

Proceedings | *Resumos*

II Jornadas CBIOS



Aprender Ensinar Investigar



Sala S.0.11

12 de Outubro 2016



Informações:

Paula Almeida, ECTS-ULHT
Campo Grande, 376,
1749-024, Lisboa

paula.almeida@ulusofona.pt
217515550

Keynotes:

José Alves, CEDOC
Nuno Silva, IMED-ULisboa, FFUL
Susana Lucas, Hovione



ects.ulusofona.pt

II Scientific Conferences CBIOS

II Jornadas Científicas CBIOS

12 October | 12 Outubro
Lisboa - Universidade Lusófona

Honor Committee /Comissão de Honra

Magnífico Reitor da Universidade Lusófona, Mário Moutinho
Presidente do Conselho de Administração da Universidade Lusófona,
Manuel de Almeida Damásio
Presidente do ILIND, Manuel José Damásio.
Direcção da ECTS/CBIOS/ALIES, Pres. das II Jornadas CBIOS, Luís
Monteiro Rodrigues.
Diretor do Curso de Ciências da Nutrição, Nelson Tavares

Scientific Committee /Comissão Científica

Luís Monteiro Rodrigues, *Direcção CBIOS*
Amílcar Roberto, *DDS*
Ana Sofia Fernandes, *PT*
Catarina Pinto Reis, *DDS*
Catarina Rosado, *DDS*
Margarida Alves, *PT*
M. Fáima Simões, *FSP*
Marisa Nicolai, *FSP*
Nuno Saraiva, *PT*
Patrícia Rijo, *FSP*
Tânia Almeida, *DDS*

Organizing Committee /Comissão Organizadora

Escola de Ciências e Tecnologias da Saúde (ECTS) da U. Lusófona de
Humanidades e Tecnologias
ALIES – Associação Lusófona para o Desenvolvimento do Ensino e
Investigação das Ciências da Saúde
CBIOS – Centro de Investigação em Biociências e Tecnologias da Saúde

Programa

12 October | 12 de Outubro

Open Session | *Sessão de abertura*

Presentation CBIOS | Apresentação CBIOS
Luís Monteiro Rodrigues

Arlinda Cabral (Direção para Ação Cultural e Língua Portuguesa
Responsável pela Educação, Ciência e Tecnologia)

1st Session | *Sessão 1*

CBIOS Group PT (Pharmacology and Therapeutics)

Keynote Lecture
José Alves, CEDOC

Speakers / Plectores
Nuno Saraiva, CBIOS
João Costa, CBIOS ((PhD Student)

Outras I&D Lusófona
António Palmeira, FEFD, ULHT
Pedro Gamito, Copelabs, ULHT

2st Session | *Sessão 2*

CBIOS Group DDS (Development Delivery Systems)

Keynote Lecture
Nuno Silva, iMED-ULisboa, FFUL

Speakers / Plectores
Tânia Almeida, CBIOS
Ana Rebelo (PhD Student)

3st Session | *Sessão 3*

CBIOS Group FSP (Food Sciences and Phytochemistry)

Keynote Lecture
Susana Lucas, Hovione

Speakers / Plectores
Marisa Nicolai, CBIOS
Catarina Garcia (PhD Student)

Open Session

Presentation CBIOS | Apresentação CBIOS

Luís Monteiro Rodrigues

Arlinda Cabral (Direção para Ação Cultural e Língua Portuguesa
Responsável pela Educação, Ciência e Tecnologia)

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

Keynote Lecture

C.01 - High-density lipoproteins beyond atherogenesis

José Delgado Alves^{1,2}

¹ CEDOC/NOVA Medical School, Departamento de Medicina, Lisboa, Portugal.

² Hospital Prof Doutor Fernando Fonseca, UDIMS/Medicina 4, Amadora, Portugal.

Contacto: jose.alves@nms.unl.pt

Abstract / Resumo da Comunicação

High-density lipoproteins (HDL) are key players in the atherogenic process. Their protective role includes cholesterol reverse transportation from the vessel wall back to the liver, anti-inflammatory, anti-thrombotic, anti-oxidant and anti-apoptotic effects.

Despite the enormous progress in the HDL-related knowledge acquired in the last decade, there is still much to be known about its properties and underlying mechanisms. A good example of this discrepancy is the paradox between the HDL potential in the context of basic-research studies and the lack of clinical response that would be anticipated, based on the former.

In the last few years, the knowledge of the wide span of HDL influence in human physiology and physiopathology has grown and it is now recognized as one of the most ubiquitous and important factors in the homeostasis of the human body.

HDL is a complex structure that includes many proteins and lipid-based molecules with multiple actions. The most studied and recognized so far as probably being the most relevant is apolipoprotein A-I which also has anti-inflammatory and anti-oxidant properties on its own.

This talk will address other fields in Medicine that have been recently recognised as relevant in the context of HDL and Apo A-I.

Alzheimer's disease, Systemic Lupus, T-cell response and regulation and vascular disease in psoriasis are some examples of the universal influence of HDL. Furthermore, HDL as a mixture of multiple proteins represents an important target for the humoral response and the presence of anti-HDL antibodies have been associated with multiple aspects of these different conditions, in addition to be a major factor in atherogenesis.

C.02 - Mind the GAAP: a tale of camels, calcium, channels and cancer

Nuno Saraiva¹

¹CBIOS, Universidade Lusófona Research Center for Biosciences & Health Technologies, Campo Grande 376, Lisbon 1749-024, Portugal.
Contacto: nuno.saraiva@ulusofona.pt

Abstract / Resumo da Comunicação

Orthologues of the human Golgi anti-apoptotic protein (GAAP) share remarkable conservation in amino acid sequence, protein size, length and hydrophobicity profile throughout eukaryotes, prokaryotes and some orthopoxviruses, suggesting a highly conserved ancestral function. Since the discovery of GAAP gene in camelpox virus, several cellular functions and structural properties of GAAPs have been described. Within eukaryotes, GAAPs regulate Ca²⁺ fluxes from the principal intracellular stores, confer resistance to a wide range of apoptotic stimuli, and promote cell adhesion and migration via the activation of store-operated Ca²⁺ entry (SOCE). In addition, human GAAP is essential for cell viability and is ubiquitously expressed amongst human tissues. Importantly, these multi-transmembrane proteins homo-oligomerize and recently were shown to form cation-selective ion channels, potentially forming the basis for the modulation of its diverse functions. Furthermore, topology mapping has uncovered a novel and unique topology for ion channels, constituting the largest currently known viral ion channel. Since increased GAAP expression can affect cellular processes associated with important hallmarks of cancer, such as broad resistance to apoptosis, increased cell migration and invasion, a link with cancer has been suggested. This is supported by dysregulation of GAAP expression in some prostate, breast, colorectal and brain cancers and its association with poor outcomes in the latter. Furthermore, the body of knowledge provided through the various functions of GAAP is centred on the Golgi, an organelle that although is attracting increased interest, remains poorly understood

C.03 - Case study: Ochratoxin A, ROS and Antioxidants

João G. Costa ^{1,2,3}, Nuno Saraiva ¹, Patrícia S. Guerreiro ², Henriqueta Louro ^{4,5}, Maria J. Silva ^{4,5}, Joana P. Miranda ², Matilde Castro ², Ines Batinic-Haberle ⁶, Ana S. Fernandes ¹ and Nuno G. Oliveira ²

¹CBIOS - Universidade Lusófona, Research Center for Biosciences and Health Technologies, Lisbon, Portugal

²Research Institute for Medicines (iMed.UL) - Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

³Department of Biomedical Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain

⁴Department of Human Genetics, National Institute of Health Dr. Ricardo Jorge, I.P., Lisbon, Portugal

⁵Center for Toxicogenomics and Human Health (ToxOmics), NMS, FCM – UNL, Lisbon, Portugal

⁶Department of Radiation Oncology, Duke University Medical School, Durham, NC, USA

Contacto: jgcosta@ulusofona.pt

Abstract / Resumo da Comunicação

Ochratoxin A (OTA) is a mycotoxin produced by several species of fungi that commonly contaminates animal feed and food. OTA has been associated with a number of diseases in both animals and humans, predominantly affecting the kidney and has been considered a recognized nephrotoxic agent and a possible carcinogen to humans. The aim of this study is to integrate several endpoints concerning OTA-induced toxicological effects in Vero cells to obtain additional mechanistic data. The characterization of OTA cytotoxicity was performed using the crystal violet, neutral red and LDH leakage assays. The genotoxicity of OTA was evaluated by the cytokinesis blocked micronucleus and the comet assays. The effects in cell cycle and apoptosis in OTA-treated cells were analyzed by DNA content and Annexin V staining, respectively. Intracellular reactive oxygen species (ROS) levels were evaluated using the DHE and DHR probes. In this work we used different antioxidants, namely the superoxide dismutase mimic (SODm) MnTnHex-2-PyP, in order to clarify the involvement of oxidative stress in OTA toxicity. Our results showed concentration and time-dependent cytotoxic effects of OTA. While the SODm mildly increased cell viability, trolox and ascorbic acid had no effect with regards to this endpoint. OTA induced micronuclei formation. Using the FPG modified comet assay, OTA modestly increased the % of DNA in tail, revealing the presence of oxidative DNA lesions. This mycotoxin increased apoptosis, which was attenuated by SODm. Additionally, the SODm decreased the ROS accumulation observed with DHE. In conclusion, our data suggest that the mechanisms responsible for OTA deleterious effects include DNA damage and the generation of intracellular ROS. However, ROS do not seem to play a central role in OTA toxicity to Vero kidney cells.

Acknowledgments

João G. Costa acknowledges his Grant PADDIC 2016 awarded by ALIES / CBIOS / ULHT.

Outras I&D Lusófona

C.04 - A investigação em Educação Física, Desporto e Exercício na Universidade Lusófona

António Labisa Palmeira¹

¹Faculdade de Educação Física e Desporto, Universidade Lusófona, Lisboa, PORTUGAL
Contacto: antonio.palmeira@ulusofona.pt

Abstract / Resumo da Comunicação

A Faculdade de Educação Física e Desporto (FEFD) integra diversas actividades direccionadas para a produção de novos conhecimentos. Desde o 1º ciclo, onde todos os alunos efectuem trabalho monográfico de final de curso, passando pelos 5 cursos de 2º ciclo, culminando no 3º ciclo que já vai no seu 5º ano. Saliente-se que a maior parte dos cursos foram avaliados pela A3ES com a melhor classificação, em parte devido à produção científica deles resultante. No entanto, estamos conscientes que a produção científica tem uma limitação latente, derivada da organização associada ao apoio à ciência da Universidade Lusófona.

Como exemplo dos trabalhos efectuados, iremos apresentar um projecto da FEFD financiado pela FCT, em parceria com a Faculdade de Medicina da Universidade de Lisboa, denominado Tratamento da Obesidade Pediátrica - TOP.

Este trabalho analisou o efeito da inclusão de pares no tratamento da obesidade em adolescentes baseado no aumento da actividade física e em sessões educativas sobre estilos de vida saudáveis, em indicadores de obesidade, saúde psicossocial, condição física e marcadores inflamatórios.

A amostra contou com 56 adolescentes, entre os 14-17 anos, com obesidade (percentil >95). Destes 29 realizaram a intervenção com um par (amigo ou familiar à sua escolha) e 27 realizaram a intervenção sem par, num desenho não-aleatorizado controlado, não cego. A intervenção teve a duração de 12 meses, com 3 cohorts, pelo que o estudo decorreu durante 3 anos. Ambas as intervenções eram idênticas, com sessões semanais de exercício e de educação para estilos de vida saudáveis. A diferença resultava da presença do par na intervenção no grupo experimental, enquanto que o grupo de comparação participava na intervenção sem par. Foram efectuadas recolhas de dados sobre a obesidade, condição física, psicossociais, hábitos alimentares e actividade física no baseline, 6 e 12 meses.

Os resultados mostraram que 84% reduziram 0,5 pontos no z-score do IMC. Os comportamentos sedentários foram reduzidos em 30 minutos por dia e a actividade física moderada a vigorosa manteve-se estável durante o programa. A qualidade de vida melhorou em média 3%, não sendo este valor estatisticamente significativo. Não se registaram diferenças entre os grupos nestes resultados.

Este foi um exemplo de um trabalho de investigação que, para além dos resultados científicos, permitiu ao grupo de investigação integrar novas aprendizagens e sinergias com outros grupos de trabalho que se espera levem ao desenvolvimento de trabalhos futuros sobre esta e outras temáticas na mesma área do conhecimento

Agradecimentos:

O autor deseja agradecer: a) à direcção da FEFD pelo esforço realizado no sentido de proporcionar apoio à investigação; b) à Professora Doutora Helena Fonseca, da Faculdade de Medicina da Universidade de Lisboa, pelo parceria no âmbito do projecto TOP.

C.05 - Systemic Lisbon Battery: dados de 5 anos de avaliação de défices cognitivos por intermédio da Realidade Virtual

Pedro Gamito,^{1,2}, J. Oliveira,^{1,2} and D. Morais,²

¹EPCV, ULHT, Campo Grande 376, 1749-024 Lisboa, PORTUGAL

²COPELABS, ULHT, Campo Grande 376, 1749-024 Lisboa, PORTUGAL

Contacto: pedro.gamito@ulusofona.pt

Abstract / Resumo da Comunicação

As medidas mais comuns para avaliar declínio cognitivo devido a envelhecimento, lesões neurológicas ou outras condições comprometedoras da função cerebral, são baseadas provas neuropsicológicas de papel e lápis. No entanto, uma das críticas que tem sido levantada relativamente a estes testes é que os mesmos podem não replicar as exigências a que um individuo está exposto no seu dia-a-dia, apresentando assim lacunas ao nível da validade ecológica. Para que os resultados do exame neuropsicológico possam ser generalizáveis à realidade do individuo, é necessário que o exame contemple a execução de exercícios contextualizados em tarefas da vida diária (e.g., fazer compras, vestir-se ou preparar o pequeno-almoço), ao invés de se considerar apenas o desempenho em testes de carácter psicotécnico. O uso de realidade virtual (RV), onde os pacientes possam realizar actividades da vida diária, exigindo diferentes capacidades cognitivas, pode ser uma solução viável para este problema. Estamos atualmente a validar um ambiente multi-proposta computadorizado em RV para exame neuropsicológico com base nos requisitos mencionados acima. Este ambiente consiste numa cidade com vários tipos de tarefas da vida diária, desde a preparação de refeições, passando pela ida às compras, até a actividades de lazer, como a visita a uma galeria de arte. Este ambiente tem sido estudado em população saudável e clínica para exame neuropsicológico e para treino cognitivo em indivíduos com patologia neurológica. Nesta sessão serão apresentados vários resultados provenientes de diferentes estudos.

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

Keynote Lecture

C.06 - Modelling and Simulation for Drug Absorption Prediction from DDS

Nuno Silva¹

¹ Faculdade de Farmácia da Universidade de Lisboa, Departamento de Ciências Farmacológicas, A. Prof. Gama Pinto, 1649-003 Lisboa, PORTUGAL.
Contacto: nmens@ff.ulisboa.pt

Abstract / Resumo da Comunicação

O desenvolvimento de formulações para administração oral necessita de um bom entendimento dos fenómenos cinéticos associados ao desempenho da formulação *in vivo*, bem como da identificação de uma potencial característica *in vitro* que reflecta o mesmo.

Os ensaios de dissolução *in vitro*, pela sua concepção e utilidade, são utilizados nos diversos passos do desenvolvimento galénico de uma nova formulação e representam muitas vezes um elemento primordial de decisão no que respeita ao estudo *in vivo* das formulações desenvolvidas.

Contudo, o processo de absorção oral de um fármaco envolve uma complexidade elevada, resultante de múltiplos passos cinéticos que incluem no tracto gastrointestinal a desintegração da formulação, a dissolução da substância activa e sua degradação, um tempo de trânsito, a permeação e/ou transporte da substância activa na parede intestinal, e ainda uma possível metabolização intestinal e hepática. Perante esta complexidade, a informação cinética fornecida pelos ensaios de dissolução poderá correr o risco de ser demasiado simplista, e assim errar na predição do desempenho da formulação *in vivo*, requeendo desta forma mais tempo para o afinamento galénico da formulação e mais estudos clínicos para demonstrar a biodisponibilidade do novo medicamento.

Com base nesta ideia, foram desenvolvidos modelos de base fisiológica para previsão da absorção, distribuição e eliminação de fármacos, utilizando informação proveniente de ensaios de dissolução, das propriedades físico-químicas das substâncias activas, das características fisiológicas do tracto gastrointestinal e do organismo no seu todo e ainda da interacção dos fármacos com o sistema biológico. Estes modelos são comercializados sob a forma de software profissional como o SimCyp[®] ou o PK-Sim[®].

Speakers / Prelectores

C.07 - Functional Ingredients in delivery systems

Tânia Santos de Almeida,¹ Ana Júlio,¹ Margarida Pereira,¹ Rita Caparica,¹ Nicole Pereira,¹ Afonso Antunes,¹ Filipa Rocha,¹ Nuno Saraiva,¹ Ana Sofia Fernandes,¹ Maria Eduarda Araújo,² André Baby,³ Catarina Pinto Reis,¹ Catarina Rosado,¹ Joana Mota¹

¹ CBIOS-Universidade Lusófona's Research Center for Biosciences and Health Technologies, Campo Grande 376, 1749-024, Lisboa, PORTUGAL.

² Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, PORTUGAL

³ School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 15, 05508-900, São Paulo, BRAZIL.

Contacto: tania.almeida@ulusofona.pt

Abstract / Resumo da Comunicação

In Pharmaceutical and cosmetic industries, the development of new delivery systems has involved the investigation of various strategies to improve the performance of drugs and cosmetic active ingredients. Hence, it is crucial to study different functional ingredients to improve/optimize the release and/or stability performance of these formulations, as well as their functional properties.

Herein, two types of substances, ionic liquids and tea infusions, were studied and their abilities as functional ingredients in different delivery systems were evaluated.

Ionic liquids (ILs) are known for their valuable properties that convey them the prospect to be incorporated in different types of formulations. In this study, several ILs have been prepared and their capacity as solubility/permeation enhancers was studied alongside with their cytotoxicity in human keratinocytes (HaCaT cell line). All studied ILs showed the ability to enhance drug solubility. On the other hand, permeation enhancement is more dependent on the ILs structure. The less toxic ILs were successfully incorporated in different delivery systems, namely in O/W emulsions, gels, lipidic implants and in protein containing formulations. The ILs proved to work as functional ingredients in all the prepared systems and did not affect negatively the formulations stability. Furthermore, the use of tea infusions as functional ingredients was also evaluated, particularly their ability as sunscreen photostabilizers. Usually two filters (UVA and UVB) are used to favour higher Sun Protection Factor (SPF) values. However, most of the formulations including both filters are unstable and require additives that enable photostabilization, such as antioxidants. Since tea infusions contain several polyphenolic compounds, with antioxidant properties, their photostabilizer effect was evaluated. Hence, O/W formulations with avobenzone and octyl methoxycinnamate were developed with substitution of water in the external phase by green or black tea infusions in the attempt to attain photostable formulations. *In vitro* antioxidant activity of the formulations was assessed by the DPPH assay. The formulations containing tea infusions presented a higher radical scavenging activity than the formulation without tea. *In vivo* photoprotection efficacy was also assessed. The formulations containing the tea infusions in association with the chemical filters, endorsed an enhancement of the SPF. Black tea showed better results, where SPF value was twice the value observed for the formulation only with the chemical filters.

In conclusion, the use of ingredients that enhance the solubility of poorly soluble drugs and/ or increase the stability of delivery systems is a topic of much relevance for the industry and consequently further studies on this subject will certainly continue to arise in the scientific community.

C.08 - Targeting Parvifloron D to pancreatic cancer using nanocarriers

Ana Rebelo¹, Patrícia Rijo^{1,2}, Jesus Molpeceres³ and Catarina Pinto Reis^{1,4}

¹CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749 -024 Lisboa, Portugal;

²iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal;

³UAH, Universidad de Alcalá de Henares, Plaza de San Diego, s/n, 28801 Alcalá de Henares, Madrid, Espanha;

⁴IBEB, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal.

Contacto: ana.rebelo@gmail.com

Abstract / Resumo da Comunicação

Pancreatic cancer is the thirteenth most common cancer and the eighth leading cause of cancer death worldwide. The prognosis of this type of cancer remains very poor with only a 5-year survival in 5% of most reports [1].

Medicinal plants studies have led to the discovery of new bioactive compounds. As example, Parvifloron D is the main component isolated from *Plectranthus ecklonii* Benth. and has a potent antiproliferative activity [2]. The aim of this work was to isolate Parvifloron D from *P. ecklonii*. and to prepare and characterize albumin nanoparticles loaded with Parvifloron D.

Herein, nanotechnology can play a crucial role by targeting Parvifloron D to the malignant cells through different mechanisms. First, nanoparticles show a tendency for accumulation in certain tumors mainly because their small particle size and their strong capability of easy escape through leaky endothelial tissue in the tumor [3]. On the other hand, nanoparticles can deliver this anti-tumoral compound to target area using special ligands onto their surface and easily achieve specific cell receptors.

In this study, Parvifloron D was extracted by an acetone ultrasound-assisted method and albumin nanoparticles were produced through the desolvation method and using different cross-linking processes [4]. Resultant particles were then characterized in terms of stability, particle mean size by photon correlation spectroscopy, zeta potential by LASER Doppler anemometry and shape by atomic force microscopy (AFM). Then, based on the previous results, optimized conditions were tested namely different concentration of cross-linking agent, presence of stabilizant agent and the time of cross-linking.

Parvifloron D was extracted 14.45 % (w/w) and isolated 0.87 % (w/w) with high yield. Particles with a small size lower than 351.2nm (+/- 100.5 SD) were successfully produced. The best cross-linking agent was glucose and albumin concentration should be 50mg. The addition of sodium chloride was not beneficial. The time of cross-linking did not influence particle size.

In conclusion, this study confirms the feasibility of producing uniform and well-defined albumin nanoparticles. Further in vitro studies will be performed with free Parvifloron D and encapsulated drug against tumor cells.

References:

[1] Silvestris, N., et al, *Curr. Med. Chem.*, 21(1): 948-965, (2014).

[2] Burmistrova, O., et al, *Phytomedicine*, 22: 1009-1016, (2015).

[3] Oliveira, C., et al, *International Journal of Pharmaceutics*, 493(2) 1-2, (2015).

[4] Niknejad, H., et al, *Iranian Journal of Pharmaceutical Research*, 14(2): 385-394, (2015).

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

Keynote Lecture

C.09 - Sustainable Chemistry by Design

Susana D. Lucas¹

¹ Hovione FarmaCiência SA, Process Chemistry Development, Sete Casas 2674 – 506 Loures, Portugal
Contacto: sdlucas@hovione.com

Abstract / Resumo da Comunicação

Drug substance production often comprises the use of reagents and processes that are not environmentally friendly. Increased concerns about sustainability arise the pressure on companies to address these issues when defining synthetic routes. A number of tools are available today for chemists to assess the impact of alternative routes and guide their efforts towards more sustainable processes. An overview of the tools used at Hovione and how they are applied to support decision making will be presented together with a case study with different challenges involved in taking an innovative drug from discovery to manufacturing

Speakers / Prelectores

C.10 - Insight Food Science and Phytochemistry Group

Marisa Nicolai^{1,2}, Diogo Matias^{1,2,3}, Filipe Pereira^{1,2,3,4}, Catarina Garcia^{1,2,3}, Joana Andrade^{1,2,4}, Íris Neto^{1,2,4}, Catarina Reis^{1,2,5} and Patrícia Rijo^{1,2,6}

¹ CBIOS - The Research Center for Biosciences & Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisbon, Portugal

² Escola de Ciências e Tecnologias da Saúde, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisbon, Portugal

³ Department of Biomedical Sciences, Faculty of Pharmacy, University of Alcalá, 28871 Alcalá de Henares, Spain.

⁴ Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal

⁵ IBEB, Faculty of Sciences, University of Lisbon, 1749-016, Lisbon, Portugal

⁶ iMed.UL, Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal
Contacto: ana.rebelo@gmail.com

Abstract / Resumo da Comunicação

The overarching research domains of the Food Science and Phytochemistry (FSP) group are related to the isolation and evaluation of biological activity of extracts and compounds, the development of new formulations and concepts for food, beverages and dietary supplements and also the study of the impact of nutrients in Human Health. Currently are ongoing several different projects, from doctoral projects, to collaborative master projects with national and international universities, but also collaborative master projects, where are being developed by integrated master's students who are experiencing the first contact with R&D.

Moreover, university-industry collaborations are also one of the highlight topics that enable both sustaining growth. It is through the Knowledge Technology Transfer (KTC) center, that provides various services from research and development to quality control, training and consultancy applied to various fields and products related to the area of human health including cosmetics, medicines, food supplements and medical devices, that partnership is achieved.

In academic research field, projects aimed at extraction, isolation, phytochemical screening and characterization of chemical constituents from *Plectranthus* spp. and also antioxidant, antimicrobial and enzymatic activities screening of these plant extracts and its isolated compounds. Some of the recent works are the description of “*Plectranthus* spp. antimicrobial and antiproliferative components and their inclusion into phytosomal formulation”; the antibacterial activity against Gram-positive strains of natural diterpene isolated from *Plectranthus* species 7 α -Acetoxy-6 β -hidroxyroyleanone and its derivatives; study of “6,7-Dehydroroyleanone as a scaffold for the development of novel anti-cancer agents”; the “Unveil new ethnopharmacological roles of *Plectranthus* species by screening biological activity”; the “Antimicrobial activity improvement of novel abietane cationic amphiphiles from dehydroabietic acid”; the “Preparation, analysis and evaluation of antimicrobial activity of extracts and essential oils of *Plectranthus* spp.”; “Chemical composition and biological activity of essential oils from plants of the genus *Plectranthus*”. Finally, the group is initiating preliminary studies related with “Determination of the antioxidant activity of medicinal plants extracts approach” and “Academic assessment of antimicrobial and antioxidant properties of ethanolic extracts from *Plectranthus madagascariensis*”.

C.11 - 6,7-Dehydroroyleanone as a Lead Molecule for the Development of Anticancer Hybrid Nanoparticles

Catarina Garcia ^{a,b}, Catarina Silvaa,^b, Tijana Stanković^c, Milica Pešić ^c, Isabel Martins^d, Patrícia Domingos ^d, Cistina Silva Pereira^d, Marisa Nicolai ^a, Carlos M. Monteiro^c, Carlos A.M. CAM^c, Catarina Pinto Reisa,^f Patrícia Rijo, P. ^{a,c}

^a Center for Research in Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal

^b Dpto. Ciencias Biomédicas, Universidad de Alcalá, Plaza de San Diego, s/n, 28801 Alcalá de Henares, Madrid, Espanha

^c Institute for Biological Research, “Siniša Stanković”, University of Belgrade, Despota Stefana 142, 11060 Belgrade, Serbia

^d Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA) Av. República EAN, 2780-572 Oeiras, Portugal

^e Instituto de Investigação do Medicamento (iMed.U LISBOA), Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal

^f IBEB, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal

Contacto: catarina.g.garcia@gmail.com

Abstract / Resumo da Comunicação

Natural products and its derivatives are notable sources of anticancer agents. Furthermore, it is currently undeniable the role of nanoparticles as loading agents, thus contributing for the success of cancer therapy. Diterpenes, such as 6,7-dehydroroyleanone, are commonly found in the *Plectranthus* genus and many are valuable sources of lead molecules, suitable for derivatization [1, 2].

Thus, and since this is a compound described as an anticancer agent [3], its isolation and derivatization is of major importance. Hence, after assessing its cytotoxicity by using the model system of sensitive non-small cell lung cancer cell line (NCI-H460) and its resistant counterpart (NCI-H460/R), we then proceeded to perform some biotransformation processes, through the use of several fungal strains representative of distinct taxa.

Also, for further conjugation of the most promising compounds, a combined system of 6,7-dehydroroyleanone with hybrid nanoparticles (NPs) were produced according to Silva et. al [4]. Both cytotoxic activities of the free diterpene and the nanosystem were compared.

In the tested cancer cell lines, the nanosystem has revealed to be approximately ten fold more cytotoxic than the 6,7-dehydroroyleanone itself. On the other hand, when applied alone, the hybrid NPs did not exert cytotoxic effect on the same cell lines. Consequently, the association of nanotechnology with this royleanone or its derivatives could potentially be a new treatment option for cancer multidrug resistance, and thus requires further assessment.

References:

[1] Lukhoba C., et al.; *Journal of Ethnopharmacology*, 103: 1–24, 2006

[2] Matos, A. M., *Development of a small library of macrocyclic diterpenes through molecular derivatization: evaluation of their activity as P-gp inhibitor*, 2013

[3] Areche C, et al., *Journal of Pharmacy and Pharmacology*, 61: 1689–1697, 2009

[4] Silva C, Request of national patent, number 20151000093093 (Portugal), “Nanossistema de ouro com revestimento biopolimérico e gama de absorção no infravermelho próximo e método para a sua preparação”, 2015.

Panel Communications | Comunicações em Posters

Abstract List | Lista de Resumos Submetidos

- P.01 **INFLUENCE OF AN IONIC LIQUID IN CAFFEINE RELEASE FROM LIPIDIC IMPLANTS.** Afonso Antunes, ¹, Joana Portugal Mota, J.^{1,*}, Tânia Santos de Almeida, ^{1,*}
- P.02 **PREPARAÇÃO E CARACTERIZAÇÃO DE NANOPARTÍCULAS POLIMÉRICAS PARA FINS COSMÉTICOS.** Ana Henriques Mota^{1,2}, Patrícia Rijo^{1,3}, Jesus Molpeceres², Lia Ascensão⁴, Luís Monteiro Rodrigues¹, Catarina Pinto Reis^{1,5}.
- P.03 **AMINO ACID BASED IONIC LIQUIDS AS FUNCTIONAL INGREDIENTS IN TOPICAL FORMULATIONS CONTAINING CAFFEINE.** Ana Júlio,¹ Cláudia Antunes,¹ Rita Caparica, Maria E. Araújo,² Nuno Saraiva,¹ Ana Fernandes,¹ André R. Baby,³ Catarina Rosado,¹ Joana Portugal Mota,^{1,*} Tânia Santos de Almeida,^{1,2,*}
- P.04 **ANTHROPOMETRIC, BODY COMPOSITION AND NUTRITIONAL INTAKE EVALUATION OF SWIMMING ATHLETES.** Ana Morgado¹, Bruno Pereira², Nelson Tavares¹
- P.05 **PLECTRANTHUS SPP. STUDY: ANTIMICROBIAL ACTIVITY OF EXTRACTS AND CHEMICAL CHARACTERIZATION OF ESSENTIAL OILS.** Anastasia Boroza¹, Catarina Garcia^{2,3}, Luís Roque^{2,3}, Ana C. Figueiredo⁴, Carlos M. Monteiro¹, Carlos A.M. Afonso¹, Catarina Reis^{1,5}, Patrícia Rijo^{1,2}
- P.06 **DESENVOLVIMENTO DE NANOPARTÍCULAS DE PRATA PARA ADMINISTRAÇÃO TÓPICA.** Beatriz Simões Janeiro¹, Ana Henriques Mota^{2,3}, Patrícia Rijo^{2,4}, and Catrina Pinto Reis^{2,5}
- P.07 **DESENVOLVIMENTO FOLICULAR NA CADELA E NA GATA AO LONGO DO CICLO ÉSTRICO.** Carolina Cardoso¹ & Daniel Murta^{1,2*}
- P.08 **EVALUATION OF RED AND PROCESSED MEAT VERSUS POULTRY MEAT CONSUMPTION BEFORE AND AFTER THE OCTOBER 2015 WHO REPORT IN A COLLECTIVE CATERING UNIT .** Catarina Afonso¹, Nelson Tavares¹.
- P.09 **DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS EXTRACTS – A DIDACTIC APPROACH.** Catarina Garcia^{1,2}, Catarina Oliveira¹, Cláudia S. Oliveira¹, Amílcar Roberto¹, Catarina Reis^{1,3}, Marisa Nicolai¹, Patrícia Rijo^{1,4}.
- P.10 **ACADEMIC ASSESSMENT OF ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF ETHANOLIC EXTRACTS FROM PLECTRANTHUS MADAGASCARIENSIS.** Catarina Garcia^{1,2}, Catarina Teodósio¹, Beatriz Janeiro¹, Catarina Reis^{1,3}, Patrícia Rijo^{1,4}.
- P.11 **ASSESSING THE EFFECT OF MANUAL MASSAGE ON THE LOWER LIMB MICROCIRCULATION.** Clemente Rocha¹, Henrique Silva^{1,2}, Inês Frazão² and Luís Monteiro Rodrigues^{1,2}.
- P.12 **MYRTLE EXTRACT AS A COSMETIC PRESERVATIVE.** Epole N. Ntungwe^{1,2}, Joana Marçalo,^{1,2} Catarina Garcia^{1,3}, Marisa Nicolai¹, Luis Monteiro Rodrigues¹, Catarina Reis^{1,4}, Patrícia Rijo^{1,2}.
- P.13 **IN VITRO INHIBITION OF ACETYLCHOLINESTERASE BY SOME PLECTRANTHUS SPECIES EXTRACTS.** M. André, C. Antunes, J. Silva, C Reis, P. Rijo and M. Nicolai.
- P.14 **EVALUATION OF THE NUTRITIONAL STATUS IN PRE-SURGICAL PATIENTS AND OUTPATIENTS: A CASE STUDY.** Flávio Jerónimo¹, Nelson Tavares¹.
- P.15 **THERMAL STABILITY OF PROTEINS IN THE PRESENCE OF IONIC LIQUIDS.** Filipa Rocha,¹ Rita Caparica,¹ Ricardo Peres,¹ Joana Portugal Mota,^{1,*} Tânia Santos de Almeida,^{1,*}.
- P.16 **CHEMICAL COMPOSITION AND BIOLOGICAL PROPERTIES OF ESSENTIAL OILS FROM PLECTRANTHUS SPP.** Francisco Sousa¹, Catarina Garcia^{2,3}, Ana C. Figueiredo⁴, Carlos M. Monteiro¹, Carlos AM Afonso¹, Catarina Pinto Reis^{1,5}, Patrícia Rijo^{1,2}.
- P.17 **EXPLORING THE MICROVASCULAR RESPONSE TO AN OXYGEN STRESS TEST IN A MURINE MODEL OF CARDIAC HYPERTROPHY.** Henrique Silva^{1,2}, Alain-Pierre Gadeau³, Luís Monteiro Rodrigues^{1,2}.
- P.18 **COMBINING PHOTOPLETHYSMOGRAPHY AND LASER DOPPLER FLOWMETRY TO CHARACTERIZE THE PERIPHERAL VASCULAR RESPONSE IN THE HUMAN LOWER LIMB.** Henrique Silva^{1,2}, Clemente Rocha¹, Beatriz Navarro Brazález³, Rita Faria¹, Raquel Ferreira¹, Luís Monteiro Rodrigues^{1,2}
- P.19 **EXPLORING THE INFLUENCE OF HEMODYNAMIC DETERMINANTS ON SKIN BARRIER FUNCTION.** Henrique Silva^{1,2}, Inês R. Frazão², Hugo Ferreira³, Luís Monteiro Rodrigues^{1,2}
- P.20 **EXPLORING THE IMPACT OF ANESTHESIA ON THE ENTROPY PROFILE OF THE DOG'S SKIN PERFUSION – A PILOT STUDY.** Henrique Silva^{1,2}, João Requicha³, Rui Assunção³, Clemente Rocha¹, Luís Lobo³, Margarida Estudante¹, L. Monteiro Rodrigues^{1,2}.
- P.21 **EVALUATION OF A NUTRITION EDUCATION PROGRAM IMPLEMENTATION AT REAL COLÉGIO DE PORTUGAL.** Inês Martins¹, Ana Abreu¹, Marta Crespo¹, Rita Faria¹, Jessica Barreira¹, Nelson Tavares¹.
- P.22 **EXPLORING THE IN VITRO ANTI-INFLAMMATORY AND SKIN-RELATED ENZYMIC INHIBITION ACTIVITIES OF PLECTRANTHUS SPP. EXTRACTS AND ISOLATED COMPOUNDS.** Joana Marçalo^{1,2}, Luísa Custódio³, Marisa Nicolai¹, Luís Monteiro Rodrigues¹, Catarina Pinto Reis^{1,4}, Célia Faustino², Patrícia Rijo^{1,2}
- P.23 **EVALUATION OF GLYCATED HEMOGLOBIN (HA1C) STATUS IN PATIENTS FROM AN INTERNAL MEDICINE SERVICE AT A LISBON HOSPITAL.** Joana Padrão¹, Nelson Tavares¹
- P.24 **NANOFORMULATIONS FOR THE TREATMENT OF YEAST INFECTIONS IN THE ORAL MUCOSA.** Luis Roque¹, Catarina Garcia¹, Patrícia Rijo¹ and Catarina Pinto Reis^{1*}
- P.25 **NANOCARRIERS FOR SKIN DELIVERY OF COSMETIC ANTIOXIDANTS.** Mafalda Almeida,¹ Patrícia Penela,¹ Ana Júlio,¹ André R. Baby,² Daniela C. Oliveira,² Catarina Rosado,^{1,*} Tânia Santos de Almeida,^{1,*} Joana Portugal Mota,^{1,*}
- P.26 **COMPARISON OF THE SENSORIAL PROFILES IN TWO DISTINCTIVE PERIODS OF TWO BRANDS OF CERVEJAS DE MOÇAMBIQUE, THE 2M AND MANICA.** Mitchell Carneiro¹, Nelson Tavares¹
- P.27 **EVALUATION OF THE AMOUNT OF ADDED SALT IN COOKING MEALS IN A LISBON HOSPITAL.** Nuno Rubio¹, Nelson Tavares¹

P1 - Influence of an ionic liquid in caffeine release from lipidic implants

Afonso Antunes,¹ Joana Portugal Mota, J.^{1*}, Tânia Santos de Almeida,^{1,*}

¹ CBIOS-Universidade Lusófona's Research Center for Biosciences and Health Technologies, Campo Grande 376, 1749-024, Lisboa, PORTUGAL.
Contacto: afonsomsantunes@gmail.com

The aim of this work was to study drug release profiles from lipidic implants in the presence of an ionic liquid (IL).

Implants were produced according to the published method of Kreye et al., 2008 with a few modifications [1].

To illustrate the feasibility of these modifications and to establish the possibility of inclusion of both lipophilic and hydrophilic substances in these kind of vehicles, a uniformity study was performed with coloring substances, either a lipophilic (Sudan III) or a hydrophilic (methylene blue). Both coloring substances showed a satisfactory diffusion and homogeneity in the entire batch.

Three batches were produced with a drug content of 10% (w/w) and a) Dysasan, b) Dynasan:Gelucire (%) e c) Dynasan:Gelucire:IL.(%)

Drug content was measured to be a) 9,62 ± 0,46 % for dynasan implants; b) 9,55 ± 0,51 % for Dynasan:Gelucire (%) and c) 10,07 ± 0,85 % Dynasan:Gelucire:IL.(%) implants. These results showed good content uniformity in all studied batches.

Drug release profiles showed the incorporation of the IL in the implants increased drug release rates when compared with implants without this ingredient.

References:

[1] Kreye, F., et al. (2008). Lipid implants as drug delivery systems. *Expert Opinion on Drug Delivery*, 5, 291–307.

P2 - SPreparação e caracterização de nanopartículas poliméricas para fins cosméticos

Ana Henriques Mota^{1,2}, Patrícia Rijo^{1,3}, Jesus Molpeceres², Lia Ascensão⁴, Luís Monteiro Rodrigues¹, Catarina Pinto Reis^{1,5}

¹ CBIOS, ULHT, Campo Grande 376, 1749-024 Lisboa, Portugal.

² Department of Biomedical Sciences, Faculdade de Farmácia, Universidade de Alcalá, Ctra. A2 km 33.600, 28871 Alcalá de Henares, Spain.

³ iMED-ULisboa, Faculdade de Farmácia, ULisboa, Av. Prof. Gama Pinto, 1749-003 Lisboa, Portugal.

⁴ CESAM, Faculdade de Ciências de Lisboa, ULisboa, Campo Grande, 1749-016 Lisboa, Portugal.

⁵ IBEB, Faculdade de Ciências de Lisboa, ULisboa, Campo Grande, 1749-016, Lisboa, Portugal.

Contacto: anavmota12@gmail.com

As nanopartículas têm sido frequentemente usadas nos últimos anos na cosmética devido a características únicas tais como a alta biocompatibilidade e o excelente perfil de segurança. Nestes sistemas de veiculação, determinadas características tais como a dimensão e forma das nanopartículas afectam significativamente a libertação do princípio activo encapsulado, a estabilidade física e a permeação cutânea. No caso das nanopartículas poliméricas, estas são geralmente preparadas com polímeros biocompatíveis e biodegradáveis (naturais ou sintéticos) e apresentam uma morfologia uniforme com diâmetros entre 10-1000 nm. Neste estudo foram preparados dois tipos de nanopartículas poliméricas para fins cosméticos: ácido poli-láctico-glicólico (PLGA) e poli-ε-caprolactona (PCL), investigando-se a sua estabilidade ao longo do tempo.

O PLGA foi adquirido à Purac Biomaterials (Gorinchen, Holanda) e o ácido oleico à FlukaChemika (Buchs, Swiss). A policaprolactona (PCL) e o Pluronic® F127 foram adquiridos à Sigma Aldrich (Steinheim, Alemanha). Todos os outros reagentes apresentavam grau analítico.

As nanopartículas de PLGA foram preparadas pelo método de emulsão/difusão de solvente. Resumidamente, estas nanopartículas foram preparadas por dissolução do PLGA numa mistura de acetona/etanol. Posteriormente, esta mistura foi adicionada sob agitação a uma solução aquosa de Pluronic® F127 a 0,1%. As nanopartículas de PCL foram preparadas pelo método de deslocação de solvente. Uma determinada quantidade de ácido esteárico em etanol foi adicionada à fase orgânica constituída por PCL e acetona, tendo sido posteriormente adicionada à solução aquosa de Pluronic® F127. Ambos os tipos de partículas foram avaliadas em termos de tamanho e potencial zeta, recorrendo à espectroscopia de correlação fotónica e anemometria de laser. Quanto à sua morfologia esta foi observada em microscopia electrónica de varrimento. Pela análise dos resultados, os tipos de nanopartículas apresentam forma esférica, sendo as de PLGA de menor dimensão (201.56 ± 3.96 d.nm) em relação às de PCL (645.70 ± 42.15 d.nm). As nanopartículas de PLGA, mais estáveis ao longo do tempo, serão alvo, num futuro próximo, de um estudo detalhado que incluirá a encapsulação de princípios activos para fins cosméticos.

Referências:

[1] Santos-Martinez, M. J. et al., *Journal of Biomedical Nanotechnology* (2014), 10, 1004–1015.

[2] Uchechi, O. et al., *Application of Nanotechnology in Drug Delivery* (2014), 193–235.

[3] Pinto Reis, C. et al., *Nanomedicine: Nanotechnology, Biology, and Medicine* (2006), 2, 8–21.

P3 - Amino Acid Based Ionic Liquids as Functional Ingredients in Topical Formulations containing Caffeine

Ana Júlio,¹ Cláudia Antunes,¹ Rita Caparica, Maria E. Araújo,² Nuno Saraiva,¹ Ana Fernandes,¹ André R. Baby,³ Catarina Rosado,³ Joana Portugal Mota,^{1,*} Tânia Santos de Almeida,^{1,2,*}

¹ CBIOS-Universidade Lusófona's Research Center for Biosciences and Health Technologies, Campo Grande 376, 1749-024, Lisboa, PORTUGAL.

² Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, PORTUGAL, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, PORTUGAL.

³ School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 15, 05508-900, São Paulo, BRAZIL.

*Shared senior authorship

Contacto: analuisajulio@gmail.com; claudia_antunes_93@hotmail.com

Pharmaceutical and cosmetic industries have a challenge with poorly and sparingly soluble drugs in water and in most pharmaceutical grade solvents. Therefore, it is fundamental to find/synthesize new biocompatible excipients that increase drug solubility and/or skin permeation.

Ionic liquids (ILs) are salts, in which the ions are weakly coordinated, resulting in solvents that are liquid below 100 °C or even at room temperature (RT). These properties allow ILs to be placed in water, oils or hydro alcoholic solutions which convey them the prospect to be incorporated in topical formulations. [1,2]

In this work, caffeine was studied as hydrophilic drug. Three choline based ILs were synthesized, characterized and investigated as solubility/permeation enhancers, namely [Cho][Phe], [Cho][Glu] and [Cho][Cys]. The active solubility in water or in water:IL mixtures (99.8:0.2) was evaluated at RT and at 32 °C. For both temperatures, ILs promoted an increment in solubility when compared to water, although [Cho][Phe] and [Cho][Glu] proved to be better solubility enhancers.

To estimate the skin permeation, in vitro permeation studies, from drug saturated solutions in water and in water:IL mixtures (99.8:0.2), were performed using pig ear skin. The results show that ILs do not influence the permeation.

Furthermore, since [Cho][Phe] and [Cho][Glu] proved to be the better solubility enhancers, these ILs were incorporated in topical formulations. Hence, caffeine (2.0 %, w/w) was incorporated into oil-in-water emulsions and gels, in the presence (or absence) of the [Cho][Phe] and [Cho][Glu] ILs, at an IL percentage that did not exhibit a marked decrease in cell viability (0.2 % w/w). All prepared formulations proved to be stable after the performed accelerated stability studies.

References:

[1] Dobler, D., et al., *Int. J. Pharm.*, 441, 620-7, 2013.

[2] Moniruzzman, M. et al., *Int. J. Pharm.*, 400, 243-50, 2010.

P4 - Anthropometric, body composition and nutritional intake evaluation of swimming athletes

Ana Morgado¹, Bruno Pereira², Nelson Tavares¹

¹ CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

² Unidade de Medicina Desportiva e Controlo de Treino - Centro de Alto Rendimento do Jamor IPDJ, Lisboa, PORTUGAL

Contacto: nelson.tavares@ulusofona.pt

Over the years we have come to realize that food intake significantly influences athletic performance. For this reason research in this specific area of Nutritional Sciences has evolved in recent years. Given that the only way the organism to obtain energy and nutrients is through food can be easily understood the importance of sports nutrition. The working tool of an athlete is his own body so it is important to practice a healthy diet adapted to the objectives in order to optimize sports performance, reducing or delaying the factors that lead to fatigue and consequently ensuring a speedy recovery between training and competition. Several studies have shown that athletes who make insufficient energy intake, or do not make a balanced distribution of nutrients, have more difficulty in adapting to the training. [1,2] The benefits of sport nutrition have been recognized by the scientific community, but also there are few studies published in Portugal that are dedicated to this area, especially swimming.

This study evaluated the anthropometric, body composition and nutritional intake of swimming athletes, belonging to the club "Os Belenenses". It was determined the height, weight, BMI, fat mass, muscle mass and body water these athletes. Evaluation of the nutritional intake using 3 days food diaries analyzed with Food Processor SQL 10.6 software. Weight and body composition were measured using bioimpedance balance Tanita BC-601 and height was measured with metric tape according to the Frankfurt plane. Ten swimming athletes participate in the study, 8 males and 2 females athletes mean age 19.9±2.0 years. Male athletes had a body fat percentage of 8.3%, which is within the recommended range, the same goes for female athletes, with a percentage of 15.2%. In addition, all athletes had high muscle mass values and adequate hydration. This study showed that athlete's energy intake was slightly above the recommended (3451.3±928.1 calories), from excessive protein intake (145.4±42.8 g) and the saturated lipids (11.7%) and sugar (139.3±52.9 g). Fiber intake was below recommended (27.9±7.9g) as well as water intake (2527.7 mL). These results could be explained by low intake of fruit, vegetables and whole grains. The results in this study suggested an inadequate nutritional and energy level for this group of swimming athletes. It becomes increasingly important to raise awareness of the athletes to the importance of nutrition for optimal health and sports performance.

References:

[1] Burke, L. *International Journal of Sport Nutrition and Exercise Metabolism*, 2003, 13, 549-552.

[2] Burke, L. *Practical sports nutrition*. Belconnen: Human Kinetics 2007.

P5 - *Plectranthus* spp. Study: Antimicrobial activity of extracts and chemical characterization of essential oils

Anastasia Borozan¹, Catarina Garcia^{2,3}, Luís Roque^{2,3}, Ana C. Figueiredo⁴, Carlos M. Monteiro¹, Carlos A.M. Afonso¹, Catarina Reis^{1,5}, Patrícia Rijo^{1,2}

¹ Instituto de Investigação do Medicamento (iMed.U LISBOA), Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal

² CBIOS, Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal

³ Dpto. Ciencias Biomédicas, Universidad de Alcalá, Plaza de San Diego, s/n, 28801 Alcalá de Henares, Madrid, Espanha

⁴ CESAM, CBV, DBV, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

⁵ IBEB, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal
Contacto: patricia.rijo@ulusofona.pt

Medicinal plants are known to be the backbone of traditional medicine, and in the last few decades, have been the subject of very intense pharmacological studies [1] [2]. Previous works on extracts and essential oils (EOs) of the *Plectranthus* genus (Lamiaceae) have demonstrated that some of their constituents hold interesting biological activities [3].

In this study, twenty-seven organic extracts of several *Plectranthus* species were prepared in order to evaluate their antimicrobial and antifungal activities. The selected plants of *P. alciace*, *P. amboinicus*, *P. barbatus*, *P. hadiensis*, *P. heroensis*, *P. japonicus*, *P. madagascariensis*, *P. madagascariensis* Lynne, *P. malvinus*, *P. ortendali*, *P. reflexus*, *P. strigosus*, *P. stylissis* and *P. zuluensis*, were submitted to an ultrasound assisted extraction using three different extracting solvents (acetone, methanol and ethanol).

The assessment of their antimicrobial activity was evaluated through the well diffusion method for Gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and yeast (*Saccharomyces cerevisiae* and *Candida albicans*). According to the highest shown antimicrobial activities, MIC and MBC values were also determined for the five selected *Plectranthus* extracts, which have revealed low antimicrobial activity.

Additionally, one of the plant extract with highest antimicrobial activity – *P. alciace* – was selected to prepare their EO by hydrodistillation, using the Clevenger apparatus. Furthermore, the chemical composition of this EO was determined and quantified by GC and GC-MS.

The cytotoxicity of the extracts was also assessed. These promising results will be interesting for further antiproliferative studies of *Plectranthus* extracts and the isolation of the compounds responsible for its bioactivity.

Acknowledgements: This study was partially funded by Fundação para a Ciência e a Tecnologia (FCT), under UID/AMB/50017/2013, FEDER PT2020-Compete 2020.

References:

- [1] Boukraâ, L. et al., *Formatex*, vol.2, pp.960–970.
- [2] Matu, E.N. & Van Staden, J., *Journal of Ethnopharmacology*, 2003, 87(1), 35–41.
- [3] Rice, L.J. et al., *South African Journal of Botany*, 2011, 77(4), 947–959.

P6 - Desenvolvimento de nanopartículas de prata para administração tópica

Beatriz Simões Janeiro¹, Ana Henriques Mota^{2,3}, Patrícia Rijo^{2,4}, and Catrina Pinto Reis^{2,5}

¹ECTS, ULHT, Campo Grande 376, 1749-024 Lisboa, Portugal.

²CBiOS, ULHT, Campo Grande 376, 1749-024 Lisboa, Portugal.

³Department of Biomedical Sciences, Faculdade de Farmácia, Universidade de Alcalá, Ctra. A2 km 33.600, 28871 Alcalá de Henares, Spain.

⁴iMED, Faculdade de Farmácia, ULISBOA, Av.Prof. Gama Pinto, 1749-003 Lisboa, Portugal.

⁵IBEB, Faculdade de Ciências de Lisboa, ULISBOA, Campo Grande, 1749-016, Lisboa, Portugal.

Contacto: beatriz.s.janeiro@hotmail.com

Nos últimos anos temos assistido a um aumento do número de casos de resistência aos antibióticos, especialmente em pediatria. Neste último grupo, a existência de otites (otite média aguda (AOM) e otite média de efusão (OME)) é cada vez mais frequente principalmente devido à ineficácia dos antibióticos tópicos na membrana do tímpano, que actua como barreira à absorção de fármacos. Aqui, administração tópica com nanopartículas poderá ser considerada uma alternativa viável neste tipo de patologias graças às suas propriedades que as tornam únicas. As nanopartículas de prata, além de atuarem como sistemas de veiculação de fármacos poderão ainda ser consideradas excelentes antibacterianos. O objectivo deste estudo consiste na preparação de nanopartículas de prata em diferentes concentrações, partindo da base da razão molar 1:2 (prata: borohidreto), a sua influência na distribuição de tamanhos.

O borohidreto de sódio (NaBH₄) e o Nitrato de prata (AgNO₃) foram adquiridos à Sigma Aldrich (Steinheim, Alemanha). Todos os outros reagentes possuíam um grau analítico.

As nanopartículas foram sintetizadas através do método de redução da prata e caracterizadas a nível de tamanho médio e polidispersividade utilizando o Delsa TMNano C (Beckman Coulter, Fullerton, CA).

Na análise de tamanhos médios das nanopartículas produzidas com a concentração descrita na literatura², estas apresentavam um tamanho e um índice de polidispersividade superior quando comparadas com a razão molar 1:1 ou 2:1, provavelmente devido a um excesso de borohidreto em reacção. Estima-se que o borohidreto aumente a força iónica da solução e a probabilidade de ocorrer aglomeração é significativamente superior². A melhor formulação foi obtida com a razão molar 1:1. Num futuro próximo, todas as formulações anteriores serão sujeitas a sua avaliação de eficácia através de ensaios in vitro.

Referências:

- [1] Guzmán, M. G. M., Dille, J. & Godet, S. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. *Proc. World Acad. Sci. Eng. Technol.* 45, 357–364 (2009).
- [2] Mulfinger, L. et al. Synthesis and Study of Silver Nanoparticles. *J. Chem. Educ.* 84, 322–325 (2007).

P7 - Desenvolvimento folicular na cadela e na gata ao longo do ciclo éstrico

Carolina Cardoso¹ & Daniel Murta^{1,2*}

¹ CBIOS-Research Centre for Biosciences and Health Technologies, Faculty of Veterinary Medicine, Lusofona University of Humanities and Technologies, Lisboa, Portugal.

² Reproduction and Development, CHISA, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

Contacto: ldanieljmcdmurta@gmail.com

A foliculogénese é morfológicamente similar nas diferentes espécies de mamíferos. Ao longo do ciclo reprodutivo, estão presentes foliculos ovários em todas as fases de desenvolvimento, que são normalmente classificados de acordo com o seu crescimento e podem estar em fases de quiescência, de crescimento ou atresia. No entanto, cada espécie tem a sua própria escala temporal de desenvolvimento, envolvendo processos como a proliferação e diferenciação das células foliculares^{2,3}. Contudo, os mecanismos que desencadeiam o início do crescimento folicular permanecem pouco compreendidos. O objetivo deste estudo consiste em determinar se o aumento da proliferação celular nos foliculos ovários está relacionado com o recrutamento e desenvolvimento folicular, comparando ainda o desenvolvimento e recrutamento folicular entre cadelas e gatas, espécies que embora filogeneticamente afastadas concentram forte interesse veterinário. Foram recolhidos ovários de 11 cadelas e 16 gatas com idades compreendidas entre os 5 meses e 2 anos de idade, agrupando os animais por fase do ciclo éstrico, proestro, estro, diestro, anestro. A fase do ciclo éstrico foi determinada por citologia vaginal, concentração sérica de progesterona e observação histológica dos respetivos ovários.

O estudo foi desenhado no sentido de utilizar a expressão de Proliferating cell nuclear antigen (PCNA) como marcador de proliferação celular nos foliculos ovários. Foi detetada forte marcação nas células do corpo lúteo em ambas as espécies, mas sempre mais evidente em diestro. As células da granulosa foram marcadas de forma diferente consoante a fase do ciclo éstrico e a fase de desenvolvimento folicular. Assim, a corona radiata e o cumulus oophorus, quando presentes, exibem pouca marcação, não estando marcados em foliculos antrais. Por outro lado, os foliculos primordiais apresentam algumas células da granulosa marcadas, sendo esta observação mais frequente em gatas, o que pode estar relacionado com o início do recrutamento folicular. Os ócitos foram marcados em todas as fases foliculares de ambas as espécies. Já as células da teca interna e externa não apresentaram marcação em foliculos pré-antrais, encontrando-se ligeiramente marcadas em foliculos antrais. De uma forma geral, as diferenças de proliferação celular encontradas tanto em células da teca como da granulosa encontradas ao longo do desenvolvimento folicular são mais evidentes em cadelas.

Estes resultados, embora preliminares, permitem concluir que a proliferação celular varia consoante as fases do ciclo éstrico e de desenvolvimento folicular tanto em cadelas como em gatas, tendo sido a primeira vez que esta avaliação foi realizada nestas espécies recorrendo a este marcador. Contudo, este trabalho será enriquecido ao ser desenvolvido a par com outros marcadores de diferenciação celular.

Referências:

- [1] Bristol-Gould S, Woodruff TK. Folliculogenesis in the domestic cat (*Felis catus*). *Theriogenology*. 2006;66(1):5-13.
- [2] Eppig JJ. Oocyte control of ovarian follicular development and function in mammals. *Reproduction*. 2001;122(6):829-838.
- [3] Smitz JEJ, Cortvrindt RG, Fujihara M, et al. The earliest stages of folliculogenesis in vitro. *Reproduction*. 2002;90(4):185-202.

P8 - Evaluation of red and processed meat versus poultry meat consumption before and after the October 2015 WHO report in a collective catering unit

Catarina Afonso¹, Nelson Tavares¹

¹ CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

Contacto: nelson.tavares@ulusofona.pt

In October, 2015, 22 scientists from ten countries met at the International Agency for Research on Cancer (IARC) in Lyon, France, to evaluate the carcinogenicity of the consumption of red and processed meat. IARC is the cancer agency of the World Health Organization (WHO). The Working Group assessed more than 800 epidemiological studies that investigated the association of cancer with consumption of red meat or processed meat in many countries, from several continents, with diverse ethnicities and diets. Overall, the Working Group classified consumption of processed meat as “carcinogenic to humans” (Group 1) on the basis of sufficient evidence for colorectal cancer. Additionally, a positive association with the consumption of processed meat was found for stomach cancer. The Working Group classified consumption of red meat as “probably carcinogenic to humans” (Group 2A). [1]

This study aims to evaluate the impact on red and processed meat consumption in a collective catering unit after the publication of the WHO report. The evaluation was made using the records of consumed meals containing poultry, red or processed meat between May to October 2015 and November 2015 to April 2016. It was also applied a questionnaire on preferences for red, processed or poultry meat and knowledge on WHO report conclusions. They were consumed on the six months before WHO report 15,501 meals containing red or processed meat and 10,090 meals containing poultry meat. During six months after report publication 15,150 meals containing red and processed meat and 9,865 with poultry meat were consumed. There were no significant differences in monthly means on red and processed meat consumption between both periods ($p=0.9886$, $p<0.01$) as well as for poultry meat ($p=0.5263$, $p<0.01$). From total collective catering unit 300 consumers, 66% were women and 34% men, aged between 22 and 62 years (mean=38 years). From total unit consumers only 141 unit answer to questionnaire. For respondents 76% poultry meat and 19% red or processed meat were the preferences. The WHO report was known by 74% and 22% considered to change meat choices after October 2015. The report recommendations were supported by 60% unit respondents.

The levels of poultry, red or processed meat consumption did not show significant changes after WHO report. Low number of respondents to the questionnaire could contributed to the not shown similarity to the registered poultry, red or processed meat consumption. Further studies must be conducted with larger quantity of consumers in order to obtain more robust conclusions.

Referências:

- [1] Bouvard, Véronique et al., *The Lancet Oncology*, Volume 16, Issue 16, 1599 - 16.

P9 - Determination of the antioxidant activity of medicinal plants extracts – a didactic approach

Catarina Garcia ^{1,2}, Catarina Oliveira ¹, Cláudia S. Oliveira ¹, Amílcar Roberto ¹, Catarina Reis ^{1,3}, Marisa Nicolai ¹, Patrícia Rijo ^{1,4}

¹Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal

²Dpto. Ciencias Biomédicas, Universidad de Alcalá, Plaza de San Diego, s/n, 28801 Alcalá de Henares, Madrid, Espanha

³IBEB, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal

⁴Instituto de Investigação do Medicamento (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal
Contacto: patricia.rijo@ulusofona.pt

Free radicals are common and popularly seen as the major problem responsible for aging. This concept, however, is not commonly associated with foremost important issues such as damaging effects on the human body. To prevent this harmful outcome, there are compounds that are able to react with free radicals, neutralizing them and therefore reduce their damaging impact on human tissues over time. Although synthetic antioxidants are currently used, there is a growing interest in finding natural ones in order to replace the use of the former, due to their detrimental long-term side effects [1]. In this work, a simple laboratory procedure was carried out to evaluate the antioxidant activity of several medicinal plants. Ethanol ultra-sound assisted extracts of *Salvia officinalis* L., *Lavandula angustifolia*, *Hypericum perforatum*, *Melissa officinalis* L., *Malva sylvestris* and *Rosmarinus officinalis* L. were obtained. Their extraction yields varied between 1.29% and 6.00% (w/w) and their antioxidant activity was assessed by TLC-DPPH and DPPH assays.

According to both assays, the extract that has revealed to have more antioxidant activity was the ethanolic extract of *Melissa officinalis* L. This showed an antioxidant activity (AA%) of 90%, which is similar to the one obtained by the positive control – rosmarinic acid – with an AA of 99%. Thus, further studies should be done as this could potentially be used as a non-synthetic antioxidant. This laboratory experiment described herein demonstrates that traditionally used plants exhibit high contents of substances with antioxidant properties.

References:

[1] Nicolai M., et al., Measurement, 89: 328–332, 2016

P10 - Academic assessment of antimicrobial and antioxidant properties of ethanolic extracts from *Plectranthus madagascariensis*

Catarina Garcia ^{1,2}, Catarina Teodósio ¹, Beatriz Janeiro ¹, Catarina Reis ^{1,3}, Patrícia Rijo ^{1,4}

¹Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal

²Dpto. Ciencias Biomédicas, Universidad de Alcalá, Plaza de San Diego, s/n, 28801 Alcalá de Henares, Madrid, Espanha

³IBEB, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal

⁴Instituto de Investigação do Medicamento (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal
Contacto: patricia.rijo@ulusofona.pt

The Lamiaceae family is commonly known for being used in traditional medicine as a source of treatment of several diseases and pathologies [1]. Belonging to this family, we can emphasize *Plectranthus madagascariensis*, which also has vast applications due to its chemical diversity and presence of bioactive natural products. Among others, we can highlight its notable antioxidant and antimicrobial properties [1,2].

In this work, a didactic laboratory procedure was carried out to assess the antioxidant and antimicrobial activity of *P. madagascariensis*. In order to do so, an ethanolic extract of *P. madagascariensis* was obtained through sonication [2]. Using the well diffusion method, the resulting extract was then screened for its antimicrobial activity against two Gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), and two yeast strains (*Candida albicans* and *Saccharomyces cerevisiae*). Additionally, it has also been qualitatively determined the antioxidant activity of the prepared extract through the TLC-DPPH assay.

As a result, the ultra-sound assisted ethanolic extract of *P. madagascariensis* has revealed antioxidant activity and also significant antimicrobial activity against Gram-positive bacteria. Thus, the results showed that *P. madagascariensis* is a promising bioactive starting material for further studies and future applications.

References:

[1] Likhoba C.W. et al., J. Ethnopharmacol., 3; 103(1):1-24, 2006

[2] Rijo P., et al., Biomed Biopharm Res., 2012; (9) 2: 225-235.

P11 - Assessing the effect of manual massage on the lower limb microcirculation

Clemente Rocha¹, Henrique Silva^{1,2}, Inês Frazão² and Luís Monteiro Rodrigues^{1,2}

¹Universidade Lusófona de Humanidades e Tecnologias, 376, 1749-024, Campo Grande, Lisboa, Portugal

²CBIOS (Research Center for Biosciences and Health Technologies), U Lusófona, Campo Grande 376, 1749-024, Lisboa, Portugal

²Pharmacol. Sc Depart, Faculty of Pharmacy, U Lisboa, Lisboa, Portugal
Contacto: c.rocha.dr@gmail.com

Body massage is one of the techniques used throughout times for relaxation, but also to improve and relief symptoms, specially those associated to circulatory dysfunction in the lower limb [1]. Although massage effects on the peripheral microcirculation have been studied, its impact on the physiological mechanisms involved are still poorly understood. Non-invasive measurement techniques, such as laser Doppler flowmetry (LDF) and photoplethysmography (PPG) currently provides easy access, also research opportunities, to microcirculatory function in vivo [2,3]. Here, we explore the impact of two massage maneuvers (upward and downward) in the peripheral microvascular response in the human lower limb. A group of seven young healthy volunteers, both sexes (35.1 ± 3.6 years old) was included in the study after informed written consent. After acclimatization, each volunteer was submitted to two massage protocols in one randomly chosen limb (test), lying supine, one applied in the ascending direction (upward) from the root of the toes until mid-leg, and another in the descending direction (downward) from the mid-leg until the root of the toes. The contralateral leg was used as control, keeping the foot in the same position. Both protocols were performed in a random order, and separated by a 30 min washout period. Each protocol consisted of three phases, 5 minute duration each - I rest, II the massage and III the recovery. Blood flow was measured in the inferior aspect of the first and second toes in both feet, by LDF (PF5010, Perimed, Sweden) and PPG (BVP sensor, Bitalino Plugged, PLUX Biosystems, Portugal). The Wilcoxon signed-rank test was used for phase comparisons (p<0.05). The upward massage caused an increase in blood flow measured by LDF and PPG in both feet, only significant in the test foot. The downward massage also increase blood flow on both feet, only significant for LDF signal in the test foot. While the increase in blood flow on the test foot seems to be due to a direct mechanical action, the increase in blood flow on the control foot suggests a different adjustment to be better investigated. The pulse rate (PR), calculated from the PPG signal in the control foot, decreased in both protocols, but only significantly during phase II of the ascending direction protocol. Although preliminary, these results suggest that massage facilitates the lower limb perfusion no matter the direction (ascending or descending) of the movement.

References:

[1] Weerapong, P et al., The Mechanisms of Massage and Effects on Performance, Muscle Recovery and Injury Prevention. Sports Med, 35(3), 235-256, 2005.

[2] Pérez-Martin, A., et al., Validation of a Fully Automatic Photoplethysmographic Device for Toe Blood Pressure Measurement, Eur J Vasc Endovasc Surg, 40, 515-520, 2010.

[3] Ubbink, D.Th., Toe Blood Pressure Measurements in Patients Suspected of Leg Ischaemia: A New Laser Doppler Device Compared with Photoplethysmography, Eur J Vasc Endovasc Surg, 27, 629-634, 2004.

P12 - Myrtle Extract as a Cosmetic Preservative

Elisabete M. Mauricio^{1,2}, Paula Pereira^{1,3}, Vanessa Martins², Maria Gabriela Gil¹, Maria João Cebola^{1,4}

¹ Universidade Lusófona Research Centre for Biosciences and Health Technologies, ULHT, Av. Campo Grande, 376, 1749-024 Lisboa, Portugal

² Elisa Câmara, Lda, Dermocosmética, Centro Empresarial de Talaide, n°7 e 8, 2785-723, S.Domingos de Rana, Portugal

³ CERENA - Centre for Natural Resources and the Environment, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

⁴ Escola Superior Náutica Infante D. Henrique, Av. Eng. Boneville Franco, 2770-058 Paço d'Arcos, Portugal

Contacto: elisabetem@netcabo.pt, p1204@ulusofona.pt, geral@elisacamara.pt, gbernardo.gil@tecnico.ulisboa.pt, mjoaocebola@enautica.pt

Cosmetics are required to include in their formulation a preservative so that their shelf life can be extended. However, some of the synthetic preservatives habitually used in most formulations are associated with causing allergies. For this reason, the cosmetic industry is increasingly looking for natural preservatives, such as natural plant extracts, to include in their formulations. Furthermore, these natural extracts may even enhance some of the cosmetic properties, like their scent, for instance. The efficacy of a preservative is tested by measuring the resistance of the formulation to contamination, through the so called challenge tests.

Our previous studies [1], as well as reports from other authors [2-3], have shown that myrtle extract possess significant antimicrobial activity in addition to a high antioxidant activity. However, its application to cosmetics as an active ingredient with antimicrobial properties are little studied. In the present work, the results of the challenge tests was performed over 28 days in a gel formulation, following the ISO 11930 standards (2012) [4], carried out show that the presence of myrtle extract at 5% (w/w) in a gel formulation reduced the microbial inoculum, thus complying with the challenge test standards criteria's for bacteria strains. The results indicate, therefore, that the myrtle extract can be recommended as a natural preservative for cosmetics by assuring protection against microbial contamination.

References:

[1] Pereira, P. et al., Supercritical fluid extracts with antioxidant and antimicrobial activities from myrtle (*Myrtus communis* L.) leaves. Response surface optimization. Journal of Supercritical Fluids (2013), 83, 57-64.

[2] Yetilmezsoy K et al., Response surface modeling of Pb(II) removal from aqueous solution by *Pistacia vera* L.: Box-Behnken experimental design, J. Hazardous Materials, 171 (2009), 551–562.

[3] Mert T., Antimicrobial and Cytotoxic Activities of *Myrtus Communis* L. J. Faculty Pharmacy Ankara, 37 (2008) 191–199.

[4] ISO 11930 (2012) Cosmetics - Microbiology – Evaluation of the antimicrobial protection of a cosmetic product.

P13 - In vitro inhibition of acetylcholinesterase by some *Plectranthus* species extracts

Epole N. Ntungwe^{1,2}, Joana Marçalo, ^{1,2}, Catarina Garcia^{1,3}, Marisa Nicolai ¹, Luis Monteiro Rodrigues ¹, Catarina Reis ^{1,4}, Patrícia Rijo ^{1,2}

¹ CBIOS-Research Center for biosciences and Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

² iMed.Ulisboa, Faculdade de Farmácia Universidade de Lisboa, Lisboa, PORTUGAL

³ Dpto. Ciencias Biomédicas, Universidad de Alcalá, Plaza de San Diego, s/n, 28801 Alcalá de Henares, Madrid, ESPANHA

⁴ IBEB, Faculdade de Ciências Universidade de Lisboa, Lisboa, PORTUGAL
Contacto: ntungweepolengolle@yahoo.com

Current research in drug discovery from medicinal plants provides new and important leads for drug development. The *Plectranthus* spp. plants are the focus of several scientific investigations, due to their ethnopharmacological use by indigenous populations [1]. The acetylcholinesterase enzyme (AChE) is an attractive target for the rational drug design and for the discovery of mechanism based inhibitors. AChE inhibitors are the most effective approach to treat the cognitive symptoms of Alzheimer disease (AD) [2,3] and other possible therapeutic applications (e.g. Parkinson's disease) [4]. AChE inhibitors such as eserine, tacrine, donepezil, rivastigmine, and galanthamine are the only drugs currently approved for the treatment of AD, which have limitations for clinical use due to their short-half-lives and/or unfavorable side-effects [5]. In this study, seven *Plectranthus* spp. were screened for their AChE inhibition since the search of plant extracts that selectively inhibit AChE is of paramount importance to find novel and more potent AChE inhibitors. The extracts were obtained from the medicinal plants under study, using acetone as the extraction solvent and the percentage yield was determined: *P. spicatus* (4.75% w/w), *P. woodii* (3.59% w/w), *P. cylindraceus* (9.68% w/w), *P. ramosior* (13.49% w/w), *P. petiolaris* (11.07% w/w), *P. swynnertonii* (3.84% w/w) and *P. welwischii* (8.51% w/w). *P. ramosior* showed the highest percentage yield (13.49% w/w). Acetylcholinesterase (AChE) in vitro inhibition was studied using the Ellman method, to evaluate the enzymatic inhibition from *Plectranthus* spp. organic extracts. The studies showed that the seven *Plectranthus* spp. did not significantly inhibited AChE ($p > 0.05$): *P. spicatus* ($24.20 \pm 0.98\%$), *P. woodii* ($24.76 \pm 0.18\%$), *P. cylindraceus* ($30.16 \pm 3.76\%$), *P. ramosior* ($19.98 \pm 3.08\%$), *P. petiolaris* ($16.65 \pm 4.14\%$), *P. swynnertonii* ($15.34 \pm 6.08\%$) and *P. welwischii* ($28.52 \pm 6.50\%$), with *P. cylindraceus* having the highest percentage inhibition of $30.16 \pm 3.76\%$. These results show that the *Plectranthus* spp. under study do not significantly inhibit the AChE enzyme. Furthermore, other biological studies of these medicinal plant extracts are under study.

References:

- [1] Likhoba, C. W., et al., Journal of Ethnopharmacology, 103:1–24, 2006
- [2] Kalauni SK, et al., Chem Pharm Bull, 50: 1423-1426, 2002
- [3] Atta-ur-Rahman, et al., Chem Pharm Bull 50: 1013-1016, 2002
- [4] Ahmad W et al., J Biol Sci, 11: 1046-1049, 2003.
- [5] Sung SY et al., Biol Pharm Bull 25: 125-127, 2002.

P14 - Evaluation of the nutritional status in pre-surgical patients and outpatients: a case study

Flávio Jerónimo¹, Nelson Tavares¹

¹ CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

Contacto: nelson.tavares@ulusofona.pt

Nutritional status is shown to be important in the recovery of health, so demystifies up hospital malnutrition as a recurring event today contributing to the emergence of co morbidities associated. Different factors affect the nutritional status of adults hospitalized as the action of the disease, diminished food intake, side effects of foods and physical inactivity. The nutritional status of a patient results from complex interactions between personal and environmental factors that influence yours health. Therefore the importance of hospital nutritional screening is a strong indicator of nutritional status. As is known one malnourished patient after undergo surgical procedure may suffer loss of functional capacity or postoperative complications resulting in more days of hospitalization.[1] [2] This case study involved hospitalized patients in the pre-surgery and outpatient unit at Clinica S. João de Deus, in Lisbon. The assessment of nutritional status is classified using the Mini Nutritional Assessment, Body Mass Index (BMI) and biochemical parameters predictors of nutritional status including total lymphocytes, hematocrit, hemoglobin, creatinine, urea and glycemia. This case study involved 67 participants (women's, n=32 and Men's, n=35) evaluated, 61 patients were classified as having adequate nutritional status, while 6 patients were classified as at risk of malnutrition. There were none individuals classified as malnourished. Regarding clinical tests it was verified that patients at risk of malnutrition had values of total lymphocyte and creatinine lower than the rated group having adequate nutritional status. In the case of BMI it was found that many individuals classified with adequate nutritional status were overweight or at obesity I level. There was not detected a large number of patients classified as malnourished or at risk of malnutrition. But there is a big number of patients in overweight condition, which causes problems for the patient's health and that can be identified with a simple nutritional screening in order to reverse the patient situation.

References:

- [1] Waitzber D, et al., Elsevier Science Inc 2001; 17:573-80.
- [2] Pablo A, et al., European Journal of Clinical Nutrition 2003; 57: 824-31.

P15 - Thermal stability of proteins in the presence of ionic liquids

Filipa Rocha,¹ Rita Caparica,¹ Ricardo Peres,¹ Joana Portugal Mota,^{1,*} Tânia Santos de Almeida,^{1,*}

¹CBIOS - Research Center for Biosciences and Health Technologies, U Lusófona, Campo Grande 376, 1749-024 Lisboa, Portugal

*Shared senior authorship

Contacto: f.filipaalmeidarocha@gmail.com, caparica.rita@gmail.com

Proteins are biomolecules that present a great pharmacological interest. Nevertheless, they are also known for their instability when exposed to certain conditions, such as high temperature, which may compromise their use in pharmaceutical formulations. In this context, ionic liquids (ILs) can be used as protein solvents in order to stabilize them [1].

In this work, lysozyme was used as a model protein and the ionic liquids (2-hydroxyethyl)-trimethylammonium-L-phenylalaninate[Cho][Phe], (2-hydroxyethyl)-trimethylammonium-L-glutamate[Cho][Glu] were used as co-solvents (0.2 %) in bidistilled water or PBS buffer 7.4.

Protein solutions with and without ILs were exposed to different temperatures (60 °C e 90 °C) from 30 minutes up to 48h.

Results show that lysozyme loses activity in the absence and presence of ILs, however the loss of activity is lower when the IL is present, for both temperatures. Results also show that [Cho][Glu] was the best protein stabilizer.

In a parallel study undergone at 90 °C, both cholinated ILs as well as 1-Hexyl-3-methylimidazolium bromide [C6mim][Br] were used. In this case results show that the halogenated IL provided the better results in stabilizing the protein. More studies, at different temperatures, are necessary to ensure these outcomes. Moreover, protein stabilization by ionic liquids was more pronounced in PBS pH 7.4 than in water.

In conclusion, the obtained results show that the use of ILs as protein stabilizers is promising, although more studies are needed to better understand the underlying mechanism in protein stabilization by these salts.

References:

- [1] Noritomi, H. et al., Thermal stability of proteins in the presence of aprotic ionic liquids, 1, 2010.

P16 - Chemical composition and biological properties of essential oils from *Plectranthus* spp.

Francisco Sousa ¹, Catarina Garcia ^{2,3}, Ana C. Figueiredo ⁴, Carlos M. Monteiro ¹, Carlos AM Afonso ¹, Catarina Pinto Reis ^{1,5}, Patrícia Rijo ^{1,2}

¹Instituto de Investigação do Medicamento (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal

²CBIOS, Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal

³Dpto. Ciencias Biomédicas, Universidad de Alcalá, Plaza de San Diego, s/n, 28801 Alcalá de Henares, Madrid, Espanha

⁴CESAM, CBV, DBV, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

⁵IBEB, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal
Contacto: patricia.rijo@ulusofona.pt

The essential oils (EOs) obtained from medicinal plants are widely used due to its therapeutic potential [1]. The Lamiaceae family is vastly known due to its broad spectrum of activities and the essential oil of some *Plectranthus* species have already been described for its antimicrobial promising activity [2, 3].

In this work, samples of the essential oils were isolated from the leaf, stems and whole plant of three different *Plectranthus* spp. (*P. neochillus*, *P. porcatius* and *P. prostratus*). In order to assess their biological activity, its antimicrobial and antioxidant properties were studied through the well diffusion and DPPH methods, respectively. Its ability for tyrosinase inhibition was also evaluated.

Thus, Gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) as well as yeasts strains (*Saccharomyces cerevisiae* and *Candida albicans*) were selected for further assessment of the selected *Plectranthus* spp. EOs' antimicrobial activity. Its chemical composition was determined by GC-MS.

As a result, *P. neochillus* and *P. porcatius* EOs revealed moderate to high antimicrobial activity, while *P. neochillus* showed potent antimicrobial activity against the tested Gram positive bacteria. The latter also demonstrated considerable activity against the yeasts strains tested. *P. porcatius* showed antimicrobial activity against not only the mentioned Gram positive bacteria, but also against *E. coli* and *S. cerevisiae*. According to the DPPH assay, *P. porcatius* EO showed the highest antioxidant properties (58%). Regardless, none of the EOs tested showed significant capacity to inhibit the tyrosinase. The GC and GC-MS analysis revealed the presence of common EO compounds to all three species, such as α -pinene, 1-octen-3-ol, β -pinene, p-cymene and limonene. α -Terpenyl acetate (28%) and p-cymene (9%) were the major compounds of *P. neochillus* EO, whereas p-cymene (21%) and 1,8-cineole (19%) dominated *P. porcatius* EO and n-heptacosane (24%) and n-hexacosane (23%) *P. prostratus* EO.

In conclusion, the studied EOs are of interest and further studies could provide more detailed information about their medicinal properties and other biological activities, such as anti-inflammatory and cytotoxicity properties, which could be tested in the future.

Acknowledgements: This study was partially funded by Fundação para a Ciência e a Tecnologia (FCT), under UID/AMB/50017/2013, FEDER PT2020-Compete 2020.

References:

- [1] Edris A., Phytotherapy research, 21 (4), 308-323, 2007.
- [2] Vladimir-Knežević S., et al., Molecules, 19(1), 767-782, 2014.
- [3] Crevelin E., et al., Evidence-Based Complementary and Alternative Medicine, vol.15, 1-6, 2015.

P17 - Exploring the Microvascular Response to an Oxygen Stress Test in a Murine Model of Cardiac Hypertrophy

Henrique Silva^{1,2}, Alain-Pierre Gadeau³, Luís Monteiro Rodrigues^{1,2}

¹ CBIOS (Research Center for Biosciences and Health Technologies), U Lusófona, Campo Grande 376, 1749-024, Lisboa, Portugal

² Pharmacol. Sc Depart, Faculty of Pharmacy, U Lisboa, Lisboa, Portugal

³ INSERM U1034, Adaptation cardiovasculaire à l'ischémie, F-33600 Pessac, France
Contacto: henrique.silva@ulusofona.pt

Changes in microvascular function can be presented as a consequence of cardiovascular diseases, but may also precede the installation of clinical signals. Cardiac hypertrophy (CO) is characterized by the enlargement of cardiomyocytes following chronic and acute morbidities which, when left unresolved, results in left ventricular dysfunction and heart failure. Our aim was to identify abnormal microcirculatory functional parameters of a CO transgenic murine model with normal cardiac contractile function to an oxygen stress test. 17 mice were used (9 with NHE1 overexpression and 8 wild type - wt). Both groups were anesthetized with a mixture of ketamine/xylazine and then subjected to a 100% normobaric hyperoxia breathing protocol – 10 min baseline, 10 min oxygen, 10 min recovery. Blood flow was measured in both hindlimb paws with laser Doppler flowmetry (LDF). All procedures involving animal experimentation were ethically supervised. The Wilcoxon signed rank test was used for phase comparisons within each group and the Mann-Whitney test for group comparisons ($p < 0.05$). Both groups responded to provocation with a significant bilateral perfusion reduction. The NHE1 group responded with a significant increase in the cardiac and sympathetic activities together with a significant decrease in the NO-dependent endothelial activity. The wt group responded with a significant increase in the cardiac activity and with a significant reduction in NO-dependent endothelial activity. The percent increase of the cardiac activity was significantly lower in the NHE1 group. These results suggest that NHE1 overexpressing mice with a still normal cardiac contractile function respond to the 100% oxygen stress test with a depressed response in comparison to wt mice.

References:

- [1] Holowatz, L.A., et al. The human cutaneous circulation as a model of generalized microvascular function. *Journal of Applied Physiology*, (2008), 105(1), 370-372.
- [2] Cecchi, F., et al. Coronary microvascular dysfunction and prognosis in hypertrophic cardiomyopathy. *New England Journal of Medicine*, (2003), 349, 1027-1035.
- [3] Kuhn, H., et al. 'The abrupt no-flow': a no-reflow like phenomenon in hypertrophic cardiomyopathy. *European Heart Journal*, (2002), 23, 91-93.
- [4] Kehat, I., Molkentin, J.D. Molecular Pathways Underlying Cardiac Remodeling During Pathophysiological Stimulation. *Circulation*, (2010), 122, 2727-2735.
- [5] Marian, A.J., et al. *Cardiovascular Medicine*, London: Springer, 2007, pp. 1177-1188.
- [6] Silva, H., et al. Regarding the quantification of peripheral microcirculation—Comparing responses evoked in the in vivo human lower limb by postural changes, suprasystolic occlusion and oxygen breathing. *Microvascular Research*, (2015), 99, 110-117.

P18 - Combining photoplethysmography and laser Doppler flowmetry to characterize the peripheral vascular response in the human lower limb

Henrique Silva^{1,2}, Clemente Rocha¹, Beatriz Navarro Brazález³, Rita Faria¹, Raquel Ferreira¹, Luís Monteiro Rodrigues^{1,2}

¹ CBIOS (Research Center for Biosciences and Health Technologies), U Lusófona, Campo Grande 376, 1749-024, Lisboa, Portugal

² Pharmacol. Sc Depart, Faculty of Pharmacy, U Lisboa, Lisboa, Portugal

³ Physical Therapy in Women's Health Research Group, Faculty of Medicine and Health Sciences, Universidad de Alcalá, Madrid, Spain
Contacto: henrique.silva@ulusofona.pt

Noninvasive techniques have been used for decades to study peripheral vascular reactivity in both physiological and pathophysiological conditions [1,2]. Photoplethysmography (PPG) was one of the first methods employed, but it was soon replaced with laser Doppler flowmetry (LDF), with few studies having been performed using both techniques. Our aim was to explore the peripheral vascular response to two postural changes using both PPG and LDF. A group of 10 young healthy subjects was selected after giving informed written consent. Subjects performed two postural changes, each consisting of three phases (10 min resting, 10 min provocation and 10 min recovery). The provocation tests consisted on lowering a random leg 50 cm below heart level (protocol 1); and raising a random leg at 30° (protocol 2). In both protocols the contralateral leg was kept unmoved. Blood flow was recorded on the inferior aspect of the first and second toes of both feet with LDF and PPG probes, respectively. In protocol 1 a significant perfusion decrease was found on both feet during provocation with both LDF and PPG. This is consistent with the venoarterial reflex on the lowered leg and with a centrally-mediated vasoconstriction on the unmoved leg. In protocol 2 a significant perfusion decrease was found on both feet during provocation with LDF. This is consistent with a gravitational transfer of blood on the raised leg and again with a centrally-mediated vasoconstrictor response on the unmoved leg. The perfusion measured with PPG did not change significantly, which suggests that the postural change produced a different response in different vascular networks. These results seem to confirm the usefulness of combining LDF with PPG for the characterization of the peripheral vascular dynamics.

References:

- [1] Kvernebo, K., et al. Response of skin photoplethysmography, laser Doppler flowmetry and transcutaneous oxygen tensiometry to stenosis-induced reductions in limb blood flow. *European Journal of Vascular Surgery*, 3(2):113-20, 1989.
- [2] Chowienicz, P.J., et al. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. *Journal of the American College of Cardiology*. 34(7):2007-14, 1999.

P19 - Exploring the influence of hemodynamic determinants on skin barrier function

Henrique Silva^{1,2}, Inês R. Frazão², Hugo Ferreira³, Luís Monteiro Rodrigues^{1,2}

¹ CBIOS (Research Center for Biosciences and Health Technologies), U Lusófona, Campo Grande 376, 1749-024, Lisboa, Portugal

² Pharmacol. Sc Depart, Faculty of Pharmacy, U Lisboa, Lisboa, Portugal

³ IBEB (Institute of Biophysics and Biomedical Engineering), Faculty of Sciences, U Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
Contacto: henrique.silva@ulusofona.pt

Transepidermal water loss (TEWL) is used as the in vivo indicator of epidermal barrier function. It is known to be influenced by age, gender and anatomical area [1] amongst others. Recent studies suggested that local perfusion conditions might also influence TEWL [2,3], although the mechanisms underlying this relationship are not yet fully understood. In the present study we explored the influence of different haemodynamic determinants on the skin barrier function of 57 healthy volunteers of both genders from 18 to 65 (36.5 ± 5.7) years old, selected following previously established inclusion criteria. After full acclimatization to the room conditions, volunteers were subjected to an oxygen stress test. The procedure involved three phases - baseline, provocation and recovery, 10 minutes duration each. The provocation consisted in breathing a saturated oxygen atmosphere applied through a facemask. Measured variables included TEWL by evaporimetry and local blood flow by laser Doppler flowmetry (LDF). The LDF signal was decomposed into its main components (cardiac, respiratory, myogenic, sympathetic, NO-dependent and NO-independent endothelial) by the Morlet wavelet transform. The Wilcoxon signed-rank test was used for phase comparison, and the Spearman test was used to explore correlations between each component's amplitude ratio and TEWL during each phase ($p < 0.05$). Two main vascular responses were recorded – a significant perfusion decrease (PD) was found in 82% of the subjects, while a significant perfusion increase (PI) was found in 18%. In the PD group, a significant increment of TEWL was found, while in the PI group the opposite was registered, although not significant. Positive correlations between perfusion and TEWL were found in the PD group in all phases. During provocation, a positive correlation was particularly found between TEWL and the respiratory and myogenic LDF components. In the PI group, however, no correlation could be found for TEWL and perfusion or any LDF components. These results are in line with previous allegations relating both variables which might open a new direction in TEWL research

References:

- [1] Pinnagoda, J., et al. Guidelines for transepidermal water loss (TEWL) measurement. *Contact Dermatitis*, (1990);22(3):164-78.
- [2] Silva, H., et al. Regarding the quantification of peripheral microcirculation—Comparing responses evoked in the in vivo human lower limb by postural changes, suprasystolic occlusion and oxygen breathing. *Microvascular Research*, (2015);99:110-7.
- [3] Rodrigues, L. M., et al. Exploring the influence of skin perfusion on transepidermal water loss. *Skin Research and Technology*, (2004), 10(4), 257-262.

P20 - Exploring the impact of anesthesia on the entropy profile of the dog's skin perfusion – a pilot study

Henrique Silva^{1,2}, João Requiça³, Rui Assunção³, Clemente Rocha¹, Luís Lobo³, Margarida Estudante¹, L. Monteiro Rodrigues^{1,2}

¹ CBIOS (Research Center for Biosciences and Health Technologies), U Lusófona, Campo Grande 376, 1749-024, Lisboa, Portugal

² Pharmacol. Sc Depart, Faculty of Pharmacy, U Lisboa, Lisboa, Portugal

³ Faculty Vet Medicine, U Lusófona, Campo Grande 376, 1749-024, Lisboa, Portugal
Contacto:henrique.silva@ulusofona.pt

Anesthesia partly suppresses the cardiovascular system function, both directly and indirectly through depression of the central nervous system. Recently the depth of anesthesia has been evaluated by analysis of the entropy (i.e. the complexity) of electroencephalography signals. The complexity of a biological system reflects its ability to adapt to changes in the environment. We reason that anesthesia may also alter the entropy of perfusion signals. Our aim was to characterize the entropy of dogs' blood flow signals measured by photoplethysmography (PPG) before and after anesthesia induction. 4 mongrel dogs (33.5 ± 32.1 months old, mean weight 17.9 ± 11.3 kg) were anesthetized with methadone and dexmedetomidine. PPG signals were recorded on the tail before anesthesia inducing and again 10 min after. Heart rate was calculated from the pulse rate of the signal and flood flow was given by its waveform amplitude. The PPG signals were then decomposed into its cardiac and respiratory activities with the Morlet wavelet transform. The entropy of the raw PPG signal and of its cardiac and respiratory components was calculated by the Multiscale Entropy Analysis (MSE). Nonparametric statistics were applied for phase (pre vs post-anesthesia) comparison ($p < 0.05$). Anesthesia produced a decrease in heart rate and blood flow, although non-significant. The entropy of the PPG signal as well as of the cardiac and respiratory components decreased also, but again non significantly. Although no statistical differences could be found due to the small sample size, this pilot study suggests that the adaptability of skin perfusion is reduced during anesthesia.

References:

- [1] Costa, M. et al. Physical review. E, Statistical, nonlinear, and soft matter physics, (2005), 71, 021906.
- [2] Liang, Z., et al. *Front Comput Neurosci*, (2015), 9, 16.
- [3] Rocha, C. et al. Application of the wavelet transform to photoplethysmography to assess the cardiorespiratory activity in the anesthetized dog [abstract]. In: International Society for Biophysics and Imaging of the Skin World Congress 2016; May 31st – June 3rd 2016; Lisbon. Abstract 12.

P21 - Evaluation of a Nutrition Education Program implementation at Real Colégio de Portugal

Inês Martins¹, Ana Abreu¹, Marta Crespo¹, Rita Faria¹, Jessica Barreira¹, Nelson Tavares¹

¹CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

Contacto: nelson.tavares@ulusofona.pt

Nutrition health education has become increasingly prominent in schools' programmes worldwide, either as part of dedicated courses such as home economics or wider skills-based programmes. There is evidence that young people develop risk factors and disease evidence for coronary heart disease early and, in Portugal [1] as elsewhere, obesity is becoming a major public health problem for children. Patterns of eating are changing with a strong reliance on frequent snacking and consumption of so-called junk foods. A variety of programmes have improved knowledge and in some cases reported behaviour. Evaluation of effectiveness can be problematic as improved knowledge may not necessarily affect long term behaviour. However, structured programmes can contribute to a wider context-based approach. [2] The aim of this study was the promotion and evaluation of nutrition education programme at the Real Colégio de Portugal, for students from the 1st to 9th year, inducing positive changes in eating habits and increase student's knowledge about healthy eating. Nutritional education program consists of 8 sessions over 12 weeks, and included also the application of a food paired validated questionnaire to measure the preference, behavior and knowledge of students regarding healthy eating. A comparative and quantitative study was developed for 12 weeks since February to June 2015. Data were used from 203 students aged 5 to 15 years old. At baseline and after 12 weeks students completed a validated food pairing questionnaire on food preference, behavior and knowledge. Significant differences were found in the intervention student's preference ($p=0.005$) and behaviour ($p=0.0002$). Knowledge levels were high at baseline and though some individual items improved, average change overall was not significant. Following the Nutrition Education Programme was seen an improvement in preferences for healthier food and eating behaviour.

References:

- [1] World Health Organization. Nutrition, Physical Activity and Obesity - Portugal. Geneva WHO 2013.
- [2] Friel S et al., Public Health Nutrition 1999; 2, 549-555.

P22 - Exploring the in vitro anti-inflammatory and skin-related enzymatic inhibition activities of Plectranthus spp. extracts and isolated compounds

Joana Marçalo^{1,2}, Luísa Custódio³, Marisa Nicolai¹, Luís Monteiro Rodrigues¹, Catarina Pinto Reis^{1,4}, Célia Faustino², Patrícia Rijo^{1,2}

¹CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

²iMed.Ulisboa, Faculdade de Farmácia Universidade de Lisboa, Lisboa, PORTUGAL

³CCMAR, Universidade do Algarve, Faro, PORTUGAL

⁴IBEB, Faculdade de Ciências Universidade de Lisboa, Lisboa, PORTUGAL

Contacto: joana-andrade@campus.ul.pt

Current research in drug discovery from medicinal plants provides new and important leads against various pharmacological targets. The *Plectranthus* spp. plants are the focus of several scientific investigations, due to their ethnopharmacological use by indigenous populations [1]. The aim of this work was to further research previous evidences on interesting biological activities of *Plectranthus* spp., directing the study for anti-skin ageing and anti-inflammatory activities [2,3].

Acetylcholinesterase (AChE) was studied in vitro using the Ellman method, to evaluate the enzymatic inhibition from the non-neuronal cholinergic system in the skin. This assay showed that the *Plectranthus* spp. organic extracts did not significantly inhibit AChE ($p>0.05$). Concerning the enzymatic inhibition of skin related enzymes, performed using colorimetric assays, in the tyrosinase inhibition assay, it was observed a high inhibition for the *P. ecklonii* methanol ($65.9\pm 3.4\%$), *P. grandidentatus* acetone ($67.9\pm 3.6\%$) and *P. porcatus* aqueous ($65.0\pm 8.7\%$) extracts. From the tested isolated compounds, abietane diterpenes, mainly present in the organic extracts of *P. grandidentatus*, *P. madagascariensis*, and *P. ecklonii* were highly active against tyrosinase, in more than 46% and up to 75%. In the collagenase assay, all tested extracts and compounds showed a high enzymatic inhibition which was in the range of 28-76% ($76.4\pm 2.1\%$ from *P. neochilus* methanol). In contrast, the elastase inhibition assay revealed that the extracts did not decrease elastase activity (30-42%). Nonetheless, the isolated compounds oleoic:curcolic acids mixture (1:4) ($63.4\pm 2.6\%$) and parvifloron D ($52.8\pm 3.8\%$), were able to highly inhibit elastase. The anti-inflammatory assay was performed by the quantification of NO production using the Griess reaction [4]. The non-cytotoxic isolated compounds (evaluated by the MTT assay) revealed to be unable to reduce NO production, after LPS stimulated inflammation (ranging from 16-23 μ M), in comparison with the normal quantities of NO production within the cells (17.7 ± 0.7 μ M), and with the positive control L-NAME that decreased NO until reaching 3.9 \pm 0.2 μ M.

Overall, the *Plectranthus* genus seem to show an interesting potential in the obtention of new leads, especially concerning skin disorders, from skin-related enzymatic inhibition in vitro activity.

References:

- [1] Likhoba, C. W., et al., Journal of Ethnopharmacology, 103:1-24, 2006
- [2] Rijo, P. et al., Measurement 58:249-255, 2014.
- [3] Rijo, P. et al., Biomedical and Biopharmaceutical Research, 9:225-235, 2012.
- [4] Rodrigues, M. J. et al., Food Chemistry, 200:322-329, 2016..

P23 - Evaluation of Glycated hemoglobin (HA1c) status in patients from an internal medicine service at a Lisbon hospital

Joana Padrão¹, Nelson Tavares¹

¹CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

Contacto: nelson.tavares@ulusofona.pt

Diabetes Mellitus (DM) is characterized by chronically elevated plasma glucose concentrations. [1] Insulin will allow glucose to enter the cells to be metabolized. This requires that the insulin is received by cell surface receptors. If the receptors on the surface provide the insulin input resistance will occur hyperinsulinemia. [2] The Direção Geral da Saúde establishes the existence of four types of DM: DM type 1 (DM1) or insulin-dependent; DM type 2 (DM2), characterized by a greater or lesser degree of insulin resistance (this corresponds to a majority of cases of DM); Gestational diabetes and other specific types of diabetes [3]. The HA1c is considered the most effective method in the diagnosis and control of DM. This value will reflect the degree of control of blood glucose levels in the 3 months prior to the analysis. Despite all the disadvantages this method, combined with the estimated average glycaemia (GME), makes foolproof method capable of preventing and delaying complications related to micro- and macrovascular DM.

This study involved patients admitted on internal medicine service at a Lisbon hospital. A cross-sectional study was elaborated for long stay hospital patients diagnosed with Diabetes Mellitus (DM). The aim of this study was the monitoring of patients with DM. The sample included 55 patients with type 2 DM, which is the most prevalent in all cases of diabetes. 65% of the patients were male and 35% female, with a mean age of 74 years. The study itself consisted in the collection of personal data, vital parameters (capillary glycaemia), biochemical test (glycated hemoglobin (HA1c)), prescribed diet, nutritional status, anthropometric and survey of food intake in the previous 24 hours. From this study it can be concluded that the majority of the sample showed that DM was under control (HA1c <7%) ($n = 29$ vs. $n = 26$) presenting fewer complications compared with the cases where the HA1c > 7%. It was found that the sample had only 13 patients with normal weight ($n = 13$); the remaining patients were overweight and obese ($n = 42$), which allow us to consider that obesity is closely connected to diabetes for this group of patients and according with findings in other studies. Probably is the reason why the term "Diabesity" has recently appeared. Although these public health problems and diagnostic methods still need some improvements, more studies and intervention measures are needed. One of those measures involves the sensitization of the general population in order to carry out a healthy diet so that it can prevent or delay not only complications such as DM itself, but to improve overall health.

References:

- [1] Drury, P. et al., Your Questions Answered. 1st ed. United Kingdom: Churchill Livingstone; 2005.
- [2] Carmo, I. et al., Diabesidade: A Obesidade na prática clínica. 1st ed. Bial 2012.
- [3] Norma da DGS N.º 002/2011. Diagnóstico e Classificação da Diabetes Mellitus. 002-(14-01-11) 1-12.

P24 - Nanoformulations for the treatment of yeast infections in the oral mucosa

Luís Roque¹, Catarina Garcia¹, Patrícia Rijo¹ and Catarina Pinto Reis¹

¹CBIOS, ULHT, Campo Grande, 376, 1749-024 Lisbon, Portugal
Contacto: catarinapintoreis@gmail.com

Introduction: Mold and yeast infections are mainly related with the large number of individuals with severe immunosuppression diseases or to some pathologies. In this case, it has been demonstrated that the leading cause of yeast infections is foremost caused by pathogens that belong to *Candida* species. Natural products are an interesting source of antifungal compounds, namely *Plectranthus* genus plants which belongs to the Lamiaceae family. Several studies regarding its extracts and isolated compounds have already been assessed for their biological activity. *P. amboinicus*, *P. japonicus* and *P. ecklonii* are the most studied species, due to their antifungal properties. [1] The aim of this work was to select which of the 16 *Plectranthus* spp. extracts studied were more effective against *C. albicans*. [2] In this specific case, the species in study were *P. aliciae* (P.A), *P. amboinicus* (P.AB), *P. barbatus* (P.BA), *P. ecklonii* (P.E), *P. fruticosus* (P.F), *P. hadiensis* (P.HA), *P. hereroensis* (P.HE), *P. japonicus* (P.J), *P. madagascariensis* (P.M), *P. madagascariensis* Lynne (P.ML), *P. malvulus* (P.MV), *P. ortendalii* (P.O), *P. reflexus* (P.R), *P. strigosus* (P.ST), *P. stylesii* (P.S) and *P. zuluensis* (P.Z). Meanwhile, several types of nanoparticles were prepared and fully characterized. Finally, the most active extracts against *Candida* were encapsulated in the most stable system of nanoparticles.

Materials and Methods: Xanthan gum, Pluronic® F-68, Pluronic® F-127, Miglyol® 812, Chitosan, Poly-(arginine) and Tripolyphosphate (TPP) were obtained from Sigma-Aldrich (St. Louis, USA). PLGA and PLA used were obtained from Purac (Gorinchem, Netherlands). All other reagents were of analytical grade. All the extracts were prepared using the ultrasound-assisted extraction method (USAE). A preliminary antifungal activity screen of the extracts was performed using *Candida albicans*. The antifungal activity was evaluated with the well diffusion method in triplicate. A preliminary general toxicity assay of the extracts was performed using *S. cerevisiae* in agar plates. The PLA and the PLGA NPs were prepared through emulsification/ solvent diffusion method. Chitosan NPs were prepared through the ionotropic gelation method. Poly-arginine NPs were prepared by using solvent displacement method and the Xanthan Gum NPs were prepared through a modified in-situ polymer cross-linking method. All preparations of the NPs were made in triplicate. The mean particle size, polydispersity index (PI) and zeta potential of the NPs were measured with a Coulter Nanosizer Delsa Nano™ (Fullerton, CA, USA).

Discussion and Conclusion: Natural products are a promising source of antifungal agents. USAE is a suitable methodology to extract bioactive compounds from the plants. PMV-A and P.S-M were the most active extracts against *C. albicans* and were encapsulated into very small systems such as PLA and PLGA NPs. Further studies such as mucoadhesion studies will be performed.

Acknowledgments: The authors would like to thank the institutions (ULHT) and the PDDIC scholarship.

References:

- [1] Reis C, Roque LV, et al., Innovative formulation of nystatin particulate systems in toothpaste for candidiasis treatment., 7450:1-6, 2015.
- [2] Likhoba CW, Simmonds MSJ, et al., *Plectranthus*: A review of ethnobotanical uses., J. Ethnopharmacol, 103:1-24, 2006.

P25 - Nanocarriers for skin delivery of cosmetic antioxidants

Mafalda Almeida,¹ Patrícia Penela,¹ Ana Júlio,¹ André R. Baby,² Daniela C. Oliveira,² Catarina Rosado,^{1*} Tânia Santos de Almeida,^{1*} Joana Portugal Mota,^{1*}

¹CBIOS - Universidade Lusófona's Research Center for Biosciences and Health Technologies, Campo Grande 376, 1749-024, Lisboa, PORTUGAL.

²School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 15, 05508-900, São Paulo, BRAZIL.

* Shared senior authorship

Contacto: mafaldagca@gmail.com , patriciapenela@gmail.com

In the last years, the topical delivery of drugs by liposomal formulations has been a subject of very high interest for the scientific community. Nonetheless, the reduced penetration on human skin by transdermal drug delivery represents a disadvantage and techniques like nanoencapsulation, have been successfully engaged to enhance transdermal delivery of several drugs to overcome the low skin permeability. [1]

Ethosomes consist in microscopic spheres of phospholipids and have many advantages since they are non-toxic, biodegradable, and they can be modulated. Also, they are nanocarriers with ability to encapsulate both hydrophilic and lipophilic drugs. The ethosomal system is composed by phospholipids, ethanol and water. The function of ethanol is to enhance permeation (by disturbance of the skin lipid bilayer), which affects intercellular region of the stratum corneum. [1]

In cosmetic science ethosomes are frequently used to increase the stability of active ingredients and enhance their topical permeation. Additionally, the use of antioxidants for topical administration is relevant when trying to decrease the production of peroxides in the skin, and to protect the skin against UV exposure.

Hence, in this study we compare the influence of encapsulating antioxidants such as Vitamin E, Vitamin C, Ferulic Acid and Rutin. [2],[3],[4]

Ethosomes were prepared with 0.05 % (w/v) of Vitamin E and Vitamin C. The synergism of these vitamins was also studied by preparing ethosomes with 0.1% (w/v). In addition, Ferulic Acid and Rutin were also encapsulated at a concentration of 0.03%. Ethanol solutions containing the same amount of antioxidants were also prepared.

The Antioxidant activity for all prepared samples was measured by DPPH assay.

For all the studied antioxidants, the encapsulated samples presented a higher antioxidant activity when compared to the non-encapsulated.

References:

[1] Ghanbarzadeh, S., Arami, S., *BioMed Research International*, 2013, 2013.

[2] Di Mambro, V. M. et al., *Internacional Journal of Pharmaceutics*, 262, 93-99, 2003.

[3] Nagarjuna, S. et al., *J. Pharm. Sci. Innov.*, 5(2), 63-65, 2016.

[4] Srinivasan, M. et al., *J. Clin. Biochem. Nutr.*, 40, 92-100, 2007.

P26 - Comparison of the sensorial profiles in two distinctive periods of two brands of Cervejas de Moçambique, the 2M and Manica

Mitchelle Carneiro¹, Nelson Tavares¹

¹CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

Contacto: nelson.tavares@ulusofona.pt

The word "cerveja", which in english translates to beer, as it origin from the latin "bibere", which equates to drink. Beer can be traced back into the culture of most of the ancient civilizations. There are two major types of beer: type Ale and type Lager. The first type, Ale, is of high fermentation, an older process on which normally the alcohol grade is superior to 5% and generally are known for their sweeter taste. As for the Lager type are of low fermentation, made from a longer and slower process which was first developed by monks from the Bavaria region. These beers, of the Lager type, are more commercialized and known for their soft flavor with an alcoholic content averaging 3,58%. The main raw materials utilized in the production of such drinks are malt, water, hop and yeast. [1-3] The present research occurred in the premisses of the Cervejas de Moçambique company in Nampula city in Mozambique.

The main objective of this work lies in the comparison of the sensorial profiles in two distinctive periods of the 2M and Manica brands of the above mentioned company. The periods being the moment just after the beer is filled (fresh beer) and 12 weeks after, in order to check if any alteration as sufficed between the two periods. The methodology translated into the analytical experimentation of samples from 3 different batches from each brand in the two defined periods. In both drinks were analyzed the alcoholic content, the bitterness, the lag-time, the real and apparent extract, the coloration and the pH.

It was also used a specific software equipment known as GTS for sensorial evaluation of beers quality.

In fresh results for 2 M brand only one batch shown Apparent Extract out of specification and 3 batches shown 3 batches lag-time out of specification. For Manica brand only two batch shown Apparent Extract out of specification. After 12 weeks only Manica shown 3 batches with pH out of specification which may result in a lower organoleptic and microbiological stability.

According to the results of GTS, it is possible to conclude that the beer reveals a loss trend of its properties over time, as can be seen when comparing a fresh beer with 12 weeks of beer, both for the two brands but higher for Manica. Physical and chemical analysis could be considered as predictive for beer behavior.

References:

[1] Galvão, MP. *Central de Cervejas SA. A Química da Cerveja*; 6-12, 1997.

[2] da Silva, P. et al., *Ciência e Tecnologia de Alimentos*, 28:4:902-906, 2008.

[3] Bokulich, N. et al., *Microbiology and Molecular Biology Reviews*, 77:2:157-172, 2013.

P27 - Evaluation of the amount of added salt in cooking meals in a Lisbon hospital

Nuno Rubio¹, Nelson Tavares¹

¹CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, PORTUGAL

Contacto: nelson.tavares@ulusofona.pt

Noncommunicable diseases are the leading cause of mortality worldwide, accounting for more deaths than all other causes combined. According to European statistics of cardiovascular diseases 2008, cardiovascular diseases are the cause of almost half of all deaths, namely 42% in the EU, which represents more than 2 million deaths per year, and the total cost of cardiovascular diseases promoted in 2006 in the EU, 192 billion euros with about 57% of the cost attributable to the costs of health care, 21% to productivity losses and 22% to informal care of cardiovascular patients.

The World Health Organisation (WHO) 2013-2020 Action Plan in the field of Food and Nutrition, approvingly, suggests strategies in the area of reducing salt intake as one of the best approaches ("best buys" - low cost and high efficiency) for prevention of noncommunicable diseases in the population in the region.[1]

The aim of this study was to evaluate the amount of added salt in cooking meals in a Lisbon hospital. During 132 working days the total amount of sea and refined salt used in cooking meals was controlled. It was made the inventory of the salt that existed in the kitchen where all meals were prepared at the beginning of the study. They were monitored and recorded all new amounts of sea and refined salt received on kitchen during the 132 days. At the end of the study it was made a new salt control salt existence in the kitchen. It was added to the 83,086 meals 420.16 kg of salt which represent per meal 5.057 grams and corresponds to 101.1% of the recommended total daily dose by WHO.

Our results support the findings of other studies, indicating the need for supervision and monitoring of meals preparations due to large amounts of added salt used. The amount of added salt by cooks for food confection is excessive when compared to the total recommended daily intake of salt by WHO and DGS. It is required local implementation strategies to reduce the founded added salt amount on meals.

Reference:

[1] Graça, P. DGS - Estratégia para a redução do consumo de sal na alimentação em Portugal. 2013.