of Pharmacognosy, Faculty of Pharmacy, Tehran university of medical science, Tehran, Iran.*
[b]*B. K. Mmody Government pharmacy college, Rajkot, Gujarat, India.*
[c]*Department Of Pharmaceutical Sciences, Saurashtra university, Rajkot, Gujarat, India*
E-mail: behzad.mohaddesi@gmail.com

Natural medicine mainly plant derived products are on high demand for combating various modern life style disease such as cancer which has a significant prevalence rate in developed world. Annonaceae is a plant family with different species which used by traditional healers of South America and East Asia. *Annona muricata* L. of Annonaceae commonly known as soursop in English or lakshmanphala in Hindi has a notable medicinal properties; various part of plant traditionally used for treating many illness. In this study seeds of plant selected for anticancer screening. Different extracts, fractions and sub fractions obtained with Bioassay guided fractionation approach and each sample separately tested on human cancer cell lines with Sulforhodamine B colorimetric (SRB) cytotoxicity assay. Phytochemichal analysis performed on most potent sample in order to find out the probable phytoconstituent which play role on the activity. Result indicated strong activity of separated sample when compared with the known standard drug of Adriamycin (Doxorubicin), Growth inhibition of 50% (GI50), drug concentration resulting in total growth inhibition (TGI) and lethal concentration 50% (LC50) calculated. In cell lines, the seeds showed significant activity of extracts and fractions with GI50 of <10 (µg/ml) and TGI value to 17.7 (µg/ml). This study showed the potential medicinal value of *Annona muricata* L. seeds phytoconstituents as anti-neoplastic agents for controlling the growth of human cancer cells and seeds which can be utilize from the edible fruit will be a good source for natural therapeutics agent.

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P 4: Evaluation of the antioxidant potential of the red seaweed *Gracilaria gracilis* (Rhodophyta, Gracilariales)

Clélia N. Afonso[a], Ana P. Correia[a], Marta V. Freitas[a], Teresa Mouga[a], Ana Pombo[a], Teresa Baptista[a]

[a]*MARE – Marine and Environmental Sciences Centre, ESTM, Politécnico de Leiria, Edifício Cetemares, Av. Porto de Pesca, 2520-641 Peniche (Portugal)*
E-mail: clelia@ipleiria.pt

Marine environments, due to their enormous biodiversity, are now arousing major interest from the scientific community. The search for new bioactive compounds, with application in the food industry, as feed additives, pharmaceuticals or cosmetics, among other applications, has recently been on the agenda. The demand for these compounds meets the wishes of consumers, who are looking for natural products that promote health and wellbeing. Seaweeds, known also as macroalgae, produce many biologically active compounds, including pigments (carotenoids, chlorophylls, phycobiliproteins), terpenoids, polyunsaturated fatty acids, polysaccharides and vitamins, and the red seaweed *Gracilaria gracilis*, is no exception [1]. These red algae live in complex habitats, exposed to cyclic conditions as a consequence of the tides rhythm, which requires a huge potential for adaptation to environmental conditions. It is therefore expected that they produce bioactive compounds with protective functions. In general, seaweeds present antimicrobial and immunostimulant effect, but also anti-viral, prebiotic and antioxidant activities, among others. The objective of the study was to evaluate the *in vitro* antioxidant activity of crude extracts of *Gracilaria gracilis* and evaluate the potential use of this red seaweed as a fish feed additive, that promotes fish health and wellbeing. The red seaweed *Gracilaria gracilis* was collected from Lagoa de Óbidos and dried at 25˚C in an oven. The aqueous and ethanolic extracts were prepared at different temperatures [2], filtered, centrifuged and concentrated in a rotary evaporator (ethanolic extracts) or freeze-dried (aqueous extracts). For the evaluation of the antioxidant activity of crude extracts, we have used complementary methods: DPPH (2,2-diphenyl-1-picryhydrazyl) radical-scavenging activity [3], and Total Phenolic Contents [4]. The total phenolic content of algal extracts was determined by spectrophotometry using the Folin-Ciocalteu method and the results expressed in % of dry weight based on a standard curve of gallic acid. Our results indicate that ethanolic extracts show higher antioxidant activity than the aqueous ones. Based on the current research, seaweed extracts show promising results, however, further research is needed.

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P 5: Action of isomers triterpenos α- and β-amyrenone isolated from *Protium sp*. (Burseraceae) in the renal parenchyma of diabetic mice

Luiz Pedro de Souza-Costa[a], Jose Fernando Marques Barcellos[a], Emerson Lima Silva[b], Fernanda Guilhon-Simplicio[b], Rosilene Gomes da Silva Ferreira[c], Maria do Carmo Queiroz Fialho[a]

[a]Federal University of Amazonas, Department of Morphological Science, Brazil; [b]Federal University of Amazonas, Faculty of Pharmaceutical Science; [c]State University of Amazonas, Higher Normal College
E-mail: souzaluizpedro@gmail.com

The policies for the maintenance of Brazilian biodiversity are of crucial importance for the development of sustainability. Among the Amazonian biodiversity are the *Protium* (Aubl.) March species that have pentacyclic triterpenes belonging to the terpene group, widely diffused and studied for medicinal purposes due to their various pharmacological activities such as hepatoprotective, antioxidant, anti-inflammatory, antitumoral and recently the hypoglycemic and lipid-lowering potential observed in mice models with Diabetes Mellitus (DM) [1], a disease in which is integrally linked with dyslipidemia which is a precursor to cardiovascular diseases where the derivative triterpene α- and β-amyrenone can be used in pharmaceutical formulations for the same result [2, 3]. The research aims to analyze the nephrotoxic effects of the triterpene mixture. As methodology, experimental methods were used, where the C57BL/6 lineage mice were used for chronic testing, kept in photoperiod of 12h light/dark and in a constant temperature of 22 ± 1 °C and humidity of 55% ± 10%, and provided with water (300 mL); normal diet (200 g). Right after, induction of diabetes was performed intraperitoneally with a nicotinamide dose (210 mg/kg) and after 20 minutes a dose of streptozotocin (STZ) a body weight of 180 mg/kg body weight [4]. The animals were divided into 5 groups of 6 animals and denominated in: basal control group (GB); positive control group (CP); standard groups (GCP) and test groups (GTA50 mg/kg and GTA25 mg/kg substance), the treatment lasted 10 days, were subsequently euthanized with xylazine and ketamine hydrochloride and the kidney was removed and fixed in 10% buffered formalin, next undergoing histological processing in paraffin [5]. Treatment with amyrenone (GTA50 and GTA25) in the kidneys demonstrated disruption in the morphology of animals treated with the test substance. The effect of the mixture in the tubules led to cellular degeneration. Treatment with the conventional drug (GCP) had similar effects to those observed with amyrenone treatment. Comparing the dosages and evaluating the tissue damages of each treatment, amyrenone 25mg had less tissue damage compared to treatment with amyrenone 50mg. In summary, the effects of α- and β-amyrenone cause toxic damage to renal tissue, a condition similar to the conventional drug for the treatment of diabetes [6].

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P 6: Antioxidant, antimicrobial and cytotoxic effect of *Menyanthes trifoliata* L. extracts from *in vitro* cultured plants

Tomasz Kowalczyk[a], Przemysław Sitarek[b], Ewa Skała[b], Patricia Rijo[c,d], Ewelina Synowiec[e], Janusz Szemraj[f], Urszula Krajewska[g], Tomasz Śliwiński[e]

[a]*Department of Molecular Biotechnology and Genetics, University of Lodz, Banacha 12/16, 90-237, Lodz, Poland*
[b]*Department of Biology and Pharmaceutical Botany, Medical University of Lodz, Muszynskiego 1, 90-151, Lodz, Poland*
[c]*Center for Research in Biosciences and Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, 1749-024, Lisbon, Portugal*
[d]*Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, 1649-003, Lisbon, Portugal*
[e]*Laboratory of Medical Genetics, Faculty of Biology and Environmental Protection, University of Lodz, University of Lodz, Pomorska 141/143, 90-236 Lodz. Poland*
[f]*Department of Medical Biochemistry, Medical University of Lodz, Mazowiecka 6/8, 92-215, Lodz, Poland*
.[g]*Department of Pharmaceutical Biochemistry, Molecular Biology Laboratory, Medical University of Lodz, Muszynskiego 1, 90-151, Lodz, Poland.*
E-mail: tomasz.kowalczyk@biol.uni.lodz.pl

Menyanthes trifoliata L. (Menyanthaceae) is a valuable medical plant which grows on peat bogs and swamps in Europe, North America and Asia. It has long been used in folk medicine as a remedy for various ailments such as loss of appetite or dyspeptic complaints [1]. Extracts obtained from this plant have been found to induce apoptosis in cancer cells and are a source of phenolic compounds such as chlorogenic, ellagic, sinapinic, syringic acids, rutin and pentacyclic triterpene- betulinic acid [2]. Many of these compounds may play an important role in medical applications. This study examines the antioxidant and cytotoxic properties of aqueous methanolic extracts derived from the aerial parts (MtAPV) and roots (MtRV) of *in vitro* grown plants on human umbilical vein endothelial cells (HUVECs). Additionally, antibacterial and antifungal properties of these extracts were tested. Our results indicate that treatment with both extracts at a concentration of 1 mg/mL was able to change the level of antioxidant genes (*HO-1*, *NQO1*, *NRF2*, *kAEP1*, *GCLC*) in H_2O_2-stimulated HUVEC cells compared to controls. Both tested extracts showed moderate antimicrobial activity against various strains at a range of MIC values (150-925 µg/mL) and for MBC/MFC (500-2500 µg/mL). The MtRV extract showed better activity than MtAPV against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Candida albicans* and *Saccharomyces cerevisiae*. It was found that the neither of the tested plant extract demonstrated a cytotoxic effect on the HUVEC cells after 24-hour incubation with the tested range of concentrations (0-5 mg/mL). Our findings demonstrate that MtRV and MtAPV extracts from *M. trifoliata* plants derived from *in vitro* cultures show antioxidant effects on human umbilical vein endothelial cells (HUVECs) stimulated by H_2O_2 and they also display antimicrobial properties against various strains. Therefore, this plant may play a potential role in the therapy and prevention of many civilization diseases.

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P 7: A molecular analysis of the transformed roots of *Leonotis nepetifolia* (L.) R.Br. for the presence of a transgene encoding the gene of DXP reductoisomerase (DXR), and the biological properties of the obtained extracts

Karol Górski[a] , Tomasz Kowalczyk[b], Ewa Skała[a] , Monika Toma[c], Joanna Wieczfińska[d], Patricia Rijo[e,f], Przemysław Sitarek[a]

[a]*Department of Biology and Pharmaceutical Botany, Medical University of Lodz Poland, ul. Muszynskiego 1, Lodz,*
[b]*Department of Molecular Biotechnology and Genetics, Faculty of Biology and Environmental Protection, University of Lodz, ul Banacha 12/16, Lodz, Poland*
[c]*Laboratory of Medical Genetics, Faculty of Environmental Protection, University of Lodz, Lodz, Poland,*
[d] *Department of Immunopathology, Chair of Allergology, Immunology and Dermatology, Faculty of Biomedical Sciences and Postgraduate Training, Medical University of Lodz, Lodz, Poland,*
[e] *Center for Research in Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, Lisboa, Portugal,*
[f] *Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal*
E-mail address: przemyslaw.sitarek@umed.lodz.pl

Leonotis nepetaefolia (L) R. Br. - Family (Lamiaceae) commonly known as 'Lion ear' is very common in India, Tropical Asia or Africa and has a long history of traditional medicinal use throughout the world for treating bronchial asthma, diarrhoea, kidney disease, rheumatism, fever, influenza and malaria and as an analgesic. It has exhibited various biological activities including antifungal and antibacterial activities [1,2]. This study describes the first *Agrobacterium rhizogenes* – mediated transformation of *L. nepetifolia* for transformed roots induction overexpressed DXP reductoisomerase (DXR). Extracts prepared from these roots were used for evaluation of its selected biological properties. The results indicate an increase in DXR gene expression in the transformed roots of *L. nepetifolia* compared to transformed roots without construct (*A. rhizogenes* A4). Both sets of extracts, i.e. from roots containing and not containing DXR construct demonstrate a cytotoxic effect against glioblastoma cells, but a stronger effect was observed for DXR extract. This extract had also stronger influence on reduction of mitochondrial membrane potential in the glioblastoma cells, indicating induction of apoptosis caused by changes in the expression of genes related to apoptosis and DNA fragmentation. The reason for the different impact of the tested extracts on cancer cells may be due to differences in the secondary metabolite content. Our results suggest new medical applications for *L. nepetifolia* root extracts.

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P 8: Multitarget mechanisms of Antitumour Hybrid Combinations integrating terpenoids

Eva María Domínguez-Martín[a,b], Ana María Díaz-Lanza[a], Patrícia Rijo[b,c], Célia M. C. Faustino[c]

[a]*Department of Biomedical Sciences, Faculty of Pharmacy, University of Alcalá, Alcalá de Henares, Madrid, Spain.*
[b]*Research Center for Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisbon, Portugal.*
[c]*iMed.ULisboa - Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal.*
E-mails: evam.dominguez@edu.uah.es; ana.diaz@uah.es

Cancer comprises a group of diseases characterized by abnormal cell growth involving cell division without control. It is one of the main causes of morbidity and mortality worldwide, with approximately 14.1 million new cases diagnosed in 2012 and 8.2 million deaths, according to the World Health Organization (WHO) latest report on cancer. Different types of treatments are been employing to overcome cancer but their usually lack of selectivity and the development of resistance result in limited efficacy or ineffectiveness of the therapies. For these reasons, the seeking of new treatment options for this disease is necessary and antitumour hybrid combinations represent a promising approach.

This review aims to provide a synopsis into Anticancer Hybrid Combinations involving terpenoids, focusing on their multi-target mechanisms of action and synergistic effects.

Antitumor hybrid combinations are the therapeutic combination of synthetic drugs with chemically defined constituents from plants (secondary metabolites) aiming to increase the pharmacological activity of the formulation and simultaneously reduce the toxic side-effects of the drugs, interaction known as synergy. The secondary metabolites used in these combinations are mainly plant-derived phenolic compounds and terpenoids, which are the focus of the present work. Their multitarget mechanisms are explained by some examples.

Antitumour hybrid combinations are a promising therapeutic strategy to reduce cancer resistance to different treatments and minimize adverse effects, while simultaneously showing selectivity to tumour cells and potentiate the activity of the drug that make them an interesting prospective option to cure cancer.

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P 9: Study of the effect of ingestion of a diet supplemented with 6.7% broccoli (*Brassica oleracea* L. var. *italica*) flour in mice

Tânia Martins[a], **Ana I. Faustino-Rocha[a,b]**, João Ferreira[c], Maria J. Pires[a,d], Carla Sobreira[a], Carlos Venâncio[a,e], Mário Faria[f], Bruno Colaço[a,e], Isabel Gaivão[c,g], Ana I. Barros[a], Luís Antunes[a,d], Paula A. Oliveira[a,d], Eduardo Rosa[a]

[a]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;
[b]Faculty of Veterinary Medicine, Lusophone University of Humanities and Technologies (ULHT), Lisbon, Portugal;
[c]Animal and Veterinary Research Centre (CECAV) and Department of Biology and Environment (DeBA), UTAD, Vila Real, Portugal;
[d]Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, UTAD, Vila Real, Portugal;
[e]Department of Animal Sciences, School of Agrarian and Veterinary Sciences, UTAD, Vila Real, Portugal;
[f]School of Life and Environmental Sciences, UTAD, Vila Real, Portugal; [g]Department of Genetics and Biotechnology, UTAD, Vila Real, Portugal.
E-mail: taniam@utad.pt

Broccoli (*Brassica oleracea* L. var. *italica*) is highly rich in sulforaphane, an isothiocyanate with anticarcinogenic properties, antibacterial effects, and protective effects against oxidative stress. Recently, studies suggest that sulforaphane may also have anti-obesity properties.[1] Animal models are used to study human diseases, nevertheless, there are no studies of oral sub-chronic toxicity of broccoli flour intake in the physiological parameters of healthy animals.

This study aims to evaluate the effects of broccoli flour (BF) supplementation in the mice's physiological parameters. After ORBEA approval, eight-months-old FVB/n male mice (n=34) were equally divided into two groups: control and 6.7% BF. Body weight, water and food intake were registered weekly during the experimental assay. After initiation of dietary exposure, animals were sacrificed at the following time points: 2h, 3h, 24h, 48h, or 21 days, and the following samples were collected to perform hematological and histopathological analysis: blood, thymus, heart, lungs, spleen, kidneys, liver, intestines, perirenal and abdominal adipose tissue. The genotoxicity of broccoli was determined by the comet assay. Animal showed no mortality and any clinical signs of disease during the protocol. The variation of body weight gain was similar between groups. Regarding water consumption, the values between groups were also similar. Concerning food intake, both groups showed at 2h and 3h lower food ingestion. Microhematocrit and the relative weight of organs, showed no significant differences between groups at all time points. Comet assay data revealed that 6.7% BF did not induce DNA damage in leukocytes at 24h, 48h and 21 days.

These results showed that the broccoli supplementation did not cause significant changes in mice's physiological parameters.

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P 10: Evaluation of the potential antifibrotic effects of *Ganoderma lucidum* extracts on renal interstitial fibrosis induced by unilateral ureteral obstruction in mice

Maria João Pires[a,b], **Ana I. Faustina-Rocha**[b,c], Oludemi Taofiq[d], Lillian Barros[d], Isabel C.F.R. Ferreira[d], Rui M. Gil da Costa[e], Rita Reis[f], Rita Ferreira[f], Paula Alexandra Oliveira[a,b]

[a] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal [b] Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), UTAD, Vila Real, Portugal [c] Faculty of Veterinary Medicine, Lusophone University of Humanities and Technologies, Lisbon, Portugal [d] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal [e] Molecular Oncology and Viral Pathology Group, IPO-Porto Research Center (CI-IPOP), Portuguese Institute of Oncology of Porto (IPO-Porto), Porto, Portugal [f] Organic Chemistry, Natural Products and Foodstuffs (QOPNA), Mass Spectrometry Center, Department of Chemistry, University of Aveiro, Aveiro
E-mail: anafaustino.faustino@sapo.pt

Chronic kidney disease (CKD) represents a global public health problem. Renal interstitial fibrosis is a common characteristic of CKD and is observed in a variety of kidney diseases that causes renal failure. The unilateral ureteral obstruction (UUO) model is a well-established CKD model marked by significant tubular injury and tubulointerstitial fibrosis [1]. In this model, tubulointerstitial fibrosis is always associated with persistent sterile inflammation [2]. *Ganoderma lucidum* (Curtis) P. Karst. is a widely studied medicinal mushroom, revealing in its composition high amounts of triterpenoids and some phenolic acids, which have been associated to anti-inflammatory activities [3]. Based on these findings we hypothesize that a *G. lucidum* hydroethanol extract (GL), obtained by an ultrasound-assisted extraction, may ameliorate renal interstitial fibrosis in a UUO mice model.

The UUO model was performed by ligating the left ureter as previously described [1]. Sham-operation was conducted for all procedures, except for ureteral ligation. After UUO/sham-operation (SO), twenty-three FVB/n male mice, eight weeks old, were randomly divided into three groups: SO-mice with simulated operation (n=7), UUO-UUO mice without treatment (n=9); and UUO+GL-UUO mice treated daily with *G. lucidum* extracts (2.0 mg/animal *per* day, n=7). Treatment was administered in drinking water for two weeks. All animals were sacrificed on day 14 after UUO or SO. Physiological parameters, histological changes and plasma inflammatory biomarkers were assessed. Procedures followed the European legislation (Directive 2010/63/EU).

Ganoderic acid H and *p*-hydroxibenzoic acid were the main triterpenic acid and phenolic acid found in the extract, respectively. Statistically significant differences concerning left kidney weight/ body weight ratio were found between SO and UUO groups (p=0.006). There were no statistically significant differences in interstitial fibrosis, interstitial inflammation and tubular dilatation scores between the UUO and UUO+GL groups. Furthermore, no statistically significant differences were found between the different groups in plasma interleukin-6 levels (p=0.692), plasma C-reactive protein levels (p=0.519) and plasma tumor necrosis factor-like weak inducer of apoptosis levels (p=0.198). These results suggested that treatment with *G. lucidum* extracts do not ameliorate renal interstitial fibrosis associated with renal tubular injury after 14 days of UUO.

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P 11: *In silico* screening of natural products and derivatives for potential inhibition of PD-1 – PD-L1 interaction

Ana Fonseca[a], Ana Carvalho[a], Pedro Bulcão[a], Stéphane Besson[a,b]

[a]*Escola de Psicologia e Ciências da Vida, Departamento de Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, 1749-024 Lisboa, Portugal*
[b]*LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal*
E-mail: stephane.besson@ulusofona.pt

PD-L1 protein, overexpressed at the surface of certain tumour cells, binds to lymphocyte protein PD-1, thus promoting immunosuppression[1]. A field of immunotherapy is currently dedicated to searching PD-1 – PD-L1 interaction inhibitors[2]. The objective of this work was to explore the potential of some natural compounds or their derivatives to interfere with the interaction between those two membrane proteins. A particular emphasis was given to the study of compounds present in food products that are traditionally used to preserve human health and fight cancer: garlic, broccoli, beet and drumstick tree (moringa tree). The screening of the selected compounds was carried out *in silico* through the use of molecular docking with the SwissDock software[3], using membrane protein PD-L1 as a target.

The most promising compound is isoquercitrin 6''-oxalate, isolated from the moringa tree (*Moringa oleifera*)[4] and closely related to the quercetin molecule encountered in garlic. Isoquercitrin 6''-oxalate was found to dock quite especifically to the PD-L1 surface area involved in PD-1 – PD-L1 binding. Two other compounds with interesting results were 6''-O-(3,4-Di-O-acetyl-alpha-L-rhamnopyranosyl)isoquercitrin and 3-[3-O-[(3R)-4-Carboxy-3-hydroxy-3-methylbutanoyl]-beta-D-glucopyranosyloxy]-4',5-dihydroxy-3',7-dimethoxy-flavone. Other selected compounds (other quercetin derivatives, sulphoraphane, glucosinolates, betacyamins, proanthocyanidins) only exhibited weak and non-specific interactions with the target.

Moringa oleifera, a traditional food in the whole of South-Asia, is considered a miracle-tree in the local pharmacopoeia with a reputation for curing several human illnesses. In this context, further study of isoquercitrin 6''-oxalate as to its potential use in cancer immunotherapy should be encouraged.

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P 12: Antioxidant and antimicrobial activities of *Saccocalyx satureioides* Coss. et Dur.

HENDEL Noui[a], SARRİ Madani[b], ALI GHAFSI Achwaq[a], BENSEGHIR Aicha[a] and SELLOUM Mounir[a]

[a] *University Mohamed Boudiaf of M'sila, Faculty of Sciences, Department of Microbiology and Biochemistry, M'sila 28000, Algeria*
[b] *University Mohamed Boudiaf of M'sila, Faculty of Sciences, Department of Nature and Life Sciences, M'sila 28000, Algeria*
Email: noui.hendel@univ-msila.dz

The aim of this work was to evaluate polyphenolic and flavonoid contents of *Saccocalyx satureioides* Coss. and Dur. (an endemic Algerian Lamiaceae) leaves and roots, as well as its antimicrobial and antioxidant activities, by different methods. The antioxidant activity was evaluated by DPPH and of β-carotene bleaching tests. The antimicrobial activity of the plant was tested by different methods: well diffusion and microdilution tests for bacteria; and incorporation, fumigation and well diffusion tests for fungi. The yields of leaves and roots, subjected to methanol extraction by Soxhlet, were 13.05 g and 11.53 g for leaves harvested in June and February periods, and 6.15g for roots respectively. Extraction of the leaves essential oil by hydrodistillation yielded 2.8% and 2.55% for June and February, respectively. The leaf polyphenolic contents were 240.72µg EAG/mg and 82.95µg EQ/mg for June and 124.40µg EAG/mg and 61.14µg EQ /mg for February, and those of the roots were 210.50µg EAG/mg and 25.394µg EQ/mg for polyphenols and flavonoids, respectively. The EO contents range from 190.31 to 265.66 µg EAG/µl and 0.38 to 0.48 µg EQ/µl, respectively. In the antioxidant activity evaluated by the DPPH test, the IC_{50} of Ext_J, Ext_F, Ext_R, EO_J and EO_F were 513.06 ± 14.08, 301.78 ± 9.44, 466.9 ± 22.83 µg/ml, 6.12 and 6.70µl/ml respectively; whereas in the bleaching test of β-carotene the IC_{50} of Ext_J, Ext_F, Ext_R, EO_J and EO_F were 266.22 ± 4.28, 257.98 ± 10.69, 236.84 ± 4.01 µg/ml, 3.64 ± 0.28 µl/ml and 5.64 ± 0.12 µl/ml respectively. In the antimicrobial activity, the EO_J MBC was 2.5 µl/ml for *S. aureus*, *P. mirabilis*, *E. coli P*, *B. subtilis*; was 5 µl/ml for *E. coli*, *P. aeruginosa*; and was > 10 µl/ml for *K. pneumoniae*. The EO_F MBC for all bacteria was 2.5µl/ml except *K. pneumoniae* (MBC> 10 µl/ml). Fungal growth was inhibited up to 55-89% for EO_J and 61-74% for EO_F, 73-90% for EO_J and 74-90% for EO_F, 26-70% for Ext_J in the incorporation and fumigation tests respectively; and 26-64% for the Ext_F and 0-54% for the Ext_R in the well diffusion test on the 7th day. In conclusion, *S. satureioides* presents an important source of natural antioxidants and antimicrobials; hence should be of a considerable industrial and economic importance.

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P 13: A brief review on patents of medicines for treating HIV containing material from algae, lichens, fungi or plants, or derivatives

Carla Pires[a], Inês Cristovão da Silva[a]

[a]*Instituto Nacional da Propriedade Industrial, Lisboa, Portugal*
Email: servico.publico@inpi.pt

Plants are widely distributed in nature and traditionally used to treat diseases, including HIV. Every year thousands of people die with HIV. Nowadays, patents are classified with the International Patent Classification (IPC) of World Intellectual Property Organization (WIPO) [1]. The classification A61K36/00 refers to *Medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof, e.g. traditional herbal medicines.*

To review patents of medicinal preparations/substances for treating HIV under the classification IPC - A61K36/00.

The advanced search of Espacenet (free database) was applied to browse patents of medicines/substances for treating HIV under the classification IPC - A61K36/00 and comprising the word "HIV" in the title or abstract. The publication date was fixed between 2010 and 2017 to cover a recent period.

Only 20 results were retrieved: 11 medicines/pharmaceutical compositions with antiviral activity (e.g. comprising capsaicinoids, didanosine, emtricitabine, zalcitabine, olive leave extract, *dovyalis abyssinica* or a dried root of *clutia robusta*); 4 substance(s) or moisture(s) of substance(s) with anti-HIV activity (e.g. hemp powder, konjac flour, walnut powder, the chrysanthemum yunnan powder, the buckwheat flour, the corn flour, a Chinese cabbage extract, flower of sulphur or rubia cordofolia) and 5 methods for treating or diagnosing patients infected with the virus HIV (e.g. dialysis membrane). The identified patents presented a date of application between 2008 and 2017 and a priority date between 2006 and 2015.

Natural extracts may be useful in the treatment or diagnosis of HIV. The number of patented natural substances and/or extracts to produce medicines involved in the treatment or diagnosis of HIV is still limited. More studies on this topic are recommended.

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P 14: A brief review on patents of medicines for treating cancer containing material from algae, lichens, fungi or plants, or derivatives

Carla Pires[a], Inês Cristovão da Silva[a]

[a]*Instituto Nacional da Propriedade Industrial, Lisboa, Portugal*
Email: servico.publico@inpi.pt

Globally, cancer is the second cause of death: 8.8 million deaths in 2015. Since the beginning of times that derivates of plants are used in the treatment of diseases, such as cancer [1]. Free access to information about inventions is available in the database Espacenet. This unpaid database comprises information on more than 100 million patent documents, which may be searched through using Keywords and the IPC - International Patent Classification from 1836 to today [2].

To review patents of medicines/substances for treating cancer/neoplasia under the classification IPC A61K36/00 - *Medicinal preparations of undetermined constitution containing material from algae, lichens, fungi or plants, or derivatives thereof, e.g. traditional herbal medicines*.

Documents of patents were browsed in Espacenet, using a publication date between 2013 and 2017, the IPC - A61K36/00 and the Keywords "cancer" and "neoplasia" in the title.

In general, USA was the country with more applications. Only 47 results were retrieved: 14 (2013); 13 (2014); 11 (2015); 8 (2016) and 1 (2017). Among the phytosubstances/phytochemicals to treat or prevent cancer are phytoestrogens (e.g. genistein, daidzein, glycitein, biochantin), papaya fermentation product, extract from the fruit of Piper cubeba L, Forsythiae fructus, Menthae Herba, Gardeniae Fructus, Scutellariae Radix, Lophatheri Folium, Glycyrrhizae Radix, Rhei Rhizoma, etc.

Plants and their derivatives may be used in the treatment and diagnosis of cancer/neoplasia with potential safety and efficacy. Given the limited number of identified patents in Espacenet on the use plants or derivates to treat cancer/neoplasia more research on this topic are advisable.

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P 15: Selective cytotoxic activity of *Thymelaea hirsuta* (L.) Endl. extract in a panel of cancer cell lines

Víctor Jiménez-González[a], José Manuel Calderón-Montaño[a], Estefanía Burgos-Morón[a], Emilio Guillén-Mancina[a], Julio José Jiménez-Alonso[a], Miguel López-Lázaro[a]

[a]*Department of Pharmacology, School of Pharmacy, University of Seville, c/Profesor García González nº2 41012 Seville, Spain*
E-mail: vjimenez3@us.es

Nowadays cancer is a serious health problem worldwide. Last year over 18 million of new cancer cases were diagnosticated and 9 millions of deaths were due to cancer[1]. The high mortality rates in patients with metastatic disease are caused by the limited selectivity of the existing therapies. Therefore, it is essential to search for new selective anticancer drugs. Considering that some of the most useful anticancer drugs are derived from plants, we decided to start a random screening of Andalusian plants[2]. Andalusia is a region in southern Spain of high plant diversity and endemism[3]. We recollected plants from this area and elaborated extracts to evaluate their possible anticancer activity on A549 human lung cancer cells and HaCaT non-malignant cells.

At the moment we have studied the selective anticancer activity of 30 plants extracts. The most interesting plant tested was Thymelaea hirsuta (L.) Endl.This plant extract displayed a marked selectivity against human A549 lung cancer cells versus human HaCaT skin non-malignant cells.

Our next aim was to assess the selective cytotoxic activity of T. hirsuta (L.) Endl. extract in a panel of cancer cell lines from different origin (gastric cancer, hepatocarcinoma, pancreatic cancer, colorectal cancer, breast cancer, endometrial adenocarcinoma, ovarian cancer, glioblastoma, head and neck cancer, non-small lung cancer, melanoma, renal cancer, bladder cancer, and prostate cancer). Cells were treated during 96 hours with the plant extract, and cell viability was estimated by the Resazurin assay.

The highest selectivity was observed in Calu-1 cell line (squamous lung cancer). These cells were approximately 170-fold most sensitive to T. hirsuta (L.) Endl. extract than HaCaT non-malignant cells. It is also worth mentioning that this extract was 10-fold more cytotoxic against Sk-Br-3 (HER2-positive breast cancer), MDA-MB-231 (triple negative breast cancer), KATO III (gastric cancer), HepG2 (hepatocarcinoma), GAMG (glioblastoma), HNO97 (tongue cancer), MeWo (melanoma) and T24 (bladder cancer) than against HaCaT (Skin non-malignant cells). Nevertheless, this plant extract showed similar cytotoxic activity against Sk-OV-3 (ovarian cancer), CAPAN-1 (pancreatic cancer) and HT29 (colorectal cancer) than against HaCaT (non-malignant cells).

Our results suggest that T. hirsuta (L.) Endl. is a promising plant for the development of new anticancer therapy. Further studies are needed to understand and evaluate the anticancer potential of this plant).

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P 16: Extraction methods of bioactive compounds from *Hermetia illucens* larvae with cosmetic application

Cíntia Almeida[a], Daniel Murta[b,c,d], Rui Nunes[b], André Rolim Baby[e], Patrícia Rijo[a], Catarina Rosado[a]

[a]*Universidade Lusófona (CBIOS – Research Center for Biosciences & Health Technologies), Lisboa, Portugal;*
[b]*Ingredient Odyssey, Santarém, PORTUGAL;*
[c]*FMV-ULHT, Lisboa, PORTUGAL;*
[d]*CIISA-FMV-ULisboa, Lisboa, PORTUGAL*
[e] *Faculty of Pharmaceutical Sciences, Universidade de São Paulo, São Paulo, Brazil*
E-mail: catarina.rosado@ulusofona.pt

P 17: Cytotoxic activity of *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim extract in a panel of cancer cell lines

Víctor Jiménez-González[a], José Manuel Calderón-Montaño[a], Elena Ogalla[a], Estefanía Burgos-Morón[a], Miguel López-Lázaro[a], José Carlos García-Gómez[b], Carmen M. Claro-Cala[c]

[a]*Department of Pharmacology, School of Pharmacy, University of Seville, c/Profesor García González nº2 41012 Seville, Spain;*
[b]*Marine biology laboratory, Seville Aquarium R + D + I Biological Research Area, Department of Zoology, School of Biology, University of Seville, Av. Reina Mercedes 6, 41012 Sevilla, Spain;*
[c]*Department of Pharmacology, Pediatrics and Radiology, School of Medicine, University of Seville, Avda. Sánchez Pizjuan, s/n 41009- Sevilla*
E-mail: vjimenez3@us.es

Exotic species *Rugulopteryx okamurae* has expanded massively on the Strait of Gibraltar since the autumn of 2015. Only a year later, five thousand tone of material had to be removed from the beaches of Ceuta[1]. This invasion directly affects tourism and fishing, the main economic activities in this area. Declining beaches quality due to the accumulation and decomposition of seaweed on the beaches caused that tourism has fallen this year. In addition fishermen are not able to catch enough fish and spend most of the time cleaning the nets from seaweeds [2]. To find a solution to this ecological problem, it is needed to know more about this species, its interaction with the ecosystem and the biological properties of this algae to find possible exploitation. Brown algae are reported to contain secondary metabolites such as terpenoids and other compounds as chloroform, methanol and sulphuric acid, interesting for their biological effects as anti-inflammatory, anti-cancer and antibiotic activities[3]. Our objective was to elaborate extracts with solvents of different polarity and decided to assess the activity of the ethanol/ethyl acetate/water extract in a panel of cancer cell lines from different origin (gastric cancer, hepatocarcinoma, pancreatic cancer, colorectal cancer, breast cancer, endometrial adenocarcinoma, ovarian cancer, glioblastoma, head and neck cancer, non-small lung cancer, melanoma, renal cancer, bladder cancer, and prostate cancer). HaCaT non-malignant skin cells and MCR-5 normal lung cells were used as normal control cells. All cells were exposed during 96 hours to the algae extract, and cell viability was evaluated by the Resazurin assay.

The cytotoxic effect of *R. okamurae* extract was observed in all the cell lines in range doses from 10 to 100 µg/ml, affecting cellular viability. The most sensible cell line to the algae extract was the human breast carcinoma BT-474 cells with an IC50 value of 18,7 ± 2,2 µg/ml. On the other hand, the colorectal cancer cell line, HT29 was the most resistant with an IC50 value of 220,0 ± 131,0 µg/ml. The extract showed similar cytotoxicity against all the other cells evaluated, with IC50 values around 30 µg/ml. Although our results point out that *R. okamurae* extract showed similar cytotoxic activity for normal and cancer cell lines, we cannot rule out that isolated compounds from this alga may have potential anticancer activity. This cytotoxic activity has to be further analyzed to understand its nature and composition.

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P 18: Synergistic inhibition of the Hedgehog pathway by newly designed Smo and Gli antagonists bearing the isoflavone scaffold

Giulia Venditti[a], Simone Berardozzi[a,b], Flavia Bernardi[c], Paola Infante[b], Cinzia Ingallina[a], Sara Toscano[a], Elisa De Paolis[a,b], Romina Alfonsi[c], Miriam Caimano[c], Bruno Botta[a], Mattia Mori[b], Lucia Di Marcotullio[c,d], Francesca Ghirga[b]

[a]*Department of Chemistry and Technology of Drugs, Sapienza University,*
[b]*Center for Life Nano Science@Sapienza, Italian Institute of Technology,*
[c]*Department of Molecular Medicine, Sapienza University,*
[d]*Istituto Pasteur-Fondazione Cenci Bolognetti, Sapienza University of Rome,*
E-mail: giulia.venditti@uniroma1.it

Hedgehog (Hh) signaling has emerged in recent years as a druggable target for anticancer therapy. [1] We previously identified Glabrescione B (GlaB), an isoflavone naturally found in the seeds of *Derris glabrescens* (Leguminosae), as a novel small molecule that proved to interfere with Gli1/DNA interaction. [2] We provided the total synthesis of GlaB based on the deoxybenzoin route, allowing the structure activity relationship elucidation through the preparation of a small-size focused library of isoflavones bearing different substitutions at the ring B. [3] Target preference has been achieved by the introduction of specific bulky substitutions at *meta* position (targeting GLI1) or *para* position (targeting SMO) of the isoflavone's ring B. Interestingly, the combined administration of two different isoflavones behaving as SMO and GLI1 antagonists, respectively, in primary MB cells has shown synergistic Hh inhibition at doses that are around 20-fold lower than individual compound doses, thus leading the way to further and innovative combination therapy for the treatment of Hh-dipendent tumors.

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P 19: Adverse effects of natural products a brief pre-systematic review

Carla Pires[a], Ana Fernandes[a]

[a]*CBIOS - Universidade Lusófona's Research Center for Biosciences and Health Technologies, Campo Grande, Lisboa, Portugal*
E-mail: p5558@ulusofona.pt

Natural products (NPs) are frequently used to treat diseases, since ancient times. Bioactive secondary metabolites may be obtained from diverse sources, such as plants or animals, but in a limited number [1]. According European Medicine Agency (EMA) guidelines Adverse Drug Reactions (ADR) or Adverse Effects (AE) are considered synonyms and may be defined as "A noxious and unintended response to a medicinal product" [2]. To identify studies describing at least one ADR/AE (or with potential to promote ADR/AE) related to the use of NPs and to describe ADR(s)/AE(s) and NPs. A pre-systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria was carried out during August 2019. keywords: "natural products" and ["adverse drug reaction" or "adverse effect"]. Screened databases: *PubMed*, *SciELO* and *DOAJ*. Inclusion criteria: papers describing at least one ADR/AE associated with the use of NPs and published between 1 January 2017 and 14 August 2019. Exclusion criteria: Repeated studies, reviews and papers written in other languages than English, Portuguese, French or Spanish. For "natural products" and "adverse drug reaction": *PubMed* (n=5); 1 included: *H. verticillate* (e.g. of pharmacologic activity: anti-inflammatory) aqueous extract showed potent inhibition of CYP1A2; 4 excluded: 2 reviews and 2 other issues [3]; *SciELO* (n= 0) and *DOAJ* (n=1); 1 excluded: repeated. For "natural products" and "adverse effect": *PubMed* (n=12); 2 included: 1 about allergic skin reactions following topical application of German chamomile (e.g. of pharmacologic activity: healing action in cosmetics), with 1,6-dioxaspiro[4.4]non-3-en-2-one identified as a potential skin sensitizer and 1 about 1'-S-1′-acetoxychavicol (ACA) (e.g. of pharmacologic activity: reduction of tumors in nude mice) from *Alpinia conchigera*, which promoted: mild pneumonitis and interstitial inflammation in kidneys (repeated doses) and induction of mild lobular hepatitis (toxicity studies in rats) [4, 5]; 10 excluded: 3 not reporting AEs; 3 reviews; 4 other products; *SciELO* (n= 0) and *DOAJ* (n=2); 2 excluded: 1 review and 1 not reporting AEs. A limited number of studies was selected, which may indicate ADR/AE related to the use of NPs are sub-reported or that NPs are safer than synthetize products. Information on inhibitors of CYP1A2 is relevant, since they predictably increase the plasma concentrations of certain drugs (e.g. clozapine or theophylline) [3, 6]. Concerning that there are more than one hundred secondary metabolites from chamomile, the elimination of skin sensitizers may be desirable when formulating cosmetics [3, 7]. Finally, the use of ACA in cancer treatment may be associated with the development of ADR/AE [5]. This review contributes for assuring patients' safety, namely by increasing the knowledge on potential interactions and skin sensitizers or contributing for the awareness on potential ADR/AE of NPs.

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P 20: Exploration of *Malva parviflora* L. fruit mucilage as biological and pharmaceutical agent

Saiqa Ishtiaq[a], Ans Munir[a]

[a]*University College of Pharmacy, University of The Punjab, Lahore, Pakistan.*
E-mail: Saiqa.pharmacy@pu.edu.pk

Flora of Pakistan is not only important medicinally but also a good source of natural materials which find applications in pharmaceutical, cosmetics, textile, paints and paper industries. Among these natural materials, mucilages are most widely used in pharmaceuticals as actives and excipients. *Malva parviflora* L. is a locally occurring medicinal herb containing mucilage. So the present research work was aimed to explore pharmaceutical and biological potential of mucilage extracted from immature fruits of *M. parviflora*. Mucilage was extracted in aqueous medium followed by precipitation with alcohol. Physicochemical, functional and micromeritic properties of extracted mucilage were determined. Antioxidant activity was done by DPPH assay. Haemolytic, DNA damage protection and solar protection factor were studied by reported protocols. The yield of mucilage was 2.46±0.15%. Preliminary phytochemical analysis showed that it was free from impurities i.e. starch, alkaloids, tannins, glycosides, saponins, steroids and lipids. Nutritional value analysis showed that it consists of carbohydrates (20.46±0.42%), protein (2.2±1.12%) and aminoacid (22.20±0.8%). Extracted mucilage was water soluble having pH (5.52±0.05), moisture content (7.67±0.16%), ash content (13.50±0.46%), swelling index (40±4.45ml), water holding capacity(6.63±0.47g/g), oil binding capacity (3.93±0.31g/g). Micromeritic properties fall within acceptable ranges of good excipients. Functional properties including emulsion capacity, emulsion stability, foaming capacity and foaming stability increased with increase in concentrations. Rheological analysis showed the psuedoplastic behavior of this mucilage. FTIR analysis demonstrated the presence of hydroxyl, methyl, carboxyl groups and glycosidic bonds. SEM (scanning electron microscope) analysis showed the agglomeration of irregular particles. DSC (Differential scanning calorimeter) analysis suggested that mucilage has good thermal stability. The results of *in-vitro* biological assays showed that mucilage is safe for human erythrocytes and can protect the body against free radicals and UV radiations. It is concluded that extracted mucilage has potential to be used as natural excepient as well as therapeutic agent in pharmaceutical industry.

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P 21: Antimycobacterial, antiplasmodial and toxicological studies on oleanolic acid and its derivative from *Syzygium aromaticum* Linn (Myrtaceae).

V.O. Fadipe[a,b], A.R. Opoku[b] M. Singh[d], NI. Mongalo[e]

[a]*Department of Chemistry, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa.*
[b]*Federal Ministry of Science and Technology, Abuja, Nigeria*
[c]*Department of Biochemistry & Microbiology, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa.*
[d]*Discipline of Biochemistry, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa.*
[e]*College of Agriculture and Environmental Sciences (CAES) Laboratories, University of South Africa, Private Bag X6, Florida, Johannesburg, South Africa.*
E-mail: victor.fadipe@scienceandtech.gov.ng

Malaria and tuberculosis are amongst the most common causes of death and enormously threatens human life globally. In a quest to find new antimalarial and anti-mycobacterial drugs, oleanoleic acid was isolated from the flower buds of *Syzygium aromaticum*. The derivative, 3-O-Acetyl-Oleanolic acid (OAA), was synthesized from the isolated product and the biological activities of the two were compared. The antiplasmodial and antimycobacterial activity of oleanoleic acid and its derivative were investigated against *Plasmodium falciparum* (Chloroquine Sensitive Strain) NF54 and *Mycobacterium tuberculosis* H37Rv respectively. OAA exhibited IC_{50} of 4.3 µg/ml against *P. falciparum* while OA exhibited IC_{50} of 0.042 µg/ml against *M. tuberculosis*. In the cytotoxicity studies, using the MTT assay, both compounds exhibited LC_{50} of ›300 µg/ml against both human embryonic kidney (HEK293) and hepatocellular carcinoma cell lines (HepG2), suggesting that the compounds may not be toxic at low to moderate concentrations.

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P 22: In vitro antioxidant and photoprotective activities of the aerial parts of Algerian Apiaceae *Capnophyllum peregrinum* (L.) Lange

Mostefa Lefahal[a,b], **Salah Akkal[b]**

[a]Department of Pharmacy, Facukty of Medecin, University of Constantine3, Algeria;
[b]Department of Chemistry, Bioactive Molecules and Biological Analysis Unit, Valorization of Natural Resources, University of Mentouri Constantine1, Constantine, Algeria
E-mail: salah62dz@gmail.com

Capnophyllum peregrinum (L.) Lange (Apiaceae) is the unique taxon of *Capnophyllum* genus in Algerian flora. It has never been investigated in regards to its total phenolic and flavonoid contents and antioxidant and photoprotective activities. Thus our study was aimed to evaluate the antioxidant and photoprotective capacities of methanolic extract of the aerial parts of this species. The in vitro antioxidant activity was evaluated through three established in vitro methods: DPPH radical scavenging assay. total antioxidant capacity (TAC) and ABTS radical scavenging assay. The in vitro photoprotective effect against UV-B radiations was performed according to the parameter SPF (Sun Protection Factor) by using UV spectroscopic technic at 290-320 nm and Mansur equation. Estimation of total phenolic and flavonoid contents were also done. Our results revealed that the methanolic extract has a significant amount of phenolics and flavonoids (74.06±1.23 µg GAE/mg DW. 44.09± 2.13 µg QE/mg DW. respectively) and exhibited good antioxidant activity in DPPH and ABTS assays. IC_{50} values were (48.68±1.71 µg/ml. 63.62±0.66 µg/ml respectively). while in TAC assay the antioxidant effect of the extract was strong (130.91±4.40 µg AAE/mg). Furthermore the methanolic extract showed high photoprotective activity with the sun protection factor (SPF) value=35.21±0.18. The findings of this study revealed that the methanolic extract of *C. peregrinum* was found to have a high phenolic and flavonoid contents as well as antioxidant and photoprotective activities. Therefore. it appears to be used a sunscreen in pharmaceutical or cosmetic preparations and as a natural source of antioxidant.

P 23: *In vitro* gastrointestinal bioaccessibility as a model for exploring the pharmacokinetics properties of natural products

Paolo Governa[a], Marco Biagi[b], Fabrizio Manetti[a]

[a]*University of Siena, Department of Biotechnology, Chemistry and Pharmacy – Department of Excellence 2018-2022.*
[b]*University of Siena, Department of Physical Sciences, Earth and Environment.*
E-mail: paolo.governa@unisi.it

The use of natural products as pharmaceutical tools is widely developing [1]. Even though their increasing popularity, the pharmaceutical applications of herbal products are limited by the shortage of clinical trials and by the inadequate knowledge of their pharmacokinetics [2].

Today, a large number of experimental models is available for investigating the pharmacokinetic properties of a drug candidate [3,4]. Together with the development of more efficient analytical techniques (i.e. HPLC, MS, NMR), *in vitro* and *in silico* systems are emerging tools to support and guide the identification of metabolites with an extremely accurate level of detail [5]. Although being largely utilized for single molecules studies, pharmacokinetic models are barely applied to more complex mixtures of molecules, such as herbal products, due to the necessity of choosing a small number of chemical markers [2]. Indeed, most pharmacokinetic principles of synthetic single molecule drugs are frequently applied to herbal products, even if this has not been validated through experimental studies [6].

In this work we applied a simple method for the analysis of the gastrointestinal bioaccessibility of 17 natural compounds, belonging to different classes of polyphenols. We also applied this method to different herbal products containing mixtures of phenolic compounds in order to verify whether the bioaccessibility of the same compounds may differ in the presence of a complex mixture of other molecules.

This work represent the first step of a broader project aimed at setting up an experimental model which, by combining *in vitro* cell models to computational techniques, will be able to predict the ADME properties of complex mixture of molecules, focusing on natural products.

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P 24: Evaluation of *Cistus ladanifer* L. effect on gastrointestinal parasites in lambs

Inês Delgado[a *], **Sara Tudela Zúquete**[a,b *], David Soldado[c], Olinda Guerreiro [a,c], Eliana Jerónimo [b,c], Ludovina Neto Padre[b]

[a] *Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal*;
[b] *ICAAM, Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Apartado 94, 7006-554 Évora, Portugal*;
[c] *Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL), Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal*.
* Both authors equally contributed to this work

E-mail: sarazuquete@fmv.ulisboa.pt

Cistus ladanifer L. (Cistaceae) is a bountiful available shrub at the western Mediterranean region, including the south of France, Spain, Portugal and the north of Morocco. It is commonly observed in uncultivated fields. This perennial shrub contains low protein levels and high levels of phenolic compounds, such as condensed tannins. It has a low organic matter digestibility, which, enhanced by its anti-nutritional components results in a poor nutritional value. However, either by natural grazing or by encouraged consumption, the incorporation of *Cistus ladanifer* L. in small ruminants' diet may simultaneously act as an important feeding complement as it can transform this shrub into a high-level end-use product. With this objective, our group wanted to determine if its composition could have an influence on gastrointestinal parasites.

Gastrointestinal nematode and coccidia pose as a highly significant economic burden for small ruminant production systems. Tannins and phenolic compounds are associated with a decrease in gastrointestinal parasites, namely, nematodes. In this study, we evaluated the tannin compound effect of both 1.25% and 2.5% of two distinct supplies of condensed tannins from *C. ladanifer*: A. leaves and soft steams and B. condensed tannin extract. Thirty-six crossbred "Merino Branco" x Romane ram lambs of approximately 60 days of age were randomly assigned to individual pens. A total of 4 stool collections were sampled from each animal at days 0, 7, 17 and at slaughter (day 35), the end of experiment. Coprological techniques included nematode egg (Epg) and coccidian count by the concentration technique McMaster and all samples were also evaluated by direct microscopic observation by the Willis fluctuation method.

Data was analyzed using a generalized linear mixed model procedure that identified a significant interaction of treatment groups over time regarding the strongylid Epg count ($Chi2 = 85.9$, $p \leq 0.001$), through the Penalized Quasi-Likelihood parameter estimation method. Pairwise contrast tests, with the Holm adjustment method, showed that the A.1.25 ($Chi2 = 21.7$, $p = 0.0006$) and B.1.25 ($Chi2 = 21.4$, $p = 0.0006$) treatments resulted in significantly lower Epg counts of strongylids over time when compared to the control group.

P 25: Evaluating natural alternatives as hypothetical nutraceuticals against gastrointestinal parasites in lactating Goats

Sara Tudela Zúquete[a,b,c *], **Inês Delgado**[b *]; David Soldado[d], Olinda Guerreiro [a,d], Eliana Jerónimo [a,d], Ludovina Neto Padre[a]

[a]*ICAAM, Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Apartado 94, 7006-554 Évora, Portugal;* [b]*Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal*; [c]*Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, 376, 1749-024 Lisboa, Portugal*; [d]*Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL), Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal*

* Both authors equally contributed to this work

E-mail: sarazuquete@fmv.ulisboa.pt

Gastrointestinal parasite burden on goats translates into high production losses due to inadequate growth and poor milk production. These parasites are also responsible for higher mortality rates when facing high infestations levels or, more frequently, in kids. During the last decades, effective anti-helminthic compounds controlled such situations. However, the approach to such problem shifted and marked animal production systems from the eighties onwards. Drugs used for treatment of severe situations started to be administered as prophylaxis at frequent time intervals. Initially, such switch had an enormous success as it allowed an unforeseen growth and intensification of animal production at a relative low cost. Later, the onset of the first anti-helminthic resistance was bypassed by new molecule research and synthesis. Notwithstanding, presently, resistance to the most recent anti-helminthics, macrocyclic lactones and monepantel, are reported at distinct regions of the globe, even though some countries have not yet approved the latter, as the US.

Clinicians are now facing a new threat as we are on the verge to lose the molecules we use to treat. Furthermore, resistance also poses as a public health threat not only for the risks implied specially for soil-transmitted helminthes subjected to the pharmaceutical compounds excreted by animal feces, but also by the environmental contamination only recently being consciously addressed.

In order to surpass such threats, new natural alternatives must be further investigated. At the present work, we evaluated the effect of two distinct condensed tannin plant extracts (*Cistus ladanifer* L. – "Esteva" (Ci) and *Schinopsis quebracho-colorado Schlecht* – "Quebracho" (Sc)) on gastrointestinal parasites in lactating goats. Eighteen "Serpentina" goats were randomly assigned to one of three groups. Animals were individually housed. Individual stool collections were sampled at days 0, 14, 21, 28 and 35. Coprological analysis included concentration McMaster technique for egg count (Epg) and Willis fluctuation assay for microscopic observation.

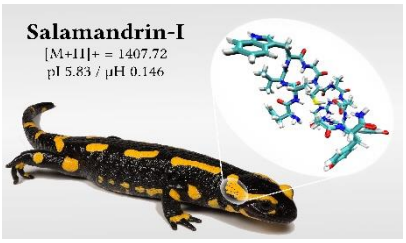
Data was analyzed using a generalized linear mixed model procedure that identified a significant interaction of treatment groups over time regarding the strongylid Epg count (Chi2 = 1355.6, $p \leq 0.001$), through the Gauss-Hermite quadrature parameter estimation method. Pairwise contrast tests, with the Holm adjustment method, showed that both Ci (Chi2 = 148.1, $p < 0.0001$) and Sc (Chi2 = 962.3, $p < 0.0001$) treatments resulted in significantly lower Epg counts of strongylids over time when compared to the control group. The Sc group also presented a significantly lower Epg counts of strongylids over time when compared to the Ci group (Chi2 = 1054.8, $p < 0.0001$).

P 26: Salamandrin-I: A novel antioxidant peptide identified in the skin secretion of the fire salamander (*Salamandra salamandra*)

Alexandra Plácido[a,b,c*], Joao Bueno[d,e], Eder Barbosa[e], Daniel C. Moreira[d], Filipe C. D. A. Lima[f], Augusto Batagin-Neto[g], Guilherme Brand[e], João Relvas[b,c], José Roberto S. A. Leite[c,d], Peter Eaton[a]

[a]LAQV/REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences of the University of Porto, Porto, Portugal. [b]Institute for Research and Innovation in Health (i3S) and Institute for Molecular and Cell Biology (IBMC) of the University of Porto, Porto, Portugal. [c]Bioprospectum, Lda, Porto, Portugal. [d]Center for Research in Applied Morphology and Immunology, NuPMIA, University of Brasilia, Brasilia, Brazil. [e]Institute of Chemistry, University of Brasilia, Brasilia, Brazil. [f]Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, Campus Matão, SP, Brazil. [g]Universidade Estadual Paulista (UNESP), Campus Experimental de Itapeva, SP, Brazil. [*]alexandra.nascimento@fc.up.pt

In addition to several physiological roles, such as water balance, respiration, ion transport and immune defense, the amphibian skin produces and secretes bioactive peptides in granular glands as a natural defense mechanism (1,2). This molecular arsenal protects these animals not only from microorganisms but also from predators and abiotic threats. For example, exposure to ultraviolet radiation and drastic changes in O_2 availability have been linked with altered reactive oxygen species (ROS) generation. Therefore, it could be predicted using a biorational approach that amphibian skin secretion could have a molecular mechanism to minimize the threat from these sources (3). In this work, we identified a novel bioactive peptide with antioxidant activity from the cutaneous secretion of the fire salamander (*Salamandra salamandra*); this is the first bioactive molecule with biotechnological application to be characterized from this species. Adult *S. salamandra* specimens (https://youtu.be/o6c01HLF3mM) were captured in the Peneda-Gerês National Park, Portugal (n°364/2018 CAPT/ICNF). The skin secretion was applied to an RP-HPLC C18 column coupled to a DAD detector. Isolated fractions were analyzed by MALDI TOF/MS for *de novo* peptide sequencing. Then, sequence was subjected to studies using molecular mechanics and semi-empiric methods and calculations were conducted with the aid of Gabedit (MD) and Gaussian 09 (DFT) computational packages. This led us to select one of the identified peptides, named Salamandrin-I (SS-I), $[M+H]^+$ = 1,407.72 Da, for solid synthesis and further analysis. SS-I showed strong free radical scavenging activity against DPPH and ABTS radicals, with values of 0.081 and 0.285 Trolox equivalents/mg. Peptides derived from natural sources are increasingly attracting interest and might also be used as leading templates for designing novel molecules for preventing neurodegeneration. This novel peptide stands as promising molecule for the design of therapeutic agents to treat ROS-related disorders.



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P 27: Isolation study of a bioactive diterpene from *Plectranthus ornatus* Codd.

Joana Tavares[a], Epole Ntungwe[a,b], Vera Isca[a,c], Eva María Domínguez-Martín[a,b], Patrícia Rijo[a,c]

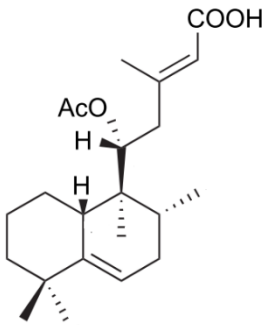
[a]CBIOS – Center for Research in Biosciences & Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisbon, Portugal
[b]Department of Biomedical Sciences, Faculty of Pharmacy, University of Alcalá, Ctra. A2, Km 33.600 – Campus Universitario, 28871 Alcalá de Henares, Spain
[c]Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto 1649-003 Lisbon, Portugal
E-mail: patricia.rijo@ulusofona.pt

Tuberculosis (TB) infects thousands of people every year and is a serious public health problem worldwide. The causative agent, *Mycobacterium tuberculosis*, is a bacterium that has elaborated survival mechanisms in the host.

The discovery of new antibiotics is essential for reducing TB deaths and natural products offer an excellent starting point for the discovery of these compounds due to their structural and functional diversity.

The *Plectranthus* genus belong to the *Lamiaceae* family, such as mint and sage, and exhibit a wide range of ethnobotanical uses. The *P.ornatus* species has diuretic, antipyretic, analgesic, antibiotic and anti-inflammatory properties and is used to relieve stomach and liver disorders.

Haliman's backbone diterpene (*11R*-acetoxy halima-5,13E-dien-15-oic* acid, **Hal**) was isolated for the first time from an acetone extract of *P.ornatus*. This compound is described as antimicrobial, namely antitubercular. Thus, in this work the large-scale compound **Hal** was isolated. Thus, acetonic ultrasound extraction was performed (extraction yield 7.082% (w/w)). Chromatographic isolation of 5.3 mg of pure **Hal**, identified by HPLC-DAD, was also performed by comparison with an authentic sample. In the future it will be possible the total isolation of the compound in this extract allowing new biological studies with potential for the development of new tuberculostatic drugs.



(**Hal,** *11R*-acetoxy halima-5,13E-dien-15-oic* acid)

P 28: Application of *Vitis vinera* L. as bioactive in food and cosmetic products

Paula Pereira[a,b], Maria Lídia Palma[a], Sara Gothe[c], Marta Rodrigues[c], Rita Gameiro[c],Patrícia Rijo[a,d], Joyce Costa[e] Pedro Fonte[a,f,g], Maria João Cebola[a], Gabriela Bernando-Gil[b], Ana Sofia Fernandes[a], Carla Palma[h], Marisa Nicolai[a]

[a]CBIOS-Research center for Biosciences & Health Teachnologies, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal. [b]Center for Natural Resources and Environment (CERENA), Instituto Superior Técnico (IST), Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal. [c]ULHT-Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal. [d]iMed.ULisboa- Research Institute for Medicines Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal. [e]Federal University of Vales do Jequitinhonha e Mucuri, Departament of Food and Technolgy, Science and Technology Institute, MG, Diamantina, Brazil. [f]LAQV, REQUIMTE, Department of Chemical Sciences—Applied Chemistry Lab, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal. [g]iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal. [h]IH – Instituto Hidrográfico, Rua das Trinas, 49, 1249-093 Lisboa, Portugal.
E-mail: marisa.nicolai@ulusofona.pt

A great quantity of residues, known as grape pomace, is produced in the wine industry. Different studies have shown that these residues are rich in bioactive compounds of great interest from a nutritional and cosmetic perspective. This work is focus on the characterization of grape pomace moisture, ash and metal contents, and antioxidant and cytotoxicity of the different extracts.

Two types of grape pomace were chosen, one used to produce white wine, the Arinto variety, and another used to produce red wine, the Touriga Nacional variety. Both samples come from the Portuguese region of Alentejo.

The grape pomace was dried, powdered and subsequently characterized for its moisture and ash content and metal composition Li, Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb in order to assess its contents and potential toxicity.

From the powder pomaces, twelve different extracts were obtained using several types of extraction methods such as ultrasound assisted, Soxhlet, and supercritical fluid extraction and different solvents such as water, acetone, ethanol, methanol and supercritical CO_2.

The antioxidant capacity of all the extracts was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. All the samples were prepared in 0.5% (v/v) DMSO (dimethyl sulfoxide) in water, with a final concentration of 0.5 $mg.mL^{-1}$. The results showed significant DPPH inhibition (I%). The lowest and highest values were obtained by Touriga Nacional ultrasonic aqueous and methanolic extracts, respectively.

For a preliminary safety assessment of the extracts as dermocosmetic ingredients, viability assay was performed in the human keratinocyte cell line HaCaT, using the crystal violet. All extracts, except Arinto obtained by Soxhlet extraction, led to cell viability a greater than 70%.

Due to the high complexity of these extracts, their profile was characterized by ATR-FTIR method. The spectra showed different extract composition depending on the solvent and the technique used to obtain the extract.

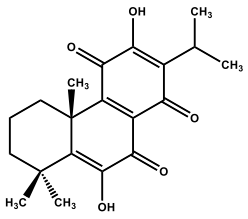
These results show that grape pomace have properties that can be applied as new food or cosmetic ingredients.

P 29: Isolation of Two Bioactive Diterpenes from *Plectranthus mutabilis* codd.

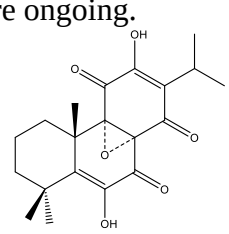
Epole Ntungwe[a,b],Vera Isca[a,c], Máté Vágvölgyi[d], Joana Tavares[a], Eva María Domínguez-Martín[a,b], Ana María Díaz-Lanza[b], Gabriella Spengler[e], Attila Hunyadi[d], Patrícia Rijo[a,c]

[a]CBIOS – Center for Research in Biosciences & Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisbon, Portugal
[b]Department of Biomedical Sciences, Faculty of Pharmacy, University of Alcalá, Ctra. A2, Km 33.600 – Campus Universitario, 28871 Alcalá de Henares, Spain
[c]Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto 1649-003 Lisbon, Portugal
[d]Institute of Pharmacognosy, University of Szeged, Eötvös str. 6. 6720 Szeged, Hungary
[e]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, H-6720 Szeged, Dóm tér 10
E-mail: patricia.rijo@ulusofona.pt

Medicinal plants continue to be an important source of compounds that could be used as drug leads for cancer treatment. It has been estimated that over 60% of all commercial anticancer agents originate from natural sources [1]. *Plectranthus* species have long been recognized for their cytotoxic and antitumor potential due to their bioactive secondary metabolites [2]. *P. mutabilis* Codd. chemical constituents have been reported to contain nepetoidins A and B [3]. We therefore aimed to study the composition and biological activity of this plant to reinforce the low phytochemical information. In this study, the air-dried *P. mutabilis* whole plant was extracted in acetone using the ultrasound assisted extraction method. Furthermore, a bio-guided fractionation was performed followed by an *Artemia salina* general toxicity assay, DPPH-antioxidant activity and antimicrobial activity testing [4]. The extract was subjected to column chromatography using silica or polyamide and gradient systems with increasing polarity to afford the metabolite coleon U quinone (**1**) and 8α,9α-epoxycoleon U quinone (**2**). The full structural characterization was performed mainly by 1D- and 2D-NMR and comparing spectral data with those in the literature. Compound **1** was tested against two Gram positive (Minimum Inhibitory Concentration - MIC values: *Staphylococcus aureus* = 1.56 µg/mL, *Enterococcus faecalis* = 25 µg/mL) and two Gram negative bacteria (*Klebsiella pneumoniae* >100 µg/mL, *Escherichia coli* >100 µg/mL). Additionally, the isolated diterpenoids showed moderate cytotoxicity in human cancer and normal cell lines (Colo 205, multidrug resistant overexpressing ABCB1 Colo 320 and MRC-5), showing slight selectivity to resistant cancer cells. Further phytochemical studies are ongoing.



Coleon U quinone (1)



8α,9α-Epoxycoleon U quinone (2)

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P 30: *Lamiaceae genus* extracts as a tool to obtain silver nanoparticles for biomedical applications

Ana Morais[a], Marisa Nicolai[a], Pedro Fonte [a,b,c], Patrícia Rijo[a,d]

[a] CBIOS - Center for Research in Biosciences & Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal. [b] LAQV, REQUIMTE, Department of Chemical Sciences - Applied Chemistry Lab, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal. [c] iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal. [d] iMed.ULisboa - Instituto de Investigação do Medicamento, Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal.
E-mail: almorais971@gmail.com

Metallic nanoparticles have been widely used in medicine as drug delivery support, diagnostic tools and for other therapeutic purposes [1,2]. Due to its antibacterial, antifungal, antioxidant and wound healing properties, silver is the most used metal in nanotechnology for biomedical applications. Yet, the production of silver nanoparticles (AgNPs) involves the use of health-hazardous reducing and stabilizing chemical compounds, so the development of more environmentally friendly approaches is needed[3].

Plant-extracts are the most commonly used *Eukarya*-domain reducing agents used on nanoparticle synthesis. Amongst plants, *Plectranthus genus* is an excellent supplier of phytochemical compounds relevant for bio-mediated nanoparticle synthesis [4,2]. Indeed, *Plectranthus amboinicus* leaf-extract has been used as an apt reducer of silver ions, as well as suited capping and stabilizer agents, giving rise to well-dispersed spherical AgNPs eligible for catalytic performance [5].

The aim of this study was to develop a method to obtain AgNPs using *Plectranthus ciliatus* and *Rosmarinus officinalis* aqueous extracts, for further use in different biomedical applications such as electroactive catalysts on biosensing electrodes. Physicochemical evaluation of prepared AgNPs was carried by dynamic light scattering (DLS), and electrophoretic light scattering (ELS) techniques. Preliminary results show *P.ciliatus*-mediated AgNPs zeta potential around -30 mV, whereas *R.officinalis*-mediated AgNPs have a lower absolute value of -26 mV. Accordingly [6], the higher zeta potential absolute value of *P. ciliatus*-based AgNPs is relevant for a more homogenous release of drug during targeted delivery. In addition, superior surface charge density fosters nanoparticle's lower aggregation avoiding their recognition by macrophages and subsequent elimination. Conversely, *R. officinallis*-mediated AgNPs afforded smaller sized particles as required for electro-catalytic performance during biosensing of health significant biomarkers. Namely, 198 nm and 409 nm. Further electroanalytic studies of prepared AgNPs are ongoing for its use in biomedical applications.

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P 31: Intracellular and extracellular mushroom polysaccharides with cytotoxic effects on human carcinoma cell lines

Diana Martinho[a] and **Amin Karmali**[a,b]

[a]*Chemical Engineering and Biotechnology Research Center and Department of Chemical Engineering of Instituto Superior de Engenharia de Lisboa, Rua Conselheiro Emídio Navarro Nº.1, 1950-072 Lisboa, Portugal*
[b]*Centre for the Research and Technology for Agro-environmental and Biological Sciences, Universidade de Trás-os- Montes e Alto Douro Quinta dos prados, Apartado 1013, 5001-801 Vila Real, Portugal*

Mushroom polysaccharides play an important role in nutraceutical and functional food because they act as biological active modifiers. The aim of the present work involved the production, purification and partial characterization of intracellular (IPS) and extracellular polysaccharides (EPS) from several basidiomycete strains. Such polysaccharides were used to investigate their effect on proliferation of human carcinoma cell lines. Mushroom polysaccharides were produced from several basidiomycete strains by submerged and solid state fermentations, assay of superoxide radical scavenging activity, purified by gel filtration chromatography, analysed by FTIR and their effect on human carcinoma cell line was investigated by MTT method. Mushroom polysaccharides have revealed scavenging activity in the range of 22 – 81 % for *Pleurotus ostreatus* (Po) and Pleurotus *eryngii*, respectively. FTIR analysis of polysaccharides showed absorption bands characteristics of these biological macromolecules. IPS inhibited cell proliferation of HeLa in the range of 16.8 – 27.01 % for Po and *Ganoderma applanatum (*Ga), respectively. EPS inhibited cell proliferation of HeLa, A459, A431 and OE21 in the ranges of 3.08 – 92.2 %, 13.8 – 97.4 %, 14.7 – 93. 8% and 25 – 94% for *Irpex lacteus* (Il) and Ga, *Ganoderma carnosum* and Ga, Il and Ga, *Lentinula edodes* and Ga, respectively. Purified preparations of polysaccharides confirmed the cytotoxic activity of these biomolecules. The present results strongly suggest growth inhibition of human carcinoma cell lines for mushroom polysaccharides and it will require a future research to understand its molecular mechanism of action.

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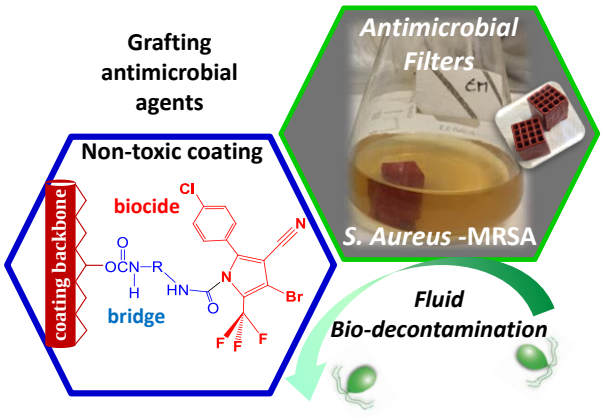
P 32: Antimicrobial fluid filtration on biocidal coated monoliths

Elisabete R. Silva[a], Olga Ferreira[a], Patrícia Rijo[b], João Gomes[c]

[a]*Biosystems & Integrative Institute, FCUL-UL, Campo Grande, Lisboa, 1749-016, Portugal* [b]*CBIOS-Center for Research in Biosciences&Health Technologies, ULHT, Campo Grande 376, Lisboa, 1749-024, Portugal* [c]*Área Departamental de Eng. Química, ISEL-IPL, Rua Conselheiro Emídio Navarro 1, Lisboa, 1959-007, Portugal*
E-mail: ersilva@fc.ul.pt

The microbial attack is one of the biggest human concerns, responsible for serious human health infections or even cause of death. The attachment and growth of this invisible threat give rise to the biofouling burden, which has been responsible for premature substrates deterioration and subsequent retrofitting penalties in a wide range of man-made devices applied in several industrial sectors. It became of particular alarm on fluid transport systems, such as air conditioning or water supply/purification. Conventionally, microorganisms' removal in those systems is provided by filtration treatments, mostly acting through the releasing of antifouling agents. Unfortunately, soon those treatments evidenced new challenges allied to their limited efficacy and intrinsic ecotoxicity into the environment.



This work aims to embrace a new generation of non-toxic antimicrobial strategies, by applying a new developed non-biocide release alternative [1], to prevent biofouling on filters surfaces. It comprised the functionalization of bioactive agents, with an isocyanate function, in order to originate its isocyanate reactive agents' derivatives with grafting ability in adequate polymeric matrices, hence providing an antifouling action by contact, and minimizing the toxic side-effects allied to the conventional releasing strategies. This strategy was particularly used to generate isocyanate Econea® biocide derivative and bioactive polyurethane and polydimethylsiloxane (PDMS) based coatings with grafted Econea, further used to coat ceramic monolithic filters. The antimicrobial activity assessment of coated filters against pathogens, namely Multi-resistant *Staphylococcus aureus* (MRSA), showed auspicious growth inhibition effects together with a bacteriostatic behavior. Complete growth inhibition was achieved on biocidal-PDMS based coated filters. This contact bioactive strategy, with tailoring ability to generate new functional agents can provide a low environmental impact and promising long-lasting antifouling effects on several industrial surfaces and become an innovative key contribution for the current bio-decontamination challenge on fluid management systems.

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P 33: Valorization of Oleuropein: Acid-promoted methanolysis

L. A. S. Cavaca[a], C. Rodrigues[a], S. P. Simeonov[a,b], R. F. A. Gomes[a], J. A. S. Coelho[a], G. P. Romanelli[c], A. G. Sathicq[c], J. J. Martínez[d], C. A. M. Afonso[a]

[a]*Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal*
[b]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences. Acad. G. Bonchev str., bl. 9, 1113, Sofia, Bulgaria*
[c]*Centro de Investigación y Desarrollo en Ciencias Aplicadas "Dr. J.J. Ronco" (CINDECA), Departamento de Química, Facultad de CienciasExactas, Universidad Nacional de la Plata Argentina Calles 47 Nº 257, B1900 AJK, La Plata, Argentina*
[d]*Escuela de Ciencias Químicas, Facultad de Ciencias, Universidad Pedagógica y Tecnológica de Colombia UPTC; Avenida Central del Norte, Tunja, Boyacá, Colombia*
E-mail: l.cavaca@ff.ulisboa.pt

Oleuropein is one of the major secoiridoids found in the olive leaf (0.5-2% (w/w) on dry basis).[1] Oleuropein structure can be divided in three subunits – glucoside, monoterpene and hydroxytyrosol (red, black and blue, respectively, Figure 1).[2] The monoterpene unit is a highly functionalized moiety that includes two esters, one alkene, one enol ether, one acetal and a stable chiral center at C-4.[3] In this context, we became interested in the valorization of oleuropein towards the synthesis of diverse and synthetically rich building blocks. The acid-promoted methanolysis of oleuropein was studied using a variety of homogeneous and heterogeneous acid catalysts (Figure 1). [4]

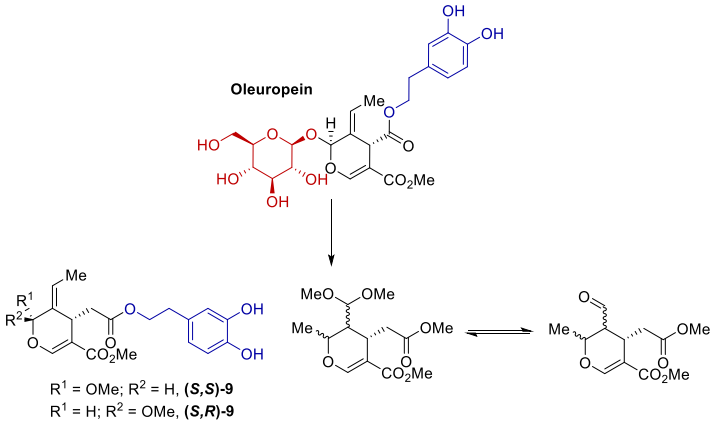

Figure 1. Tunable acid-promoted methanolysis of oleuropein.

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P 34: Bioactivity guided isolation anticancer principle from *Celosia trigyna* Linn (Amaranthaceae): The ridules of vegetable to drug

Bunyamin Adesina Ayinde[a], **Oluwasegun Adedokun[b]**, Luciano Barboni[c], Filippo Maggi[d], Patricia Rijo[e]

[a]Department of Pharmacognosy, University of Benin, Nigeria; [b]Department of Pharmacognosy, Igbinedion University, Nigeria; [c]School of Science and Technology, Chemistry Division, University of Camerino, Italy; [d]School of Pharmacy and health products, University of Camerino, Italy; [e]CBIOS Research Center for Biosciences and Health Technologies, Lusófona University, Portugal
E-mail: adedokunsegun05@yahoo.com

Celosia triygna is a well-known vegetable used in preparation of many indigenous soups in south-western Nigeria. Apart from its delicious and nutritive values, *C. triygna* usage in treatment of tumour related ailments is also known among locals. However, not may studies have been done to establish the anticancer ethnomedical claim as well as isolate the bioactive principle(s). The aim of this study was to evaluate the anticancer property of *C. triygna* using bench top models and cell line experiments, evaluate the antioxidant property as well as isolate the cytotoxic principle(s) by using bioactivity guided model. Preliminary phytochemical screening of crude extract was done using standard method. Total phenolics and flavonoids were done using Folin Ciocalteau's and aluminium chloride methods respectively for the crude extract (CE) and aqueous fraction (CAF). Moreover, bench top cytotoxicity was carried out using *Saccharomyce cerevisiae* and *Raniceps ranninus* models as well as cytotoxicity studies against breast (MCF-7), lung (H460) and colon cancer (HCT116) cell lines; radical scavenging potential against DPPH was likewise performed. Bioactivity guided isolation of cytotoxic principle was done using combination of different chromatographic techniques. Presence of steroids, terpenoids, glycosides, reducing sugars, flavonoids and phenolics were present in CE. Phenolic and flavonoid contents were estimated to be 87.52± 2.45 and 81.35± 3.70 as well as 52.81± 1.97 and 39.11± 2.86 respectively for CE and CAF. A concentration non-dependent cytotoxicity against *S. cerevisiae* was observed in CTA, with lowest inhibition of organism growth at 31.2 µg/ml (26.40±1.92%) and highest activity at 250 µg/ml (56.00±2.12%). Concentration dependent inhibition was observed in CTA with 84.80± 1.97 % at 250 µg/ml, which is significantly different from values observed in DMSO (negative control) 33.84± 1.03% at $P<0.01$. 100% motility of *R. ranninus* (Tadpoles) were recorded in all concentrations (20 - 40 µg/ml) in CE and CAF significantly different $P<0.05$ from values obtained for vehicle (distilled water). Concentration dependent DPPH radical scavenging potential was likewise noted both in CE and CTA at 20 – 100 µg/ml. lowest inhibition was observed at 20 g/ml (41.35% and 32.31%) while highest noted at 100 µg/ml (63.26% and 41.73%) for CE and CTA respectively. Cytotoxic effects against cancer cell lines are on-going. Moreover, CTA was further subjected to bio-guided isolation resulting to bioactive bulked column fractions BVLC1 and CTCF1. Compound CTCF1A (Spinasterol) colourless crystals with melting point 168 – 1690C. Spinasterol exhibited motility of tadpoles at all concentrations examined (20 – 100 µg/ml). Cytotoxic effect against cancer cell lines is on-going. Findings from this research experimentally justify the ethnomedicinal claim of usage of *C. triygna* in treatment of cancer in south-western Nigeria. Spinasterol was likewise reported from *C. triygna* for the first time. Likewise, reduced cancer rate among south-western population of Nigeria might be attributed to constant consumption of vegetables for local soup, most especially *C. triygna* and melting point of Spinasterol above 1000 C indicate the bioavailability of the active principle despite subjecting the plant to cooking temperature.

P 35: Antiproliferative, antioxydant activties and chemical profile of chloroformic extract of *Euphorbia cornuta* PERS.

Amar Zellagui[a], Agena Ghout[a] Noureddine Gherraf[b], Ibrahim Demirtas[c], YagliogluAyse Sahin[c], Hallis youcef [d] and Mesbah Lahouel[e]

[a]*Laboratory of Biomolecules and Plant Breeding, Life Science and Nature Department, Faculty of Exact Science and Life Science and Nature, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria.*
[b]*Department of Chemistry, Faculty of Exact Science and Life Science and Nature, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria.*
[c]*Laboratory of Plant Research, Department of Chemistry, Faculty of Science, Gaziosmanpaşa University, Uluyazi Campus, Cankiri Karatekin University, Cankiri, Turkey* [3]*Department of Chemistry*
[d]*Center for Arid Areas (CRSTRA), Biophysical Station, Touggourt, Algeria*
[e]*Laboratory of Molecular Toxicology, University of Jijel 18000 Algeria*
*E-mail: zellaguiuniv@yahoo.com

The present study was aimed mainly to identify the bioactive compounds in chloroformic extract of *Euphorbia cornuta* Pers. by using HPLC-TOF-MS technique. The inhibitory effects of extract on two different cancer cell lines and various antioxidant assays including scavenging effect against DPPH, reducing power and inhibition of lipid peroxidation, were studied and were compared to standards such as quercetin, gallic acid and ascorbic acid. The contents of the chloroformic extract on phenolics and flavonoids (144.77±14.85 mg GAE/g and 14.52±1.73mg QE/g respectively), showed a high activity against DPPH and by reducing power and a high lipid peroxidation inhibition activity. On the other hand the chloroform extract showed the highest antiproliferative properties than 5-FU against C6 cells at 250 µg/mL.

P 36: Chemical Composition and Antibacterial Activity of *Launaea nudicaulis*

Hamza Kabouche

Life Science and Nature Department, Faculty of Exact Sciences and Life and Nature Sciences, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria.
E-mail: allamagpc@gmail.com

The analyses of *Launaea nudicaulis* essential oils by GC/MS allowed the identification of 94.1% of the total crude oil. The major components are: β-caryophyllene: 7.9%, (E)-β-farnesene: 7.6%, β-selinene: 9.9%, Spathulenol: 4.9%, α-cadinol: 5.9%, haxadecanoic acid: 17.3%. The scavenging activity using the DPPH essay showed an IC_{50} = 1.94 mg/mL.

Moreover, the extract revealed in vitro antibacterial activity on the some bacterial strains namely *E. coli*, *Staphylococcus aureus*, *Proteus sp*, *Klebsiella sp and Candida albicans* on nutrient agar plates using disc diffusion method confirmed by the inhibition zone diameter ranging from 11 to 15 mm depending on the microorganism being tested and concentration.

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P 37: Phenolic Contents and Anti-Proliferative Activity of EtOAc extract of *Rosmarinus officinalis* Collected from Three Geographic Origins in Algeria

Noureddine Gherraf[a], Ouroud Fellah[a], Samir Hameurlaine[a], Amar Zellagui[b],

[a]*Laboratory of Natural Resources and Management of Sensitive Environments, university, Oum ElBouaghi, Algeria*
[b]*Laboratory of Biomolecules and Plant Breeding, Life Science and Nature Department, Faculty of Exact Science and Life Science and Nature, University of Oum El Bouaghi, Algeria,*
E-mail: ngherraf@yahoo.com

The aerial parts of *Rosmarinus Officinalis* L. collected from three different environments in Algeria were extracted using water. The crude extracts were exposed to total phenolics and flavonoids assessment in addition to anti-proliferative valuation against rat brain tumor (C6) and human cervix carcinoma (HeLa) using BrdU (bromo-deoxyuridine) ELISA (Enzyme-linked immune-sorbent assay) and xCELLigence assay. The total phenolics yield was found between 16.14 and 39.32 mg GAE (Gallic acid equivalent)/g of extract and a flavonoids yield ranging between 16.51 and 20.35 mg QE (quercetin equivalent)/g. The various phenolics were identified using HPLC-TOF/MS to highlight hesperidin and rosmarinic Acid as major components. Furthermore, the extracts displayed diverse levels of antitumor effectiveness against C6 and HeLa cell lines depending upon the concentration.

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P 38: In vitro antioxidant and anti-inflammatory activities of Algerian Hypericaceae *Hypericum afrum* Lam. aerial parts extracts and Phenolics

Farida Larit[a,b], Samira Benyahia[c], Francisco León[a], Stephen J. Cutler[a]

[a]*Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677-1848, USA.*
[b]*Département de Chimie, Faculté des Sciences Exactes, Université des Frères Mentouri, Constantine, Route d'Aine El Bey 25000, Constantine, Algeria.*
[c]*Laboratoire de Synthèse Organique, Modélisation et Optimisation des Procèdes (LOMOP), Université Badji Mokhtar, Faculté des Sciences, Département de Chimie, 23000 Annaba, Algeria*
E-mail: laritfarida@umc.edu.dz

The aim of this research was to determine the phytochemical composition, the *in vitro* antioxidant and anti-inflammatory activities of the Algerian species *Hypericum afrum* aerial parts extracts and its isolated phenolics. The structures were determined using 1D- and 2D-NMR spectroscopy and HREIMS as well as comparison with literature data. The antioxidant properties of *H. afrum* extracts and isolated pure compounds were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DDPH) and cellular antioxidant (CAA) assays. In addition, the extracts and isolated compounds were evaluated toward multiple targets related to inflammation and metabolic disorder such as NF-κB and iNOS.

The chloroform, ethyl acetate and *n*-butanol extracts of *H. afrum* showed all high antioxidant activity in term of radical scavenging activity, using DPPH assay. In addition, these extracts were shown to decrease cellular oxidative stress by inhibiting ROS generation with inhibition ranged between 63 and 75% at 1000 µg/mL. Furthermore, Bioassay-guided fractionation of the aerial parts of *H. afrum* yielded seven known flavonoids, quercetin (1), myricetin (2), Myricitrin (3), Hyperoside (4), myricetin-3-*O*-β-D-glucopyranoside (5), myricetin-3'-*O*-β-D-glucopyranoside (6), Biapigenin (7). The isolated phenolics showed all good antioxidant activity. Compounds (1) and (2) from ethyl acetate extract of *H. afrum* aerial parts showed the highest effect against oxidative stress with inhibition values of 93 and 78%, respectively.

Regarding the results of the evaluation of anti-inflammatory activity, *H. afrum* extracts did not show any inhibition of iNOS or NF-κB and therefore did not affect cellular nitric oxide levels in lipopolysaccharide (LPS)-treated macrophages. The isolated pure compounds did not show any inhibition of iNOS or NF-κB except for compounds (1) and (7) which showed both moderate inhibition of iNOS with IC_{50} values of 12 and 22 µg/mL, respectively.

The studied plant extracts and its isolated phenolics did not show good anti-inflammatory activity. However, *H. afrum* aerial parts extracts and pure isolated phenolics reveal high antioxidant activity that requires further phytochemical and pharmacological investigations.

P 39: Phenolic compounds from an algerian endemic species of *Hypochaeris laevigata* var. *hipponensis* and investigation of antioxidant activities

Nabila SOUILAH[a,b], Zain ULLAH[c], Hamdi BENDIF[d], Mehmet ÖZTÜRK[c], Salah AKKAL[a] and Kamel MEDJROUBI[a]

[a]*Department of Chemistry, Faculty of Exact Sciences, University of Constantine 1, Constantine, Algeria.*
[b]*Department of Natural and Life Sciences, Faculty of Science, University of Skikda, Algeria.*
[c]*Department of Chemistry, Faculty of Sciences, University of Muğla Sitki Koçman, Muğla, Turkey.*
[d]*Department of Natural and Life Sciences, Faculty of Science, Mohamed Boudiaf University, M'sila, Algeria.*
E-mail: souilah_n_phyto@hotmail.fr

Hypochaeris laevigata var. *hipponensis* (Asteraceae) is an endemic plant from Algeria. In the current study, We analyzed for the first time the chemical composition especially phenolic constituents of dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH) fractions of the aerial parts of *Hypochaeris laevigata* var. *hipponensis* by liquid chromatography-mass spectrometry (LC-MS/MS). The number of phenolic compounds detected in DCM, EA and BuOH fractions were found to be 9, 20 and 15; respectively. More specifically, 12 phenolic acids were detected among them, quinic acid, chlorogenic acid and caffeic acid were the most abundant ones. While only 7 flavonoids were detected among them, rutin, apigetrin and isoquercitrin were the majors. We also determined the total phenolic and flavonoid contents, and BuOH fraction showed the highest values, followed by EA and DCM fractions. Furthermore, the antioxidant action was dictated by six methods and the tested plant fractions demonstrated a noteworthy antioxidant action.

P 40: Evaluation of antifungal activity of commercial essential oils (CAPTAIN Company) against different species of *Candida*

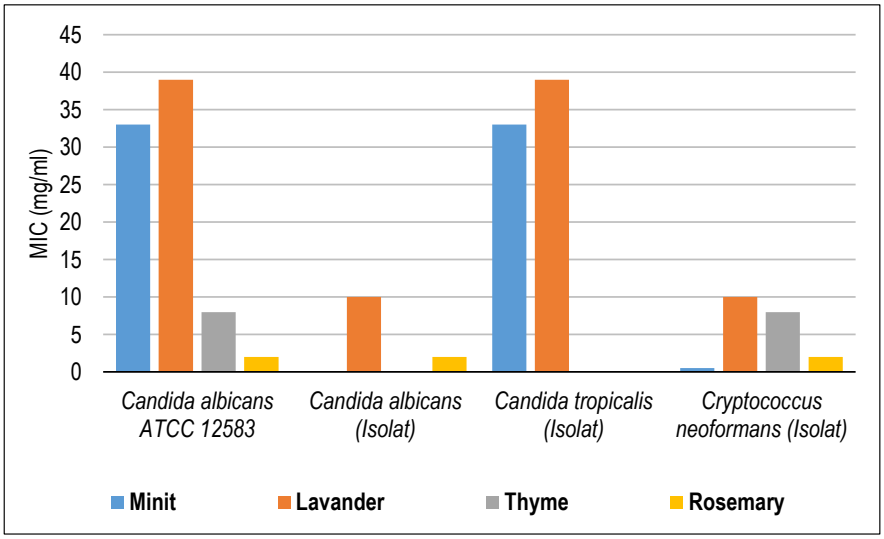
Ibrahim SIFI[a], Chaima ABOUTALEB[b], Amel BOUZOUADA[b] and Fatna FERMELI[c]

[a]Laboratory of Fundamental Science, University of Amar Telidji –Laghouat 03000, Algeria
[b]Laboratory of Microbiology, University of Amar Telidji –Laghouat 03000, Algeria
[c]Regional Veterinary Laboratory, Laghouat 03000, Algeria
E-mail: sifi_ibrahim@yahoo.fr or *i.sifi@lagh-univ.dz*

Essential oils have important antifungal activities, this led us to carry out the study of the antifungal activity of a commercial essential oils (*Rosmarinus officinalis*, *Thymus vulgaris*, *Mentha piperita*, *Lavandula officinalis*) of the "CAPTAIN" Egyptian company, against deferent strains (*Candida albicans* (ATCC 12583), *Candida albicans* (isolate), *Candida tropicalis* (isolate), *Cryptococcus neoformans* (isolate)).

Our results show that the MIC varies between 0.5 and 10 mg / ml, for *Candida albicans* (Isolate), and from 514 to 10 mg / ml, for *Cryptococcus neoformans*. These strains are the most sensitive from essential oils tested. On the other hand, the strain *Candida albicans* (ATCC 12583) presented low sensitivity with MIC values ranging from 2 to 39 mg / ml; it is the most resistant. The strain *Candida tropicalis* is presented with a high sensitivity to essential oils of thyme and rosemary, with a MIC of 2µg / ml and 32µg / ml respectively. However has been revealed a resistance against the essential oils of mint and lavender.

The synergy between the essential oil of mint and the essential oil of lavender can be evaluated by calculating a factor called "*fractional inhibitory concentration index*" (FICI), according to Naghmouchi et al. (2013), the synergy is considered negative because the FICI index is greater than 1.



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P 41: Supercritical CO_2 extraction as a tool to isolate potential medicinal sesquiterpenes from chicory taproots by-products

J. Baixinho[a], M. Matos[a], A. Alexandre[a], N. Fernández[a], C. Nunes dos Santos[a], A. A. Matias[a]

[a] *iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal*
E-mail: jbaixinho@ibet.pt

Some varieties of chicory (*Cichorium intybus L.*) are used as leafy vegetables (witloof, radicchio). However, the most common chicory species is an under-utilized root variety which taproots are currently used for the commercial production of inulin. This compound is widely used in food products as dietary fiber and substitute of sugar. The current world market of chicory inulin is 120 - 150 kton/year extracted from about one million tons of chicory taproots, which generates a substantial amount of residue. After the inulin extraction with hot water, the remaining parts, where sesquiterpene rich fractions can be found, are currently treated as waste. Many of those sesquiterpenes have potential as antimicrobial, anti-inflammatory and anti-parasitic compounds.

This work is part of a major European project which one of the main goals is to develop an exploitation strategy to convert the current chicory waste into valuable products for use in human health-promoting applications. Within this context, green methodologies are being explored for the extraction and fractionation of sesquiterpenes and further exploitation of their use as valuable health-promoting products.

Taproots of the *Cichorium intybus L.* were extracted by supercritical CO_2 to recover sesquiterpene rich fractions. The response surface methodology was used to model the extraction of sesquiterpene and optimize the effect of different process parameters on the extraction performance (extraction yield and chemical composition). These extractions were carried out following two different central composite face-centered design as a function of: pressure (10 - 55 MPa), temperature (25 - 60°C), flow rate (10 - 30 g/min) and co-solvent percentage (EtOH 10 - 40 %). The chemical composition of obtained extracts were analyzed by HPLC-UV[1]. For comparison, conventional S-L extractions using ethyl acetate were performed. The extraction with supercritical CO_2 resulted to be more selective for the sesquiterpene extraction than the conventional method[2].

Cytotoxicity screening of the sesquiterpene-rich extracts was performed in human intestinal epithelial cells (Caco-2), mimicking the intestinal mucosa, in order to evaluate further for health applications. For a concentration of 1 mg of dry extract/mL none of the chicory extracts revealed a cytotoxic effect on the tested cell line, nevertheless the sesquiterpene-rich extract obtained by supercritical fluid extraction revealed the highest value of EC_{50}, therefore being less cytotoxic than the conventional ethyl acetate extract.

Acknowledgements: This work was conducted under the project "CHIC" (H2020-NMBP-BIO-2017). This project has received funding from the EU Horizon 2020 research & innovation programme under grant agreement N. 760891.

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P 42: Antioxidant, Antimicrobial and Antiproliferative Activities of Medicinal plant *Cytisus villosus pourr*.

Amel Bouziane, Boulanouar Bakchiche

Laboratory of Process Engineering, BP 37G Ghardaia Road, 03000, University Laghouat, Algeria.

E-mail: a.bouziane@lagh-univ.dz; b.bakchiche@lagh-univ.dz

In order to enhance the value of Algerian flora, we are interested in a species of the Fabaceae family (*Cytisus villous*). It's frequently grows in Algeria, France, Italy, Spain, Portugal, and Tunisia. In Algeria, it is common in the region of the Tell Algéro-Constantinois [1].

This work evaluated the antioxidant and biological activities of Cytisus villous pour extract.

Both extracts exhibited good ativities against DPPH radical (EC50 µg/mL of 59± 2 and 31± 2 for aqueous and ethyl acetate extracts, respectively). However, the ethyl acetate extract demonstrated more potent quenching activities than the aqueous extract. The antimicrobial activities were assessed on selected Gram-positive (Staphylococcus epidermidis) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria, as well as on pathogenic fungus Candida glabrata. The extracts possesses powerful antimicrobial properties against gram-positive bacteria (IC50 of 186 ± 9 µg/ml and IC50 = 92 ± 3 µg/ml for aqueous extract and ethyl acetate extracts, respectively).

C.villosus extracts were tested for their antiproliferative potential on three human cancer cell lines representing breast and colon cancers. Although both extracts demonstrated sufficient growth inhibition of the three different cell lines, the ethyl acetate extract exhibited higher activity (**LD_{50}** values of **1.57 ± 0.06** mg/ml, **2.2 ± 0.1** mg/ml and **3.2 ± 0.2**mg/ml for **T47D**,**MCF-7**, and **HCT-116** cell lines) [2].

The results indicate that extracts obtained from the aerial parts of *C.villosus* revealed to have promising bioactivities and could be applied as functional agents in the food, pharmaceutical, and cosmetic.

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P 43: Novel alginate-chitosan aerogel fibres for potential wound healing applications

M. P. Batista[a], V.S.S. Gonçalves[a], F. B. Gaspar[a], P. Gurikov[b], A. A. Matias[a]

[a] *iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal;*
[b] *Institute of Thermal Separation Processes, Eißendorferstr. 38, 21073 Hamburg, Germany;*

E-mail: mbatista@ibtet.pt.

Aerogels are very interesting materials with high porosity whose wound healing applications are arousing great interest. In particular, aerogels produced from marine polymers are of particular interest due to their attractive properties such as the antimicrobial activity of chitosan or the capacity to provide a moist environment of alginate. The aim of this work was to evaluate the potential for wound healing applications of alginate-chitosan aerogels in the form of fibres and the influence of chitosan's content on the fibre characteristics. The aerogel fibres were prepared with different mass ratios of alginate-chitosan: 99:1, 19:1 and 9:1 (w/w). To produce them, a hydrogel of both polymers was made by the emulsion-gelation method. Through solvent exchange an alcogel was obtained, removing the water from the hydrogel by ethanol, which was then dried with supercritical CO_2, leaving behind a solid structure that greatly resembles original wet gel. Once the fibres were produced, the characterization of its solid state, biocompatibility, cell migration stimulation and antimicrobial activity were carried out. To characterize the solid state, determination of the fibre's chitosan content was first performed. Then, the morphology and its textural properties were also analysed. Fibres biocompatibility and stimulation of cell migration were evaluated by two *in vitro* methods, the direct contact method described in ISO 10993-5 and the scratch assay, respectively, using in both methods the mouse fibroblast NCTC clone 929 cell line. The antimicrobial activity was evaluated against *Staphylococcus aureus* and *Klebsiella pneumoniae* by two standard methods (dynamic and static) described in ASTM E 2149-01 and in ISO 20743:2013, respectively. In addition, in order to compare with a consumer product already on the market, cell and antibacterial assays were also performed for a dry calcium-sodium alginate wound dressing. The obtained results suggest that these alginate-chitosan aerogel fibres are good candidates for wound healing applications.

P 44: Supercritical CO_2 extraction as a green approach for the valorization of microalgae

S. García-Poza[a], A. Alexandre[a,b], A. Paiva[c], N. Fernández[a], A.A. Matias[a]

[a] *iBET, Instituto de Biología Experimental y Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal*
[b]*ITQB NOVA, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*
[c]*Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Departamento de Química, REQUIMTE/CQFB, Caparica, Portugal*
E-mail: sara.garcia@ibet.pt

Microalgae are recognized as an important renewable source of bioactive compounds including proteins, valuable pigments, vitamins and lipids with a high proportion of polyunsaturated fatty acids (PUFAs) which have been shown to be effective in preventing or treating several diseases [1].

Traditional methods for the extraction of lipids and carotenoids from microalgae frequently require more than one extraction step with organic solvents, which are of limited usage in the processing of food ingredients or additives. Lipophilic compound extraction from microalgae using supercritical carbon dioxide (SC-CO_2) is considered relevant as an industrial process. SC-CO_2 is safer than organic solvents, is a non-flammable solvent with a tunable selectivity, usually shorter extraction times are required with this technology and high-quality final products are obtained without any trace of toxic solvent [2]. SC-CO_2 extraction technology can be considered as a feasible alternative to traditional solid-liquid extraction methods to obtain potential natural bioactive compounds, which could eventually be used in nutraceutical formulations and functional foods.

Here we present an alternative to conventional oil extraction from microalgae by SC-CO_2 extraction. For this purpose four microalgae (*Tetraselmis striata*, *Phaeodactylum tricornutum*, *Chlorella vulgaris* and *Nannochloropsis oceanica*) were selected for the study. They are well known for their high content on lipid compounds especially PUFAs (about 20% of the total lipids are fatty acids) [1].

Freeze-dried samples of the above mentioned microalgae were tested under different operating conditions: pressures of 300 and 550 bar, dynamic extraction time of 2, 4 and 6 hours, and 5, 10 and 20 g/min of CO_2 flow rate. Temperature was fixed at 50ºC. The best operating conditions within the tested range to obtained highest extraction yield were achieved at 300 bar, 4 hours of dynamic time and 5 g/min of CO_2 flow rate. Among the microalgae species tested, *Tetraselmis striata* showed the highest extraction yield of 16.4 mg/g $_{\text{dry biomass}}$.

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P 45: The one pot synthesis of some α-aminophosphonates derivatives and investigation for their biological activities

Addoum Boutaina[a], Elkhalfi Bouchra[a], Harrati Suhayla[a], Sakaoui Souraya[a], Derdak Reda[a], Elmakssoudi Abdelhakim[b], Soukri Abdelaziz[a].

[a]*Laboratory of Physiopathology, Genetics, Molecular and Biotechnology (PGMB), Department of Biology, Faculty of Sciences Ain Chock, University Hassan II, Casablanca, Morocco*
[b]*Laboratory of Organic Synthesis, Extraction and Valorization, Department of chemistry, Faculty of Sciences Aïn Chock, University Hassan II, Casablanca, Morocco.*
E-mail: addoum636@uni.com

Nowadays essential oils are known as a hugely valuable natural resource due to their clinical applications and multiple biological functions including antifungal, antiviral, herbicidal, antioxidant, antitumorale, and antibacterial potential[1]. However, the extensive use of natural antioxidants is being ruled out owing to their minimal amount, the processes of extraction, and the purification of their bioactive molecules. For that reason scientific have used green chemistry[2] as a new tool to conflict several infections and especially to treat several oxidative stress-related diseases such as carcinogenesis, inflammation, and aging in aerobic organisms[3].

The purpose of this work was the synthesis and characterization of four α-aminophosphonates derivatives: **4 (a-d)** via the conventional spectroscopic methods (MNR, IR) [4]. Then the synthesized compounds were investigated for their antibacterial and antioxidant potential. Firstly, we tested the antibacterial potential of these products against 3 pathogenic strains (***E. coli, S. Aureus, and L. Monocytogenes***); their antimicrobial action was carried out by using the well diffusion method[6]. Afterwards we screened the antioxidant activity in vivo after inducing the oxidative and nitrosative stress by using *Tetrahymena thermophila & pyriformis*[5] cells as model organism. The results of the antibacterial test demonstrated that the three multi-resistant strains are sensitive to all compounds synthesized (**4a**, **4b**, **4c**) except of **4d** product which have a moderate antibacterial activity.

In the other hand we demonstrate that both products **4.a** and **4.c** own a protective effect against oxidative and nitrosative stress. These two derivatives played an antioxidant role but at the same time endowed with a powerful antimicrobial activity.

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P 46: Marine biomolecules activity against multiresistant bacteria

Asmaa Chbel[a], Jorge Rodriguez Castro[b], Javier Quintero[b], Manuel Rey Mendez[b], Abdelaziz Soukri[a], Bouchra El khalfi[a]

[a]Laboratory of Physiopathology, Molecular Genetics & Biotechnology, Faculty of sciences Ain Chock, Research center of Health & Biotechnology. University of Hassan II Casablanca, Morocco.
[b]Laboratory of Molecular Systematics, Department of Biochemistry & Molecular Biology, CIBUS University of Santiago de Compostela, Spain.
Email: ab.soukri@gmail.com

The marine environment is one of the main sources of bioactive natural substances among which molecules produced by marine bacteria. They are distinguished by genetic and metabolic characteristics that lead to production of a large number of bioactive molecules.

In this work, we have isolated and identified marine bacteria collected from the Atlantic deep ocean water in Morocco. Then, we induced them to produce biomolecules within four culture conditions. Furthermore, released molecules have been tested for their antibacterial activity against three multiresistant pathogenic bacteria: *Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes*.

Our results show that marine bacteria produce biomolecules showing high inhibition diameters against the three pathogenic bacteria that have been tested. Thus, these biomolecules may be exploited in pharmaceutical industries in order to restrict bacterial multiresistance spreading nowadays.

P 47: Extraction and bioactivities of phycoerythrin obtained from *Gracilaria gracilis* (Rhodophyta)

Teresa Mouga[a], Inês Silva[a], Ana P. Correia[a], Clélia N. Afonso[a], Marta V. Freitas [a], Ana Pombo[a], Teresa Baptista[a]

[a]*MARE – Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria, Edifício CETEMARES, Av. Porto de Pesca, 2520-641 Peniche (Portugal)*
E-mail: mougat@ipleiria.pt

Seaweeds, as any other photosynthetic organism exhibit pigments to harvest photons, required to perform photosynthesis. In addition to chlorophyll *a*, red seaweeds present phycobilins which mainly include phycoerythrin, a pink pigment that confers these seaweeds their pink/red/purple color. These organisms live mainly on the inter-tidal rocky shore, where they are periodically exposed to drought, high temperatures, and direct sunlight. Being marine organisms, seaweeds must have protective mechanisms that reduce inhibition of photosynthesis [1]. It is also well known that these stress stimuli increase the production of protective compounds that have important bioactivities. Phycobiliproteins, namely phycoerythrin, are used in food, pharmaceutic and biomedical industries, as well as in cosmetics and as a fluorescent indicator due to their interesting anti-cancer, antioxidant, antimicrobial, anti-inflammatory, neuroprotective, hepatoprotective, hypocholesterolemic, sun-protecting, as well as fluorescence properties [2]. As such, we investigated the phycoerythrin extraction methods in *Gracilaria gracilis*. The best results were achieved through a pre-treatment to allow mechanical cell wall rupture, allowing a more efficient cell content extraction. Then, an aqueous extraction was performed, using fresh biomass, and phycoerythrin yield was measured. The crude extracts, rich in phycoerythrin, were then scanned for antimicrobial and antioxidant properties. Results show that bioactivities in these extracts are low, compared to other red seaweeds and further purification of phycoerythrin might be an important step to increase bioactivities.

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P 48: Evaluation of the anti-Listeria monocytogenes effects of some biomolecules produced by bacteria isolated from donkey milk

Derdak Reda[a], Sakoui Souraya[a], Addoum Boutaina[a], Soukri Abdelaziz[a] and EL Khalfi Bouchra[a]

[a]Laboratory of Physiopathology Molecular Genetics & Biotechnology, Faculty of Sciences Ain Chock, Health and Biotechnology Research Center, Hassan II University of Casablanca
E-mail: ab.soukri@gmail.com

Recently, the interest in donkey milk has increased considerably because of its nutrients and convincing functional elements, although its composition is well known, its microbiota remains less studied. This study aims to analyze donkey milk, isolate, characterize its bacterial community and evaluate its ability to produce biomolecules with antibacterial activity against Listeria monocytogenes. All the investigations that we carried out made it possible to isolate and identify 44 strains. Five have been selected to screen for bioactive molecules. Various induction methods were used, among which tween, which was the most effective, making it possible to have a listeria inhibition halo which is of the order of 20 mm, thus showing that these biomolecules can be a promising target for neutralize bacterial multidrug resistance.

P 49: Chemical Constituents of The Volatile, Nonvolatile and Free Radical Scavenging activity of Medicinal Plants *Acanthus dioscoridis* and *Ranunculus millefoliatus*

Fuad O. Abdullah[a,b], Baram A. Hamah Ameen[c], **Dara Dastan**[d,e]

[a]*Department of chemistry, College of Science, Salahaddin University, Erbil, 44001, Kurdistan-Iraq.*
[b]*Research center, Salahaddin University, Erbil, 44001, Kurdistan Region of Iraq.*
[c]*Charmo research center, Charmo University, Chamchamal-Sulaimaniyah, Kurdistan region of Iraq.*
[d]*Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.*
[e]*Department of Pharmacognosy and Pharmaceutical Biotechnology, School of Pharmacy, Hamadan*
University of Medical Sciences, Hamadan, Iran.
E-mail: fuad.abdullah@su.edu.krd; d.dastan@umsha.ac.ir

The first reported investigation of the two medicinal plants *Acanthus dioscoridis* and *Ranunculus millefoliatus* used as traditional herbal remedies since antiquity to treat human diseases in the Iraqi Kurdistan is described. The hydro-distilled volatile oil from leaves and aerial parts (A. dioscoridis and R. millefoliatus), respectively, were investigated by GC-MS and GC-FID. In total 78 constituents, 46 and 44 compounds representing 92.5 and 92.57% of the volatile oil were identified from A. dioscoridis and R. millefoliatus respectively. The main volatile constituents of ADL-Oils were caryophyllene oxide (3.1%), E-phytol acetate (3.6%), (E)-β-ionone (3.7%), spathulenol (8.8%), phytol (15.7%), palmitic acid (23.0%). The major compounds of RMA-Oils were palmitic acid (4.89%), (E)-nerolidol (6.89%), α-copaen-11-ol (11.96%), γ-eudesmol (12.84%) and α-eudesmol (35.98%). The preliminary phytochemical tests for methanolic extracts (ADL and RMA) were studied by a standard method and their antioxidant activity by using DPPH assay. The results showed that the methanolic extract from RMA was stronger than ADL, with IC_{50} values (54 and 118 µg/ml) respectively. Likewise, the antioxidant activity of RMA oil (RMA-Oils and ADL-Oils) was found to be stronger than ADL oil (43 and 105 µg/ml) respectively, compared with BHT (IC50, 24 µg/ml), as a positive control. The data of this study suggest that A. dioscoridis and R. millefoliatus have potent antioxidants and contain interesting bioactive compounds which can be isolated from both plants.

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P 50: Preventive role of *Nigella sativa* seed extracts and Thymoquinone in Diabetes and its secondary complications

Ahmad Ali[a], Prairna[a]

[a]*University Department of Life Sciences, University of Mumbai, Vidyanagari, Santacruz (E), Mumbai*
Email: - ahmadali@mu,ac,in

Metabolic disorders have become major concerns for the world population. Diabetes is one of major disorder among the metabolic disorders. The consequences of diabetes are due to the accumulation of glucose which reacts with other molecules of the body and generates a group of harmful molecules commonly known as advanced glycation end products (AGEs). These products have been implicated in diabetes, cataract and neurodegenerative disorders. In the present study the extracts of Nigella sativa (black cumin) were used to assess their role in the prevention of formation of AGEs. The amount of glycation products, generated in vitro by interaction between proteins and sugars, were measured by established methods like NBT, carbonyl content and total AGEs by flourimetry in the presence and absence of black cumin extracts after an incubation of four weeks at 37 °C. The effect of glycation induced aggregation and glycoxidation were also measured by Thioflavin T and agarose gel electrophoresis methods. The results indicate that aqueous extract of black cumin could significantly prevent the formation of early and advanced glycation end products. It was also observed that these extracts prevented the glycation induced aggregation of BSA. The glycoxidative damage of DNA was also prevented by black cumin seed extracts. This study clearly indicates that black cumin seed extract have bioactive compounds which have potential antiglycating properties. A comparison with the pure thymoquinone suggested that these antiglycating potentials are attributed due to this major phytoconstituent of black cumin. Further characterization needs to be performed to analyze the exact mechanism of action of these phytoconstituents and/or black cumin seed extracts in the prevention of glycation products.

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P 51: Preservation of antioxidant activity on NADES

Liane de Meneses[a], Ana Rita C. Duarte[a], Alexandre Paiva[a]

[a]*LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Univesidade Nova de Lisboa, Campus da Caparica*
E-mail: alexandre.paiva@fct.unl.pt

Ascorbic Acid (AA) or α-tocopherol, also known as vitamins C and E, respectively, are strong antioxidants that are present in many fruits and vegetables. Due to their strong antioxidant activity both compounds are widely used in food, cosmetics and pharmaceutical industries. Citric acid (CA) is also used as food preservative or flavoring agent in the industries mentioned [1]. Industry is always seeking ways to extend the shelf-life of such products, since they are highly sensitive to environment conditions, such as light. When combined with other metabolites in specific molar ratios, both AA and CA have the ability to form Natural Deep Eutectic Systems (NADES). NADES have been described for the extraction and stabilization of phenolic compounds [2]. In this work, CA-based NADES were prepared according to reported examples [3]. Using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay the system's ability to preserve the antioxidant effect of Ascorbic AA was tested. For comparison, the system comprising choline chloride (ChCl) and AA in a molar ratio 2:1 was tested under the same conditions, since it has been reported that it can stabilize AA for at least six months period [4].

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P 52: Chemical constituents from *Ardisia kivuensis* displayed cytotoxic effects in carcinoma cells and multi-factorial drug resistant cancer cells

Yves M.M. Nguekeu[a], Blanche L. Ndontsa[a], Armelle T. Mbaveng[b,d], Victor Kuete[b,e], Maurice D. Awouafack[a,c], Roukayatou Mbouangouere[a], Takuya Ito[c], İlhami Çelik[f], Oğuzhan Karaosmanoğlu[e,g], Pierre Tane[a], Hiroyuki Morita[c], Hülya Sivas[e], Thomas Efferth[d]

[a]Department of Organic Chemistry, Faculty of Science, University of Dschang, Cameroon; [b]Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon; [c]Institute of Natural Medicine, University of Toyama, 2630-Sugitani, Toyama 930-0194, Japan; [d]Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, University of Mainz, Staudinger Weg 5, Mainz 55128, Germany; [e]Department of Biology, Science Faculty, Anadolu University, Eskişehir, Turkey; [f]Department of Chemistry, Faculty of Science, Anadolu University, Tepebaşı, Eskisehir 26470, Turkey; [g]Department of Biology, KamilÖzdağ Science Faculty, KaramanoğluMehmetbey University, Karaman, Turkey;
E-mail: my_nguekeu2014@yahoo.com; yves.nguekeu@univ-dschang.org

Ardisia kivuensis Taton (Myrsinaceae) is an evergreen shrub encountered at Mount Oku and Mount Kupe in Cameroon [1,2]. The fruits are used in folk medicine to treat venereal diseases. This work was designed to carry out the chemical study of the MeOH extract of the fruits of *A. kivuensis*, as well as the cytotoxicity of some isolated compounds.

Successive silica gel open column chromatography followed by Sephadex LH-20 was used to isolate six compounds and standard acetylation reaction was done on one of the compounds to afford its derivative. The structures of these compounds were determined using spectroscopic analysis (NMR and MS) data, and by comparison with published data. The cytotoxicity of samples was performed by using the Neutral Red uptake (NR) and Resazurin reduction assays against a panel of six carcinoma cell lines and nine cancer cell lines including various sensitive and drug-resistant phenotypes.

The phytochemical study led to the isolation of one new alkenylmethylresorcinol named ardisinol III (**1**) along with five known compounds, oleanolic acid (**2**), *β*-sitosterol (**3**), pentacosanoic acid (**4**), N-lignoceroyltriptamin (**5**) and ardisiacrispin B (**6**). A new acetylated derivative, 1,3-di-*O*-acetylardisinol III (**7**) was obtained after standard acetylation procedure applied to **1**. Among the isolated compounds, **1** and **6** displayed significant cytotoxic effects with IC_{50} values below 10 µM against all the selected human cancer cell lines. The IC_{50} values ranges were 0.88 µM (against SPC212 mesothelioma cells) to 8.36 µM (against hepatocarcinoma HepG2 cells) for ardisinol III (**1**) and 1.20 µM (towards leukemia CCRF–CEM cells) to 6.76 µM (against heptocarcinoma HepG2 cells) for ardisiacrispin B (**6**).

The results obtained indicated that *A. kivuensis* had excellent cytotoxic molecules that deserve more detailed exploration in the future, to develop novel cytotoxic drugs for chemotherapeutic use.

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P 53: Isolation and biochemical characterization of cellulase and xylanase activities from thermophilic *Bacillus*

Rawand Gharsalli[a]

[a]*Tunisia*

The new technologies used in industries focus on respect for the environment and human health, hence the use of enzyme-producing microorganisms of industrial interest. The present work is part of this approach since it aims to isolate and characterize new strains of thermophilic *Bacillus* producing cellulases and xylanases. We began by isolating thermophilic bacteria from different samples of thermal waters collected from several regions of Tunisia. The isolated strains were identified macroscopically, microscopically and biochemically. Of the 130 strains of *Bacillus* isolated, 38 strains producing cellulase and 27 strains producing xylanase. In fact, a new thermostable cellulasewas detected in *Bacillus licheniformis* strain R101 isolated from the Djabel El West hot spring. After optimizing the production of cellulase produced by strain R101, the enzyme was purified and its size was determined which is about 12kDa by polyacrylamide gel analysis (10%) under denaturing conditions. The conditions for maximum activity of this enzyme were determined according to the optimum pH is about 5 and the optimum temperature is 70 ° C. Activity was stimulated by $MnSO_4$, DTT, PMSF, SDS and Triton x100.Analysis by Maldi TOF-MS has shown that it has a strong homology with an uncharacterized protein of a *Bacillus licheniformis* strain. This significant homology between our cellulase and the uncharacterized protein produced by *Bacillus licheniformis* CG-B52 deposited in the NCBInr database under accession number T5HLP8_BACLI shows that this uncharacterized protein is a cellulase. After treatment with cellulase, the denim fabric changed color as the control retained its color. Also, a thermostable xylanase was purified and characterized from the strain *Aeribacillus pullidis* R68 isolated from the El Hamma hot spring. After optimizing the production of xylanase produced by strain R68, the enzyme was purified. Subsequently, to confirm the purity of the xylanase and to determine the size of this enzyme, the polyacrylamide gel proteins (10%) were electrophoresed under denaturing and zymogram conditions. The xylanase has a molecular weight of about 50 KDa and the optimum temperature and pH for maximum activity were 80 ° C and 9, respectively. The activity was stimulated by $CaCl_2$, DTT, Triton x100, PEG 6000 and Tween 80.

P 54: Impact of the food bioactive erucin on human renal cancer cells

Bojana Vidovic[a], **Irís Guerreiro**[b], João G. Costa[b], Nuno G. Oliveira[c], Nuno Saraiva[b], Ana S. Fernandes[b]

[a]*Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia*
[b]*CBIOS, Universidade Lusófona Research Center for Biosciences & Health Technologies, Lisbon, Portugal*
[c]*Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal*
E-mail: ic.guerreiro@campus.fct.unl.pt

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P 55: Anti-cancer properties of Parvifloron D in breast cancer cells

Nuno Saraiva[a], **João G. Costa**[a], Patrícia Rijo[a,b], Nuno Almeida[a], Catarina Reis[a,b], Ana S. Fernandes[a]

[a]*CBIOS, Universidade Lusófona Research Center for Biosciences & Health Technologies, Lisboa, Portugal;*
[b]*Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal*
E-mail: jgcosta@ulusofona.pt

Medicinal plants are an abundant source of new bioactive compounds with anticancer properties. Parvifloron D (ParvD) is an abietane diterpenoid isolated from *Plectranthus ecklonii* acetonic maceration extract. ParvD was previously shown to have cytotoxic and pro-apoptotic effects in leukemia and melanoma *in vitro* models. In the present work, the anticancer effects of ParvD were evaluated in a human model of triple negative breast cancer (MDA-MB-231 cells).

ParvD (0.1–10 μM) decreased cell viability in a concentration-dependent manner (crystal violet assay). Treatment with ParvD increased the percentage of apoptotic nuclei (DAPI staining) and of the sub-G1 population (PI staining). Cell exposure to a low concentration of ParvD (1 μM) had no effect on cell-substrate attachment (EDTA-induced detachment assay). ParvD (1 μM) significantly reduced directed cell migration and invasion (transwell assay), which are determinant processes for the formation of metastases. In summary, the natural compound ParvD showed interesting anticancer potential waranting further studies.

P 56: Compliance with the Codex alimentarius of kefir beverage produced from Portuguese cow milk

Emília Alves[a], Patrícia Rijo[a], Luís Monteiro Rodrigues[a,b], Catarina Rosado[a]

[a]CBIOS – Universidade Lusófona's Research Center for Health Science and Technologies, Campo Grande, 376, 1749-024, Lisboa, Portugal
[b]Dep.Pharmacol Sciences – Universidade de Lisboa (Faculty of Pharmacy)
E-mail: catarina.rosado@ulusofona.pt

Fermented dairy products are the most common fermented foods worldwide being kefir one of the most interesting due to its probiotic content and subsequent health benefits. Kefir is a viscous, slightly carbonated dairy beverage obtained from the fermentation of milk with kefir grains that contain a symbiotic association of lactic-acid bacteria, acetic-acid bacteria and yeasts in a polysaccharide matrix. The presence of yeasts in the mixture, together with fermentation temperature, encourages the growth of aroma-producing bacteria. As fermentation proceeds, growth of lactic-acid bacteria is favored over growth of yeasts and acetic-acid bacteria. As stated by the Food and Drug Administration (FDA), if there is a long history of using poorly defined microbiological mixtures in food production (eg mixtures used to make dairy products like kefir) and if the fermentation substrate is consistent with this history, its use is acceptable for human consumption. Thus, kefir is considered a safe food for human consumption, given its history of use for hundreds of years. According to the *Codex Alimentarius*, in a typical kefir beverage (fermented milk obtained from kefir grains) the total number of microorganisms should be at least 10^7 colony-forming units (CFU)/ml and the yeast number not less than 10^4 CFU/ml.

The objective of this work was to evaluate the compliance with the Codex Alimentarius guidelines of a kefir beverage obtained from the fermentation of semi-skimmed cow's milk, of Portuguese provenance, with a CIDCA kefir grain inoculum.

Kefir beverage samples were produced by fermentation of a commercial ultra-high temperature pasteurized (UHT) semi-skimmed cow's milk, of Portuguese provenance, for 24h at a temperature of 20ºC with CIDCA kefir grains (10%w/v).

Conventional culture techniques were used to determine latic-acid bacteria and yeast count. Coliform determination was also performed by conventional culture techniques.

The microbiological analysis of the kefir CIDCA fermented drink obtained revealed $7x10^7$ CFU/ml of lactic-acid bacteria and $2x10^6$ CFU/ml of yeast. Furthermore, the absence of coliforms (E.coli) was also confirmed. According to these results the kefir CIDCA fermented beverage complies with the recommendations of *Codex Alimentarius*.

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P 57: Characterization of Kefir beverage produced from Portuguese cow milk

Emília Alves[1], Marisa Nicolai[1], Pedro Fonte[1,2,3], Joyce Costa[4], Luís Monteiro Rodrigues[1,5], Patrícia Rijo[1], **Catarina Rosado[1]**

E-mail: catarina.rosado@ulusofona.pt

[1] *CBIOS – Universidade Lusófona's Research Center for Health Science and Technologies, Campo Grande, 376, 1749-024, Lisbon, Portugal*

[2] *LAQV, REQUIMTE, Department of Chemical Sciences—Applied Chemistry Lab, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal*

[3] *iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal*

[4] *Federal University of Vales do Jequitinhonha e Mucuri, Departament of Food and Technolgy, Science and Technology Institute, MG, Diamantina, Brasil*

[5] *Dep. Pharmacol Sciences – Universidade de Lisboa (Faculty of Pharmacy), Lisbon, Portugal*

One of the most interesting sources of natural probiotics is kefir, the milk based beverage produced by fermentative activity of kefir grains that contain a symbiotic association of lactic acid bacteria, acetic acid bacteria and yeasts in a polysaccharide matrix. The milk type, origin/composition of the grains, time and temperature of fermentation and storage conditions influence the nutritional composition of kefir. Fermentation process can modify the distribution of essential elements in kefir. During fermentation, proteins are breakdown into peptides more easily digestible, lactose is hydrolyzed becoming a good option for lactose-intolerant individuals and lactic-acid bacteria grow rapidly increasing the acid-lactic concentration and causing an initial drop in pH. According to the *Codex Alimentarius*, a typical kefir (fermented milk obtained from kefir grains) should contain at least 2.7 % of protein, 0.6 % of lactic acid, and less than 10% of fat.

The aim of the study was to characterize and evaluate the composition of kefir beverage produced by fermentation of cow milk of Portuguese provenance.

Kefir beverage samples were produced by fermentation of a commercial ultra-high temperature pasteurized (UHT) semi-skimmed cow's milk, of Portuguese provenance, for 24h at a temperature of 20ºC with CIDCA kefir grains (10%w/v).

The kefir beverage samples were characterized in relation to particle size, viscosity, pH, total solids and fat content, according to the AOAC methodology. A qualitative analysis was made using ATR-FTIR.

The fermented kefir beverage obtained showed a pH value of 4.56, 9.27% of fat and 9% solids. This beverage revealed a viscosity of 230 mPas. The medium particle size found was 234.20±3.86 nm with a medium polydispersity index of 0.15±0.02. The FTIR spectra showed that the physicochemical properties of kefir may change during the fermentation process.

The CIDCA kefir fermented Portuguese milk achieved the expected chemical proprieties. The growing preference for health-promoting foods and increasing disease resistance has led to the inclusion of fermented milk products as part of a diet benefiting from their potential health effects. Due to its nutritional attributes, traditional kefir can be regarded as an interesting option as a functional food.

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P 58: Amarallidaceae-type alkaloids from *Narcissus* and *Pancratium* species

Shirley Sancha[a], Silvia Priska[a], Maria-José U. Ferreira[a]

[a]*Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.*
E-mail: shirleysancha@ff.ul.pt

Plants from Amaryllidaceae family, traditionally used to treat cancer, can synthesize a high content and a wide variety of bioactive alkaloids [1]. The main goal of this study is to develop a library of amaryllidaceae-type alkaloids, through isolation and molecular derivatization, for reversing multidrug resistance (MDR) in cancer cells, the major obstacle for cancer treatment.

The phytochemical study of the methanol extract of bulbs and flowers of *Narcissus bulbocodium* L. subsp *obesus* (Salisb) (Amaryllidaceae) led to the isolation of an alkaloid, an alkamide and four steroids. In addition, the study of the alkaloid fraction of the methanol extract of the bulbs of *Pancratium maritimum* L. (Amaryllidaceae) led to the isolation of several amaryllidaceae-type alkaloids. The structures of the compounds were established from their physical and spectroscopic data (IR, MS, 1D and 2D NMR -COSY, HMQC and HMBC and NOESY experiments).

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P 59: Antioxidant properties and toxicity of Portuguese centenarians herbal teas

Alda Pereira Silva[a,b,c,d], Epole Ntungwe[a], M Céu Costa[a,e], J.P. Gorjão-Clara[g,h], M. Bicho[c,d], Patrícia Rijo[a]

[a]*Research Center in Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal*
[b]*Lapa Health Center - Personalized Health Care Unit, Ministry of Health, Lisbon, Portugal*
[c]*Genetics Laboratory, Environmental Health Institute - ISAMB, Faculty of Medicine, University of Lisbon, Portugal*
[d]*Institute of Scientific Research Bento da Rocha Cabral, Lisbon, Portugal*
[e]*ERISA- High School of Health Ribeiro Sanches*
[f]*Phytochemistry and Food Sciences-Group Coordinator - CBIOS*
[g]*Geriatric Universitary Unit of Faculty of Medicine, University of Lisbon, Portugal*
[h]*Academic Medical Center of Lisbon - North of Lisbon Hospital Center*
E-mail: alda_pereira@hotmail.com

Medicinal plants traditionally consumed can contribute to longevity. A sample of 253 centenarian individuals in Portugal, both sexes, median age 100 years was study, to verify past habits in relation to medicinal-interest in plants use. It was compared with a control group median age 67 years, with a reduced theoretical probability of reaching 100 years. Amongst the 8 most cited plants, the lemon-balm (*Melissa off.*), barley (*Hordeum vulgare*), lemon-verbena (*Aloysia triphylla*), orange (*Citrus sinensis*, leaf and flower), linden (*Tiliae platyphyllos*), whig-plant (*Chamamelum nobile*), pennyroyal (*Mentha pulegium*) and mount-carqueja (*Pterospartum-tridentatum*) were most used by the centenarian's group and lemon-balm, lemon-verbena, chamomile (*Matricaria chamomille*), linden, prince-herb (*Cymbopogon citratus*), green-tea (*Camellia sinensis*), lemon-tea (*Citrus Limonium*) and mint-tea (*Mentha piperita*) used by the control (decreasing frequency order). Moreover, 28% of the control subjects reported not using infusion plants, whereas in the centennial group, only 9.1% reported not routinely using them ($\chi 2=30,42$, $p<0.001$). Among the eight plants most cited by the centenarians that were not mentioned by the controls were barley, whig-plant, pennyroyal and mount-carqueja. The plants were prepared using the infusion extraction method and the extraction yield in percentage determined. All extracts were prepared in 10% (w/v) and evaluated for their antioxidant activity (DPPH method) and general toxicity (*Artemia salina* model. The results showed that barley coffee (5.66%) and barley (5.39%) had the highest % of extraction yields. All extracts have a high direct scavenging ability with the mint-tea having the highest value of antioxidant activity (98.35%). All samples found to be not toxic against the *A. salina* model, compared to the positive control. Further studies are on going in order to investigate if the high antioxidant activity determined and the lower general toxicity of all herbal teas consumed by centenarians can help to explain the successful use these plants by centenarian individuals, which may have contributed in achieving exceptional longevity.

P 60: *In vitro* safety assessment of rutin, of a choline-based ionic liquid and of their combination in renal cells

Rita Caparica[a,b], Ana Júlio[a,b], André Rolim Baby[c], Maria Eduarda Machado Araújo[d], João Guilherme Costa[a,*] and Tânia Santos de Almeida[a,d,*]

[a]CBIOS-Universidade Lusófona's Research Center for Biosciences & Health Technologies, Campo Grande 376, 1749–024 Lisboa, Portugal; [b]Department of Biomedical Sciences, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain; [c]Department of Pharmacy, School of Pharmaceutical Sciences, University of São Paulo, 580 Prof. Lineu Prestes Av., Bl. 15, São Paulo, SP 05508-900, Brazil; [d]CQB, CQE and Department of Chemistry and Biochemistry, Faculty of Sciences, University of Lisbon, Campo Grande 1749-016 Lisbon, Portugal.
E-mail: rita.caparica@ulusofona.pt, tania.almeida@ulusofona.pt
*Shared senior authorship

P 61: Properties of ximenia oil used as cosmetic in Angola

Gabriel Satoto[a], Fernando Santos[b], Nuno Neng[a,b], José Manuel Nogueira[a,b], Nuno Saraiva[c], Ana Sofia Fernandes[c], Tânia Santos de Almeida[c], **Maria Eduarda M. Araújo[a,b]**

[a]CQB and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal
[b]CQE and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal
[c]CBIOS - Universidade Lusófona's Research Center for Biosciences and Health Technologies, Campo Grande 376, 1749-024, Lisboa, Portugal
E-mail: mearaujo@fc.ul.pt

Ximenia *(Ximenia americana* L.) is native from tropical Africa but spread across different continents [1] and local populations in Angola use seeds oil mainly for cosmetic uses [2]. Herein an oil produced in Angola [3], as well as two other samples from seeds of the same region extracted in laboratory, were studied and their composition and chemical properties, viscosity, UV transmission and cytotoxicity were evaluated.

Results showed that the saponification, iodine and peroxide values of the three oils, as well as the GC-MS analysis performed, are quite similar.

Additionally, results also showed that a thin film, of any of the three oils, almost blocks the transmission of UV light. At normal temperature of the skin, viscosity studies showed that each oil can be spread over the skin as a thin film. In terms of toxicity studies, at concentrations up to 10 µg/mL, the oil obtained from the local producer is not toxic to human keratinocytes, thus suggesting the safety of the studied oil

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P 62: Ionic liquids as vehicles to deliver poorly soluble antioxidants for biomedical applications

Ana Júlio[a,b], Rita Caparica[a,b], Maria E. M. Araújo[c], André Rolim Baby[d], Pedro Fonte[a,e,f,*], Tânia Santos de Almeida[a,c,*]

[a]CBIOS-Research Center for Biosciences and Health Technologies, Lusófona University, Campo Grande 376, 1749-024 Lisbon, Portugal; [b]Department of Biomedical Sciences, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain; [c]CQB, CQE and Department of Chemistry and Biochemistry, Faculty of Sciences, University of Lisbon, Campo Grande 1749-016 Lisbon, Portugal; [d]School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 15, 05508-900, São Paulo, Brazil; [e]LAQV, REQUIMTE, Department of Chemical Sciences-Applied Chemistry Lab, Faculty of Pharmacy, University of Porto, Porto, Portugal: [f]iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, 1049-001 Lisbon, Portugal.
E-mail: tania.almeida@ulusofona.pt
[*]Shared senior authorship

P 63: Assessing the anti-inflammatory properties of lycopene-enriched extracts formulated in topical microemulsions

Ana Costa[a], Andreia Ascenso[a], Joana Marto[a], Manuela Carvalheiro[a], Lídia Gonçalves[a], Helena Margarida Ribeiro[a], Sandra Simões[a]

[a]*Research Institute for Medicines (iMed.ULisboa), Nano2B, Faculty of Pharmacy, Universidade de Lisboa, 1609-003 Lisbon, Portugal*.

E-mail: acosta @farm-id.pt

Lycopene is a natural carotenoid presents in red-colored fruits and vegetables. Due to its well-known antioxidant properties, this natural compound is being used in cosmetic formulations for antiaging and skin care purposes [1]. In this work, lycopene-enriched extracts (LEE) from tomato residues (peel and seeds obtained from tomato processing industry) were obtained by CO_2 Supercritical Extraction. By modifying the extraction conditions (temperature and pressure), different LEE were obtained. Those LEE were screened by evaluating the reduction of reactive oxygen species in HaCaT cell line. The LEE with the highest antioxidative properties was further encapsulated into microemulsions [2].

Microemulsions were prepared by forming a blending of monoacylglycerol and diacylglycerol (oily phase) and by the combination of surfactant/co-surfactant in a proportion of 1:1. Afterwards, water was added, and the mixture was stirred for 15 minutes at room temperature, leading to the formation the formation of a spontaneous transparent and isotropic microemulsion. The selected formulation, constituted by 20% of oil, 50% of a mixture of surfactant/co-surfactant in a ratio of 1:1 and 30% of water, displayed a small droplet size and a monodisperse population (mean size lower 100 nm and a polydispersity index of 0-15-0.30). Different concentration of LEE were incorporated into the microemulsions (1%, 2.5% and 5% (w/w) related with the mass of oil) and it was observed that incorporations of 5% (w/w) of LEE changes the initial physical-chemical properties of microemulsions.

The *in vitro* cytotoxicity, anti-oxidative and anti-inflammatory properties of LEE and LEE-loaded microemulsions will be further investigated.

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P 64: Adherence to the Mediterranean diet in Portuguese university students

Cíntia Ferreira-Pêgo[a,b], Joana Rodrigues[b], Adriana Costa[b], Bruno Sousa[a,b]

[a]*CBIOS Lusófona's Research Center for Biosciences and Health Technologies, Av. Campo Grande 376, 1749-024 Lisbon, Portugal;*
[b]*School of Sciences and Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal*
E-mail: cintia.pego@ulusofona.pt

Mediterranean diet (MedDiet) is one of the most recognized healthy dietary patterns. Although the well-known beneficial effects of healthy eating habits on academic performance, the university students used to present unhealthy food choices. Cross-sectional information regarding MedDiet adherence was collected in 305 students from the *Universidade Lusófona de Humanidades e Tecnologias*, from different academic courses, related or not with health sciences. Nutrition students presented significantly higher MedDiet adherence compared to those studying pharmaceutical sciences and also from other courses not related to health sciences. 28.90% of the total population presented poor MedDiet adherence, 58.70% presented an average adherence and only 12.50% presented a good MedDiet adherence. Nutrition sciences degree presented more students in the highest category of MedDiet adherence compared to the rest. Pharmaceutical students and other students not related to health sciences presented a significantly higher risk of poor MedDiet adherence.

Nutrition students presented the highest MedDiet adherence of all the students analyzed. Pharmaceutical students, although being health professionals, showed poor adherence to the MedDiet, similar to students from courses not related to health sciences.

P 65: Development of an alginate hydrogel for topical delivery of chitosan-coated PLGA nanoparticles

Patrícia Filipe[a,b], Ana S. Macedo[a,c], Catarina Rosado[a], Pedro Fonte[a,c,d]

[a]CBIOS - Universidade Lusófona Research Center for Biosciences & Health Technologies, Campo Grande 376, 1749 - 024 Lisboa, Portugal [b]Department of Biomedical Sciences, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain [c]LAQV, REQUIMTE, Department of Chemical Sciences-Applied Chemistry Lab, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal [d] iBB -Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal
E-mail: patricia.cfilipe@gmail.com

Alginate is an anionic polymer extracted from brown seaweed (class *Phaeophyceae*), used to prepare wound dressings due to its favorable properties, such as biocompatibility, non-toxicity, ability to absorb wound exudate and limit bacterial infections at the wound site [1]. Low molecular weight chitosan is another natural polymer able to stimulate the expression of matrix metalloproteinases in primary human dermal fibroblasts, preventing collagen IV hydrolysis [2]. Chitosan also exhibits mucoadhesive properties, hemostatic, antimicrobial features and is an angiogenesis promoter. Overall, the aim of this work was to develop an alginate hydrogel containing chitosan-coated nanoparticles to deliver therapeutic proteins on the skin for wound healing.

Chitosan-coated PLGA nanoparticles were produced by a water/oil/water double emulsion technique developed by our group [3]. The nanoparticles coating process with chitosan at different concentrations (0.25%, 0.5% and 1%) was effective and the particles were further incorporated in the hydrogel containing alginate, carboxymethylcellulose and propylene glycol. It was observed that the size of chitosan-coated PLGA nanoparticles is augmented by the increase of chitosan proportions, showing a size of 1300 nm in comparison with empty nanoparticles that exhibits a size of 280 nm. The chitosan-coated PLGA nanoparticles exhibits a charge of 30 mV due to natural positive charge of chitosan, while the uncoated PLGA nanoparticles shows a zeta potential of -14 mV. The model therapeutic protein was loaded into the nanoparticles with an association efficiency of about 85%, and still the hydrogel had a suitable viscosity for topical administration. In conclusion, the developed formulation allowed to yield a chitosan-coated PLGA nanoparticles hydrogel system alongside satisfactory uniform structure, as well as capability to load the drug. It is expected that both alginate and chitosan presence afford a stimulation of the wound recovery.

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P 66: Skin compatibility assessment of sodium alginate hydrogels loaded with PLGA nanoparticles for wound healing

João Vieira[a], Patrícia Filipe[a,b], **Ana S. Macedo**[c], Catarina Rosado[a], Pedro Fonte[a,c,d]

[a]CBIOS - Universidade Lusófona Research Center for Biosciences & Health Technologies, Campo Grande 376, 1749–024 Lisboa, Portugal
[b]Department of Biomedical Sciences, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain
[c]LAQV, REQUIMTE, Department of Chemical Sciences—Applied Chemistry Lab, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[d]iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal
Email: ana.macedo@ulusofona.pt

Wound healing is a long complex process involving the recovery of the function and tissue regeneration [1]. The process of wound healing might be accelerated in the presence of some natural products that have anti-inflammatory, antioxidant antibacterial, and pro-collagen synthesis properties. Sodium-alginate is a naturally occurring polymer extracted from the brown seaweed and is widely employed in wound dressings due to its water absorbing and antibacterial properties, and ability to maintain a physiologically moist environment. In this work, we propose the use of PVA/alginate hydrogels loaded with PLGA nanoparticles as carriers for growth factors for wound healing.

Poly (lactic-co-glycolic acid) (PLGA) was used as the matrix of the nanoparticles and PVA was the surfactant. PLGA nanoparticles were produced following a protocol developed by our group [2] Sodium alginate, glycerin, and urea were added, and the hydrogels were formed after freeze-thawing cycles. It was observed that the hydrogel production method did not change the particle size of 300 nm and obtained a narrow PdI of 0.25. The ATR-FTIR analysis showed an interaction between PLGA and glycerin (1075 cm^{-1}) and urea (1650 cm^{-1}). The skin compatibility was assessed by Laser Doppler Flowmetry, and values were similar for water and the gels containing no glycerin, 5% glycerin, and 10% glycerin (16.7, 11.0, 17.6, 13.0 AU, respectively), showing evidence of not causing erythema. In contrast, the positive control methyl nicotinate showed an increase in flowmetry to 124.8 AU.

Overall, it was obtained a stable and biocompatible polymer-nanoparticle based hydrogel for wound healing purposes. Such system is intended to load growth factors in the future and assess its *in vitro* and *in vivo* performances.

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P 67: Development of lipid-based nanoparticles for delivery of caffeine and curcumin

Joyce Costa[a], Ana Júlio[b], Jocilane Oliveira[a], Pedro Fonte[b,c,d]

[a]*Federal university of Jequitinhonha and Mucuri Valleys, JK Campus, Rodovia MGT 367. Km 583, No. 5000, 39100-000 Diamantina, Minas Gerais, Brazil,* [b]*CBIOS-Universidade Lusófona's Research Center for Biosciences & Health Technologies, Campo Grande 376, 1749–024 Lisboa, Portugal* [c]*LAQV, REQUIMTE, Department of Chemical Sciences - Applied Chemistry Lab, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal,* [d]*IBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal*
E-mail: pedro.fonte@ulusofona.pt

The aim of this work was to develop and characterize nanostructured lipid carriers (NLC) co-encapsulated with Curcumin (diferuloylmethane), a yellow lipid-soluble polyphenol which is present in the rhizome of turmeric (Curcuma longa L.) with Caffeine as hydrophilic compound, a white and water-soluble compound. Such system may used in different food and biomedical applications. The physicochemical characteristics of developed CV-SLN (unloaded SLN) and CC-SLN (curcumin-caffeine-loaded SLN) including average particle size, polydispersity, FTIR and association efficiency (AE) were investigated. The CV-SLN were about 650 nm, with a low polydispersity index (<0.30), whereas the CC-SLN were 720 nm. This size increase demonstrates de association of the drugs within the nanoparticles. The results of FTIR analysis confirmed the association of curcumin and caffeine within the nanoparticles, demonstrating the co-encapsulation of both drugs. Also, the association efficiency (AE) of curcumin and caffeine were about 91.6 and 97.6%, respectively. This result was a good achievement, demonstrating that the co-encapsulation of drugs was not detrimental to their AE. The promising results of our work could lead a further exploration of this nanoparticle formulation to be used in food and pharmaceutical industry.

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List of participants

Adedokun Oluwasegun, University of Benin, Nigeria
Alda Pereira Sila, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Alexandra Plácido, Faculadade de Ciências da Universidade do Porto, Portugal
Alice Santos, Portugal
Amar Zellagui, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria
Amilcar Roberto, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Amin Karmali, Instituto Superior de Engenharia de Lisboa, Portugal
Ana Costa, iMed, Universidade de Lisboa, Portugal
Ana Cristina Fonseca, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Ana Cruz, Portugal
Ana Faustino, Portugal
Ana Júlio, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Ana Lúcia Morais, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Ana Macedo, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Ana Teresa Belo, INIAV, National Institute of Agrarian and Veterinarian Research, Portugal
Ana Sofia Fernandes, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Andreia Rostella, iMed, Universidade de Lisboa, Portugal
Andreia Ascenso, FF, Universidade de Lisboa, Portugal
Anshalika Sachaphibulkij, Thailand
Attila Hundayi, University of Szeged, Hungary
Bruno Sousa, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Carlos A. M. Afonso, iMed, Universidade de Lisboa, Portugal
Carmen Freire, CICECO, Universidade de Aveiro, Portugal
Catarina Batista, Portugal
Catarina Rosado, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Celso Almeida, FCUL, Portugal
Celso Alves, IPL, Instituto Politécnico de Leiria, Portugal
Cíntia Almeida, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Cíntia Pêgo, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Clélia N. Afonso, IPL, Instituto politécnico de Leiria, Portugal
Daniel Santos, iMed, Universidade de Lisboa, Portugal
Diogo Carregosa, iBET, Instituto de Biologia Experimental e Tecnológica, Portugal
Elisabete Silva, FCUL, Portugal
Elizabeth A. Lopes, iMed, Universidade de Lisboa, Portugal
Emília Leitão, HOVIONE, Portugal
Epole Nyungwe, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Eva María Domínguez Martín, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Fátima Piedade, FCUL, Portugal
Filipa Siopa, iMed, Universidade de Lisboa, Portugal
Francesco Esposito, Università degli Studi di Napoli "Federico II", Italy
Giulia Venditti, Sapienza University, Italy
Graça Mariano, DGAV, Portugal
Hamza Kabouche, University of Larbi Ben Mhidi Oum El Bouaghi, Algeria
Inês Cristovão da Silva, Instituto Nacional da Propriedade Industrial, Lisboa, Portugal
Inês Delgado, FMV, Universidade de Lisboa, Portugal
Iris Guerreiro, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Isabel Ferreira, CIMO, Portugal
Jaime Coelho, iMed, Universidade de Lisboa, Portugal

Joana Mota, Lecifarma, Portugal
Joana Tavares, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
João Baixinho, iBET, Instituto de Biologia Experimental e Tecnológica, Portugal
João Costa, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
João Rosa, iMed, Universidade de Lisboa, Portugal
Joyce Costa, Federal university of Jequitinhonha and Mucuri Valleys, Brazil
José Prieto, UCL, University College London, London
Karol Górski, University of Lodz, Poland
Kamonrat Chantra, Thailand
Kessia Andrade, iMed, Universidade de Lisboa, Portugal
Liane Meneses, FCT, Universidade Nova de Lisboa, Portugal
Lídia Palma, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Luis Monteiro Rodrigues, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Luísa Custódio, CCMAR, Universidade do Algarves, Portugal
Margarida Madureira, Portugal
Maria Eduarda Araújo, FCUL, Portugal
Maria M. M. Santos, iMed, Universidade de Lisboa, Portugal
Maria Céu Costa, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Maria J. Umbelino Ferreira, iMed, Universidade de Lisboa, Portugal
Maria João Neves, Portugal
Mariana Almeida, Medinfar, Portugal
Mariana Reis, CIIMAR, Portugal
Marco Teixeira, Portugal
Marisa Nicolai, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Marta Borges, DGAV, Portugal
Mattia Mori, Università di Siena, Italy
Miguel Batista, iBET, Instituto de Biologia Experimental e Tecnológica, Portugal
Miguel González, CSIC, Universitat Politècnica de Valencia-Consejo Superior de Investigaciones Científicas, Spain
Mihayl Varbanov, Université de Lorraine, France
Nelson Tavares, Natiris, Portugal
Nguekeu Mba Yves Martial, University of Dschang, Cameroon
Noureddine Gherraf, University of Oum ElBouagh, Algeria
Nuno Saravia, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Patrícia Filipe, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Patrícia Rijo, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Paula Bico, DGAV, Portugal
Paula Castilho, UMA, Universidade da Madeira, Portugal
Paula Pereira, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Pedro Barbosa, ICAAM - Universidade de Évora, Portugal
Pedro Bulcão, Portugal
Pedro Fonte, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Ricardo Ferreira, Uppsala University, Sweden
Rita Caparica, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Rita Jessus, Portugal
Rita Pereira, iMed, Universidade de Lisboa, Portugal
Rui Mineiro, Portugal
Salah Akkal, University of Mentouri Constantine 1, Algeria
Sandra Ferreira, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Sara García Poza, iBET, Instituto de Biologia Experimental e Tecnológica, Portugal

Sara Zúquete, FMV, Universidade de Lisboa, Portugal
Sarah Gothe, Portugal
Shirley Sancha, iMed, Universidade de Lisboa, Portugal
Stephania Moreira, Portugal
Stephanie Almada, Portugal
Stéphane Besson, EPCV, Universidade Lusófona de Humanidades e Tecnologias, portugal
Tânia Almeida, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Teresa M. Santos Lemos, Portugal
Teresa Mouga, Instituto politécnico de Leiria, Portugal
Tiago Rodrigues, IMM, Faculdade de Medicina, Universidade de Lisboa
Vanessa Gonçalves, Lecifarma, Portugal
Vera Isca, CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Portugal
Víctor Jiménez González, University of Seville, Spain
Victor O. Fadipe, University of Zululand, South Africa/Federal Ministry of Science and Technology, Nigeria

List of virtual participants

Addoum Boutaina, University of Hassan II, Morocco
Ahmad Ali, University of Mumbai, India
Amel Bouziane, University Laghouat, Algeria
Ana Faustino, CITAB, University of Trás-os-Montes and Alto Douro, Portugal
Asmaa Chbel, University of Hassan II, Morocco
Behzad Mohaddesi, Tehran university of medical science, Iran
Carla Maria Batista Ferreira Pires, Instituto Nacional da Propriedade Industrial, Portugal
Dara Dastan, Hamadan University of Medical Sciences, Iran
Farida Larit, University of Mentouri Constantine 1, Algeria
Ibrahim Sifi, University of Mentouri Constantine 1, Algeria
Inês Cristovão da Silva, Instituto Nacional da Propriedade Industrial, Portugal
Lina María Ruíz Hincapié, Universidad Autónoma de Chile, Chile
Luiz Pedro de Souza Costa, Federal University of Amazonas, Brazil
Nabila Souilah, University of Mentouri Constantine 1, Algeria
Noui Hendel, University Mohamed Boudiaf of M'sila, Algeria
Paolo Governa, University of Siena, Italy
Rawand Gharsalli, Tunisia
Reda Derdak, University Hassan II, Morocco
Saiqa Ishtiaq, University of The Punjab, Pakistan
Tomasz Kowalczyk, University of Lodz, Poland
Yarabin De Icaza, Faculty of Bioscience Engineering, University of Gent, Belgium