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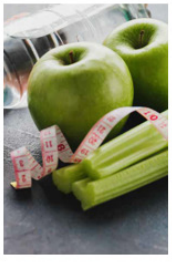
Proceedings | *Resumos*

III CBIOS Seminar

October 4, 2018

Auditorium Armando Guebuza

Universidade Lusófona de Humanidades e Tecnologias



Keynote lecture

Maria La Sallete Reis

LAQV – REQUIMTE , FFUP

Invited lectures

Ana Margarida Abrantes

CIMAGO, FMUC

André Baby

USP, Brasil

Daniel Santos

BioISI, FCUL

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III Scientific Conferences CBIOS
III Jornadas Científicas CBIOS

4 October | 4 Outubro
Lisboa - Universidade Lusófona

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Programa

4 October | *4 de Outubro*

Open Session | *Sessão de abertura*

Presentation CBIOS | Apresentação CBIOS
Luís Monteiro Rodrigues

Maria La Sallete Reis, LAQV - REQUIMTE; FFUP
Keynote Lecture

1st Session |*Sessão 1*

CBIOS Group PT (Pharmacology and Therapeutics)
Invited speaker
Ana Margarida Abrantes, CIMAGO, FMUC

Speakers / *Prelectores*
Margarida Alves, PT-CBIOS
Clemente Rocha, CBIOS (PhD Student)

R&D Lusófona Beyond CBIOS
Susana Santos, DREAMS, ULHT
Pedro Fernandes, IBB, DREAMS, ULHT
Stephane Besson, LAQV-REQUIMTE, FCT-UNL, ULHT

2st Session |*Sessão 2*

CBIOS Group DDS (Development Delivery Systems)
Invited speaker
André Baby, USP, Brazil

Speakers / *Prelectores*
Pedro Fonte, DDS-CBIOS
Rita Caparica, CBIOS (PhD Student)

3st Session |*Sessão 3*

CBIOS Group FSP (Food Sciences and Phytochemistry)
Invited speaker
Daniel Santos, BioISI, Biosystems and Integrative Sciences Institute, FCUL

Speakers / *Prelectores*
António Raposo, PFS-CBIOS
Joana Andrade, CBIOS (PhD Student)

Open Session

Presentation CBIOS | Apresentação CBIOS
Luís Monteiro Rodrigues

1st Session | Sessão 1
CBIOS Group PT (Pharmacology and Therapeutics)

Keynote Lecture

C.01 - Molecular Biophysics contribution for Innovative therapies and delivery strategies

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Abstract / *Resumo da Comunicação*
Many natural bioactive compounds are described as potentially effective for many conditions, including cancer, diabetes, cardiovascular and cerebrovascular diseases and inflammatory illnesses. However, the mechanisms by which many of these exert such diverse bioactivities and potential benefits as pharmacological agents remains unclear. Notwithstanding the possibility of having specific targets, natural bioactive compounds must interact and permeate through cell membranes in the body. Indeed, it was suggested that those molecules insert into the membranes and thereby may have an activity by changing the structural properties of lipid bilayers. In this context, this work focuses the use of biophysical approaches to measure the effects of bioactive compounds on critical membrane properties in order to correlate those effects with the attributed pharmacological actions.
The knowledge acquired from interaction studies can be used to design and develop efficient drug delivery systems. In fact, it is well known that the therapeutic effects of bioactive natural compounds are often hindered by the high concentration levels required. In this context, the development of nanodelivery systems to protect natural bioactive compounds from degradation, enhancing its bioavailability, for further use as supplements or nutraceuticals, is presented. The nanoformulations are based on lipid nanoparticles, namely solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). These are produced using a modified hot homogenization technique with no use of organic solvents, which promotes sustainability and is economically affordable to scale-up. The developed nanodelivery systems were physico-chemically characterized according to their surface morphology, entrapment efficiency (EE), average diameter, polydispersity index (PI), zeta potential, degree of crystallinity, and in vitro release studies in the shelf conditions of storage and gastrointestinal simulations. Additionally, biocompatibility, cellular uptake, intestinal permeability and transcytosis of lipid-based nanoparticles across the intestinal barrier were assessed. The developed nanosystems reveled to be a promising strategy for enhancing the efficacy of natural bioactive compounds.

Invited speaker/ *Prelector convidado*

Ana Margarida Abrantes

C.02 - Biophysics in metabolic radiotherapy using Radium-223

Ana Margarida Abrantes[1,2,3] Ana Salomé Pires[1,2,3], Inês Marques[1,2,3], Gonçalo Balteiro[1,4], Isabel Meireles[1,4], Maria Filomena Botelho[1,2,3]
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Abstract / *Resumo da Comunicação*
More than 120 years after the discovery of the emission of ionizing radiation by the atoms of some elements, with the possibility of being used in diagnosis and in therapeutics, the knowledge about the effects of this type of radiation on living beings is increasingly important. Biological effects can be classified into two types, direct effects and indirect effects, taking into account how the macromolecules, namely the deoxyribonucleic acid (DNA) molecule, are affected.
The direct effects are obtained mainly by the use of radiation composed of particles, such as alpha radiation, beta radiation and auger electrons. Radiation with high linear energy transfer have the potential to easily create severe cellular damage in biological systems, most of the time unable to repair these damages being cell death inevitable. Metabolic radiotherapy, a therapeutic approach using particle-emitting radionuclides, is currently one option for cancer treatment. Examples of these emitters β-particles used in the clinic are Iodine-131, Samarium-153, Lutécio-177, Yttrium-90, Strontium-90 and Radio-223, this particle emitter.
Clarification of the molecular mechanisms underlying the use of metabolic radiotherapy using Radio-223 was the primary cause of the development of a set of experimental work.
The mechanism of transport and the factors involved in the kinetics of this radiopharmaceutical are other important aspects capable of providing relevant information in optimizing the use of Radio-223. Despite the proven increased survival of patients receiving Radio-223 therapy, side effects to this therapy are also reported. The study of the biological effects of this alpha emitter on the level of genotoxicity, cellular proliferation, protein synthesis and cell viability with characterization of the type of predominant cell death allowed a better understanding of the effects of this therapy.
The study of the effects of this radionuclide on different cancer cell lines, such as human cell lines of metastatic prostate cancer of different stages, in addition to normal prostate cells, clarify the possibility of extending its clinical use.
Data obtained suggest that this therapy may be extended to other malignant tumors as well as justify its use in other stages of prostate cancer. In addition, and taking into account all the criteria that must be considered for the administration of Radio-223, it may also be relevant to study the effect of the combination of this radiopharmaceutical with other drugs. In this context, it is especially important to associate with other chemotherapeutics and with drugs used in pain therapy. Our results point to a synergistic effect with some cytostatic / cytotoxic drugs and some drugs for pain.
Acknowledgments: CNC.IBILI UID/NEU/04539/2013; POCI-01-0145-FEDER-007440. Centro Hospitalar e Universitário de Coimbra
References: Marques, IA et al. Cancer Treat Rev. (2018) 68:47-54..

Speakers / *Prelectores*

C.03 - The Veterinary Sciences research at Lusófona University

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Abstract / *Resumo da Comunicação*
The main goal of Faculdade de Medicina Veterinária at Universidade Lusófona de Humanidades e Tecnologias (FMV-ULHT) is to generate knowledge and contribute to the development of Veterinary Sciences, in its various areas of intervention: Medicine and Pathology, Animal Production, Public Health and Food Safety.
Presently, several projects are being developed as a result of collaborations with other Research Centers and Universities, where, wherever possible, students are encouraged to take part, in particular those who are doing the Curricular External Training, thus developing research leading to the elaboration of their Integrated Master Dissertation.
The presentation will include a brief presentation on some research projects in Veterinary Sciences taking place at FMV-ULHT:
- "Histological evaluation of the effect of insect diet on chickens", in collaboration with Entogreen and INIAV.
- "Three-dimensional movement analysis for rehabilitation and training for high performance in equine athletes" (PTDC / CVT-CVT / 32613/2017), in collaboration with CICANT and Movlab/ULHT.
- "Searching for the origins and evolution of the dog in Iberia and North Africa", in collaboration with the School of Communication, Architecture, Arts and Information Technologies, LABTEC (ULHT) and the Archaeological Laboratory (LARC) / DGPC and CIBIO / InBIO - ArchGen and EnvArch groups.
- "Anaplasma phagocytophilum: repercussion of wild animals' infection in dogs and cats of an endemic area. Potential for implication in Public Health ", in collaboration with the Center for Vector and Infectious Disease Studies (CEVDI) of the National Institute of Health Dr. Ricardo Jorge - INSA.
- "Follicular dynamics of the female dog and cat.", in collaboration with the Center for Interdisciplinary Research in Animal Health (CIISA), FMV-UL.
- "Bone involvement in stomatologic oncology of dogs and cats", in collaboration with the University of Trás-os-Montes e Alto-Douro and the University of Bern.
- "Characterization of the ovarian inflammasome of obese females", in collaboration with the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland.
- "Evaluation of the prognostic potential of the identification of microcalcifications in mammary tumors of the female dog and cat", in collaboration with Évora University.

C.04 - Does ageing influences the hindlimb response to massage in human?

Clemente Rocha[1], Henrique Silva[1,2], Hugo Ferreira[3], Ana Macedo[1], and L. Monteiro Rodrigues[1,2]

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Abstract / *Resumo da Comunicação*
Non-invasive optical technologies, such as laser Doppler flowmetry (LDF) and photoplethysmography (PPG) are robust and reliable non-invasive instruments to study human microcirculation. In this study, we access the response of older healthy volunteers to massage, and its physiologic implications.
Two groups, one of 34 young healthy volunteers (19.86 ± 1.58 years old) and 9 older, also healthy volunteers (55.22 ± 6.04 years old), both sexes, were submitted to two massage protocols (applied on the ascending and descending direction) in one randomly selected limb, using the contralateral as the control. Each protocol consisted in three phases - resting (I), massage (II) and recovery (III). PPG and LDF signals were registered in both feet (toes) and decomposed by the wavelet transform (WT) ($p<0.05$).
All features were similarly revealed by PPG and LDF. The younger group evidenced an increase of blood flow in both limbs (massaged and non-massaged) during massage. The same was observed in older group although with lesser expression and not so obvious in the control limb. In any case a similar homeostatic control, probably of central origin, might be evoked to reestablish the local circulation setpoint.
By WT analysis we can see that, with the younger volunteers, the myogenic and sympathetic components seem to vary most with massage. In the older group focus stays in the sympathetic and the endothelial components. This study suggests that microcirculatory homeostasis is influenced by aged and that the WT helps to look deeper into the potential mechanisms here involved.
This study was supported by FCT – Fundação para a Ciência e a Tecnologia, IP, project UID/DTP/04567/2016.

R&D Lusófona Beyond CBIOS

C.05 - Using DNA testing to Improve Wellness

Susana Santos[1]

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Abstract / *Resumo da Comunicação*
I: Health DNA testing has come a long way in the last decade. Factors such as increasing incidence of genetic diseases, increasing awareness of personalized medicine and the decreasing prices of DNA sequencing are driving the demand for DNA analysis.
The integration of the human genome project with health and wellness outcomes has led to the emergence of gene-based diagnostic tests, gene-drug interaction tests (pharmacogenetics) and more recently to gene-diet interaction tests (nutrigenetics) and gene-exercise interaction tests (athletic performance) [1, 2, 3,4].
M: DNA testing requires a sample of DNA, usually extracted from a saliva sample, and the genotyping analysis by using a microchip. The microchip target specific genetic polymorphisms (Single Nucleotide Polymorphisms, SNPs) that are compared to reference databases. An approach for converting genetic data to a predictive measure is to aggregate the contribution of multiple SNPs related to a specific feature. Machine-learning algorithms based on scientific evidence related to SNPs-effect-intervention are applied for the predication of an individual's response to certain drugs, susceptibility to nutrients or even to enquire the potential for certain types of sports [5].
D: When people have access to their genetic information, along with insight into how it affects health, it is possible to make better decisions for the future. For instance, by using a pharmacogenetics test it is possible to prescribe safe and effective drug dosages. A nutrigenetics test will help to understand how to manage an ideal and healthy weight. Nutrigenetics could reveal carbohydrate and fat sensitivities, the need for an increased ingestion of PUFA or antioxidants, or the predisposition for a high Body Mass Index (BMI) or insulin resistance. A genetic test to determine the athletic potential provides information to understand if the body is predisposed to get great results from high-intensity cardio workouts or if prolonged aerobic exercise is a better choice. All of these tests are focused on providing individuals with useful health-related information. Hence, by learning more about our unique genetic composition, it is possible to take steps towards living a healthier life [1, 3-6].
References:
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[2] Grimaldi K.A. et al., Genes & Nutrition (2017) 12:1
[3] Yael T. J. and Christine A. H., Curr Oncol Rep (2016) 18:43.
[4] Papadimitriou et al., BMC Genomics (2016) 17:285.
[5] Guillaume P. et al., Scientific Reports (2017) 7: 12665.
[6] Wang T. et al., BMJ (2018);360:j5644

C.06 - Microscale tools for rapid evaluation of biocatalytic systems

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Abstract / *Resumo da Comunicação*
The introduction of miniaturized tools has been contributing to improve the competitiveness of bioprocesses. The ability to rapidly screen bioconversion reactions for characterization and optimization is a key asset in biocatalyst selection, definition of operational conditions and bioprocess development. Studies at lab-scale are typically time consuming, labor intensive and have a low throughput. The introduction of microscale tools has allowed high levels of parallelization, while requiring low amounts of consumables, and are prone to automation, therefore overcoming the limitatins of the traditional approaches. Microscale scale abridge different configurations and modes of operations, from microwell plates with diverse levels of complexity, e.g. fit with built-in sensors for on-line process monitoring, to mini- and microreactors, that ultimately operate in microfluidic environment. While the former are mostly fit for the early stages of biocatalyst selection and process characterization, the later also allow for a paradigm shift in production technology, by replacing conventional cumbersome scale-up strategies by scale-out/numbering up approach. The present work aims to provide an overview on the use of microscale tools in the development of biocatalytic systems, with their inherent advantages and limitations, supported by illustrative examples. These abridge different reaction models, all of them with practical application, such as whole cell biocatalysis for the production of corticoid intermediates, single enzyme catalyzed processes for the production of sweeteners, enzyme cascades and multi-enzyme systems for the deconstruction of waste biomass towards the production of fermentable sugars as carbon sources for microbial cell factories.

C.07 - Protein Biochemistry: Case-studies of proteins involved in the respiratory systems of microorganisms

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Abstract / *Resumo da Comunicação*
The scientific work performed to this date may be resumed as PROTEIN BIOCHEMISTRY:
This covered essentially the study of proteins involved in the respiratory systems of microorganisms, in particular the denitrification pathway, a key process of the Biological Nitrogen Cycle: this phenomenon is important regarding its applications (fight against pollution, biotechnology, agriculture) but also from a fundamental point of view (original catalytic centres, ecological importance of the biological nitrogen cycle). Enzymes and electron carriers are purified from bacterial cultures, and then characterized (enzymology, spectroscopy,...) [1-4]. Another aspect of the work deals with the proteomic studies of animal venoms. Fast evolving proteic components of venoms represent an excellent biological material for the study of structure/function relationships.
Present research interests:
1) Structure/function studies of metalloproteins involved in the respiratory system of microorganisms.
2) Biodiversity and structure/function studies of natural animal toxins
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[3] Correia, C. et al. J. Biol. Inorg. Chem., 13, 1321-1333 (2008).
[4] Brown, K. et al. Nat. Struct. Biol., 7, 191-195 (2000).

2st Session | Sessão 2
CBIOS Group DDS (Development Delivery Systems)

Invited speaker/ *Prelector convidado*

André Baby

C.08 - Suncare delivery systems: from development to performance evaluation

Camila Areais de Oliveira[1], Rafael Sauce[1], Tânia Santos de Almeida[2], Ana Sofia Fernandes[2], Maria Valéria Robles Velasco[1], Catarina Rosado[2], André Rolim Baby[1]

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Abstract / *Resumo da Comunicação*
Cosmetic product market and consumers demand a special dynamicity from Cosmetic industry to introduce new ingredients and delivery systems. They should present robust innovations that should be rapidly perceived by the users 1. Several physicochemical and functional properties of active substances may be expressively improved by using micro/nanotechnology platforms that will, directly or indirectly, influence the ingredient/product cosmetic attributes. In this work, we summarized some of the achievements of our Research Group that concerned the development and performance evaluation of suncare products.
Oliveira et al. (2016) developed rutin-loaded gelatin-based nanoparticles of 398±1 nm, as average size diameter; zeta potential of 24.6±0.6 mV; and 51.8±1.4% of active entrapment efficiency. A sunscreen system containing this ingredient increased 48% its Sun Protection Factor (SPF) in vitro determined. Pre-clinical and clinical tests claimed this nanostructure system as safe 2.
Graziola et al. (2016) developed gelatin-based microparticles crosslinked with rutin (average size of 19.7±1.6µm) and they were used as adjuvants in sunscreens. Distinctively from the nanoparticles obtained by Oliveira et al. (2016), the microparticles were not effective as a SPF booster. Their safety profile was evidenced by in vivo cutaneous compatibility assay (skin superficial hydration, redness and transepidermal water loss) 3.
Daneluti et al. (2017) synthetized ordered mesoporous silica type SBA-15 encapsulating/entrapping the ultraviolet B (UVB) filter ethylhexyl methoxycinnamate (EHMC). According to the differential thermal analysis, the UVB filter demonstrated higher thermal stability. Particles developed mean diameter of 15.7±0.5 µm and the entrapment efficiency, determined by thermogravimetric analysis, ranged from 33 to 48%. SBA-15 was considered a potential UV filter delivery system with high ability to stabilize the EHMC 4.
This study was financed by FAPESP 2016/24360-4 and in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.
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[2] CA Oliveira et al., 2016. Eur. J. Pharm. Sci. 81:1-9.
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2st Session | Sessão 2 (Cont.)
CBIOS Group DDS (Development Delivery Systems)

Speakers / *Prelectores*

C.09 - Applications of ionic liquids in drug delivery: a nanotechnological approach

Ana Júlio[1], Rita Caparica[1,2], Ana Rita Gonçalves[1], Sofia Costa Lima[3], Salette Reis[3], Tânia Santos de Almeida[1], Pedro Fonte[1,3]

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Abstract / *Resumo da Comunicação*
The development of new drug delivery systems is usually focused to overcome some drawbacks such as poor drug release and solubility, low physico-chemical stability and systemic side effects, in order to improve drugs bioavailability and enhance therapies. The ionic liquids are functional excipients that can help on solving problems such as poor drug solubility and stability [1]. In addition, the use of nanosystems made of biopolymers is a strategy to protect the loaded drugs and deliver it in a sustained, controlled or targeted manner, enhancing drug bioavailability and avoiding systemic side effects and toxicity. The exploitation of the synergic effects of ionic liquids and nanoparticles is a promising strategy to develop a multipotent drug delivery system. Using a modified w/o/w double emulsion technique, ionic liquid-nanoparticle hybrid systems were developed to encapsulate several drugs such as clofazimine and dapsone for leprosy treatment and rutin [2]. It was observed the ability of ionic liquids to increase the loading of those poorly soluble drugs, which ultimately improve their bioavailability and improve therapy. Also, the ability of ionic liquids to act as cryoprotectant upon freeze-drying of nanosystems was also explored, and it was observed that the ionic liquids were able to improve the stability of nanosuspensions upon freeze-drying. Finally, the effect of ionic liquids on the stabilization of therapeutic proteins encapsulated into nanoparticles is also addressed.
Acknowledgments: The authors would like to thank to Fundação para a Ciência e a Tecnologia, Portugal (FCT/MCTES (PIDDAC), UID/DTP/04567/2016) and also to COMPETE 2020 (PTDC/MEC-DER/32610/2017).
References:
[1] Santos de Almeida, T. et al., Drug Development and Industrial Pharmacy, 43 (2017) 1858-1865.
[2] Fonte, P. et al., Biomacromolecules, 15 (2014) 3753-3765.

C.10 - Choline-amino acid ionic liquids as green functional excipients for topical delivery of poorly soluble drugs

Rita Caparica[1,2], Ana Júlio[1], André Rolim Baby[3], Maria Eduarda Araújo[4], João Guilherme Costa[1,5,#], Tânia Santos de Almeida[1,#]

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# Both authors contributed equally to the senior authorship
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Abstract / *Resumo da Comunicação*
The development of alternative and efficient forms to deliver poorly soluble drugs remains a problem. In this context, ionic liquids (ILs), due to their specific properties, may be useful by acting as functional excipients in drug delivery systems to improve the solubility of sparingly soluble drugs. Nonetheless, to determine their usefulness it is vital to evaluate not only the influence of the ILs and the drugs individually, on cell viability, but also the co-treatment with the systems ILs-drug.
Hence, two choline-amino acid ILs were prepared and their usefulness as functional excipients was assessed by evaluating their influence on the solubility of two poorly water soluble, ferulic acid and rutin, while considering both safety and efficacy.
Results showed that in the presence of the ILs, the solubility of both compounds under study, was always higher than in water. Additionally, [Cho][Phe] proved to be the best solubility promoter. Further studies showed that the studied ionic liquids did not affect significantly the cell viability or the radical scavenging activity (RSA) of the drugs.
Stable O/W emulsions, in the presence of the ILs, were prepared allowing a significantly higher drug loading.
Thus, this study revealed the usefulness of choline-based ILs as green functional excipients, since, at nontoxic concentrations, they improve drug solubility and allow a higher drug loading in topical formulations without affecting the potential effect of the studied drugs.
Acknowledgment: This work was financially supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal), through funding UID/DTP/04567/2016 to CBIOS. Part of the work was also supported by FCT to CQB through project UID/MULTI/00612/2013.

3st Session | Sessão 3
CBIOS Group FSP (Food Sciences and Phytochemistry)

Invited speaker/ *Prelector convidado*

Daniel Santos

C.11 - Targeting Cancer: New Findings to Solve Old Problems

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Abstract / *Resumo da Comunicação*
At least 11 transporters of the ABC family have well-characterized roles in human diseases while others are expressed in many tissues and appear to be particularly related with multidrug resistance (MDR) in cancer. Although multifactorial in nature, multidrug resistance to chemotherapy regimens can be achieved by selecting phenotypes that over-express ABC transporters. The over-expression of P-glycoprotein (P-gp/ABCB1) was found to be marker of overall poor chemotherapy response and prognosis in various cancers.
P-gp is a single polypeptide of ~170 kDa. It effluxes a wide-range of substrates out of the cell through an ATP-dependent mechanism and is found at the apical surface of kidney proximal tubule cells, canalicular membrane of hepatocytes, pancreas, villous intestinal cells, and blood–tissue barriers (e.g., brain, placenta, testis).
We developed a new research line to discover the main physicochemical features responsible for modulation through a new pharmacophore/QSAR1 with a better classification capability and with the appearance in 2009 of the murine P-gp structure, this structural information was used through simulations to study the dynamics of the transporter, how drug efflux occurs and how drug adsorption may affect P-gp activity. The characterization of three drug binding sites was achieved by matching experimental information with extensive docking results to unravel elusive drug/P-gp recognition, interactions and modulation mechanisms. A computational classification scheme was proposed to organize molecules in different class types (modulators, substrates, non-substrates). The access of drugs to the drug-binding pocket through a hypothesized gate was also studied.2
Unfortunately, all P-gp modulators entering phase III clinical trials failed by showing a dramatic increase in cellular toxicity (tariquidar) or reduced in vivo effectiveness (zosuquidar, laniquidar). Thus, the problem resides elsewhere, namely in the polyspecificity of the binding sites. All these findings will be reviewed, discussed and linked with very recent findings as new emerging strategy to overcome the problems found in clinical trials.3 Finally, applications and relevance of computer modeling in other areas of Science than Pharmacology like Food Science, Toxicology and Phytochemistry will be presented and discussed.
Acknowledgements: We thank Fundação para a Ciência e Tecnologia for financial support (PTDC/QEQ-MED/0905/2012, UID/DTP/04138/2013). This work also received financial support by national funds, and was co-financed by the European Union (FEDER) over PT2020 Agreement (projects UID/QUI/50006/2013 and POCI/01/0145/FEDER/007265).
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Speakers / *Prelectores*

C.12 - Edible jellyfish: safety, chemical and sensory properties

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Abstract / *Resumo da Comunicação*
People's preference for fish with a high trophic level, like Atlantic cod and tuna, leads to a large food footprint. Responsible seafood consumption should include underutilised local products; hence the culinary use of edible jellyfish can be an effective contribution. The present work focused on Catostylus tagi to contribute to the consumption of edible jellyfish in the West.
A questionnaire conducted with 192 young people showed an interest in tasting jellyfish-based food (64.6%). The resulting product, obtained by an alternative cooking process to traditional Asian ones, was chemically characterised and underwent microbiological and heavy metals control. The results indicated its non-toxicity. Patients who were allergic to seafood as well as non-allergic volunteers revealed no allergic reaction to the jellyfish umbrella product (intakes up to 5 mg/kg body weight and 8 mg/kg, respectively). Seafood-trained panellists defined the product's main impact on the mouth as freshness (72 mg/kg body weight). The preliminary snack, a pâté, was positively accepted by allergic (7 in 9; n = 20) and non-allergic volunteers (6 in 7; n = 21).
The present study confirmed that jellyfish intake is safe, even for allergic individuals, and its organoleptic properties were accepted by the study population.

3st Session | Sessão 3 (Cont.)
CBIOS Group FSP (Food Sciences and Phytochemistry)

C.13 - Cytotoxicity in human lung cancer cells of natural halimane and labdane diterpenes by mitochondrial dysfunction

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Abstract / *Resumo da Comunicação*
Medicinal plants of the Plectranthus genus are a valuable source of bioactive natural products such as diterpenes (1,2). Mitochondrial dysfunctions have been associated with several malfunctions such as increase of reactive oxygen species (ROS) and uncontrolled Mycobacterium tuberculosis replication (3,4). The electrochemical gradient produced by mitochondria generates the mitochondrial membrane potential (MMP), which is a key parameter for evaluating mitochondrial function (4).
Previous works have reported the cytotoxic activity and have proven to be interesting antitubercular agents against Mycobacterium smegmatis (2,5).
Since the coexistence of tuberculosis and lung cancer has remained controversial since the middle of the 19th century, one halimane (HAL: 11R*,13E)-11-acetoxyhalima-5,13-dien-15-oic acid) and two labdane diterpenes (PLEC: Plectrornatine C and the MRC: 1:1 mixture of 1,6-di-O-acetylforskolin:1,6-di-O-acetyl-9-deoxyforskolin), previously isolated from P. ornatus Codd. (1,2), were evaluated for their cytotoxic effect against human lung adenocarcinoma cells (A549 cell line) and for the mechanisms of cell death associated with mitochondrial dysfunction.
The ROS level and evaluation of the MMP revealed that only HAL decreased mitochondrial membrane potential. In conclusion, the halimane diterpene (HAL) seems to particularly have a cytotoxic effect associated with mitochondrial dysfunction on lung cancer cells that may be further evaluated on the uncontrolled Mtb replication mechanism.
To the best of our knowledge, this is the first report of these activities for the halimane and labdane diterpenes under study.

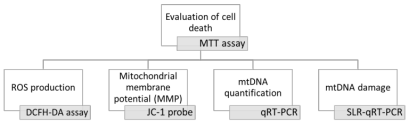

Figure 1 Schematic representation of the studies performed herein, regarding the evaluation of cell death through mitochondrial dysfunction.

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Panel Comunications | Comunicações em Posters

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P1 - Ionic liquids-polymer nanoparticles hybrid systems as tools to deliver poorly soluble drugs

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Two major concerns in drug delivery are poor drug solubility and low skin permeation, which are particularly important for poorly soluble drugs. Hence, it is vital to develop new strategies to overcome these challenges. In this context, the encapsulation of drugs into nanoparticles may be a suitable approach to avoid such problems, at the same time that allow a targeted or controlled manner [1]. Ionic liquids (ILs) may be another useful tool since they are salts, weakly coordinated, liquid below 100 ºC and may act as functional excipients to enhance drug solubility and permeation [2]. Consequently, the combination of nanocarriers and ILs may be an interesting approach on the delivery of poorly soluble drugs. Therefore, this work has as aim the development an IL-polymer nanoparticle hybrid system as a new strategy to deliver a poorly soluble drug, rutin.
Poly(lactic-co-glycolic acid) (PLGA) 50:50 or 75:25 were used to produce nanoparticles by a modified solvent-evaporation W/O/W double emulsion technique [3]. The inner phase was an aqueous solution of 0.2 %(v/v) of a choline-based IL [2], (2-hydroxyethyl)-trimethylammonium-L-phenylalaninate [Cho][Phe] or (2-hydroxyethyl)-trimethylammonium-L-glutamate [Cho][Glu], dissolving rutin to its maximum solubility. This phase was also prepared at pH 6.7, the isoelectric point of rutin [4]. The physico-chemical proprieties of the hybrid nanosystems were evaluated and drug association efficiency (AE) was also performed. Nanoparticles without pH adjustment had a diameter of 250-300 nm with acceptable polydispersity index (between 0.2-0.4) and good colloidal stability (-35 to -45 mV). However, when the inner phase was adjusted to pH 6.7, those systems showed a significant enhancement in particle size while maintaining good PdI and zeta potential results. Concerning the AE, [Cho][Phe] allowed a superior AE (75 %) then [Cho][Glu] (50 %), with no relevant differences found between PLGA ratios in the formulations without pH adjustment. In the hybrid systems with the inner phase at pH 6.7, there is an improvement on the AE with [Cho][Glu] to 60 %. These results demonstrated the potential of the IL-PLGA nanoparticles hybrid systems to deliver poorly soluble drugs.
Acknowledgments: The authors would like to thank to Fundação para a Ciência e a Tecnologia, Portugal (FCT/MCTES (PIDDAC), UID/DTP/04567/2016) and also to by COMPETE 2020 (PTDC/MEC-DER/32610/2017). A special acknowledgment is given to Professor André Rolim Baby for providing rutin.
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P2 - Ionic liquids as tools to improve the leprosy treatment

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Clofazimine and dapsone are drugs widely used in the treatment protocols of leprosy. Ionic liquids (ILs) are salts that are liquid below 100 ºC and may be placed in water, oils or hydroalcoholic solutions for pharmaceutical purposes, mainly due to its ability to improve the solubility of poorly soluble drugs [1]. Additionally, the encapsulation of such drugs into nanoparticles may be a good strategy to improve their bioavailability [2]. In this work, ILs, 1-butyl-3-methylimidazolium bromide [C4mim][Br], 1-hexyl-3-methylimidazolium bromide [C6mim][Br], (2-hydroxyethyl)-trimethylammonium-L-phenylalaninate [Cho][Phe] and (2-hydroxyethyl)-trimethylammonium-L-glutamate [Cho][Glu], were studied as solubility promotors of the poorly soluble clofazimine and dapsone. Therefore, IL-polymer nanoparticles hybrid systems were developed to deliver those drugs.
The solubility studies were performed in water and in water:IL mixtures at room temperature for 72 hours. The ILs were used at concentrations where cell viability is maintained [1]. It was observed that, for both drugs, [Cho][Phe] was the best solubility enhancer, and particularly important for clofazimine, where a 10-fold improvement was obtained.
Poly(lactic-co-glycolic acid) (PLGA) 75:25 nanoparticles were produced by a modified solvent-evaporation W/O/W double emulsion technique [2]. In the inner phase was incorporated an aqueous solution of 0.2 % (v/v) of IL [1], dissolving each drug to its maximum solubility, and the system was physico-chemically characterized.
The nanoparticles had close diameter with around 580 nm for clofazimine and 640 nm for dapsone. Additionally, polydispersity index (PdI) was around 0.3 for both drugs, which is the recommended value of PdI for delivery nanosystems [2]. Regarding the poor solubility of the drugs, the AE of 64.0 ± 4.0 % and 58.6 ± 10.0 % for clofazimine and dapsone, respectively are promising results that prospects the ability of the hybrid IL-nanosystems to improve the bioavailability of the drugs and the leprosy treatment.
Acknowledgments: The authors would like to thank to Fundação para a Ciência e a Tecnologia, Portugal (FCT/MCTES (PIDDAC), UID/DTP/04567/2016).
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P3 - Comparison of the Neutropenic Diet with the Normocaloric Diet in Hospital Environments

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The neutropenic diet consists of a diet low in microorganisms, it is composed of foods that always undergo any method of confection, except for the grilled method, that is, they are not allowed raw foods like salads and raw fruit. In addition to these, the consumption of sausages and squids is also not allowed [1,2]. The diet under study is mainly prescribed to patients who have compromised immune systems, such as oncology patients. With dietary restrictions, the patient's diet contains a lower microbial load, thus reducing the risk of infection [3].
The present study was carried out at the ITAU unit of the São José Hospital, in the months of April and May of 2018. This consists in comparing the neutropenic diet with the normocaloric diet in the existing four-week rotating table. The data obtained were analyzed with the objective of verifying the differences between the diets in question, which patients had a prescription of the neutropenic diet and for what reason, and also the proportion of meals served in relation to the other diets offered at the hospital. It was concluded that the neutropenic diet represents only 4% of the diets served, which is due to its specificity for the Hematology service due to the unique needs of the patients in question. Soup and dessert do not differ between diets compared. The meat and fish dishes are mostly the same (87% and 80%), presenting differences only when the grilled confection method is used or salad is served to accompany. For fruit, unlike what happens in the normocaloric diet, there is no choice between raw and cooked or roasted fruit, always being made. It was found that most of the dishes present in the menu are the same in the diets compared. This is because, in the ITAU HACCP Handbook, there are already some foodstuffs that are not allowed, and hygienic care is compulsory so that food safety can be guaranteed.
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P4 - Exploitation of cryoprotectant properties of ionic liquids on the PLGA nanoparticles stabilization upon lyophilization

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Ionic liquids (ILs) are salts, with an organic cation and organic or inorganic anion, liquid below 100 ºC and even at room temperature and may act as water substitutes [1]. So, they could be used as functional excipients and/or cryoprotectants in the lyophilization of nanoformulations mitigating freezing stress [2]. The aim of this work was the evaluation of ionic liquids as cryoprotectants on the stability of polymer nanoparticles after lyophilization.
BSA-loaded poly(lactic-co-glycolic acid) (PLGA) 50:50 nanoparticles were produced by a modified solvent-evaporation W/O/W double emulsion technique [2]. As cryoprotectants, two choline-aminoacid ILs, (2-hydroxyethyl)–trimethylammonium-L-phenylalaninate [Cho][Phe] and (2-hydroxyethyl)–trimethylammonium-L-glutamate [Cho][Glu], were used at 0.1, 0.2, 5 and 10 % (w/v). The nanoformulations were characterized regarding its diameter and association efficiency after and before the freeze-drying.
Results showed that ILs at 0.1 and 0.2 % (w/v) maintained better physico-chemical properties of the nanosystems when compared with nanoparticles without cryoprotectants, which was a promising result. To assess such property, it was tested a higher IL concentration with 5 and 10 % (w/v). These ILs concentrations demonstrated that the nanoparticles kept its native features, particularly in the presence of [Cho][Phe].
This study showed that ILs may be used as cryoprotectants, since they indicated the ability to protect nanoparticle systems upon lyophilization. The developed system may be used to stabilize the nanoformulations after freeze-drying and, consequently, maintained the stability of encapsulated conventional drugs and biopharmaceuticals.
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[1] Santos de Almeida, T. et al., Drug Development and Industrial Pharmacy, 43 (2017) 1858-1865.
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P5 - Development of drug-loaded PLGA nanoparticle hydrogel for treatment of ulcers

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Hydrogels offer excellent potential as therapeutic systems due to inherent biocompatibility, diversity of both natural and synthetic material options and tuneable properties. In this work we developed a multipotent hydrogel carrying poly-lactic-co-glycolic acid (PLGA) nanoparticles to deliver drugs for ulcers treatment. PLGA nanoparticles were prepared by a solvent evaporation double emulsion technique previously developed by our group [1]. The hydrogel was produced by adding 1.5% (w/v) of sodium alginate to the nanoparticles and cooled at -20ºC for 12 hours. Then, the hydrogel thawed for 4h. The particle size and the rheological properties of the hydrogel were evaluated. The PLGA nanoparticles had 272±12 nm and a PdI of 0.16±0.01. Nanoparticles size and polydispersity increased after hydrogel production into 622±66 nm and PdI of 0.32±0.01. Viscosity increased from 500 mPa to 12.000 mPa after freeze-thawing. The increase in size is desirable to prevent particle permeation and the increase in the viscosity is expected to improve drug delivery due increased residence time and mucoadhesivity. The developed may be a promising system to deliver several drugs for the treatment of ulcers.

Acknowledgments:
The authors would like to thank to Fundação para a Ciência e a Tecnologia, Portugal and COMPETE 2020 (PTDC/MEC-DER/32610/2017) for financial support.

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P6 - Exploring texture analysis to improve skin sonography image analysis

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Skin sonography is a useful imaging tool to assess skin structure, both in healthy and in compromised skin (e.g. lesions, edema, tumors, etc). However, the image interpretation is a difficult process since it largely depends on the observer's experience, and is further complicated by the limited image resolution of commercial scanners [1]. Texture analysis (TA) offers a range of analysis tools that assess the spatial variation and distribution of gray levels within a given image and translates them into quantitative parameters [2]. Our aim was to explore the TA tools to quantify and compare different skin layers. Images from the volar forearm were taken using a 20-MHz ultrasonography (Dermascan C, Cortex Technology, Denmark) in 6 young healthy volunteers (aged 18-30) of both sexes. We compared non-treated skin with hydrated skin with a 5% solution of glycerin under occlusion for 24 hours. All procedures complied with the principles of the Helsinki declaration and subsequent amendments. The probe was placed on the skin in a fixed standard position, with the echo recorded at a velocity of 1580 m/s, to obtain a two dimensional-image. The color image was converted to a grey scale image for further analysis. TA parameters (entropy, contrast, correlation, energy, homogeneity) were calculated from the epidermis and dermis selected regions of interest. Entropy, contrast and homogeneity were higher in the more echogenic epidermis, while correlation and energy were higher for the more homogenous dermis. The echo, entropy, contrast and correlation were found to be consistently lower on both layers of the hydrated skin, while energy was higher. Finally, homogeneity decreased in the hydrated epidermis, but increased in the dermis. These results suggest that TA is useful to quantitatively describe the differences in skin layers based on their echogenic profile, justifying further studies.

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P7 - Physical activity and nutritional status of users of nutrition appointments

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INTRODUCTION: Currently, obesity and sedentary lifestyle are relevant problems for public health that contribute to the appearance of several diseases and a reduction in the quality of life. The aim of this study was to analyse physical activity and nutritional status of users of nutrition appointments.
METHODS: This study included 43 patients of both genders (83.7% females) and ages between 18-71 years, with an average age of 39.6 (±15.0) years old. Weight (kg) and height (cm) were evaluated using standards procedures and the body mass index (BMI) (kg/m2) was calculated. To evaluate the nutritional status it was used the World Health Organization (WHO) classification using BMI.
To evaluate the physical activity the International Physical Activity Questionnaire (IPAQ) was applied and the WHO recommendations used.
RESULTS: More than half of our population shows to be sedentary (53.4%), while 20.9% show moderate activity and 25.6% are vigorous practitioners. Relatively to the nutritional status, about 60.4% is obese or overweight, while 34.9% were within the normal range and only 4.7% were underweight. We also verified that 78.2% of the sedentary individuals were overweight or obese and 81.8% of subjects with vigorous activity had an adequate weight.
CONCLUSION: The high percentages of sedentary individuals and with overweight or obesity show the need to promote healthy eating habits and physical activity.

P8 - Antibacterial activity of a supercritical fluid extract of Myrtus communis L. for topical pharmacological formulations

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Myrtus Communis L. is an evergreen shrub belonging to the Myrtaceae family that grows wild around all the Mediterranean region, Portugal included. Many medicinal and nutraceutical properties have been attributed to myrtle, which has been used since ancient times in folk medicine. It is traditionally used as an antiseptic and wound-healing, disinfectant, hypoglycaemic agent, with anti-hemorrhagic, antimicrobial and antioxidant properties. The antibacterial activity of an extract of myrtle obtained by supercritical fluid extraction has already been ascertained [1] but in this study, we are testing the supercritical fluid extract of myrtle against different bacteria. The extract was obtained using supercritical CO2 at 230 bar, 45ºC and a flow of 0,3 kg.h-1 and using a co-solvent, ethanol, at a flow rate of 0,09 kg.h-1. This study aims to investigate the applications of supercritical myrtle extract as a possible antimicrobial ingredient in cosmetic, pharmacological or functional food formulations.
The conventional well diffusion and the broth microdilution method was employed for assessment of antibacterial potential of the myrtle extract against three gram positive bacteria: Enterococcus faecalis (ATCC® 29212), methicillin-resistant Staphylococcus aureus (MRSA ATCC® 33591) and Propionibacterium acnes (ATCC® 6919).
Results clearly demonstrated that myrtle extract has antimicrobial activity against all tested microorganisms. The minimum inhibitory concentration (MIC) and inhibition zones values obtained showed significant inhibitory effect against the gram-positive bacteria tested.
The tested extract, sourced from a wildly available plant, seems, then, a promising low-cost antimicrobial agent. Further studies will be conducted to address their potential use as a functional ingredient to be employed in cosmetic, food or pharmaceutical industries.

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P9 - Abietane diterpene 7α-Acetoxy-6β-hydroxyroyleanone from Plectranthus hadiensis: Isolation and cytotoxicity study

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The Plectranthus spp. plants are the focus of several scientific investigations, due to their ethnopharmacological use by indigenous populations. The Plectranthus genus is a known source of bioactive diterpenes with antitumor potential [1]. Abietane diterpenoids display an array of biological activities including cytotoxic and antiproliferative activities against human tumor cells. [2].
In this work, we evaluated the general toxicity of acetonic extracts of sixteen Plectranthus spp. The extracts of the different Plectranthus spp. were obtained by sonication (10% (w/v) of dry plant and the highest yield obtained was from P. mutabilis (30 %, w/v). The general toxicity was initially screened using the Artemia salina (brine shrimp) lethality assay and the LC50 determined. P. swynnertonnii was the most toxic using A. salina model (0.036 µg/mL). The antitumor potential of the most toxic extracts was further explored in different cancer cell lines: colon colorectal carcinoma (HCT116), human breast adenocarcinoma (MCF-7) and lung cancer carcinoma (NCI-H460). The results showed that P. hadiensis with IC50 values 3.45±0.35, 2.9±0.10 and 3.00±0.10, respectively, was the most cytotoxic.
The cytotoxic P. hadiensis extract was purified by preparative chromatography (silica gel; n-hexane/AcOEt; 8:2) to afford 7α-acetoxy-6β-hydroxyroyleanone. The royleanone compound was structural elucidated based in physicochemical data (melting point), spectroscopic data (1D- and 2D-NMR experiments) and comparison with bibliographic data. This cytotoxic compound showed significant selectivity towards cancer cells (NCI-H460 sensitive non-small cell lung carcinoma cell line; NCI-H460/R multidrug resistant non-small cell lung carcinoma cell line with P-glycoprotein overexpression; MRC-5 normal human embryonal bronchial epithelial cells) ranging IC50 values of 2.7-8.6 µM.
This compound is the potential responsible for the cytotoxicity of the extract, however other compounds or its synergetic effect could account for the extract cytotoxicity. More studies are ongoing to unveil the cytotoxicity of this extract.
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P10 - Assessing lower limb microvascular reactivity to dynamic posture changes by the Tissue Viability Imaging® system – a preliminary approach

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Postural changes are among the most commonly used tests to assess microvascular reactivity [1]. These changes frequently implicate movement of the assessed region, leading to artefacts on the recorded physiological signals, rendering them unusable. Recently, the Tissue Viability Imaging® system (TiVi) was introduced for the quantification of the concentration of moving red blood cells (CMBC) over large skin areas, with the advantage of not being affected by movement [2]. Our aim was to assess the usefulness of the TiVi system for the assessment of lower limb perfusion indifferent postures and during dynamic postural changes. After giving their informed consent, five (26.0±6.5 y.o.) young healthy subjects performed the following three protocols: (A) lying supine for 5 min; (B) sitting upright for 3 min then standing up straight for another 3 min; (C) standing up straight for 3 min, then performing a 15 repetitions of squatting exercise for 1 min and returning to the initial position for a further 3 min. CMBC was higher during sitting and standing postures, which relates to differences in blood distribution due to gravity. On protocol B, standing up caused a bilateral perfusion increase, although with considerable variability between feet, which might be related to an unequal load distribution, possibly affecting the intra-tissue pressure at ankle level. On protocol C, squatting exercise promoted a bilateral perfusion increase, comparable between limbs. TiVi seems to be a useful system to further explore the possible effect of posture and intra-tissue pressure on the lower limb microcirculation.
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P11 - Anti-tumor properties of Parvifloron D in breast cancer cells

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Medicinal plants are an abundant source of new bioactive compounds with anticancer activity. Parvifloron D (ParvD) is an abietane diterpenoid isolated from Plectranthus ecklonii acetonic maceration extract. ParvD has previously shown cytotoxic and pro-apoptotic effects in leukemia and melanoma models. However, to the best of our knowledge, there are no studies in breast cancer models. Breast cancer is a leading cancer type in women in the developed countries accounting for approximately 20% of female cancer deaths and has a complex and heterogeneous pattern. Triple negative breast cancer (TNBC) is generally more aggressive and is associated with weak overall therapeutic response and poor clinical outcomes. Herein, the anticancer effect of ParvD was evaluated in MDA-MB-231 cells, a model of human TNBC. ParvD (0.1–10 µM) decreased cell viability in a concentration-dependent manner (crystal violet assay). Treatment ParvD (5 µM) significantly increased the percentage of apoptotic nuclei (DAPI staining). Cell exposure to 3 µM ParvD increased the sub-G1 population (flow cytometry). Treatment with ParvD (1 µM) had no effect on cell-substrate attachment when cell detachment was induced with EDTA. As cell migration and invasion are determinant processes for the formation of metastases, a transwell assay was carried out. ParvD (1 µM) significantly reduced cell chemotaxis and invasion. In summary, the natural compound ParvD showed interesting anticancer properties, warranting further studies towards a potential therapeutic application.
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P12 - Local microvascular changes detected by Tissue Viability (TiVi®) imaging during an isometric calf contraction in human

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The calf muscle pump activity is a known determinant of peripheral circulation on the lower limb. The recently developed Tissue Viability Imaging® System (TiVi, Wheels Bridge, Sweden) is a non-contact method for assessing microcirculatory viability even during movement, and over wide areas. This study aims to test this system during active isometric plantar flexion in the standing position. Four healthy subjects (31± 9 years old) participated in this study after giving informed consent. All performed a protocol with isometric plantar flexion, divided into 3 phases - standing upright for 5 minutes (phase I), isometric plantar flexion for 1 minute (phase II), followed by a recovery period of 5 min in the initial position (phase III). We evaluated pulse rate (PR) with photoplethysmography (PPG) probe placed on the second finger of the right hand and the Concentration of Moving Red Blood Cells (CMBC) of the dorsum of both feet (ankle joint area). The 4 subjects had stable CMBC values during phase I (209.3 ± 16.0) and during recovered in phase III (209, 98 ± 9,6). In phase II the CMBC decreased (166.6 + 22.8). PR did not change during the protocol. These results are in line with previous studies published on these issues. The TiVi is useful for the quantification of the microvascular response to isometric tension, and can be used to explore movement related microcirculatory behaviour. This study was supported by FCT – IP project UID/DTP/04567/2016.

P13 - The human Golgi anti-apoptotic protein induces cancer cell invasion by an H2O2-dependent mechanism

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The severity of most cancer types is related to their ability to spread and invade to other parts of the organism. The pharmacological approaches for oncological diseases rely mainly in anti-proliferative drugs and only few and poorly effective strategies are available to control cancer spread. Therefore, it is essential to understand the mechanisms involved in cancer cell spread so that therapeutic strategies can be improved.
Human GAAP (hGAAP) is a novel highly conserved Golgi cation channel that modulates Ca2+ fluxes, inhibits apoptosis and increases cell motility via store operated Ca2+ entry induced-calpain2 activation and focal adhesions turnover. Bioinformatics analyses suggest a link between dysregulation of hGAAP expression and several human cancers. This work aims at exploring the role of hGAAP on cancer cell invasion. Unpublished data indicate that hGAAP overexpression increases in vitro and in vivo cell invasion, extracellular proteolytic degradation and specifically MMP2 activity. Conversely, hGAAP KD reduces cell invasion and MMP2 activity, while the overexpression of an hGAAP null mutant has no effect on cell invasion or proteolytic degradation. Moreover, the overexpression of hGAAP induces intracellular ROS (CellROX) accumulation and specifically H2O2 (HyPerRed). The reduction of both hGAAP-induced in vitro cell invasion and extracellular proteolytic degradation when cells were treated with catalase suggests that H2O2 plays a central role in this mechanism. The mitochondria are a very likely source of the ROS that accumulate when hGAAP is overexpressed. To investigate this possibility we are currently performing assays to measure O2 consumption and ATP production.
Together, these data suggest that hGAAP is a novel regulator of cancer cell invasion. Although intimately associated, the mechanisms related to the interplay between Ca2+ and ROS signalling involved in tumour cell migration/invasion are still not clear. A deeper understanding of hGAAP roles in cell biology will contribute to dissect this complex interplay and might provide new cancer biomarkers and/or druggable targets to be explored for anti-metastization therapeutic strategies.
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P14 - Impact of a Superoxide dismutase mimic in cancer cell migration

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Manganese(III) porphyrins (MnPs) are a class of superoxide dismutase mimics that scavenge a plethora of reactive species (RS) and modulate redox signalling pathways. MnPs are currently in clinical trials in cancer patients submitted to chemo- or radiotherapy, due to their ability to boost anticancer treatments while protecting off-target tissues. Although many cellular processes implicated in the metastatic process may undergo redox regulation, only scarce studies have addressed the impact of MnPs in metastases. In this work we characterized the impact of non-cytotoxic levels of the MnP MnTnHex-2-PyP5+ in cell migration and invasion, two processes closely related with metastases. This MnP (0.25 μM) decreased the chemotactic migration of renal cancer cells 786-O. A concentration of 5 μM of MnP was also studied in MCF7 and MDA-MB-231 breast cancer cells, alone and in combination with the anticancer drug doxorubicin (dox; 0.1 μM). The co-treatment decreased the collective motility of MCF7, the chemotaxis of both cell lines, and the proteolytic invasion of MDA-MB-231 cells. MnP also counteracted the increase in random MDA-MB-231 cell migration induced by dox. To unravel the mechanisms underlying the observed effects, differences in cell spread/area, focal adhesions, intracellular RS levels, and NF-kB activation were studied. Overall, our results suggest that MnP may have a beneficial impact in reducing cancer cells migration.
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P15 - Vending Machines in the Workplace - The Relationship Between Food Options and BMI

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2.8 million annual deaths worldwide are a result of overweight or obesity. An unbalanced diet with excessive consumption of heavily energetic foods and sedentary lifestyle are the key factors in triggering weight gain. In this study it was our ambition to relate the body mass index (BMI) of workers of a factory to the consumption of vending machine products. We analyzed the orders of products available in the vending machines and identified the nutritional compositions of the top selling foods. Due to the sample size and lack of data it was not possible to relate BMI, to food consumption from vending machines existing in the workplace, although user's BMI was higher than non-users, it was not possible to establish a relationship.
Key words: Vending machines; Body mass index; Consumption habits; Obesity;
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P16 - Development of hydrogels containing chitosan-coated PLGA nanoparticles for topical delivery of insulin

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Insulin is a therapeutic protein that may be use as growth factor in skin regeneration. Every year, in United States, over six million people suffer from chronic wounds and around 25 billion dollars are spent in therapy. In Europe, the cost for cutaneous regeneration oscillate between 6.000€ and 10.000€ per patient and year [1]. The aim of this work was to develop a hydrogel containing insulin-loaded chitosan-coated PLGA nanoparticles for wound healing. Chitosan-coated PLGA nanoparticles were produced by a modified solvent evaporation method based on a water/oil/water double emulsion technique described by Fonte et al. [2]. The incorporation of chitosan was forged at different concentrations (0.25%, 0.5% and 1%) and was evaluated the physical-chemical properties to obtain particles in the nanosized range, bottom polydispersity and good colloidal stability (Table 1). Ultimately, it was produced a semi-solid hydrogel to deliver the nanoparticles for topical administration for wound healing. The production of a semi-solid hydrogel, composed of sodium carboximethylcellulose and sodium alginate both suitable for wound regeneration, with good uniformity and rheological properties. The developed protocol granted to produce chitosan-coated insulin loaded PLGA nanoparticles with good colloidal stability and no need of high temperatures. It is foreseen that these formulations may be used for topical application for wound healing.

Table 1. Physical-chemical properties of PLGA nanoparticles at different concentrations of chitosan

	Diameter	PdI	ZP
PLGA NP uncoated	270 ± 15	0.207 ± 0,008	-14.12 ± 1.17
PLGA NP coated chitosan 0.25%	749 ± 30	0.289 ± 0.006	–
PLGA NP coated chitosan 0.5%	1072 ± 47	0.343 ± 0.022	31.72 ± 2.58
PLGA NP coated chitosan 1%	1289 ± 24	0.248 ± 0.037	–

PdI: Polydispersity Index; ZP: Zeta Potential

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P17 - Cytoxicity profile of a Myrtus communis L. extract obtained by Supercritical Fluid Extraction

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The use of bioactive ingredients in cosmetic formulations is on the rise in response to an increasing public demand for natural ingredients instead of synthetic ones. This can be achieved through the inclusion of bioactive vegetable extracts in the cosmetic formulations. These extracts can act, for example, as preservatives or as antiageing agents, if they exhibit antimicrobial or antioxidant properties, respectively. Myrtus communis L., or myrtle, is an evergreen shrub, typical of the Mediterranean flora, which grows wildly across most of Portugal. It is, since ancient times, considered as a medicinal plant and used as such, which is probably related to its antioxidant and antimicrobial activities. We have already ascertained these properties for a supercritical fluid myrtle extract [1]. These qualities make myrtle a very interesting plant for the cosmetic industries. The supercritical fluid extract was obtained at 230 bar and 45ºC, with a flow of CO2 of 0,3kg h-1. The fact that CO2 is a non-polar solvent and the compounds to extract are polar makes it necessary to use a co-solvent, in this case, ethanol. The flow of ethanol was 0,09 kg h-1. Myrtle extract obtained through conventional extraction methods is already considered safe for use in cosmetics. Because the myrtle extract used in this study was obtained through a different method, supercritical fluid extraction (SFE), its safety needs to be proved. Therefore, as a first approach for the safety assessment of this extract, cytotoxicity assays were carried out. Since a dermatological application is sought, studies were carried out in human keratinocytes (HaCat cell line). HaCat cells were exposed to a range of concentrations of the extract up to 60 µg/mL for 24 h, and cell viability was evaluated by the MTT assay [2]. No cytotoxic effects were observed for concentrations up to 10 µg/mL. Concentrations of 30 and 60 µg/mL decreased cell viability to around one half of that of non-treated control cells ($p<0.05$). However, as cell lines have commonly higher sensitiveness relatively to animals [3], this result does not mean necessarily that toxicity will occur in an in vivo scenario. Although further studies are needed to duly assess the safety of SFE myrtle extract, namely mutagenicity and genotoxicity assays, this work constitutes a step forward towards the potential use of this extract as a cosmetic ingredient.

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P18 - The perception of first-cycle teachers regarding the therapeutics of the Attention Deficit Hyperactivity Disorder

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The Attention Deficit Hyperactivity Disorder (ADHD) is characterized by a persistent pattern of inattention and / or hyperactivity-impulsivity, with personal, academic, family and social impact, affecting 5-7% of the school-age population. Methylphenidate is the first-line drug, and its use had a large increase in recent years. The management of the pathology, including the monitoring of the response to therapy, should involve health professionals, family members and teachers. Teachers have a greater contact with children and can thus easily detect behavioral changes upon the beginning of medication. However, few studies have focused role of teachers in the management of ADHD, especially in the context of therapeutics.

The present work aims to characterize the perception of 1st cycle teachers regarding the impact of ADHA therapeutics on their students. In the absence of an adequate instrument to collect these data, a specific questionnaire was constructed. The questionnaire was focused on teachers' training regarding ADHD and its therapy; the experience with students with ADHD; the changes upon beginning of medication; and the observation of adverse drug reactions (ADR) and possible notification to the physicians, family members and National Pharmacovigilance System (SNF). The feasibility of the questionnaire was verified in a pre-test applied personally to a convenience sample of 12 teachers from a school of Lisboa e Vale do Tejo region. Subsequently an online version of the questionnaire was developed and distributed to the teachers from the schools of this region. A total of 107 responses was received. In addition, pediatric psychiatrists were interviewed by telephone, in order to gather experiences regarding their interaction with teachers in the management of ADHA therapeutics.

The results indicate that more than 40% of the inquired teachers have received training in ADHA, but in most cases the theme of therapeutics was not included. About 87% of the teachers mentioned the need for more training. The vast majority of teachers (91.6%) have had students with ADHD. Most of the teachers observed alterations in their students upon starting medication, generally in a beneficial way. More than 60% of the teachers answered that they are aware of the ADR and 24% have already detected them in their students. The teachers reported the observed ADR to the parents in 93% of the cases and to the doctors in 28% of the cases, but never to the SNF. From the preliminary results of the interviews, pediatric psychiatrists highlighted the interaction with teachers. Importantly, physicians mentioned that they do not report ADR to the SNF due to lack of information about the notification process.

In conclusion, the results show the need to reinforce teachers' training in ADHD and its therapeutics, as well as the need to disseminate the SNF amongst teachers and physicians. This study allows to characterize the perception of the teachers towards the ADHD therapeutics, contributing to the identification of strategies for the better follow up of the children with this pathology.

P19 - Ex vivo characterization of taped stripped stratum corneum treated with multifunctional sunscreen by HPLC TBARS method

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Unprotected chronic exposure to ultraviolet (UV) radiation can contribute to the development of many diseases and sunscreens are relevant to avoid such harmful consequences. However, traditional sunscreens do not provide enough protection against cutaneous oxidative stress. Therefore, it is expected the development of multifunctional photoprotective formulations, acting not only in the absorption and/or reflection of UV radiation but also assisting in cutaneous homeostasis, with the presence of antioxidant agents. In the present study, a single and fast quantitative method for high performance liquid chromatography (HPLC) was developed for a formulation containing ferulic acid in conjunction with two UV filters, bemotrizinol and ethylhexyl triazone [1]. The method will be used for skin penetration assay by applying the formulation on the stratum corneum of volunteers, with consequent withdrawal by a tape stripping method (ex vivo). In addition, a lipid peroxidation test, TBARS (thiobarbituric acid reactive substances), was developed using HPLC. The ex vivo assay used the stratum corneum to evaluate the potential of cutaneous lipid peroxidation, with or without artificial irradiation stress, stimulating the production of malondialdehyde (MDA), an biomaker of lipid peroxidation. An irradiation dosage of 2753 KJ.m2 raised the lipid peroxidation by 34%, whereas 5506 KJ.m2 raised by 110%. To date, it is the first time that a HPLC TBARS method was used to characterize the stratum corneum (ex vivo). The protocol will be used to test if the photoprotective formulation containing ferulic acid decrease the MDA concentration. Also, this study may aid the efficacy of antioxidant agents, as well as elucidate the level of lipid peroxidation caused by drugs and cosmetics, and even in carrying out baseline studies characterizing different ethnicities and genders.

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P20 - Development of nanostructured lipid carriers for delivery of therapeutic proteins

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Nanostructured lipid carriers (NLCs) have been presented as advantageous over solid lipid nanoparticles, mainly due to their drug loading capacity and controlled drug delivery. In this work we propose a NLC system produced without heating, thus allowing the incorporation of therapeutic proteins for oral delivery. BSA was used as model of therapeutic protein. The BSA-loaded NLCs were prepared by an adapted solvent-evaporation double emulsion technique [1]. SLNs were produced as controls. Different solid and liquid lipid combinations were used at a 70:30 w/w ratio, with the surfactant Tween 80 at 1%, 2% and 3% w/v. After formulation optimization the best lipids were Precirol (soli) and oleic acid (liquid). The SLNs and NLC had size range of 360±58 nm (PdI of 0.29±0.01) and NLC at 311±13.3 nm (PdI of 0.30±0.02) respectively. The measured encapsulation efficiency was about 64% for SLNs and 81% for NLCs. The results showed the ability of the developed methodology to encapsulate therapeutic proteins into NLCs, with an increased efficiency in NLC when compared SLN. This system may be a good carrier to enhance the bioavailability of therapeutic proteins.

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P21 - In vitro antimicrobial and cytotoxic activities of natural and hemi-synthetic isopimaranes

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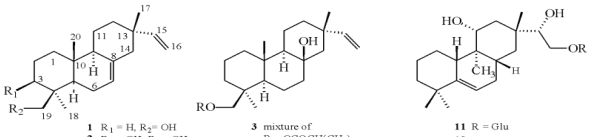
A growing list of infections related to methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE) are becoming more difficult, and sometimes impossible, to treat [1]. An important strategy to overcome this need is the continuous research on natural products and derivatives. Compounds 1 – 6 and 9 were isolated from Aeollanthus rydingianus aerial parts [2]. Five new acyl (7, 8) and glycosyl (10 – 12) derivatives of these natural isopimaranes were prepared and their structures elucidated by NMR (Figure 1). These new derivatives and the natural ones (Figure 1) were assayed against an enlarged panel of bacteria, which included two MRSA and one VRE strains. Only natural compounds 1 and 6 showed promising results. Compound 1 was the most active, showing MIC values between 1.95 µg mL-1 against S. aureus (ATCC 43866) and 62.50 µg mL-1 against E. faecalis (FFHB 427483) and E. flavescens (ATCC 49996). In addition, the cytotoxicity of compounds 1 – 3, 5, 6, 8 – 12 was evaluated in the human breast cancer cell line MDA-MB-231. Compound 2 was the only compound showing relevant cytotoxicity. The IC50 value of compound 2 was 15 µM, suggesting a mild antiproliferative activity against these breast cancer cells [3].



1 R_1 = H, R_2= OH
2 R_1 = OH, R_2 = OH
4 R_1 = OAc, R_2= CH_3
5 R_1 = OAc, R_2= OH
6 R_1 = OH, R_2 = OAc
7 R_1 = H, R_2= OAc
8 R_1 = R_2= OAc
10 R_1 = H, R_2= OGlu

3 mixture of R = $OCOCH(CH_3)_2$ and $OCO(CH_2)_2CH_3$
9 R = H

11 R = Glu
12 R = Man

Figure 1 – Natural and hemi-synthetic isopimarane compounds.

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