4TH MEETING OF
MEDICINAL
BIOTECHNOLOGY

BOOK OF ABSTRACTS

P.PORTO
ESCOLA SUPERIOR DE SAÚDE

4TH MEETING OF MEDICINAL BIOTECHNOLOGY

17 DE MAIO DE 2019

ESCOLA SUPERIOR DE SAÚDE
POLITÉCNICO DO PORTO

4TH MEETING OF MEDICINAL BIOTECHNOLOGY

ORGANIZING COMMITTEE

Ana Rita Costa
Ana Rita Dias
Cristina Prudêncio
Cristina Borges
Dulce Teixeira
Joana Almeida
Mónica Vieira
Pedro Coelho
Ricardo Ferraz
Sofia Cunha

SCIENTIFIC COMITTEE

Cristina Prudêncio
Mónica Vieira
Pedro Coelho
Ricardo Ferraz

4TH MEETING OF MEDICINAL BIOTECHNOLOGY

17 DE MAIO DE 2019

ESCOLA SUPERIOR DE SAÚDE
POLITÉCNICO DO PORTO

CONTENTS

4TH MEETING OF
MEDICINAL
BIOTECHNOLOGY

FOREWORD

Dear colleagues,

It is with great pleasure that we welcome you at the 4th Meeting on Medicinal Biotechnology (4EBtM). These meetings have been held once a year since 2015 and have played an important role as a point of contact among several professionals and students in Medicinal Biotechnology.

Like the Medicinal Biotechnology field itself, these meetings have also been expanding, not only in the number of participants, but also in their geographical origin. Therefore, it is our goal to act as an interface between students and biotechnology companies/researchers, as well as to increasingly promote networking, projects and collaborations within this field for the upcoming years.

We hope you enjoy the Meeting and we are looking forward to welcoming you back next year at the 5th Meeting on Medicinal Biotechnology.

The Organizing Committee

4TH MEETING OF MEDICINAL BIOTECHNOLOGY

17 DE MAIO DE 2019

ESCOLA SUPERIOR DE SAÚDE
POLITÉCNICO DO PORTO

13:30 Registration

14:00 Opening Session

14:20 Panel I

- **Paula Parreira** (Invited Speaker 1)
  "Bioengineering applied to gastric cancer management"
- **Emanuel V. Capela** (Oral Communication 1)
  "Thermoreversible aqueous biphasic systems composed of protic ionic liquids for biotechnological applications"
- **Joana Silva** (Oral Communication 2)
  "Recombinant production and purification of the R248W mutant of human p53 in *Escherichia coli*"
- **Cristina Cabral-Dias** (Invited Speaker 2)
  "Downstream processing of biomolecules with pharmaceutical interest"

15:40 Coffee Break and Poster

16:10 Panel II

- **João Paulo Noronha** (Invited Speaker 3)
  "Algae the Food of the Future? Challenges for Conservation and Introduction in the Portuguese Diet"
- **Ana Luísa Machado** (Oral Communication 3)
  "KRAS mutation as an alert for immunotherapy in colorectal cancer"
- **Jorge Neves** (Oral Communication 4)
  "Cone Snails Natural Products: Neuronal Pharmacology and the Potential for Medicinal Application"
- **Luísa Aguiar** (Oral Communication 5)
  "Towards the development of novel antimalarial strategies: coupling chloroquine to the cell-penetrating peptide TP10"

17:30 Plenary Session

Cledir Santos
"Fungi And Mycotoxin Contamination In *Capsicum* Pepper And In Its Derivatives"

18:15 Closing Session

PLENARY SESSION

Fungi and Mycotoxin Contamination In *Capsicum* Pepper And In Its Derivatives

JÉSSICA COSTA[1], RODRIGO RODRIGUEZ[1,2], CARLA SANTOS[2], CÉLIA SOARES[2], NELSON LIMA[2], CLEDIR SANTOS[1*]

1-Chemistry of Fungi Laboratory, Department of Chemical Science and Natural Resources, Universidad de La Frontera, Temuco 4811-230, Chile
2-CEB-Centre of Biological Engineering, Micoteca da Universidade do Minho (MUM), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
*Correspondence: cledir.santos@ufrontera.cl

Mycotoxins are low-molecular-weight secondary metabolites produced by filamentous fungi. These grow in a wide range of agriculture crops (e.g., cereals, soybeans, grapes, tree nuts, groundnuts, coffee, cocoa and spices) and can produce one or more mycotoxins (Costa et al., 2019).

In Chile, berry fruits of *Capsicum annuum* L. cv. "Cacho de Cabra" are used for the manufacture of a traditional smoky flavour pepper powder known as Merkén. This is a product intrinsically associated with the ancestral Mapuche Amerindian Ethnicity and, in the year 2015, the total Chilean exportations of Merkén reached 4.4 million US dollars, representing an increase of 11.3% compared to 2014.

The agricultural practices used by Merkén local producers are empirical and do not consider the prevention of mycotoxigenic fungi (Costa et al., 2019). In January 2017 mycotoxin contaminations in Merkén, mainly Ochratoxin A (OTA), has been reported by the Chilean Ministry of Health (Minesal, 2017).

In this context, in the present work the results of the mycotoxigenic potential of the mycobiota belonging to the genus Aspergillus and Penicillium isolated in both the different points of the traditional production chain of *Capsicum annuum* L. cv. "Cacho de Cabra" and in the Merkén powder will be presented and discussed. Moreover, the possible points of contamination with OTA will be presented and the ecological interactions between mycotoxigenic fungi and *Capsicum annuum* L. cv. "Cacho de Cabra" and Merkén powder will be discussed.

Acknowledgements:
J.C. thanks to the CONICYT Chile for the PhD grant no 21181445. C.S. thanks to the Universidad de La Frontera (Temuco, Chile) for financial support. N.L. thanks to the Portuguese Foundation for Science and Technology for financial support.

References:
Costa, J.; Rodríguez, R.; Garcia-Cela, E.; Medina, A.; Magan, N.; Lima, N.; Battilani, P.; Santos, C. (2019). Overview of Fungi and Mycotoxin Contamination in Capsicum Pepper and in Its Derivatives. Toxins, 11, 27.
Minesal (2017). Chilean Ministry of Health. https://www.minsal.cl/nueva-alerta-por-contaminacion-de-alimento-merken/ (Accessed at 10/04/2019).

4TH MEETING OF
MEDICINAL
BIOTECHNOLOGY

INVITED SPEAKERS

4

INVITED SPEAKER 01

Bioengineering applied to gastric cancer management

PAULA PARREIRA[1,2]; CATARINA SEABRA[1,2,3*]; M. CRISTINA MARTINS[1,2,3]

1-i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal;
2-INEB, Instituto de Engenharia Biomédica, Universidade do Porto, Portugal;
3- ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal;
*Current Affiliation: CBQF- Centro de Biotecnologia e Química Fina, Universidade Católica Portuguesa.

Gastric cancer remains the 5th most common cancer worldwide and the 3rd deadliest. It was recently estimated that 89% of all gastric cancers are linked to *Helicobacter pylori* infection and, consequently, *H. pylori* eradication would allow reducing the burden of gastric cancer.The available treatment for *H. pylori* eradication relies on long time antibiotherapy, which combines at least two antibiotics (clarithromycin plus amoxicillin or metronidazole) and an acid-suppressive drug (e.g. proton pump inhibitor). This therapeutic scheme besides failing in around 20% of the infected patients, also presents several antibiotics-associated secondary effects, such as development of bacterial resistance and dysbiosis (destruction/unbalance of normal healthy gut microbiota). In fact and according to the World Health Organization, *H. pylor*i is one of the 16 antibiotic-resistant bacteria that pose the greatest threat to human health. Therefore it is essential to develop novel antibiotics or strategies to eradicate this gastric pathogen.
Over the last years, allying Bioengineering to the use of non-antibiotic derived compounds, our group has developed several innovative strategies aiming *H. pylori* eradication, with particular emphasis to the use of *H. pylori* specific glycosylated receptors immobilized onto biomaterials, biomaterials decorated with antimicrobial peptides and lipid nanoparticles loaded with docosahexaenoic acid (DHA). These 3 strategies will be briefly highlighted to demonstrate the Bioengineering potential for gastric infection management and consequently, its role in reducing mortality/morbidity rates linked to gastric cancer.

Keywords: Biomaterials, Bioengineering, New Therapies Development

INVITED SPEAKER 02

Downstream processing of biomolecules with pharmaceutical interest

A. CRISTINA DIAS-CABRAL [1,2]

1 - CICS-UBI - Health Sciences Research Centre, University of Beira Interior, 6200-506 Covilhã, Portugal
2 - Department of Chemistry, University of Beira Interior, 6200 - 001 Covilhã, Portugal

In recent years, the major driving force for pharmaceutical industry growth has been the production of biopharmaceuticals. Still, their purification continues to be considered the bottleneck of the manufacturing process. Despite the competition of nonchromatographic techniques, preparative chromatography remains to be the dominant technique in the purification of these biomolecules due to its high resolution. Nevertheless, the retention and separation mechanisms involved, and that are of extreme importance for predicting and controlling adsorptive behavior, are not fully understood. To overcome this limitation and to seed light onto the mechanisms of interaction between biomolecules and chromatographic resins is important the biophysical characterization of the adsorption process. This presentation intends to provide the state-of-the-art in experimental approaches to monitor biomolecule - surface interactions. Focus will be put on flow microcalorimetry (FMC) and small-angle X-ray scattering (SAX), as two non-labeling techniques capable of simulating a dynamic chromatography system allowing online and in situ monitoring of the adsorptive process. Present applications of these in situ monitoring techniques to better understand separation of antibodies with pharmaceutical interest will be presented [1-3].

[1] G.F. L. Silva, J. Plewka, R. Tscheließnig, H. Lichtenegger, A. Jungbauer, A.C. Dias-Cabral, "Antibody binding heterogeneity of Protein A resins", Biotechnology Journal, doi: 10.1002/biot.201800632, 2019.
[2] G.L. Silva, J. Plewka, H.C. Lichtenegger, A.C. Dias-Cabral, A. Jungbauer, R. Tscheließnig, "The pearl necklace model in protein A chromatography: Molecular mechanisms at the resin interface", Biotechnology and Bioengineering, 116, 76–86, 2019.
[3] S.A.S.L. Rosa, C.L. da Silva, M.R. Aires-Barros, A.C. Dias-Cabral, A.M. Azevedo, "Thermodynamics of the adsorption of monoclonal antibodies in phenylboronate chromatography: Affinity versus multimodal interactions", Journal of Chromatography A, 1569, 118–127, 2018.

Keywords: Affinity chromatography, Monoclonal antibodies, Flow microcalorimetry, Small - angle X - ray scattering.

INVITED SPEAKER 03

Algae the Food of the Future? Challenges for Conservation and Introduction in the Portuguese Diet

J. P. NORONHA

LAQV-REQUIMTE, Departamento de Química
Faculdade de Ciências e Tecnologia/**Universidade NOVA** de Lisboa, 2829-516 Caparica, Portugal

E-mail: jpnoronha@fct.unl.pt

In a world where food is becoming scarce due demand and population, and in which there is a growing concern about sustainable food production, and health, nutrition and innovation are increasingly valued, algae can be a viable solution to overcome some of the challenges. The introduction of algae in food is seen as being of major importance in economic, nutritional, health and environmental terms.
Project ALGA4FOOD (https://alga4food.wixsite.com/page) aims to increase the diversity and quality of the algae from the Portuguese coast available for human consumption. This involves the development of new conservation techniques allowing to maximize the organoleptic and nutritional characteristics of the finished product, as well as allowing for more convenient forms of use by final consumers. In addition, it is intended to develop new strategies and products which can contribute to change Portuguese dietary habits. The evaluation of the suitability of the new conservation techniques and the products obtained will be made through the quantitative determination of parameters such as color, texture, umami content and volatile release, these will be further correlated with sensory analysis data.
Alga4Food can constitute a significant contribution to effectively promote the introduction of algae in the Portuguese diet.

Keywords: Edible seaweeds from Portugal; Food science; New products.

4TH MEETING OF
MEDICINAL
BIOTECHNOLOGY

ORAL COMMUNICATIONS

4

ORAL COMMUNICATION 01

Thermoreversible aqueous biphasic systems composed of protic ionic liquids for biotechnological applications

BOJAN KOPILOVIĆ[1,2]; EMANUEL V. CAPELA[1]; ALEKSANDAR MARIĆ[1,3]; JOÃO A.P. COUTINHO[1]; SLOBODAN GADŽURIĆ[3]; MARA G. FREIRE[1]

1 - CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal2 - Faculty of Science, Department of Chemistry, Biochemistry and Environmental Protection, University of Novi Sad, Serbia3 - Institute of Food Technology in Novi Sad, University of Novi Sad, 7 Bul. cara Lazara 1, 21000 Novi Sad, Serbia

The use of organic solvents in liquid-liquid separation processes is often associated to several disadvantages, such as high volatility and toxicity. Due to their high water content, aqueous biphasic systems (ABS) can be considered a viable alternative to conventional liquid-liquid extraction techniques. These systems are composed of water and two non-volatile solvents (usually two polymers, a polymer and a salt or two salts). Based on their advantages and water-rich media, their potential for the extraction and purification of several (bio)molecules with high interest in biotechnological applications was already reported. In addition to traditional polymer-based ABS, the use of ionic liquids (ILs) in the design of such processes has great advantages since they can be tailored for a specific task/application by a proper combination of the cation/anion used. Herein, we studied several protic ionic liquids (PILs), which are of simple synthesis, low preparation cost and possible eco-friendly character if properly designed, to create novel IL-polymer and IL-co-polymer ABS. All ILs were synthesized and characterized by us, and the respective ABS phase diagrams were determined at two different temperatures of interest for biotechnological applications – 25 and 37 ºC. Based on the gathered results, it was found that systems composed of ILs and both polymers and co-polymers present a thermoreversible ability, allowing to define regions in which monophasic mixtures can be used at 25 ºC and the promotion of two-phases formation occurs by an increase in temperature up to 37 ºC. The systems developed in this work are of upmost importance for the development of processes and applications in the field of biotechnology.

This work was developed within the scope of the project CICECO (UID/CTM/50011/2019), financed by national funds through the FCT/MCTES. E.V. Capela acknowledges FCT for the PhD grant SFRH/BD/126202/2016. M.G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-337753.

Keywords: Biopharmaceuticals, monoclonal antibodies, manufacturing processing, ionic-liquid-based aqueous biphasic systems

ORAL COMMUNICATION 02

Recombinant production and purification of the R248W mutant of human p53 in *Escherichia coli*

JOANA SILVA[1], LUCÍLIA DOMINGUES[1], CARLA OLIVEIRA[1]

1 - CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

The p53 protein is a tumour suppressor that specifically binds to DNA sequences to control gene expression. By controlling DNA repair, cell cycle arrest, and apoptosis, p53 prevents the dissemination of mutations within the genome. In about 50% of human cancer cases this protein is mutated. The mutation R248W in p53 (p53R248W) is designated by a contact mutation, which means that it compromises the DNA binding domain and prevents the correct activity of p53 as a transcriptional factor. This mutant promotes tumorigenesis and is related with poor prognosis and poor overall survival. In this work, the p53R248W was produced and purified in *Escherichia coli* aiming at developing novel therapeutic approaches targeting this protein. The coding sequence of the p53R248W core domain was cloned into the pETM-20 vector without any fusion patterns and expressed in E. coli BL21 (DE3). The conditions for the soluble production and purification of the recombinant protein were optimized. Some specific compounds were included in the process to improve protein stability. Yields of about 32 mg of pure p53R248W per litre of culture were obtained. The recombinant protein migrated in SDS-PAGE electrophoresis with its predicted molecular weight for a monomer (~25 kDa), which was corroborated by size-exclusion chromatography (SEC). Nonetheless, dynamic light scattering (DLS) analyses revealed high propensity for protein aggregation. Subsequent work includes an in-depth characterization of the protein at the level of its thermodynamic stability. The advances in recombinant production, purification and characterization of p53R248W to be gathered from this work will be certainly important for the progress of cancer research involving p53 proteins.

Acknowledgements: Study supported by FCT under the scope of the strategic funding of UID/BIO/04469/2019 and BioTecNorte operation (NORTE-01-0145-FEDER-000004), funded by the European Regional Development Fund under the scope of Norte2020.

Keywords: p53; mutant R248W; recombinant production; *Escherichia coli*

ORAL COMMUNICATION 03

KRAS mutation as an alert for immunotherapy in colorectal cancer

ANA LUÍSA MACHADO[1,2,3]; S MENDONÇA[1,2]; P DIAS CARVALHO[1,2], F MARTINS[1,2], MJ OLIVEIRA[1,3,4], S VELHO[1,2]

[1]i3S
[2]IPATIMUP
[3]INEB
[4]FMUP

Immunotherapy has recently thrived in cancer treatment, emerging as a therapeutic solution to patients which did not respond to the conventional chemotherapy and/or radiotherapy. The immunotherapy main goal is the activation of the host immune system to efficiently attack the cancer cells. This therapeutic approach is based on overcoming mechanisms that support cancer immunosurveillance escape, for example some mutations that might reduce cancer cell immunogenicity and lead to tolerance.
KRAS is a crucial oncogenic mutation present in about 30% of colorectal cancer cases and which confers a greater potential for malignancy. It is known that KRAS mutant cancer cells regulate the recruitment, activation, and differentiation of immune cells, promoting tumour evolution by ensuring leakage to the immune system. Few evidence highlights an association between KRAS mutations and myeloid cells, mainly macrophages and neutrophils infiltration. However, the mechanism and implications of this interaction remains unclear.
In this work, we decided to investigate whether KRAS mutations may modulate the expression of immunomodulatory molecules, namely immune checkpoint inhibitors, and therefore impair an efficient immune response. Therefore, a series of immunomodulatory molecules were analysed by flow cytometry in a panel of KRAS mutant colorectal cancer cells in which KRAS was silenced by small interfering RNA. In addition, the effect of chemotherapy and of IFN-g administration in the expression of those immune checkpoints molecules was also evaluated.
Our results suggest that the silencing of KRAS leads to the alteration of some molecules involved in the crosstalk with the immune cells, such as macrophages and lymphocytes. Additionally, we observed that 5-FUand IFN-gadministration promote the expression of some immune checkpoint inhibitors of Tcell function, which was abrogated by KRAS gene silencing.
In conclusion, the KRAS activation seems to be capable to regulate the expression of surface markers, which can modulate and suppress the immune response against the cancer cells. Thus, this mutation can have implications in the stratification of the CRC patients which may benefit from immunotherapy.

ORAL COMMUNICATION 04

Cone Snails Natural Products: Neuronal Pharmacology and the Potential for Medicinal Application

JORGE NEVES[1]; VITOR VASCONCELOS[1]

1 - Centro Interdisciplinar de Investigação Marinha e Ambiental da Universidade do Porto, Matosinhos, Portugal

The natural products from Cone Snails (Conus) venoms – "conotoxins" – have received much attention over the last decades due to the biological activity, extraordinary diversity, and molecular studies, opening a window for biomedicine applications. Conotoxins, are currently being developed as analgesics for the treatment of neuropathic pain. In December 2004, the synthetic version of the peptide ω-conotoxin MVIIA (commercial name Prialt®; generic name Ziconotide; N-type calcium channel) from C. magus has been approved by the United States Food and Drug Administration (FDA) to treat chronic pain in humans. Others promising natural products that have reached human clinical trials as therapeutically drugs include, α-Conotoxin Vc1.1 (Molecular Receptor: Nicotinic Ach Receptors; Therapeutic Target: Neuropathic pain; Clinical Phase: Phase II), κ-Conotoxin PVIIA (Molecular Receptor: Potassium Channels; Therapeutic Target: Cardioprotection; Clinical Phase: Preclinical), ω-Conotoxin CVID (Molecular Receptor: N-type Calcium Channels, Therapeutic Target: Cancer pain, Clinical Phase: Phase I). Our research has been focused on Cabo Verde venomous marine snails particularly those of the genus Conus. This work comprises analysis of two species, one endemic – Conus alteralbus –, and one non-endemic Conus genuanus. On C. ateralbus we purified, and biochemistry characterized a new peptide. An excitatory activity was manifested by the peptide on a majority of mouse lumbar dorsal root ganglion neurons and homology which include conserved sequence elements with δ-conotoxins (pharmacology family) from other worm and fish-hunters. Although research on cone snails has been ongoing for nearly half a century by researchers around the world, leading to discovery of hundreds of highly pharmacological active venom peptides, the presence of bioactive small molecules was never demonstrated. On the Conus genuanus instead purified a peptide, we purified a novel small-molecule, a guanine derivative with unprecedented feature; we named it genuanine. Genuanine was neuroactive when injected into mice – having paralytic activity.

Keywords: Bioactive Natural Products, Conotoxins, Pharmacological potential

Towards the development of novel antimalarial strategies: coupling chloroquine to the cell-penetrating peptide TP10

LUÍSA AGUIAR[1]; FÁTIMA NOGUEIRA[2]; NUNO VALE[3]; PAULA GOMES[1]

1 - LAQV-REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal
2 - Laboratory of Pharmacology, Department of drug science, Faculty of Pharmacy, University of Porto, Portugal
3 - Institute of Hygiene and Tropical Medicine, Lisbon, Portugal

Malaria remains a worldwide threat, and despite all efforts against this parasitic disease, it afflicted over 200 million people in 2015. Although malaria cases and deaths have declined globally over the years, antimalarial drug resistance poses a huge burden for controlling this infection and the development of new drugs is a pressing matter.
Recycling classical drugs is a cost-effective reduced-risk strategy for developing new drugs. While many of the latest efforts on antimalarial drug development are focused on finding more potent derivatives, or to develop dual-action hybrids by linking two antimalarials together, the targeted delivery of known antimalarial drugs may be a promising alternative, as it may increase drug's concentration at site of action, reducing side effects associated to high doses. More importantly, drug masking with a suitable carrier may elude parasite resistance.
Cell-penetrating peptides (CPP) are becoming prominent shuttles for intracellular drug delivery as they are able to ability to be uptaken by diverse cell types and carry different cargo sizes or types without significant toxicity. Interestingly, increased CPP uptake into Plasmodium-infected erythrocytes (PiRBC) as compared to healthy erythrocytes (hRBC) has been reported, as RBC undergo significant changes upon infection, including acquired adhesion properties, which improves cell permeability towards cationic amphipathic CPP. Besides, amphipathic peptides specifically targeting PiRBC have been identified. Hence, carefully chosen peptides may act as selective shuttles for intracellular delivery of antimalarials into PiRBC. The approach herein proposed, unprecedented in the literature, addresses this hypothesis, toward a paradigm shift in AC.
In this work, the classic antimalarial chloroquine was conjugated through different spacers to TP10, a CPP active against blood-stage Plasmodium falciparum, for subsequent assessment of conjugates' in vitro antimalarial activity. Results thus far obtained will be presented.

Keywords: Malaria; Cell-Penetrating Peptides; Chloroquine; Peptide-Drug Conjugates.

4TH MEETING OF
MEDICINAL
BIOTECHNOLOGY

POSTERS

POSTER 01

Anti-*EFG1* oligomer able to control *Candida albicans* filamentation in human body fluids

ANA BARBOSA[1]; DANIELA ARAÚJO[1]; MARIANA HENRIQUES[1]; SÓNIA SILVA[1]
1 Biological Engineering Center of the University of Minho, Braga, Portugal

Antisense oligomers (ASOs) and their analogues have been successfully utilized to silence gene expression for the treatment of many human diseases, however the control of yeast's virulence determinants has never been exploited before. In this sense, this work is based on the key hypothesis that if a pathogen's genetic sequence is a determinant of virulence, it will be possible to synthesize a nucleic acid mimic that will bind to the mRNA produced and will degrade it, blocking its translation into protein and consequently reduce its phenotype. *EFG1* is an important determinant of virulence that is involved in regulation of *Candida albicans* filamentation.
Thus, our main goal was to validate the in *vitro* applicability of an ASO, previously synthesized, targeting the *EFG1* mRNA. For that, the performance and stability of the anti-*EFG1*oligomer in human body fluids (artificial saliva and urine) was evaluated by determining its ability to inhibit *C. albicans* filamentation and to reduce *EFG1* gene expression. The results demonstrated that the anti-*EFG1* oligomer is capable to reduce not only the rate of *C. albicans* filamentation but also the size of their filaments. RT-PCR assays demonstrated *EFG1* gene expression reduction of 80% and 60% in the artificial saliva and urine, respectively. Since, the anti-*EFG1* oligomer maintains its activity and performance after 24h in human body fluids, this work reinforces a possible applicability of ASOs for controlling virulence genes and thus reduce *C. albicans* virulence factors, such as filamentation.

Keywords: *Candida albicans*; Candidiasis; Virulence factors; Antisense therapy.

POSTER 02

Bacteriophage origin peptide biomarker for mastitis producing *Streptococcus* spp. identification and characterization by LC-ESI-MS/MS.

A.G. ABRIL[1], M. CARRERA[2], K. BÖHME[3], J. BARROS-VELÁZQUEZ[4], J.L.R. RAMA[1], C.SIEIRO[5], T. G. VILLA[1], CALO-MATA P.[4]

1-Department of Microbiology and Parasitology, Faculty of Pharmacy, Campus Sur 15782.Universidad de Santiago de Compostela. Spain. E-mail:tom.villa@gmail.com
2-Institute for Marine Investigation of the Spanish Research Council, R/ Eduardo Cabello 6, 36208 Vigo, Spain
3-Agroalimentary Technological Center of Lugo, C/ Montirón 154, 27002 Lugo, Spain
4-Department of Analytical Chemistry, Nutrition and Food Science, Food Technology Division, School of Veterinary Sciences, University of Santiago de Compostela, Campus Lugo, Lugo. Spain.
5-Functional Biology and Health Sciences Department, Microbiology Area, Faculty of Biology, University of Vigo, Campus Lagoas-Marcosende, Vigo, Spain

Streptococcus includes numerous mastitis-causing species, being responsible for high economic losses as well as human health issues. Recently, new rapid molecular microbial diagnostic methods based on genomics and proteomics have been developed in order to achieve faster and more effectively species identification than classical and culture-based methods which are labour-intensive. Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) has been used for the analysis of bacterial pathogen strain-specific diagnostic peptides. As bacteriophages are high specific to their host pathogens, their nucleic acids, antibodies, phage-display peptides (PDPs), and most recently phage's receptor binding proteins (RBPs) have been studied as biosensor for pathogen detection. In addition, LC-ESI-MS/MS techniques have been employed for the identification and detection of bacterial bacteriophages. However, any study has been published for *Streptococcus* phage detection and identification by LC-MS-MS so far.
In this study, *Streptococcus* spp. tryptic digestion peptides have been analysed by LC-ESI-MS/MS to search specific biomarkers useful for a rapid identification. A total of 100µg protein extraction was digested with trypsin, cleaned on a C18 microSpinTM, following by LC-MS/MS analyse. The data was processed by SEQUEST (Proteome Discoverer 1.4 package, Thermo Scientific) against Bacteria in the UniProt/ TrEMBL database. The study of bacteriophage resulting peptides of bacterial pathogen led to the discovery of some specific biomarker peptides.

Keywords: *Streptococcus* detection, LC-ESI-MS/MS, mass spectrometry, phage peptide biomarker.

POSTER 03

Purification of antileukemic biopharmaceuticals using supported ionic liquid materials based on silica

JOÃO CLÁUDIO FONSECA NUNES, MAFALDA R. ALMEIDA, MARGARIDA ROSMANINHO, MARGARIDA CASTRO RIBEIRO, MÁRCIA C. NEVES, MARA G. FREIRE, ANA P. M. TAVARES

Department of Chemistry, CICECO - Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

Acute lymphoblastic leukemia (ALL) accounts with approximately 5,000 new cases in the United States and 4,000 in Europe each year. The first-line biopharmaceutical being used to treat acute ALL, Oncaspar, is based on L-asparaginase (LA), and accounts with approximately USD $100 million in annual sales, with its purification accounting for up to 80% of its total production cost. Therefore, it is crucial to optimize the purification of LA in order to decrease its current cost and allow their routinely use by a widespread population.

Supported ionic liquid materials based on silica (SILs) are already reported in the literature and have been mainly used in the separation of natural compounds from vegetable biomass. Although SILs represent a class of materials with high potential in the purification of proteins, this particular application has been scarcely considered.

In this work, the search for SILs able to establish (non-covalent) specific interactions with LA, allowing therefore its purification from the fermentation broth in which it is produced was investigated. Commercial LA was used in a first set of studies in order to understand the adsorption behavior of the enzyme into SILs. Experimental conditions, such as pH, contact time and SILs/LA ratio were evaluated and optimized in what concerns the LA purity and yield. With this strategy, process costs, energy consumed, and waste generated, may be significantly decreased, which may lead to this biopharmaceutical price decrease and wider application.

This work was developed within the scope of the project CICECO (UID/CTM/50011/2019), financed by national funds through the FCT/MCTES, and project POCI-01-0145-FEDER-031268 - funded by FEDER, COMPETE2020, POCI and OE. A.P.M. Tavares and M.C. Neves acknowledge FCT for the research contracts IF/01634/2015 and CEECIND/00383/2017, respectively.

Keywords: Acute lymphoblastic leukaemia, L-Asparaginase, Purification, Supported Ionic Liquids

POSTER 04

Development and characterization of solid-in-oil-in-water systems containing proteins

D. STAEL[1]; A. C. MARQUES[1,*]; P. C. COSTA[1,2]; J. M. SOUSA LOBO[1,2], M. H. AMARAL[1,2]
1 - Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto (FFUP), 4050-313 Porto, Portugal;
2 - UCIBIO/REQUIMTE, Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto (FFUP), 4050-313 Porto, Portugal
*micf12191@ff.up.pt

Introduction

In recent years, proteins and peptides have emerged as an important class of biomolecules due to their unique biological, chemical and physical properties. However, these biomolecules are very sensitive to external aggressions, which can easily lead to conformational changes and loss of biological activity. Nanotechnology can be applied to overcome such limitations, by developing nanosystems that stabilize and protect these biomolecules from damage, as well as deliver them to the therapeutic target. Therefore, protein formulations based on nanodispersions constitute a promising option for drug delivery applications.
The aim of this work was to develop solid-in-oil-in-water (S/O/W) nanodispersions containing bovine serum albumin (BSA), used as a model protein.

Material and Methods

Firstly, the solid-in-oil (S/O) nanodispersion was produced by: i) BSA-sucrose ester complex formation by lyophilization and ii) nanodispersion of the complex in isopropyl myristate. Then, the S/O nanodispersion was emulsified with water by high shear homogenization followed by sonication, to obtain the S/O/W nanodispersion.
The size of S/O nanodispersion droplets was evaluated by Dynamic Light Scattering (DLS). The BSA-sucrose ester complex association efficiency (AE) was also assessed by UV/Vis spectrophotometry using the bicinchoninic acid (BCA) protein assay method.

Results and Conclusions

The S/O internal phase of the S/O/W system presented droplets in the nanometric size range, with an average size of 639.3 nm (± 34.9%). Considering the BSA-sucrose ester complex AE a mean value of 72.6% (± 17.5%) was obtained. Thus, it is possible to conclude that the S/O/W nanodispersion may be a suitable alternative for dermal administration of peptides and proteins.
In the future, this system will be incorporated in semisolid formulations and the obtained S/O/W emulgel or cream will be characterized.

Keywords: Nanotechnology; drug delivery; solid-in-oil-in-water nanodispersions; proteins.

POSTER 05

Laccase extraction and activity enhancement using ionic-liquid-based strategies – toward its application in the medicinal field

EMANUEL V. CAPELA[1]; ANA I. VALENTE[1]; OSCAR RODRIGUEZ[2]; JOÃO A.P. COUTINHO[1]; ANA SOTO[2]; MARA G. FREIRE[1]; ANA P.M. TAVARES[1]

1 - CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal;
2 - Department of Chemical Engineering, Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

Oxidative enzymes, such as laccases, catalyze reactions in a wide range of biotechnological processes, being in the forefront of the emerging field of medicinal biotechnology. For instance, laccases can be used in the manufacturing of anticancer drugs and anti HIV-1, in the development of antibiotics, and as medical diagnostic tools. However, large amounts of enzyme with high purity and concentration are required for these applications. Microbial fermentation is the most viable production method; however, the enzyme recovery and purification involve several steps. Thus, their production costs still remain high due to the lack of cost-effective purification techniques. Ionic-liquid-based aqueous biphasic systems (IL-based ABS) have emerged in recent years as promising platforms for the extraction and purification of a large range of (bio)molecules. Thus, they can be foreseen as viable and cost-efficient tools for the extraction and purification of enzymes from the fermentation broth, allowing the intensification of the downstream processing. Accordingly, in this work several IL-based ABS for laccase extraction and preservation of its activity were evaluated. Studies on the enzyme partitioning were carried out with ABS formed by several imidazolium-, pyridinium-, phosphonium-, ammonium- and cholinium-based ILs combined either with salts or polymers. ILs were also investigated as adjuvants in traditional ABS. In general, remarkable extraction efficiencies of laccase with enhanced activity were obtained in a single-step, allowing to conclude that laccase presents higher activity and affinity to the most hydrophilic phases.
This work was developed within the scope of the project CICECO (UID/CTM/50011/2019), financed by national funds through the FCT/MCTES. E.V. Capela and A.P.M. Tavares acknowledges FCT for the PhD grant SFRH/BD/126202/2016 and the research contract IF/01634/2015, respectively, and also the financial support from COST-IL for the Short Term Scientific Mission grant (ECOST-STSM-CM1206-110116-068796). M.G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-337753.

Keywords: Oxidative enzymes, laccase, ionic liquids, aqueous biphasic systems

POSTER 06

Ionic-liquid-based aqueous biphasic systems for the recovery of monoclonal antibodies

ANA F.C.S. RUFINO[1], ALEXANDRE E. SANTIAGO[1], EMANUEL V. CAPELA[1,2], ANA P.M. TAVARES[1], MATHEUS M. PEREIRA[1], AMINOU MOHAMADOU[3], JOÃO A.P. COUTINHO[1], M. RAQUEL AIRES-BARROS[2], ANA M. AZEVEDO[2] AND MARA G. FREIRE[1]

1 - CICECO – Aveiro Institute of Materials, Aveiro, Portugal
2 - iBB – Institute for Bioengineering and Bioscience, Lisboa, Portugal
3 - ICMR- Institute of Molecular Chemistry of Reims, Reims, France

Monoclonal antibodies (mAbs) have a therapeutic role for the treatment of diseases.The upstream processing of mAbs suffered improvements in recent years, being now the downstream processing the limiting stage of mAbs manufacturing. Protein A affinity chromatography is the standard technique of the pharmaceutical industry to purify mAbs. However, it is an extremely expensive technique and ionic-liquid-based aqueous biphasic systems may be considered as alternatives based on the already reported use on the purification of several (bio)molecules.

Aiming to develop purification routes for mAbs from cell culture supernatants, novel ABS formed by glycine-betaine analogous ILs and K_2HPO_4/KH_2PO_4 were studied. Recovery yields up to 100% with purification factors up to 1.6 were obtained in a single-step for the IL-rich phase. Additionally, the purification factor can be increased up to 2.7 (final purity of 60.9%) by optimizing the IL concentration in order to obtain three phase partition (TPP) systems in which mAbs tends to precipitate at the interphase. The recovery was finally performed by an ultrafiltration step; this final step further allowed to increase the purity up to 67.2% and to simultaneously carry out a buffer exchange to an appropriate final formulation. Allowing conclude that novel ABS studied are promising alternative strategies in the downstream processing of mAbs.

Keywords: Monoclonal antibodies, aqueous biphasic systems

Acknowledgements: This work was developed within the scope of project CICECO-Aveiro Institute of Materials (UID/CTM/50011/2019), financed by national funds through the FCT/MCTES. E.V. Capela, A.F.C.S. Rufino, A.P.M. Tavares acknowledge FCT for the grants SFRH/BD/126202/2016 and SFRH/BD/138997/2018, and the research contract IF/01634/2015. M.G. Freire acknowledges the European Research Council (ERC) for the Grant ERC-2013-StG-337753.

POSTER 07

Bioactivity and radical scavenging capacity of *Castanea sativa* shell extract obtained by subcritical water extraction

DIANA PINTO[1], ANDREIA F. PEIXOTO[2], CRISTINA FREIRE[2], CRISTINA DELERUE-MATOS[1], FRANCISCA RODRIGUES[1]

1 - REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Porto, Portugal2 - REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Chestnut fruit market is increasing worldwide. *Castanea sativa* Mill. is the main chestnut species in Europe, being Portugal one of the main producer countries. During chestnut processing, huge amounts of by-products are generated, mainly shells. Chestnut shells are excellent sources of valuable compounds, namely antioxidants, vitamin E and amino acids. The bioactive compounds of *C. sativa* shell might prevent oxidative stress-mediated disorders, such as aging and chronic diseases. The aim of this study was to evaluate the total phenolic and flavonoid contents (TPC and TFC, respectively), antioxidant activity (DPPH and FRAP assays) and scavenging capacity against reactive oxygen (ROS) species of *C. sativa* shells. Shells were randomly collected in November 2018, in Bragança (Portugal), dried in a dehydrator at 41 °C/24 h and milled to a particle size of 1 mm. The extract was obtained by subcritical water extraction and lyophilized. The TPC and TFC were, respectively, 183.9 mg GAE/g dw and 71.4 mg CE/g dw. A remarkable antioxidant activity was observed (IC_{50} values of 55.2 µg/mL and 89.6 µg/mL for FRAP and DPPH assays, respectively). Additionally, *C. sativa* shell extract showed a high ability to scavenge $O_2^{\cdot-}$ (IC_{50} = 47.2 µg/mL). These results support the bioactivity and radical scavenging ability of *C. sativa* shells.

Acknowledgments: The authors are grateful for the financial support from FCT/MCTES through national funds (UID/QUI/50006/2019). The authors would also like to thank the EU and FCT for funding through projects: FOODnanoHEALTH - Qualidade e Segurança Alimentar – uma abordagem (nano)tecnológica (Portugal2020, Norte-01-0145-FEDER-000011) and FFCastanea - Cascas de *Castanea sativa* como fonte de ingredientes ativos para Alimentos Funcionais e Cosméticos: uma abordagem sustentável (PTDC/ASP-AGR/29277/2017) (POCI-01-0145-FEDER-029277).

Keywords: chestnut shells; subcritical water extraction; bioactivity.

POSTER 08

Unlocking the potential of kelp-associated Actinobacteria to produce novel bioactive compounds

MARIANA GIRÃO[1]; INÊS RIBEIRO[1,2]; TIAGO RIBEIRO[1]; ISABEL C. AZEVEDO[1]; FILIPE PEREIRA[1]; RALPH URBATZKA[1]; PEDRO N. LEÃO[1]; MARIA F. CARVALHO[1];
1 - Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto, Portugal2 - Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal

The increase in cancer pathologies and infections caused by antibiotic multi-resistant bacteria urgently demands new therapeutic solutions. Searching for novel drugs in underexplored environments, such as marine environments, represents a promising approach to tackle this problematic. Actinobacteria living in association with marine organisms, such as macroalgae, represent a valuable resource for the discovery of chemical novelty. Under the scope of this work, actinobacteria associated with the macroalgae *Laminaria ochroleuca* were isolated and screened for their potential to produce compounds with antimicrobial and/or anticancer properties. This kelp species was collected in a rocky shore in northern Portugal. A total of 90 actinobacterial strains were isolated, most of which affiliated with the genus *Streptomyces*. Other genera were also identifed such as *Isoptericola, Rhodococcus, Nonomuraeae, Nocardiopsis, Microbispora* and *Microbacterium.* Forty-five isolates showed antimicrobial activity, against *Candida albicans* and/or *Staphylococcus aureus.* The minimum inhibitory concentration (MIC) was determined for these active extracts and values ranged from < 0.487 to 1000 µg mL-1. The actinobacterial extracts were also tested for their anticancer potential on two human cancer cell lines (breast carcinoma T-47D and neuroblastoma SH-SY5Y) and cytotoxicity was tested in a non-carcinogenic endothelial cell line (hCMEC/D3). Twenty-eight extracts affected the viability of the three cell lines, but seven extracts only compromised the viability of cancer cells. Dereplication data indicated that most active extracts contained antimycins, but two of them might contain novel bioactive compounds. The results obtained in this study revealed that kelp-associated actinobacteria have a promising potential for the production of novel drugs, highlighting their importance and biotechnological value.

Keywords: marine actinobacteria, antimicrobial, anticancer, kelp

POSTER 09

Screening of pathogenic variants of the *DMD* gene in female patients with undetermined muscular dystrophy

MARA MORAIS[1]; ANA GONÇALVES[2]; SANDRA MOTA[1,3]; MANUELA AMORIM[1,3]
1 - Área Científica de Análises Clínicas e Saúde Publica, ESS/IPP, Porto, Portugal2 - Centro de Genética Médica Jacinto Magalhães, Centro Hospitalar do Porto, Porto, Portugal3 - Centro de Investigação em Saúde e Ambiente, ESS/IPP, Porto, Portugal

Introduction: Dystrophinopathy (Duchenne/Becker muscular dystrophy, DMD/BMD), a progressive neuromuscular disease with an X-linked recessive inheritance, is caused by pathogenic variants in the *DMD* gene, including large deletions (68%), duplications (11%), point variants (20%) and others. Although the majority of females heterozygous for these variants are asymptomatic, about 2.5-7.8% may have some symptom manifestations.
Objective: Identify symptomatic carriers of dystrophinopathy.
Methods: In this study, 80 randomly selected female patients with progressive muscular dystrophy of unknown genetic cause, were screened for deletions and duplications in the *DMD*gene, using Multiplex Ligation-dependent Probe Amplification (MLPA) technique. In the positive cases the X-chromosome inactivation (XCI) pattern was studied resorting to the HUMARA assay. Additionally, it was conducted a review of all the manifesting female carriers (n=18), previously characterized in the *Unidade de Genética Molecular do Centro de Genética Médica Jacinto Magalhães (Centro Hospitalar do Porto).*
Results: The present study allowed the identification of 4 new cases of symptomatic female carriers. In the overall analysis of all patients (n=22), 50% of cases had deletions in the *DMD*gene, followed by point variants (27.3%) and duplications (22.7%). Skewed XCI, performed in leukocytes, was observed in 9 of the 18 heterozygous patients.
Conclusions: Symptomatic carriers of dystrophinopathy, although rare, are underdiagnosed and preferentially included in other subtypes of muscular dystrophies. The results obtained corroborate that the skewed XCI can be one of the main mechanisms involved in these DMD/BMD phenotypic expression patients. However, further genetic research is required, namely, systematic XCI analysis in muscle biopsy and patient´s family studies to determine alelism. Due to the high risk of transmission to offspring, referring these patients to genetic counselling is essential.

Keywords: Duchenne/Becker muscular dystrophy; symptomatic female carriers; *DMD* gene; X-chromosome inactivation.

POSTER 10

Novel enzymes for the analysis and glycoengineering of therapeutic glycoproteins

MARLENE BAPTISTA[1]; CARLA OLIVEIRA[1]; LUCÍLIA DOMINGUES[1]; TATIANA Q. AGUIAR[1]

1 - CEB-Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

Many proteins of clinical and pharmaceutical interest are *N*-glycosylated, being their bioactivity, pharmacokinetics and pharmacodynamics affected by the glycan structures they carry. Thus, one of the biggest challenges in the pharmaceutical and biomedical areas is the manufacturing of glycopeptides and glycoproteins with homogenous and defined oligosaccharide structures.
Endo-β-*N*-acetylglucosaminidases (ENGases) of the glycoside hydrolase (GH) family 85 are a class of enzymes (EC 3.2.1.96) that, in addition to hydrolytic activity against the diacetylchitobiose core of *N*-glycans, can also display transglycosylation activity. These enzymes have increasingly become a focus of interest due to their useful applications in the analysis and glycoengineering of therapeutic glycopeptides and glycoproteins.
Although family GH85 currently contains 768 members, only 11 GH85 ENGases have been characterized thus far. Envisioning the identification of new GH85 ENGases with useful action, this study focused on two new putative ENGases of this family: Q752H6 from the filamentous fungus *Ashbya gossypii* and C5DRB8 from the yeast *Zygosaccharomyces rouxii*. Previous results hinted at the existence of ENGase activity in these organisms, and therefore this work aimed at using *in silico* approaches to assess about the potential activity of their putative ENGases. Data obtained from multiple alignments and homology-based models allowed obtaining indications on possible 3D structures and catalytic residues. The closest structural homolog in the Protein Data Bank (PDB) for the putative ENGases from *A. gossypii* and *Z. rouxii* was Endo-A from *Arthrobacter protophormiae* (PDB: 2VTF.1.A). Considering the 3D models generated with SWISS-MODEL (ExPASy), only the ENGase from *Z. rouxii* presents the typical $(\beta/\alpha)_8$-TIM-barrel structure of ENGases, indicating that this protein likely presents ENGase activity. Ongoing work comprises the recombinant production, purification and characterization of this novel enzyme.

Keywords: Endo-β-*N*-acetylglucosaminidase, *N*-glycans, *Ashbya gossypii*, *Zygosaccharomyces rouxi*

Acknowledgements: Study supported by FCT, Compete2020 and Norte2020 through the strategic funding of UID/BIO/04469/2019, Project EcoBioInks4SmartTextiles (PTDC/CTM-TEX/30298/2017–POCI-01-0145-FEDER-030298) and BioTecNorte operation (NORTE-01-0145-FEDER-000004).

POSTER 11

Identification And Bioactivity Screening Of Actinobacteria Isolated From Deep-Sea Environments

INÊS RIBEIRO[1,2], MARIANA GIRÃO[1], ADRIANA REGO[1,3], CÍNTIA RIBEIRO[1,3], FRANCISCA BRANCO[1,3], EDUARDA ALMEIDA[1,3], ANA P. MUCHA[1], ANDREIA HENRIQUES[4,5], RALPH URBATZKA[1], PEDRO LEÃO[1], MARIA F. CARVALHO[1]

1 - CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal
2 - Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 – Porto, Portugal
3 - Faculty of Sciences, University of Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal
4 - MARE-Marine and Environmental Sciences Centre, Estação de Biologia Marinha do Funchal, Cais do Carvão, 9900-783 Funchal, Madeira Island, Portugal
5 - ARDITI-Regional Agency for the Development of Research, Technology and Innovation, Oceanic Observatory of Madeira (OOM), Madeira Tecnopolo, Caminho da Penteada, 9020-105 Funchal, Portugal

In the last years, the investigation of actinobacteria in terms of their biotechnological potential has been quite exhaustive due to their well-known ability to produce compounds with important properties. New approaches for the discovery of novel molecules from these microorganisms target the prospection of unexplored environments, where the chances of finding new taxa and metabolic characteristics are greater. Deep-sea regions represent about 90% of the marine environment and many of these regions have never been explored before in terms of their biotechnological potential. The unique and extreme characteristics found on deep-sea habitats, such as high pressure, low temperature, lack of light and variable salinity and oxygen concentrations are excellent evolutionary drivers, making deep-sea environments a promising source of novel microorganisms and bioactive compounds.
In this study, several deep-sea samples (431-3199 m depth) collected in the Portuguese continental platform (Madeira and Azores) and in the Arctic Mid-Ocean Ridge were used for the isolation of actinobacteria. In order to maximize the isolation of actinobacteria two pre-treatments and three selective culture media supplemented with different antibiotics were used. The actinobacterial strains recovered so far are distributed by eight genera - *Microbacterium, Rhodococcus, Micrococcus, Streptomyces, Dietzia, Brevibacterium, Actinotalea* and *Tsukamurella*. These strains are being tested for their antimicrobial and anticancer activities. Up to the moment, crude extracts of two *Streptomyces* strains showed ability to inhibit the growth of *Candida albicans* and one strain of the same genus inhibited the growth of *Staphylococcus aureus*. Anticancer screening assays revealed that extracts of two *Streptomyces* strains were capable of reducing the viability of the human cancer cell line HepG2 in ca. 40%. Future steps will focus on the identification of the remaining isolates and on the screening of their bioactivities.

Keywords: Deep-sea; Actinobacteria; Secondary metabolites

POSTER 12

Ecotoxicological effect of 1-(2-hydroxyethyl)-3-methylimidazolium chloride and cetylpyridinium chloride towards the microalgae *Chlorella vulgaris*

ELISABETE PACHECO[1,2]; RICARDO FERRAZ[1,2,3]; CRISTINA PRUDÊNCIO[1,2,4] ; PIEDADE BARROS[1,2]

1 - ESS|IPP – School of Health, Polytechnic Institute of Porto, Porto, Portugal2 - CISA – Research Centre in Health and Environment, School of Health, Polytechnic Institute of Porto, Porto, Portugal3 - LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Portugal
4 - i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

Ionic liquids (ILs) are salts that are stable over their melting temperature and are made exclusively of ions. ILs have received considerable interest due to their unique properties. The growing interest in ILs predicts an increase of their manufacture and use at industrial scale, which may result in the increased release of these compounds into the environment. In the past years, ILs have been used as a greener alternative to hazardous conventional solvents.
Microalgae play an important role in the equilibrium of aquatic ecosystems. Since they belong to the first level of the trophic chain, perturbations to its welfare may have repercussions on the higher levels of the ecosystem.
In the present work, we assessed the ecotoxicological effect of two ILs, 1-(2-hydroxyethyl)-3-methylimidazolium chloride ([C2OHMIM][Cl]) and cetylpyridinium chloride ([C16Pyr][Cl]), to the microalgae *Chlorella vulgaris* growth, according to the OECD guidelines number 201.
Growth inhibition was quantified from measurements of the algal biomass as a function of time by optical density and the effective concentration that causes a 50% inhibition in the algae growth (EC50) was determined.
The results demonstrated that *C. vulgaris* growth rate inhibition increased with the increase of the tested concentration. The 96h EC50 mean value of [C16Pyr][Cl] was 0.011 mM, being classified as moderately toxic to *C. vulgaris*. The 96h EC50 mean value of [C2OHMIM][Cl] was 34.03 mM, which classifies it as relatively harmless to *C. vulgaris*.
It is possible to conclude that [C16Pyr][Cl] presents a higher toxic effect than [C2OHMIM][Cl] towards *C. vulgaris*.

Keywords: Ionic liquids, 1-(2-hydroxyethyl)-3-methylimidazolium, cetylpyridinium, *Chlorella vulgaris*

POSTER 13

Purine removal for a healthier beer

ANA SANTOS, JOHANNA SIMÕES, MÁRCIA NEVES, MARA FREIRE
Department of Chemistry, CICECO - Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

Of all fermented alcoholic beverages, beer is the one that offers higher purine content, mostly derived from yeast and malt DNA1. When ingested by humans can be catabolized into uric acid contributing for its serum level increase leading to hyperuricemia, known to be a risk factor of gout2. Therefore, hyperuricemic and gouty patients are adviced to restrict beer from their diets3. In order to contradict the relation between beer and these diseases, an adsorptive technique to selectively and efficently remove purines from beer using functionalized materials: graphene-based composite (NGr) and supported ionic liquids materials (SILs) was developed. The NGr was synthetized from a protein-rich biomass and a deep eutectic solvent, and two SILs with diferent cations (SilPrMImCl, SilPrNEt3Cl) were used as adsorbents for purine compounds and Saccharomyces cerevisiae DNA in aqueous solutions. While NGr demonstrate high removal efficencies for guanosine, adenine and adenosine, SILs demonstrates no capacity to remove purines. However these materials shows a removal efficiency for DNA (approx. 50%).
These results prove the suitability of NGr for the efficient and selective removal of purine compounds through mainly non-covalent interactions. SILs are more efficient for DNA removal through ionic bonds with phosphate group of DNA leading to a high selectivity and extraction efficiencies. Therefore, these materials are good options to remove these target molecules and avoid beer restriction.

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, FCT Ref. UID/CTM/50011/2019, financed by national funds through the FCT/MCTES. Márcia C. Neves acknowledges the FCT, I.P. for the research contract CEECIND/00383/2017 under the CEEC Individual 2017.

Keywords: Beer, purine compounds, DNA, uric acid, gout, adsorption, graphene-based composites, supported ionic liquids

1 - Cortacero-Ramírez, S., Hernáinz-Bermúdez.De Castro, M., Segura-Carretero, A., Cruces-Blanco, C. & Fernández-Gutiérrez, A. Trends Anal. Chem. 22, 440–445 (2003).
2 - Ragab, G., Elshahaly, M. & Bardin, T. J. Adv. Res. 8, 495–511 (2017).
3 - R. Preedy, V. The World Economy 30, (Elsevier, 2008).

POSTER 14

Voltammetric Magnetoimmunoassay for the Determination of Cystatin C

C. VARELA[1], M.M.P.S. NEVES[1], H.P.A. NOUWS[1], A. SANTOS-SILVA[2], C. DELERUE-MATOS[1*]

1 - REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Porto, Portugal
2 - REQUIMTE/UCIBIO, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal
*cmm@isep.ipp.pt

Chronic kidney disease (CKD) is a health problem with increasing prevalence and mortality. CKD is a silent, asymptomatic disease and the established biomarkers to evaluate kidney injury are unreliable for its diagnosis at the initial stages. Therefore, patients are usually diagnosed at advanced phases, when irreparable renal damage already occurred. Cystatin C (CysC) is a valuable early predictive biomarker for the evaluation of kidney function.

The established methods for the determination of CysC are usually expensive and require routine laboratory analyzers, which difficult the decentralization of the analysis to a point-of-care (POC) setting. Thus, the development of methods that ensure real-time results at a POC level is a constant demand in clinical practice. Furthermore, the development of new technologies to improve the diagnosis, prognosis and self-management of CKD is highly demanded. Therefore, in this work an electrochemical magnetoimmunoassay for the detection of CysC was developed.

The method was based on a sandwich immunoassay performed on carboxylic acid-modified magnetic beads. The antigen (CysC) was detected employing an alkaline-phosphatase labelled antibody. A screen-printed carbon electrode was employed as transducer surface and a mixture of 3-indoxyl phosphate/silver ions was used as substrate. The analytical signal was recorded through the voltammetric stripping of enzymatically deposited silver.

Aspects such as the miniaturized size, the need of low reagents/sample volumes, and portability, facilitates the transfer of the developed methodology to a POC approach. Hence, the proposed immunoassay could contribute to new paths for the decentralized and front-line diagnosis of CKD.

Acknowledgements

The work was supported by UID/QUI/50006/2019 with funding from FCT/MCTES through national funds and by the European Union through projects Norte-01-0145-FEDER-000024 and Norte-01-0145-FEDER-000011, co-funded by FEDER in the scope of CCDR-N and NORTE2020 Partnership Agreement.

Keywords: chronic kidney disease, cystatin C, magnetoimmunoassay, electrochemistry

POSTER 15

Amphibian ocellatin-PT peptides as novel anti-parasitic, anti-inflammatory and neuroprotective agents

ANABELA MOREIRA[1,2,3]; MARIA VIEIRA[1]; MARIA INÊS ROCHA[1]; JAIME FREITAS[1]; MARCELO BEMQUERER[4]; J.R.S.A. LEITE[5]; ANA M. TOMÁS[1]; JOÃO B. RELVAS[1,6]; ALEXANDRA PLÁCIDO[1,6]

1 – Instituto de Investigação e Inovação para a Saúde (i3S), Porto, Portugal
2 – Faculty of Engineering, University of Porto, Portugal
3 – Institute of Biomedical Science Abel Salazar, University of Porto, Portugal
4 – Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil
5 – Universidade de Brasília (UnB), Brazil
6 – Bio*prospectum* Lda., Porto, Portugal

Bioprospection can be defined as the search in natural products for compounds with pharmaceutical, industrial or cosmetic applications. Ocellatin peptides, first obtained from the frog *Leptodactylus ocellatus*, have been raising interest in the last decade due to their antimicrobial properties. In particular, ocellatin-PT peptides, recently isolated from the species *Leptodactylus pustulatus*, are active against several bacterial species and *Leishmania infantum* parasites. Interestingly, these peptides appear to act selectively in microbial membranes, since no cytotoxic effects have been detected in murine cells and no hemolytic activity was observed in human erythrocytes. Until now, eight different ocellatin-PT peptides (PT1 to PT8) have been described. The objective of the present work is to characterize and assess the anti-parasitic, anti-inflammatory and neuroprotective potential of ocellatin-PT4 and the newly isolated ocellatin-PT9 peptides. Both were synthesized using a solid-phase method, purified by RP-HPLC, and structurally characterized by mass spectrometry (nanoLC-MS/MS) and circular dichroism analysis. Studies conducted in human monocyte-derived macrophages did not reveal any cytotoxic effects, indicating that both peptides present favourable safety profiles. Preliminary experiments suggest that ocellatin-PT4, but not ocellatin-PT9, is also active against *Leishmania amazonensis*. The neuroprotective activity of ocellatins PT4 and PT9 was measured in live microglial cells by fluorescence resonance energy transfer (FRET) microscopy, including their effect on the production of reactive oxygen species (ROS) and activation of the NF-κB pathway. Future experiments will include atomic force microscopy (AFM) imaging to visualize the effect of ocellatin-PT4 in the cell membranes of *L. amazonensis* parasites, as well as hemolytic studies with human erythrocytes for ocellatin-PT9. Moreover, the neuroprotective role of both peptides will be further evaluated with additional FRET analysis, namely their performance in excitotoxic conditions.

Keywords: bioprospection, ocellatin peptides, neuroprotection, FRET

POSTER 16

The two sides of the use of supported ionic liquids (SILs) materials

JÉSSICA BAIROS; MARIA SANTOS; EMANUEL CAPELA; MÁRCIA NEVES; ANA TAVARES; MARA G. FREIRE
CICECO, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal

Solid-phase extraction (SPE) is used for purification and removal techniques for target molecules in liquid extracts. In recent years, the introduction of ionic liquids (ILs) in SPE, have emerged as SILs materials and considering their characteristics[1] SILs can be excellent adsorbents of a wide range of bioactive compounds, ranging from small organic compounds to complex molecules like antibodies (Abs). Despite the studies that have already been carried out, that prove their role in the extraction and purification of proteins[2], there are no indications regarding their performance when applied to Abs. However, it seems to be a viable and sustainable strategy to be used in the downstream processing of Abs. Also, these materials could have the ability to adsorb and remove emerging contaminants that are not being successfully removed from wastewaters by conventional treatment systems. In this work three SILs materials were synthesized and used in IgG purification and in adsorption studies of a pesticide (imidacloprid) from aqueous solutions, being $SilPrN(C_8)_3Cl$ the more promising SIL for both applications. The development of a new platform such as SILs, showed tremendous potential for the purification of IgG and removal of imidacloprid from water, thus this technique appears to be a viable option for the downstream processing of Abs and to be applied as a filter for the elimination of pesticides from wastewaters.
This work was developed within the scope of the project CICECO (UID/CTM/50011/2019), financed by national funds through the FCT/MCTES, project POCI-01-0145-FEDER-031106 (IonCytDevice) and project POCI-01-0145-FEDER-031268 - funded by FEDER, COMPETE2020, POCI and OE. Emanuel Capela acknowledges FCT for the PhD grant SFRH/BD/126202/2016. Ana Tavares and Márcia Neves acknowledge FCT for the research contracts IF/01634/2015 and CEECIND/00383/2017, respectively.

Keywords: SILs, IgG purification, pesticide removal

1. Belchior, V. et al. Advances in Biochemical Engineering/Biotecnology, (2018)
2. Song, H. et al. RSC Adv 6, (2016).

POSTER 17

Spoilage fungi and mycotoxins contamination risk in *Capsicum* products

JÉSSICA COSTA[1]; NELSON LIMA[2]; CLEDIR SANTOS[1]

1 - Department of Chemical Science and Natural Resource,Universidad La Frontera, Temuco,Chile2 - Micoteca da Universidade do Minho, Centre of Biological Engineering, University of Minho, Braga, Portugal

Due to food quality and safety issues, the increased worldwide commercialisation of *Capsicum* products has triggered an international alert. *Capsicum* and derivative-products are substrates with unusual nutritional characteristics for microbial growth. Despite this, the presence of spoilage fungi and the occurrence of mycotoxins in the pepper production chain have been commonly detected. The potential contamination of pepper and pepper-based products with mycotoxins represents a serious risk to consumer health and affects negatively this agribusiness sector. The main aim of this work is to discuss the critical control points, with a focus on mycotoxin contamination, during the production, storage and distribution of *Capsicum* products from a safety perspective; outlining the important role of ecophysiological factors in mycotoxin biosynthesis in these food commodities and the human health risks caused by the ingestion of peppers contaminated with mycotoxins.
Overall, *Capsicum* and its derivative-products are highly susceptible to contamination by mycotoxins. The control of water activity (a_w), temperature and moisture content are essential to avoid the growth of potential mycotoxigenic spoilage fungi, such as *Aspergillus* and *Penicillium* species. Concerning classes of mycotoxins, aflatoxins and ocratoxina A are among the most important contaminants from a consumer point of view. In addition, citrinin, deoxynivalenol, patulin, sterigmatocystin and zearalenone have also been detected in *Capsicum*products. Pepper crop production and further transportation, processing and storage are crucial for production of safe food. The presence of capsaicinoids in *Capsicum* plant and in powdered pepper can select and delay fungal infection. However, further research is needed to elucidate the ecophysiological conditions that support fungal growth in this substrate, as well as the role that mycotoxins play during the infection process. Moreover, studying the effect of substrate composition on the mycotoxin production can open new avenues in knowledge of how potential mycotoxigenic fungi can be controlled in terms of their metabolism.

Keywords: Mycotoxins, Pepper, Spoilage fungi

POSTER 18

Portuguese vine canes as source of active compounds for skin purposes

OLENA DOROSH[1,2]; DIANA PINTO[1]; JAROSLAVA ŠVARC-GAJIĆ[3]; MANUELA M. MOREIRA[1]; FRANCISCA RODRIGUES[1]; CRISTINA DELERUE-MATOS[1]

1 - REQUIMTE/LAQV, ISEP, Porto, Portugal
2 - ITQB, Oeiras, Portugal
3 - Faculty of Technology, Novi Sad, Serbia

Grapes are one of the major fruit crops produced throughout the world, and despite from the richness of grapes wastes in polyphenols, usually they end up discarded; in the case of vine-canes they are typically incorporated in the soil or incinerated. Depending on the vine varieties, it is estimated that for each hectare of vineyard 1,75 tons of vine-cane wastes are produced. Considering that polyphenols possess a powerful antioxidant capacity with a wide range of health benefits, vine-canes could be used to obtain bioactive extracts which could be further used in the cosmetic industries.
The aim of this work was to evaluate the antioxidant properties from vine-cane extracts, in order to investigate their potential use in different industries. For that, six different Portuguese vine cane varieties, namely, *Alvarinho* and *Loureiro* from Minho region, *Touriga Nacional* (TN) and *Tinta Roriz* (TR) both from Douro and Dão regions were subjected to subcritical water extraction to recover bioactive compounds. The extracts phenolic content and antioxidant activity were evaluated through spectrophotometric methods. All extracts presented similar antioxidant activity and the highest phenolic content was reported for TR and TN varieties from Douro region (33.7 ± 1.9 and 35.2 ± 1.7 mg_{GAE}/g dry sample, respectively). The capacity of vine cane extracts to capture reactive oxygen species superoxide ($O_2^{\cdot-}$) was also studied. The highest IC_{50} value was obtained for *Alvarinho* variety (IC_{50}= 56.68 ± 2.60 µg/mL). Furthermore, the cellular viability in the HaCaT (keratinocytes) dermal cell line was tested, being possible to conclude that none of the extracts presented cytotoxicity. Work is in progress to select the most promising extracts to be applied into a topical formulation.

Acknowledgements: FCT/MCTES financial support through national funds (UID/QUI/50006/2019) and to the projects PTDC/BII-BIO/30884/2017, PTDC/ASP-AGR/29277/2017 and SFRH/BPD/97049/2013 (MMM).

Keywords: vine-canes, antioxidants, cytotoxicity, cosmetics products

POSTER 19

Natural Broad-Spectrum Anti-adhesive Coating

B. COSTA,[1, 2,3] R. MOTA,[1,4] M.C.L. MARTINS,[1,2,5] P. TAMAGNINI1,[3,4] & F. COSTA[1,2]

[1]i3S - Instituto de Inovação e Investigação em Saúde, Porto, Portugal
[2]INEB – Instituto de Engenharia Biomédica, Porto
[3]FCUP- Faculdade de Ciências da Universidade do Porto
[4]IBMC – Instituto de Biologia Molecular e Celular, Porto
[5]ICBAS- Instituto de Ciências Biomédicas Abel Salazar, Porto

Medical device-associated infections are a major health threat, imposing a high human and economic burden. Most these infections are caused by biofilms that are very difficult to eradicate [1]. Current strategies to prevent this problem, based on coatings with antibiotics or antiseptics, have proven to be insufficient, often toxic, and even promoters of bacterial resistance [2]. Therefore, new biotechnological approaches capable of preventing biofilm formation, are being explored [3].
Previously, we have reported the development of an infection preventive coating (CyanoCoating) based on an extracellular polymer released by a marine cyanobacterium. This coating exhibited a smooth topography, low thickness, high hydrophilic properties and anti-adhesive properties against *Staphylococcus epidermidis*.
Herein, we report CyanoCoating broad-spectrum of activity (against Gram-positive *Staphylococcus aureus*, Gram-negative *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*and fungi *Candida albicans*, in comparison to medical grade polyurethane), blood compatibility (tested with platelets in the presence of plasma proteins), and stability after ethylene oxide sterilization.
CyanoCoating prevented adhesion of all the bacteria tested (≤80%) and platelets (<87%), without inducing platelet activation. This effect was not hindered by the presence of human plasma proteins. Importantly, this coating performance was not compromised after ethylene oxide sterilization.
The development of this anti-adhesive coating is an important step towards the establishment of a new technological platform capable of preventing medical device associated-infections, without inducing thrombus formation in blood-contacting applications.

Keywords: cyanobacteria; extracellular polymer; anti-adhesive coating; medical device associated-infections

References:

1. del Pozo, J.L. and R. Patel. Clin Pharmacol Ther, 2007. 82(2): p. 204-209.
2. Salwiczek, M., et al. Trends Biotechnol, 2014. 32(2): p. 82-90.
3. Cloutier, M., et al.Trends Biotechnol, 2015. 33(11): p. 637-52.

POSTER 20

Optimization and validation of a new cytology stain – CytoPath line®

TEIXEIRA C1, FERNANDES S1 AND SILVA RA1

1 - Centro de Investigação em Saúde e Ambiente da Escola Superior de Saúde do Instituto Politécnico do Porto (CISA-ESS|P.PORTO), Rua Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal

Cytology plays an important role in the screening and diagnosis of cervical cancer. The correct staining of cervical smear allows the recognition of essential characteristics for the diagnosis of some pathologies. However, given the existence of flaws in the current staining procedures, the development of new staining techniques as well as the optimization of the existing ones is important. Recently, a new staining line - the CytoPath line® is being developed by DiaPath S.p.A. The objective of this work is to evaluate its performance in the staining of gynecological cytology samples.
For this purpose, 60 gynecological liquid-based samples were used, were observed microscopically and evaluated by 3 independent evaluators. At the end, the staining under test was compared with the traditional technique.
The evaluation was performed taking into account parameters for both nuclear and cytoplasmic staining. The microscopic evaluation allowed to identify differences in the two stains at the level of color intensity of nuclei and cytoplasm. The statistical analysis performed on nuclear staining evaluation reveals significant differences on parameters of "differentiation" and "hematoxylin color". Concerning cytoplasmic staining evaluation, statistically significant differences were only found on "cyanophilia intensity" parameter. In addition, the overall evaluation of both staining techniques showed statistically significant differences, being superior the performance of the staining under test, with a reduction of 10% of the total time of the technical procedure.
In conclusion, the new CytoPath line® staining line proved to be superior to the traditional Papanicolaou staining in some of the evaluated parameters, so its utilization is recommended for diagnostic purposes in gynecological cytology.

Keywords: Cervical cytology, Papanicolaou staining, CytoPath line®, screening

POSTER 21

Phytochemical profile, antioxidant activity and cytotoxicity against keratinocytes, fibroblasts and endothelial cells of picoplanktonic marine cyanobacteria

JANAÍNA MORONE[1,2], GRACILIANA LOPES[1,2], MARCO PRETO[2], VÍTOR VASCONCELOS[1,2], ROSÁRIO MARTINS[2,3]

[1]Faculty of Sciences, University of Porto, Porto, Portugal
[2]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Matosinhos, Portugal
[3]Health and Environment Research Centre, School of Health, Polytechnic Institute of Porto, Porto, Portugal

Combining the increase demand for natural products in skin care formulations, and the bioactive arsenal of cyanobacteria, we aimed with this study to evaluate the potential of a 70% ethanolic extract of picocyanobacteria strains of the genera *Cyanobium* and *Synechocystis* for skin care applications. The cyanobacteria extract was analyzed for the phytochemical profile including carotenoid and phenolic content, for the antioxidant potential, and for the *in vitro* cytotoxicity against keratinocytes (HaCat), fibroblasts (3T3L1) and endothelial cells (hCMEC/D3). The total carotenoid content ranged from 162.43 to 383.89 μg g^{-1} of dry biomass and the total phenolic content (TPC) from 1.09 to 2.45 mg GAE g^{-1}. Identified carotenoids consisted in zeaxanthin, lutein, canthaxanthin, echinenone and β-carotene, being zeaxanthin and lutein the most representative (49.82 and 79.08 μg g^{-1}, respectively). The antioxidant potential assessed by the DPPH• and superoxide anion radical ($O_2^{\bullet-}$) scavenging assays resulted in an IC_{50} of 863.82 μg mL^{-1} and 1275.86 μg mL^{-1} respectively. An increase in cell viability was registered, particularly for fibroblasts and keratinocytes. From all the strains, both the species *Synechocystis salina* LEGE06099 and *Synechocystis salina* LEGE06155 evidenced an interesting potential for further exploitation.

Keywords: cyanobacteria, phytochemicals, antioxidant potential, cytotoxicity

Acknowledgments:
This work was done in the framework of the project BLUEHUMAN - BLUE biotechnology as a road for innovation on HUMAN's health aiming smart growth in Atlantic Area - EAPA_151/2016 of the Interreg Atlantic Area Programme funded by the European Regional Development Fund and ALGAVALOR - MicroALGAs: integrated production and valorization of biomass and its various applications, supported by the PORTUGAL 2020 through the European Regional Development Fund (ERDF).
This work was supported by the FCT Project UID/Multi/04423/2019 and by the Atlantic Interreg Project – EnhanceMicroAlgae - High added-value industrial opportunities for microalgae in the Atlantic Area (EAPA_338/2016).

POSTER 22

Development of a Disposable Paper-Based Potentiometric Immunosensor for Real-time Detection of a Foodborne Pathogen

NÁDIA F.D. SILVA[1,3]; CLÁUDIO M.R. ALMEIDA[2,4]; JÚLIA M.C.S. MAGALHÃES[2]; MARIA P. GONÇALVES[2]; CRISTINA FREIRE[3]; CRISTINA DELERUE-MATOS[1]

1 - REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal
2 - REQUIMTE/LAQV, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal
3 - REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto (FCUP), Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.
4 - Present address: CIEPQPF, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, 3030-790 Coimbra, Portugal

The detection and effective prevention of foodborne illnesses caused by bacteria still stand today a worldwide public health issue. Among other bacterial pathogens, *Salmonella spp.* was one of the most common causes of foodborne outbreaks, originating many hospitalizations and deaths every year [1, 2]. The last summary of foodborne outbreaks from EFSA reported an unexpected increase of 11.5% compared to 2015 data [3]. Accordingly, a newest demanding for lab-on-chip infield testing devices for real-time detection of foodborne pathogens has been observed [4], in which electrochemical biosensors reached a prominent place by rapidness, sensitivity, portability, low-cost and user-friendly interface which have shown. Despite this, a perfect method which can be used onsite in different matrices is still needed [2].
In this work an innovative paper-based sensing platform and its application in a label-free potentiometric immunosensor for *Salmonella typhimurium* detection based on the blocking surface principle is reported. The sensor device consists simply in a paper-based strip electrode integrated with a filter paper pad which acted as a reservoir of the internal solution. This design offers a convenient platform for antibody immobilization and sampling, proving also that it is a simple and affordable methodology to control an ionic flux through a polymer membrane.
Two different immunosensing interfaces were assembled on the developed paper-strip electrode and the analytical performance of the resulting immunosensors was compared. The simplest interface relied on direct conjugation of the antibody to the polymer membrane and the second one resorted to an intermediate layer of a polyamidoamine dendrimer, with an ethylenediamine core from the fourth generation. For such, the potential shift derived from the blocking effect of the ionic flux caused by antigen-antibody conjugation was correlated with the logarithm of the *Salmonella typhimurium* concentration in the sample. In optimized conditions, a limit of detection of 5 cells mL^{-1} was achieved. As a proof-of-concept the proposed method was applied to apple juice samples with a recovery value of 54 %, demonstrating to be a suitable prototype to be used in real scenarios in useful time (< 1h assay).

Keywords: paper-based, immunosensor, label-free, foodborne pathogens

POSTER 23

Novel Anticancer Metallodrugs

LUÍS MONTEIRO [1]; ADRIANA GOMES [1]; C. SILVA [3,4]; F. MARTEL [3,4]; R. MENDES [1,2]; F. PAZ [1,2]; B.J.M. LEITE FEIRREIRA [1,2]; T.M. SANTOS [1,2]

1- Department of Chemistry, University of Aveiro, Aveiro, Portugal
2- CICECO, Aveiro Institute of Materials, Aveiro, Portugal
3- Department of Biomedicine - Unit of Biochemistry, Faculty of Medicine, University of Porto, Porto, Portugal
4-Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

Chemotherapy still depends on platinum-based drugs, although facing toxicity and spontaneous or acquired resistance problems. Aiming to overcome these limitations, a vast number of other metal complexes have been synthesized and their bio-action investigated over the years.[1] The combination of transition metals and drugs, such as NSAIDs, has demonstrated to be very promising due to synergistic effects between metals and the drugs, which improve their anti-ulcerous, -tumor and -bacterial activities. [2] NSAIDs possess N and/or O donor atoms (ammine and/or carboxylic groups) fundamental to obtain stable complexes with a variety of transition metal ions. [3], [4] With the objective of taking advantage of the above-described characteristics, novel complexes with several N-donor ligands and different NSAID's have been synthesized. With cobalt, nickel, and copper, diclofenac and ibuprofen (two NSAIDs) and different N-donors, seven different complexes were obtained and characterized and interaction studies of these complexes with BSA and DNA were undertaken as well as MTT and anti-oxidant activity assays.

References:
[1] N. Muhammad and Z. Guo, "Metal-based anticancer chemotherapeutic agents," Curr. Opin. Chem. Biol., vol. 19, no. 1, 2014.
[2] J. M. Suh, G. Kim, J. Kang, and M. H. Lim, "Strategies Employing Transition Metal Complexes to Modulate Amyloid-β Aggregation," Inorg. Chem., vol. 58, no. 1, 2019.
[3] W. W. Brandt, F. P. Dwyer, and E. C. Gyarfas, "Chelate complexes of 1,10-phenanthroline and related compounds," Chem. Rev., vol. 54, no. 6, 1954.
[4] C. N. Banti and S. K. Hadjikakou, "Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in Metal Complexes and Their Effect at the Cellular Level," Eur. J. Inorg. Chem., vol. 2016, no. 19, 2016.

Keywords: Metal complexes, Non-steroidal anti-inflammatory drugs (NSAID's), Metallodrugs, Chemotherapy

POSTER 24

Bioactivity screening of Cyanobacteria for repression of intestinal lipid absorption

SUSANA LEMOS DA COSTA[1-3]; TIAGO RIBEIRO[1]; FILIPA ROSA[1]; VÍTOR VASCONCELOS[1,2]; RALPH URBATZKA[1]

1 - Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Porto, Portugal
2 - Faculty of Sciences, University of Porto, Porto, Portugal
3 - Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, Portugal

Obesity is a worldwide epidemic that affects over 600 million people, regarded as a critical health risk to develop associated diseases such as diabetes, cardiovascular or even several types of cancer. Cyanobacteria are known for a high production of secondary metabolites that may reveal bioactivities for the treatment of obesity. In this work, we aimed to study cyanobacteria for their beneficial effects on obesity, by repressing intestinal lipid absorption using zebrafish larvae as a whole small animal model. Freshwater, estuarine and marine cyanobacterial strains were obtained from CIIMAR's Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC). After exposure to the cyanobacterial fractions, the ability to inhibit intestinal lipase and protease activity in the context of a whole organism was assessed. The activity was visualized in zebrafish larvae utilising a fluorescence-based method with PED6 and EnzChek, as phospholipase and protease reporters, respectively. The use of both reporters creates a more physiologically relevant readout of the complexity of digestive processes. The screening of cyanobacterial fractions allowed the identification of a few promising strains with over 60% inhibition of lipase activity. Those fractions with bioactivity and absence of general toxicity or malformations were selected for future works. Here, we intend to isolate the responsible compounds repressing intestinal lipid absorption.

Acknowledgments: This work was supported by the European ERA-NET Marine Biotechnology project CYANOBESITY (ERA-MBT/0001/2015), financed by national funds through FCT (Foundation for Science and Technology, Portugal).

Keywords: Anti-obesity drugs; Lipase activity; Natural products

POSTER 25

Effects of glyphosate and cylindrospermopsin at environmental concentrations on growth, photosynthesis and mineral content in lettuce plants

SOUMI SENGUPTA[2,3], MARISA FREITAS[1,2], EDGAR PINTO[4], JOANA AZEVEDO[2], FLÁVIO OLIVEIRA[2], ALEXANDRE CAMPOS[2], VITOR VASCONCELOS[2,5]

1-School of Health (ESS)-Polytechnic of Porto (P.Porto)/CISA-ESS-P.Porto, Porto, Portugal
2-CIIMAR-Porto University, Matosinhos, Portugal
3-School of Bio-sciences and Technology-VIT University, Tamil Nadu, India
4-REQUIMTE-FFUP, Porto, Portugal
5-FCUP, Porto, Portugal

Glyphosate is the most widely used herbicide, mainly due to the extensive cultivation of glyphosate-resistant plants. The intensification of agriculture has increased water eutrophication and the presence of natural cyanobacterial toxins, such as cylindrospermopsin. Previous studies support the hypothesis that glyphosate and cylindrospermopsin can affect the yield of crop plants, depending on the exposure concentration. Lettuce (*Lactuca sativa* L.) is a commercial leafy vegetable, extensively consumed worldwide with major importance for human nourishment and economy. A study investigating the effects on lettuce exposed to these contaminants simultaneously is necessary to predict their potential interactions. This study aimed to assess the effects of environmentally relevant concentrations of cylindrospermopsin (50µg/L), glyphosate (750 µg/L) and the cylindrospermopsin/glyphosate mixture on growth, photosynthesis and mineral content in lettuce plants grown in soil and hydroponic system. In general, for all the treatments, the plants exposed in soil system resulted in a decrease in fresh weights of the shoots and roots; however an increase in the fresh weight of roots was observed in plants exposed to cylindrospermopsin. The plants in hydroponic system showed an increasing trend in shoot weight and negligible differences in root weight. No negative effect on photosynthesis was observed, even leading to an increase in this parameter in lettuce in soil system. In both the soil grown and hydroponic plant leaves, a general decline in mineral content was observed in majority of the macro and micro elements, with a few elements showing an enhanced concentration. Our results suggest that glyphosate and cylindrospermopsin can change yield and nutritional quality of lettuce when present in relevant concentrations in the environment. Further research is needed to understand the under-lying impacts.

Keywords: cylindrospermopsin · glyphosate · *Lactuca sativa*

POSTER 26

Antimicrobial peptides and food preservation

SORAIA ALVES[1]; PAULA GOMES[1]; CÁTIA TEIXEIRA[1]

1 - LAQV-REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências Universidade do Porto, Porto, Portugal

Food is normally susceptible to physical, chemical and microbiological deterioration throughout storage and distribution, leading to a constant search for new strategies to increase food's shelf-life. One of the most fashionable trends consists of the development of innovative biopolymers based on natural polysaccharides, proteins or lipids obtained from by-products of the food industry. These biopolymers can act as carriers for antimicrobial additives to extend food's shelf-life and safety of packaged foods, by reducing and/or preventing growth of pathogenic and spoilage microorganisms and, thus, leading to active edible films and coatings. Noteworthy, the introduction of natural active additives to packaging materials provides advantages compared to the direct addition to food, such as the lower amount of active substances required, controlled release to food, and elimination of additional steps on processing.
In current food industry approaches to active edible films and coatings, additives are usually added as free components to the edible film and coating mixture, such as synthetic antibiotics (e.g. enilconazole). Recent trends point out antimicrobial peptides (AMP) as promising alternatives to current food preservatives. AMP are well-known components of the innate immune system that are rapidly gaining relevance, as opposed to conventional antibiotics whose effectiveness is declining. This is explained by a group of special features, including wide activity spectrum, high efficacy at low concentrations, and low propensity for eliciting resistant microbial strains. This work focuses on the mechanism of action of selected natural antimicrobial peptides and their potential application in food preservation.

Acknowledgements: Thanks are due to Fundação para a Ciência e Tecnologia (FCT, Portugal) for funding through grants UID/QUI/50006/2019 and PTDC/BAA-AGR/31798/2017.

Keywords: antimicrobial peptides, food preservation, spoilage microorganisms, active edible films

POSTER 27

Modulation of oxidative stress with Vitamin E in *Sacharomyces cerevisiae*

ALVES M.[1]; CUNHA, S.[2]; COSTA, A.[2]; NASCIMENTO, A.[3];VIEIRA, M.[2,4] ; PRUDÊNCIO, C.[2,4]

1 - Mestrado Bioquímica em Saúde, ESS-P.PORTO
2 - Ciências Químicas e das Biomoléculas, CISA, ESS-P.PORTO
3 -Biomatemática, BioestatísticaeBioinformática,ESS-P.PORTO4 - i3S, Universidade do Porto

Neurodegenerative diseases, such as Parkinson's Disease or Alzheimer's Disease, are characterized by the death of a subset of neurons over long periods of time. These age-related diseases are becoming more prevalent with the generalized increase of life expectancy and have been linked by many authors with increased oxidative stress levels. Indeed, oxidative stress effects can be accounted as cumulative damage, which associates well with the delayed onset and progressive nature of these conditions. Moreover, various results on life extension research strongly support the hypothesis that enhancing the cell protective systems against oxidative stress can extend life span. In view of this, the present study aimed at evaluating the role of the antioxidant α-tocopherol (vitamin E) on induced oxidative stress conditions. α-tocopherol (vitamin E) was chosen for its principal role in scavenging lipid peroxyl radicals, at lipoproteins and cell membranes, hence breaking the chain of lipid peroxidation initiated by ROS. The toxic effect of hydrogen peroxide (H_2O_2) and the antioxidant role of vitamin E were investigated using Saccharomyces cerevisiae as a model for cell viability. A High-Performance Liquid Chromatography analysis was also performed to assess 3-nytrotirosine and GSH/GSSG production levels, due to their relevance as oxidative stress biomarkers. Altogether, the results presented here demonstrated that H_2O_2 exposure decreased yeast cells viability equally, independent of dose, and that the adverse effects were, at least, partially rescued by the combined exposure with vitamin E. The results from redox biomarkers were, however, shown to be inconclusive. This preliminary study helped to understand the dual nature of vitamin E, under the conditions tested. However, future studies should be able to further explore vitamin E antioxidant role in pathological models of neurodegenerative diseases.

Keywords: neurodegenerative diseases, oxidative stress, *S. cerevisiae*, vitamin E.

POSTER 28

Study of oxidant and anti-oxidant molecular alterations in an eukaryotic model

ALLEN A.[1]; CUNHA, S.[2]; PRUDENCIO C.[2,3]; VIEIRA, M.[2,3]

1 - Mestrado Bioquímica em Saúde, ESS-P.PORTO
2 - Ciências Químicas e Biomoléculas, CISA, ESS-P.PORTO
3 - i3S, Universidade do Porto

Mitochondrial dysfunction and protein aggregation are two phenomena that have been correlated with neurodegenerative diseases and are both interlinked with cellular oxidative stress. Mitochondrial dysfunction is associated with nucleic acid damage and protein aggregation with mistakes in protein folding. However, aggregation has not yet been defined as a cause or consequence of neurodegenerative diseases. Therefore, this thesis' main objectives were to analyze the influence of different concentrations of hydrogen peroxide, an oxygen-free radical produced by our cells, in protein aggregation and genetic material as well as the role of vitamin E as an antioxidant agent. For that purpose, S. cerevisiae cellular samples were collected throughout the exponential growth phase. Analysis of insoluble aggregates was performed using the electrophoretic technique SDS-PAGE followed by protein lysis and DNA breakage bands through 1,5% agarose electrophoresis followed by RAPD-PCR. Exposure to the oxidizing agent over time has enhanced protein aggregation, namely ovalbumin. However, the anti-oxidant did not appear to reverse the oxidizing effect and seems to have a pro-oxidant effect. Although differences in protein aggregation were observed over time, there were no breaks in the genetic material.

Keywords: Neurodegenerative diseases, DNA damage, protein aggregation, oxidative stress.

POSTER 29

Isolation of novel compounds from marine cyanobacteria with lipid reducing activity

BEGOÑA ASTRAIN[1*], FILIPA ROSA[1], NATÁLIA SILVA[1], VÍTOR VASCONCELOS[1,2], RALPH URBATZKA[1]

[1] – CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros de Leixões, Av. General Norton de Matos s/n, 4450-208, Matosinhos, Portugal
[2] – FCUP, Faculty of Science, University of Porto, Rua do Campo Alegre 1021/1055, 4169-007, Porto, Portugal
*E-mail address: bastrain@ciimar.up.pt

Obesity is an increasingly global health problem and novel treatments are urgently needed. This condition raises the risk of developing several chronic diseases such as diabetes, cardiovascular diseases, musculoskeletal disorders and some forms of cancer. Cyanobacteria, known as blue-green algae, are an ancient group of gram-negative photosynthetic prokaryotes recognized to produce many secondary metabolites and may represent an interesting source of novel compounds. The aim of this study is to discover new secondary metabolites produced by strains of cyanobacteria with activities towards obesity and steatosis. Following a bioactivity screening of many strains, the marine cyanobacteria *Cyanobium* sp. was selected for compound isolations and cultured on a large-scale. Their freeze-dried biomass was extracted using a mixture of dichloromethane and methanol (2:1). The resultant organic extract was submitted to vacuum liquid chromatography to generate fractions. The lipid reducing capacity of these fractions was tested by the zebrafish Nile red fat metabolism assay. Steatosis was induced in HepG2 cells by fatty acid overloading and the potential to reduce lipid accumulation was analyzed. Afterwards, more (sub)fractions were originated with normal phase solid-phase extraction, followed by a reverse-phase HPLC. The chemical nature of the fractions was studied by spectroscopic techniques (NMR) and mass spectrometry (LC-HRMS). At the moment, some fractions showed strong lipid reducing activities (40% to 70%) in zebrafish larvae. This strong bioactivity was additionally confirmed in HepG2 cells, with more than 80% of lipid reduction. In the ongoing work, we intend to isolate novel bioactive compounds and elucidate their structures.

Keywords: *Cyanobium* sp., lipid reducing capacity, zebrafish Nile red fat metabolism and hepatic steatosis

POSTER 30

Isolation of a novel compound, 13(2)-hydroxy-pheofarnesin a, with lipid reducing activity

NATÁLIA GONÇALVES SILVA[1,*]; MARIA LÍGIA SOUSA[1]; MARIANA ALVES REIS[1]; VÍTOR VASCONCELOS[1,2]; RALPH URBATZKA[1]

1 - Interdisciplinary Center of Marine and Environmental Research (CIIMAR), Matosinhos, Portugal;2 - Faculty of Sciences, University of Porto, Portugal;* - Corresponding author: nsilva@ciimar.up.pt

Obesity is a complex metabolic disease that became one of the major public health concerns in our societies. Currently, research has focused on natural product discovery for health treatments and cyanobacteria are regarded as a prolific source of biologically active natural compounds. In this work, a bioassay-guided isolation strategy was used to explore the potential of secondary metabolites from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGEcc) with beneficial activities towards obesity. Cyanobacterial strain *Nodosilinea* sp. LEGE 06001 was cultured and harvested. The freeze-dried biomass was extracted by repeated percolation with mixtures of DCM/MeOH (2:1, v/v). The resultant organic crude extract was fractionated by normal-phase vacuum liquid chromatography, and the chemical nature of the fractions was followed by spectroscopic techniques (NMR). After several fractionation procedures a novel chlorophyll derivative was successfully isolated. The structure elucidation of 13^2-hydroxy-pheofarnesin a (hfa) was established based on one- and two-dimensional NMR spectroscopy and mass spectrometry. After 48 h of exposure, hfa showed significant neutral lipid-reducing activity in the zebrafish Nile red fat metabolism assay with an EC_{50} value of 15.5 ± 1.3 µM. This novel compound did not cause any general toxicity or malformations. Chlorophylls a and b were analyzed using the same assay, to establish the structure-activity relationships, and did not show any effect. These results highlight that the structural differences of hfa are crucial for the lipid reducing activity. This bioactivity was additionally confirmed in differentiated 3T3-L1 multicellular spheroids of murine preadipocytes. Future studies are needed to elucidate the mechanisms of action of hfa and its suitability to be developed as nutraceutical with lipid reduction activity.

Acknowledgments: Financed by national funds through FCT (CYANOBESITY, ERA-MBT/0001/2015).

Keywords: Chlorophyll derivative; Lipid reducing activity; Nile red fat metabolism assay; murine pre-adipocytes

POSTER 31

Antimicrobial activity of new lactic acid bacteria: potential as biocontrol and biofertilizer agents

JACOBO LÓPEZ-SEIJAS[1]; BELÉN GARCÍA-FRAGA[1]; ABIGAIL F. DA SILVA[1]; CARMEN SIEIRO[1]

1 Department of Functional Biology and Health Sciences, Microbiology Area, University of Vigo, Vigo, Spain

Food preservatives, pesticides and fertilizers of chemical origin are used for food conservation, to protect agricultural crops from pests and to stimulate plant growth, respectively. All these products have been shown to be highly polluting and above all very harmful to human health. Due to these problems there is a tendency to reduce and restrict progressively the use of the more toxic pesticides. In line with this approach, there is a growing need and demand, for new pesticides and preservative agents that are healthy for humans and friendly with the environment. Biological control, using microorganisms with antimicrobial properties as bipesticides and bio-preservatives, is the most widely accepted alternative. In addition, often, these microorganisms can act as plant growth promoters, thus behaving as plant probiotics. In the present work the potential of wine lactic bacteria as bio-preservatives, biocontrol and biofertilizer agents was evaluated. By using the DNAr 16S and *recA* gene sequences, ARDRA analysis and ISR-16S/26S RFLPs, twenty-two different strains of lactic acid bacteria were identified and assigned to *Lactococcus lactis*, *Lactobacillus plantarum*, *L. hilgardii* and *L. paracasei* species. The different strains showed, to varying degrees, antimicrobial activity against species of the genera *Staphylococcus* and *Bacillus*. The greatest effectiveness, considering both the number of inhibited species and the degree of inhibition, was presented by strains of *L. lactis* and *L. paracasei*. Strains belonging to these species were also tested for their ability to inhibit the growth of fungi showing all of them, in different degree, activity against *Fusarium oxysporum*. Finally, the ability of two strains (UV10 and UV12) was evaluated to protect *Lycopersicon esculentum* plants against *F. oxysporum* and promote its growth. Strain *L. plantarum* UV10 was able to significantly inhibit the harmful effect of *F. oxysporum* in tomato plants as well as to significantly stimulate their growth.

Keywords: Lactic acid bacteria, antimicrobial activity

POSTER 32

Actinobacteria from the Marine Sponge Hymeniacidon perlevis: isolation, phylogenetic identification and bioactive potential

ANA CATARINA FONSECA[1,2,3*], INÊS RIBEIRO[1,2], ANA REGUEIRAS[1], RUI S. OLIVEIRA[4], RALPH URBATZKA[1], PEDRO LEÃO[1], M. FÁTIMA CARVALHO[1]

[1]CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208 Matosinhos Portugal
[2]Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[3]Faculty of Sciences, University of Porto, Rua do Campo Alegre 1021/1055, 4169-007, Porto, Portugal
[4]Centre for Functional Ecology – Science for People & the Planet, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

The discovery of new strategies to fight serious diseases, such as cancer and multiresistant bacterial infections, is an urgent issue. Actinobacteria are a group of microorganisms known for their high capacity to produce molecules with several biotechnological applications, including important compounds with antimicrobial and anticancer activity. Marine environments represent an underexplored source of bioactive compounds and marine actinobacteria have proven to be a valuable source of novel biotechnologically important molecules.
This work aimed to investigate the cultivable actinobacteria associated with the marine sponge Hymeniacidon perlevis, collected in the intertidal zone of Praia da Memória, in Northern Portugal, and to study the potential of these microorganisms to produce antimicrobial and anticancer compounds. Different pre-treatments and selective culture media were used for the isolation of actinobacteria. The selection process allowed the isolation of 83 actinobacterial strains distributed by 11 genera: Nocardia, Nocardiopsis, Gordonia, Tsukamurella, Micrococcus, Micromonospora, Rhodococcus, Arthrobacter, Brachybacterium, Dietzia and Streptomyces, with the latter genus being the most represented. Screening of bioactivity in these isolates is being investigated. The first results, showed that some actinobacterial extracts have antimicrobial activity against Candida albicans and anticancer activity against the human liver cancer cell line HepG2.

Keywords: Actinobacteria; Marine Sponge; Bioactive Compounds; Biotechnological applications

POSTER 33

Antimicrobial bioactivity screening in *Croton betaceus* Baill. native of Brazil

ANDRÉ ROCHA[1,3,4]; JOSÉ LEITE[2]; ALEXANDRA PLÁCIDO[3]; FRANCISCO SANTOS-FILHO[4]; HERBERT SOUSA[4]; FRANCISCA LIMA[4]; ELIANA LAGO[1,2]

1University State of Maranhão, UEMA, Caxias, Brazil.
2NuPMIA, Faculty of Medicine, University of Brasília, Brasília, Brazil.
3Bioprospectum, Lda, UPTEC, Porto, Portugal.
4GERATEC, University State of Piauí, UESPI, Terezina, Brazil.

Croton betaceus Baill. is an endemic sub-bush of South America and native from Brazil, occurring mainly in the northeastern region of the country. Its use in traditional Brazilian medicine, in the form of infusions, includes treatments for cancer, diabetes, digestive problems, hypertension and inflammation. It has strong economic potential, especially for the pharmaceutical industry. In imbalance, commensal microorganisms, such as *Candida albicans* and *Enterococcus faecalis* can cause infections such as candidiasis or gingivitis..The aim of this work was to evaluate the antimicrobial potential of foliar ethanolic extract and fractions of *C. betaceus*.The leaves (450g), after drying (26°C), were ground to powder. The extraction was carried out in 95% ethanol at 26°C. The extract (15%) was suspended in a methanol/water (MeOH/H2O) mixture and submitted successively to the fractionation process, obtaining: hexanic, chloroform, ethyl acetate and hydrometanolic fractions. *Candida albicans* (ATCC 10231) and *Enterococcus faecalis* (ATCC 29212) were tested by the disc-diffusion assay in Muller-Hinton agar for 24h/36°C, at concentrations of 1.0; 2.5; 5.0; 10; 20; 30 and 100mg/mL. After analysis, in C. albicans, inhibition halos were observed from the first concentration (1.0mg/mL) in hexanic and chloroform fractions, both with 8mm. In the hydomethanolic fraction, inhibition halo formation was observed from 5.0mg/mL. In all other fractions tested, they were found capable of inhibiting the growth of yeast in 10mg/mL. Higher diameter halos were observed in the highest extract concentrations (30 and 100mg/mL), in all, ranging from 9.3 to 12mm. *E. faecalis*, was resistant, no halos were observed at any of the concentrations tested. The results suggests the use of extracts of *C. betaceus*, is a promising source for the development of products, like natural mouthwashes for candidiasis.

Key-words: Antimicrobial. Candidiasis. Natural product. Plant extracts.

POSTER 34

VALORISATION OF GUSTAVIA GRACILLIMA MIERS: EXPERIMENTAL EVIDENCE ON ITS ANTIDIABETIC PROPERTIES

CATARINA ANDRADE[1]; NELSON G. M. GOMES[1]; FEDERICO FERRERES[2]; ANGEL GIL-IZQUIERDO[2]; DAVID M. PEREIRA[1]; RUNGCHARN SUKSUNGWORN[3]; SUTSAWAT DUANGSRISAI[3]; PATRÍCIA VALENTÃO[1]; PAULA B. ANDRADE[1]

1 - REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal
2 - Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), Murcia, Spain
3 - Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand

Gustavia gracillima Miers is a flowering tree found in Thailand lacking reports on its chemical profile and biological activity. As result of our *in vitro* screening of Thai species with antidiabetic potential, the methanolic extract obtained from *G. gracillimia* flowers displayed a strong α-glucosidase inhibitory effect (IC_{50}=4.06 µg/mL), being more potent than the reference drug acarbose (IC_{50}=106.30 µg/mL). Kinetic studies of the enzyme activity indicated that the extract acts as a mixed inhibitor. *G. gracillima* flowers' extract was not able to inhibit α-amylase at the highest tested concentration (1 mg/mL), suggesting its selectivity against α-glucosidase. We assessed the effect of the extract on aldose reductase, an enzyme associated with the reduction of diabetes complications, a strong inhibitory effect being observed (IC_{50}=78.48 µg/mL). Furthermore, given the strong relation between oxidative stress and diabetes physiopathology, the extract's antiradical activity was evaluated, with significant scavenging effects detected, particularly against superoxide radical (IC_{50}=66.44µg/mL). Relevantly, no significant changes on the hepatocyte cell line (HepG2) viability were observed through the MTT assay, discarding the hepatotoxicity of the extract.
HPLC-DAD-ESI-MS^n characterization of the phenolic profile of the methanolic extract was also performed, allowing the identification of twelve kaempferol derivatives and eight ellagic acid derivatives, which might be related with the observed biological activities.
G. gracillima is biologically valorised for the first time, the current study providing initial proof of its antidiabetic activity and encouraging further *in vitro* and *in vivo* studies.

Keywords: *Gustavia gracillima*; antidiabetic actity; antiradical; phenolic profile

POSTER 35

Total phenolics and antioxidant activity of a functional beverage with seaweed

ANA BARROS, MARIA SOUSA, ANA ALMEIDA, CRISTINA SOARES, HELENA CORREIA, STEPHANIE MORAIS. M. FÁTIMA BARROSO, PAULA PAÍGA, CRISTINA DELERUE-MATOS, CLARA GROSSO

REQUIMTE–LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal

The aim of this project is to produce a functional beverage with bioactivity based on seaweed, carob, and oats. Preliminary tests were carried out on three abundant seaweeds on the Portuguese coast in order to choose the seaweed with pleasant sensory properties. The seaweed was washed and dried before being roasted at 200ºC. For oats and carob, the roast procedure used was the same. All samples were ground in a coffee mill.

The total phenolics content, the total phlorotannins content, the total flavonoids content, the total acidity and the anti-radical activity of the beverages were determined. The results were compared with other drinks found in the market. The obtained results showed approximately one-third of the total phenolics of coffee and decaf, a greater amount of total flavonoids than chicory and a low amount of phlorotannins. On the other hand, the functional mixtures and chicory are the samples with the highest anti-radical activity, which is even greater in the mixture without coffee.

This beverage intends to take advantage of the bioactive characteristics of the seaweed, simulating the sensory properties (body, aroma, and taste) of coffee drink.

Acknowledgments: The authors would like to thank the EU and FCT for funding through the project PTDC/OCE-ETA/30240/2017-SilverBrain - From sea to brain (POCI-01-0145-FEDER-030240)

Keywords: seaweed, bioactivity, total phenolics, total phlorotannins

POSTER 36

Development of a functional drink with a neuroprotective activity using abundant raw materials

MARIA SOUSA, ANA ALMEIDA, ANA BARROS, CRISTINA SOARES, HELENA CORREIA, STEPHANIE MORAIS. M. FÁTIMA BARROSO, PAULA PAÍGA, CRISTINA DELERUE-MATOS, CLARA GROSSO

REQUIMTE–LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal

In order to provide alternatives to existing coffee substitutes in the market, it is intended to evaluate new abundant raw materials not valued in Portugal, such as carob, seaweed, and oats, and develop functional beverages.
Preliminary tests were carried out on three abundant seaweeds on the Portuguese coast, in order to choose the seaweed with pleasant sensory properties. The seaweed was washed and dried before being roasted at 200ºC. For oats and carob, the roast procedure used was the same. All samples were ground in a coffee mill. The neuroprotective potential of the beverages was assessed by testing their capacity to inhibit the enzyme acetylcholinesterase (AChE). Thus, it was found that the developed mixtures presented a fair inhibition capacity of the AChE enzyme, while the mixture with the addition of 20% of coffee has a greater neuroprotective capacity. The elderly population avoids coffee due to the potential health problems it may cause. Therefore, this beverage simulates all the sensory properties of the coffee, while taking advantage of its neuroprotective characteristics.

Acknowledgments: The authors would like to thank the EU and FCT for funding through the project PTDC/OCE-ETA/30240/2017-SilverBrain - From sea to brain (POCI-01-0145-FEDER-030240)

Keywords: algae, neuroprotective capacity, acetylcholinesterase (AChE)

POSTER 37

Development of a functional beverage rich in health important minerals and iodine

ANA ALMEIDA, MARIA BARROS, MARIA SOUSA, CRISTINA SOARES, HELENA CORREIA, STEPHANIE MORAIS. M. FÁTIMA BARROSO, PAULA PAÍGA, CRISTINA DELERUE-MATOS, CLARA GROSSO

REQUIMTE–LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal

The goal of this project is to produce a functional beverage based on seaweed with carob and oats, which presents beneficial mineral properties for human health Preliminary tests were carried out on three abundant seaweeds in the Portuguese coast, in order to choose the seaweed with pleasant sensory properties. The seaweed was washed and dried before being roasted at 200ºC. For oats and carob, the roast procedure used was the same. All samples were ground in a coffee mill. The content of the minerals: calcium, magnesium, potassium, and sodium was evaluated by atomic absorption spectroscopy while the iodine content was analyzed by the Sandell-Kolthoff method and the obtained results were after compared with other beverages. In comparison with other coffee substitutes, the beverage created has a higher content in minerals such as iodine. Since there is an iodine deficit in the population, the ingestion of two doses of 100 mL of any of our mixtures almost reaches the recommended daily dose of iodine. The substitutes produced showed similarities with coffee in terms of color, flavor, body, and texture, revealing itself even more similar than other substitutes already available in the market, such as barley and chicory.

Acknowledgments: The authors would like to thank the EU and FCT for funding through the project PTDC/OCE-ETA/30240/2017-SilverBrain - From sea to brain (POCI-01-0145-FEDER-030240)

Keywords: seaweed, iodine, minerals, coffee substitute

POSTER 38

Development of a chromatographic method for simultaneous analysis of glutathione forms

DULCE TEIXEIRA[1,2,3]; PEDRO COELHO[1,4]; CRISTINA PRUDÊNCIO[1,2,4]; MANUEL AZENHA[5]; MÓNICA VIEIRA[1,2,4]

1-Ciências Químicas e das Biomoléculas, Escola Superior de Saúde do Porto, Porto, Portugal
2-Centro de Investigação em Saúde e Ambiente, Escola Superior de Saúde do Porto, Porto, Portugal
3-Departamento de Biología Funcional y Ciencias de la Salud, Universidad de Vigo, Vigo, España
4-I3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
5-CIQ-UP – Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, Portugal

Reduced glutathione (GSH)is the most abundant low molecular weight thiol-containing tripeptide (glycine, cysteine, and glutamate) which is synthesized in the cells. GSHplays critical roles in protecting cells from oxidative damage and the toxicity of xenobiotics. Besides, it is also involved in the regulation of intracellular redox homeostasis, which leads to its oxidation into oxidized glutathione (GSSG). Determining the ratio of GSH/GSSG in different biological samples is a major procedure for the evaluation of an individual's oxidative status and can be a potential biomarker of oxidative stress.
The aim of the present study was to developa modified HPLC-DAD that allows simultaneous quantification of both glutathione forms.
All experiments were performed on a HPLC system and separation was carried out using a RP-18 column. Throughout the experiments, the influence of the following parameters was evaluated: 1) mobile phase composition, 2) wavelength settings, 3) pH, 4) temperature and 5) flow rate. Different protocols for sample preparation was also assessed in blood cells and plasma samples.
From all the protocols tested, the best results were obtained using a mobile phase composed by sodium perchlorate acidized with orto-phosporic acid, and a flow rate of 1.5 mL/min at 40ºC. The meta-phosphoric acid was the one that showed better results in the sample preparation.
The proposed method, which was successfully developed for GSH and GSSG quantification, is simple, cheap and easy to perform. It would be interesting to assess whether this method is suitable for the quantification of other biological specimens.

Keywords: Reduced glutathione-GSH, oxidized glutathione-GSSG, HPLC-DAD, oxidative stress

POSTER 39

Assessing anti-obesity and anti-cancer activity of cyanobacteria extracts from the LEGE CC culture collection

JOÃO MAGALHÃES[1], MARIA LÍGIA SOUSA[2,3], TIAGO SILVA[2,3], FILIPA ROSA[2,3], VÍTOR VASCONCELOS[2,3], RALPH URBATZKA[3]

[1] Catholic University of Porto, Porto, Portugal
[2] FCUP - Faculty of Sciences of University of Porto, Porto, Portugal
[3] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal

Cyanobacteria ubiquitously inhabit on earth and are well known producers of secondary metabolites. These metabolites are often unknown and with particular bioactivity, which can be used for the development of new drugs. According to World Health Organization, obesity is a crescent disease affecting people worldwide that, as a secondary effect, can lead to cancer.
Thirteen cyanobacterial strains from LEGE CC culture collection were tested both for anti-obesity and anti-cancer assays. For anti-obesity testing, lipid content of zebrafish embryos was quantified after exposure to cyanobacteria extracts, aided by Nile Red staining. For anti-cancer potential activity, the same extracts were tested on colon carcinoma cell line HCT116 cultured in monolayer, evaluating the viability after exposure using MTT assay.
So far, in the lipid reduction assay, extracts 303, 307, 314 and 373, corresponding to *two Calothrix sp., Plectonema cf radiosum* and *Cuspidothrix issatchenkoi*, respectively showed activity with 307 being the most effective with about 80% and 303, 314 and 373 with about 40%.
Regarding the viability test on HCT116 cell line, after 48h of exposure, about 40% viability decreased on the extracts 293, corresponding to *Nodularia sp.*, 298, to *Gloeocapsopsis sp.*, 301, to *Gloeocapsopsis sp.*, 317, to *Rivularia sp.* and 372, to *Cuspidothrix issatschenkoi.*; about 50% in 370 corresponding to *Nostoc sp.*
Taken all the results together, the active extracts that decreased lipid content did not show toxicity on zebrafish larvae and very little cytotoxicity on HCT116 cell. For the viability assay, 12 extracts demonstrated some activity. For future work, the extracts with higher activity, both in lipid reduction and cytotoxicity should be fractionated pure active compounds and isolated. Also, the more cytotoxic fractions should be tested on a more relevant model, such as spheroids, which have higher similarities to an *in vivo* tumour.

Keywords: cyanobacteria, obesity, zebrafish, HCT116

POSTER 40

New anthocyanin derivatives for technological applications in the cosmetic industry

BEATRIZ VALADARES[1]; NUNO MATEUS[1,2]; IVA FERNANDES[1]

1 -COLÉGIO INTERNATO DOS CARVALHOS, RUA DO MOEIRO, S/Nº. CARVALHOS, Vila Nova de Gaia, PORTUGAL
2- REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

There is a growing market demand for the incorporation of plant-derived ingredients in new products of the cosmetic industry. Anthocyanins are polyphenols arising from plant secondary metabolism that have been shown to display many bioactive properties such as free radical scavenging, metal-chelating, antimicrobial, wound healing and chemopreventive activities. The ability to prevent oxidative damages has led to the incorporation of natural bioactives in lotions and facial creams to prevent skin diseases and premature ageing, therefore the biological activities of anthocyanins make them potential novel compounds for cosmetic formulations. However, native anthocyanins present a low solubility in lipophilic media, which compromises their effective application.
In this work, anthocyanins from industrial wastes were recycled and used in their genuine forms. Enzymatic lipophilization was performed by addition of selected chain fatty acids to improve their solubility in lipophilic systems. Their biological activities were then assessed by developing a new skin barrier model using keratocytes living cells. The behavior of the cells incubated with the lipophilized anthocyanins was also monitored continuously with a microeletrode-based biosensor device, referred to as Electric Cell-Substrate Impedance Sensing (ECIS). This new system allowed the determination of compounds cytotoxicity as well as to understand their effects on cell morphology and behavior. Wound healing assays were also performed to analyze the effect of the compounds towards skin care. In addition, the effect of lipophilized anthocyanins on the activity of some key skin enzymes (MMP-9, elastase and tyrosinase) was investigated to understand the ability of these compounds in preventing wrinkle formation.

Keywords: anthocyanins, lipophilization, Skincare, Wound-healing

POSTER 41

The biotechnological potential of cyanobacteria bioactive extracts

DUARTE NUNO CLARINHA [1,2] , MARCO PRETO [1] , VÍTOR VASCONCELOS [1,2] , GRACILIANA LOPES [1,2 *]

1- CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, Novo Edifício do Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, 4450-208, Matosinhos, Portugal
2- FCUP, Faculty of Sciences University of Porto, Department of Biology, Rua do Campo Alegre, 4169-007 Porto, Portugal

Cyanobacteria, formerly known as blue-green algae, represent a vast group of organisms, still underexplored for their potential biotechnological applications in the many branches of biological sciences. The relatively ease of handling regarding cyanobacteria biomass production, and the non-need of arable land occupation, makes these organisms very attractive in the diverse scientific fields. Known for their richness in secondary metabolites, different from those found in higher plants, cyanobacteria are emerging as a possible source of bioactive compounds with promising beneficial effects in humans´ health.
In order to screen the potential biotechnological applications of cyanobacteria, diverse strains from the LEGE culture collection (lege.ciimar.up.pt) were selected for pigment profiling and biological activity assessment. With the aim of screening a wide range of cyanobacteria, strains belonging to the genera *Cyanobium* (LEGE 12431), *Nodosilinea* (LEGE 13457), *Cuspidothrix* (LEGE 03282), and *Alkalinema* (LEGE 15481) were cultured and scaled-up to 4L culture. After harvesting, biomass was lyophilized and extracted with different solvents or solvent mixtures (acetone 100% and ethanol 70%). The extracts were analysed by High Performance Liquid Chromatography (HPLC) with Photo Diode Array (PDA) detection, in order to establish their carotenoids profile, and with the Folin-Ciocalteu assay for total phenols quantification. The antioxidant capacity of the extracts was evaluated against superoxide anion radical, through an *in vitro* cell-free assay, and the biological activity appears to be correlated with the total phenols content and carotenoids profile. In order to determine the potential of cyanobacteria bioactive extracts to be used in the treatment for chronic skin inflammatory diseases, the extracts will be screened for their anti-inflammatory capacity using the macrophage cell line RAW 264.7. This work aims to enrich the current knowledge in underexplored cyanobacteria strains, both regarding their metabolome, and the potential biotechnological application of their bioactive extracts in the treatment of inflammatory process-based diseases.

Keywords: Cyanobacteria, Carotenoids, Inflammation, Oxidative Stress

Acknowledgments: This work was done in the framework of the project BLUEHUMAN –BLUE biotechnology as a road for innovation on HUMAN's health aiming smart growth in Atlantic Area –EAPA_151/2016 of the Interreg Atlantic Area Programme funded by the European Regional Development Fund.

POSTER 42

Antibacterial activity of Ionic Liquids Based on Beta-lactam antibiotics Against Resistant Bacteria

VITORINO DIAS[1]; MIGUEL SANTOS[2]; CRISTINA PRUDÊNCIO[1,3]; JOÃO P. NORONHA[2]; INÊS R. GRILO[4]; RITA G. SOBRAL[4]; ANA RITA DIAS[1,5] LUÍS C. BRANCO[2]; RICARDO FERRAZ[1,5]

1 - Ciências Químicas e Biomoléculas, Centro de Investigação em Saúde e Ambiente (CISA), Escola Superior de aúde do Instituto Politécnico do Porto;
2- LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa,
3- Instituto de Inovação e Investigação em Saúde (I3S);
4 -UCIBIO-REQUIMTE, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa,
5- LAQV- REQUIMTE

The cases of antibiotic resistance are increasing and becoming more and more common, giving rise to a new problem for public health. Therefore, the discovery of new antibiotics is important and necessary.
Active pharmaceutical ingredient-ionic liquid (API-IL) concept could be a new strategy to fight antibiotic resistance. Recently, a buffer neutralization method was developed and applied for the synthesis of ampicillin-based API-ILs. In this work we show the application of the buffer neutralization method on synthesis of ionic liquids derived from β-lactam antibiotics (amoxicillin and penicillin) as well as their activity against sensitive (*Escherichia coli* and *Staphylococcus aureus*) and resistant bacteria (MRSA, *E. coli* CTX M9 and *E. coli* CTX M2). To evaluate the antibacterial activity we used the broth microdilution method. Results showed that these compounds could be active against resistant bacteria and be a good alternative to traditional methods

Keywords: Ionic Liquids, antibiotic resistance, antibiotics, Active Pharmaceutical Ingredients

POSTER 43

Oxidative stress in noradrenergic neurons of the Locus Coeruleus neurons in an animal model of hydrocephalus

MARTA LOUÇANO[1-4], JOANA OLIVEIRA[5], ISABEL MARTINS[1-4], RUI VAZ[5], ISAURA TAVARES[1-4]

[1]Department of Biomedicine, Faculty of Medicine, University of Porto;
[2]Unit of Experimental Biology, Faculty of Medicine, University of Porto;
[3]IBMC-Institute of Molecular and Cell Biology, University of Porto, Portugal;
[4]I3S- Institute of Investigation and Innovation in Health, University of Porto, Portugal;
[5]Department of Neurosurgery, São João Hospital Center, Porto, Portugal

Hydrocephalus is a congenital or acquired disease characterized by an enlargement of the cerebral ventricles usually caused by the obstruction of cerebrospinal fluid flow which leads to a distortion of periventricular tissues. Usually, patients suffering from hydrocephalus present dysfunctions in learning and memory and motor deficits but pain modulation from the brain was never studied. We used a validated animal model of hydrocephalus (the rat injected in the cisterna magna with kaolin) to study descending modulation of pain from a circumventricular noradrenergic region: the Locus Coeruleus (LC). In order to evaluate the effects of hydrocephalus in pain modulation, three weeks after induction of hydrocephalus, animals were sacrificed and immunodetection of the noradrenaline-synthetizing enzyme tyrosine hydroxylase (TH) was performed, at the LC and spinal cord. Hydrocephalic rats presented increases in the levels of TH both in the LC and spinal cord. The expression of a validated oxidative marker (8-Hydroxyguanosine; 8-OHdG) was studied in noradrenergic LC neurons by double immunodetection of TH and 8-OHdG. Hydrocephalic animals presented increases of 8-OHdG in the population of TH-immunoreactive neurons of the LC. Hydrocephalic animals presented increases in the expression of TH-immunoreactive fibers at the spinal dorsal horn. Pain-related parameters were measured namely behavioural responses in a validated pain inflammatory test (the formalin test) and nociceptive activation of spinal cord neurons (Fos immunoreaction). Hydrocephalic animals presented decreases of behavioral responses in the second phase of the test (inflammatory phase) along with decreases in Fos expression, indicating that hydrocephalus decreases nociceptive responses. Since the LC has higher levels of TH in rats with kaolin-induced hydrocephalus, which also appears to increase the noradrenergic innervation in the spinal dorsal horn, it is possible that an increase in the release of noradrenaline at the spinal cord accounts for pain inhibition. The increases in noradrenaline levels at the LC in hydrocephalic animals may represent neuroprotective responses to increased oxidative stress in periventricular regions. Collectively, the present study indicates that there are disturbances in descending noradrenergic pain modulation in hydrocephalic conditions.

Keywords: Hydrocephalus, Descending pain modulation, Noradrenaline, Oxidative Stress

POSTER 44

Anticholinesterase activity of two Chinese medicinal plants

ADRIANA PEREIRA[1], CATARINA GONÇALVES[1], MÓNICA DIAS-TEIXEIRA[1,2], CRISTINA DELERUE-MATOS[1], CLARA GROSSO[1]

[1] REQUIMTE/LAQV, Instituto Superior de Engenharia do Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4249-015, Porto, Portugal
[2] NICiTeS–Núcleo de Investigação em Ciências e Tecnologias da Saúde, Escola Superior de Saúde Ribeiro Sanches, Rua do Telhal aos Olivais, Lisboa, Portugal

With the increase in population and life expectancy, Alzheimer's disease has become a global health challenge. Current treatments only provide symptomatic relief, thus encouraging the search for new drugs. Chinese Herbal Medicine has been a valuable source of drugs for centuries and research has grown in recent years to understand the scientific basis for its use. Since most of the drugs available in the market are cholinesterase inhibitors and one of Alzheimer's hallmarks is acetylcholine depletion, this study evaluated the anticholinesterase activity of aqueous extracts obtained from two Chinese medicinal plants, *Ginkgo biloba* L. and *Scutellaria baicalensis* Georgi.
Concerning acetylcholinesterase inhibition, *S. baicalensis* extract (IC_{50} = 892 µg/ml; IC_{25} = 229 µg/mL) was more potent than that of *G. biloba* (IC_{50}> 2100 µg/ml; IC_{25} = 983 µg/ml). The same pattern was observed for butyrylcholinesterase inhibition (IC_{25} = 503 µg/ml for *S. baicalensis* and IC_{25} = 1197 µg/ml for *G. biloba*). In order to correlate these bioactivities with the chemical composition of these extracts, HPLC-DAD analyses were performed. Three flavonoids were identified in *S. baicalensis* extract - baicalin, baicalein and wogonin. *G. biloba* extract is composed by phenolic acids and flavonoids, including gallic acid, catechin, epicatechin-3-*O*-gallate and derivatives of quercetin, kaempferol and isorhamnetin.
These results are in accordance with studies reporting the neuroprotective activity of flavonoids through different mechanisms of action, including cholinesterase inhibition.

Keywords: Chinese medicinal plants; cholinesterase inhibition; flavonoids

Acknowledgments: This study was financed by: FCT/MCTES through national funds (UID/QUI/50006/2019); the EU and FCT through PTDC/OCE-ETA/30240/2017- SilverBrain - From sea to brain: Green neuroprotective extracts for nanoencapsulation and functional food production (POCI-01-0145-FEDER-030240); and FCT through FCT investigator IF/01332/2014/CP1255/CT0001.

POSTER 45

Discovery of a New Xanthone against Glioma: Synthesis and Development of (Pro)liposome Formulations

ANA ALVES [1,2], MARTA CORREIA-DA-SILVA [2,3], CLAÚDIA NUNES [4], JOÃO CAMPOS [1], EMÍLIA SOUSA [2,3], PATRÍCIA M.A. SILVA [5], HASSAN BOUSBAA [3,5], FRANCISCA RODRIGUES [6], DOMINGOS FERREIRA [1], PAULO C. COSTA [1] AND MADALENA PINTO [2,3]

1 UCIBIO, REQUINTE, Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Rua Viterbo Ferreira, 228, 4050-313 Porto, Portugal
2 Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
3 Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Terminal de Cruzeiros do Porto de Leixões Avenida General Norton de Matos P 4450-208 Matosinhos, Portugal
4 LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
5 CESPU, Institute of Research and Advanced Training in Health Sciences and Technologies (IINFACTS), Rua Central de Gandra, 1317, 4585-116 Gandra, Portugal
6 REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Portugal

Current treatment for invasive brain glioma is still inadequate, and prognosis upon diagnosis tends to be very poor. Several factors contribute to these limitations, such as the highly invasive, non-localized and diffuse characteristics of the tumors, and the difficulty of local drug activity. Conventional surgical methods and/or radiotherapy alone cannot eliminate cancer cells from the brain, and the relapse is, most of the time, inevitable. Temozolomide, an alkylating agent, remains the standard-of-care in glioma chemotherapy. However, chemotherapy for gliomas is difficult due to two major obstacles such as the blood-brain barrier and the heterogeneity of the brain cancer. A new acetylated xanthonoside, 3,6-bis(2,3,4,6-tetra-O-acetyl-β-glucopyranosyl)xanthone (2), was synthesized and discovered as a potent inhibitor of tumor cell growth. The synthesis involved the glycosylation of 3,6-di-hydroxyxanthone (1) with acetobromo-α-D-glucose. Glycosylation with silver carbonate decreased the amount of glucose donor needed, comparative to the biphasic glycosylation. Xanthone 2 showed a potent anti-growth activity, with GI50 < 1 μM, in human cell lines of breast, lung, and glioblastoma cancers. Current treatment for invasive brain glioma is still inadequate and new agents against glioblastoma with high brain permeability are urgently needed. To overcome these issues, xanthone 2 was encapsulated in a liposome. To increase the well-known low stability of these drug carriers, a proliposome formulation was developed using the spray drying method. The results showed that the liposomes obtained from the proliposomes have an average diameter of 200 nm. In comparison, the traditional liposomes presented an average size in the order of 100 nm. Both formulations presented negative zeta potential values. The stability of the proliposomes was tested and showed no significant changes in liposome properties after 15, 30, and 90 days. While the proliposome formulation showed significantly higher stability, it was at the expense of losing its biocompatibility as a drug carrier in higher concentrations. More importantly, the new xanthone 2 was still able to inhibit the growth of glioblastoma cells after liposome formulation. This result highlighted that it will be worthy to explore new formulations and/or drug carriers to fight glioma growth with this promising xanthone.

Keywords: xanthone; glioblastoma; liposomes; proliposomes.a

4TH MEETING OF
MEDICINAL
BIOTECHNOLOGY

ACKNOWLEDGEMENTS

This meeting is only possible due to the support, effort and dedication of all who have worked on it. To those who have been involved in this work, to the members of the Technical-Scientific Area of Chemical Sciences and Biomolecules, to our students, in particular, the 2nd year students of the BSc in Medicinal Biotechnology, to our sponsors, and to the collaborators of the School of Health, a very big thank you!

The Organizing Committee would also like to thank the Presidency of the School of Health - P.Porto and its associated services ("Gabinete de Empregabilidade, Alumni, Formação e Atividades na Comunidade", "Gabinete de Relações Externas, Projetos e Comunicação", "Centro de Informática", and "Área de Segurança, Serviços Técnicos e Suporte" for all the support and availability

4TH MEETING OF MEDICINAL BIOTECHNOLOGY

SPONSORS

Frilabo

VWR

Sociedade Portuguesa de Biotecnologia

Bebé Vida

Cerveja Nortada

Associação de Estudantes da ESS|IPP

Câmara Municipal do Porto

INSTITUTIONAL SUPPORT

P.PORTO ESCOLA SUPERIOR DE SAÚDE

BIOTECNOLOGIA MEDICINAL
Licenciatura

BIOQUÍMICA EM SAÚDE
Mestrado

BIOTECNOLOGIA AVANÇADA
Doutoramento

INDEX

4TH MEETING OF
MEDICINAL
BIOTECHNOLOGY

BOOK OF ABSTRACTS

P.PORTO
ESCOLA SUPERIOR DE SAÚDE