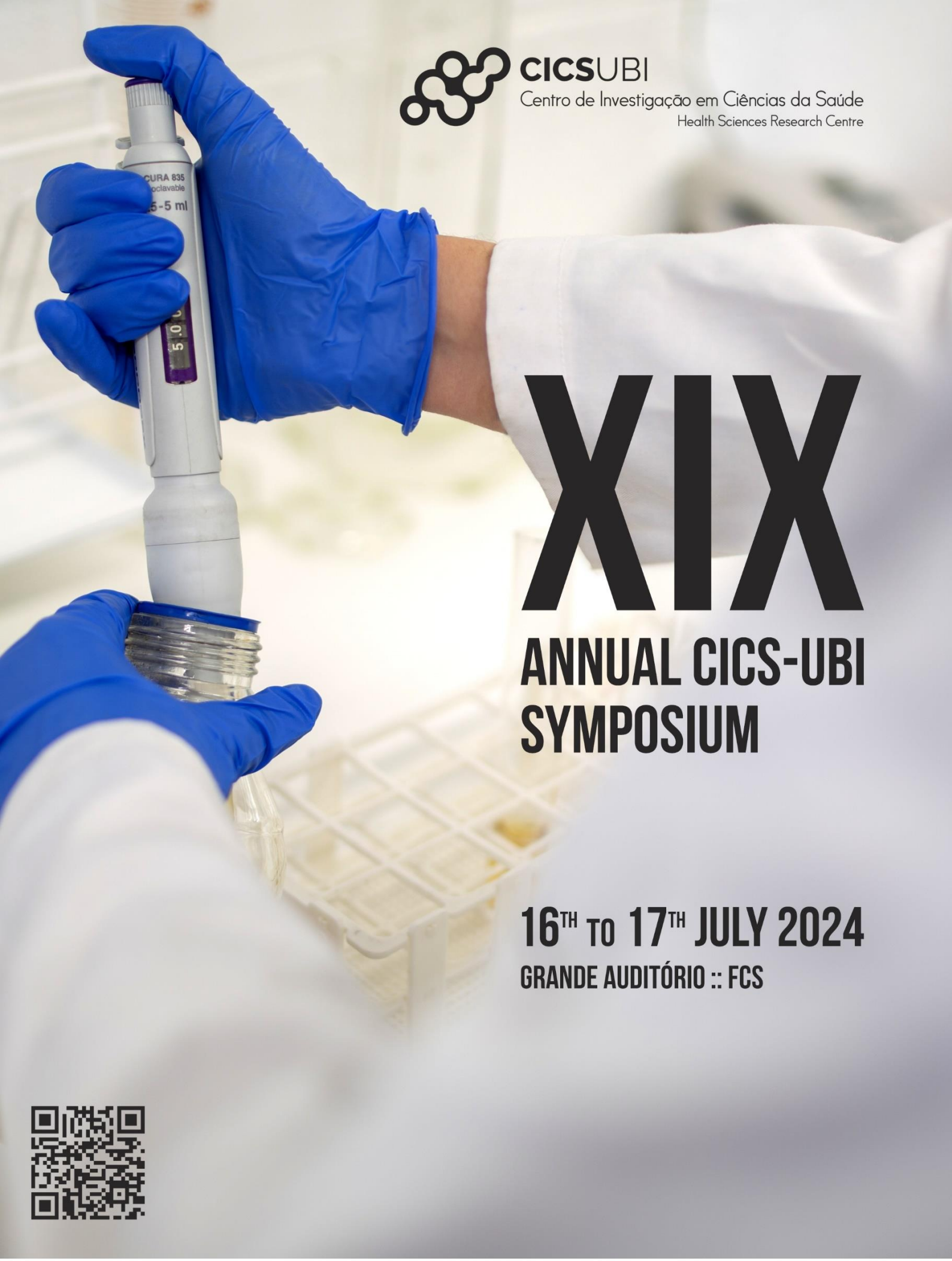

CICSUBI
Centro de Investigação em Ciências da Saúde
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XIX

ANNUAL CICS-UBI SYMPOSIUM

16TH TO 17TH JULY 2024

GRANDE AUDITÓRIO :: FCS

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XIX ANNUAL CICS-UBI SYMPOSIUM 2024

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WELCOME MESSAGE FROM THE CICS-UBI COORDINATOR

Dear researchers,

I am pleased to send the invitation for you to attend the XIX Annual CICS-UBI Symposium, which will be held on 16-17 July 2024.

The Symposium is a unique opportunity for younger researchers to present their work, and an important venue to foster interdisciplinarity as well as the development of internal and external collaborations.

Now back to its original on-site format, the CICS-UBI symposium will be held at the great auditorium of the Faculty of Health Sciences of the University of the Beira Interior, in Covilhã, Portugal. This will be a wonderful opportunity to discuss results, Science in general, and to plan novel research.

The programme of the Symposium is quite exciting and posters, oral communications and main lectures will hopefully draw everyone's attention and promote ample discussion. Awards will be given to the best communications in each category.

We look forward to your invaluable contributions and your attendance at the meeting.

Looking forward to meeting you at the XIX Annual CICS-UBI Symposium!

Luís Taborda Barata

Coordinator of CICS-UBI

WELCOME MESSAGE FROM THE SYMPOSIUM PRESIDENT

22 Years of CICS-UBI!

19 Years of Sharing Science!

And 19 years have passed since the first edition, for which I was responsible as its mentor. The CICS-UBI annual symposium takes place once again in the city of Covilhã at the Faculty of Health Sciences.

It is open to the entire scientific community, with themes within the CICS-UBI research areas: Biopharmaceuticals and Biomaterials; Drug Discovery, Development and Safety; Natural Products & Microbial Research; Neurologic and Neurovascular Disorders; Hormones and Metabolism and Respiratory and Allergic Diseases. The symposium is always a high point in the life of CICS-UBI, where "new blood boils", and where the most recent ones can show and discuss their results at the highest level. I have no doubt that this will be an extraordinary edition, as coordinator I can only thank all the teams, participants, and support from the vice-rector for research, CICS-UBI and UBI.

On the other hand, the social and cultural program has been enriched so that everyone can participate. Welcome everyone.

To all, I wish you a good symposium

Eduardo Cavaco - Chairman Vice-Coordinator of CICS-UBI

PROGRAMME

16th July – Tuesday

08h30 – 09h00 – Check In

09h00 – 09h30 – Opening Ceremony

Rector Mário Raposo, PhD

Vice-Rector Sílvia Socorro, PhD

FCS-UBI President Miguel Castelo Branco, MD, PhD

CICS-UBI Coordinator Luís Taborda Barata, MD, PhD

Symposium Chairman Eduardo Cavaco, PhD

CADBI-CICS-UBI

09h30 – 10h00 – *CICS-UBI, Numbers, Present and Future*

Luís Taborda Barata, MD, PhD – CICS-UBI

Plenary Session I

Chairperson: Cecília Santos

10h00 – 10h30 - *A DIFFERENT PERSPECTIVE ON BIOMOLECULE ADSORPTION MECHANISM DURING PREPARATIVE CHROMATOGRAPHY*

BB- Biopharmaceuticals and Biomaterials

Ana Cristina Dias Cabral, PhD

10h30 – 11h00 - Oral Communications A

1. *Flash communication*

EVALUATION OF G4-FORMING MOTIF IN PRE-MIR150

Pedro Lourenço, Joana Figueiredo, Diogo Dias, Carla Cruz

2. *Flash communication*

EVALUATION OF SPECIFIC ANTHRAQUINONES AS NEW CATECHOL-O-METHYLTRANSFERASE INHIBITORS: VIRTUAL DOCKING, MOLECULAR DYNAMICS AND INHIBITION IN VITRO STUDIES

Fábio A. Proença, João P. Batista-Silva, Samuel Silvestre, Luís A. Passarinha

3. *Flash communication*

OPTIMIZATION OF BSA-COATED PEPTIDE NANOCOMPLEXES USING DESIGN OF EXPERIMENTS FOR ENHANCED CO-DELIVERY OF TEMOZOLOMIDE AND p53 GENE

Inês Afonso, Ana R. Neves, Dalinda Eusébio, Tânia Albuquerque, Eric Vivès, Prisca Boisguérin, Adriana O. Santos, Ângela Sousa, Diana Costa

11h00 – 11h30 – Coffee Break

Plenary Session II

Chairperson: Susana Ferreira

11h30 – 12h00 - *UNLOCKING THE HEALTH BENEFITS OF NATURAL PRODUCTS: WHAT ABOUT THE BIOACCESSIBILITY AND BIOAVAILABILITY OF PHYTOCHEMICALS?*

NPM - Natural Products & Microbial Research
Ana Paula Duarte, PhD

12h00 – 12h30 - Oral Communications B

1. *Short communication*

PORTUGUESE NATURAL RESOURCES AS MODULATORS OF *Acne vulgaris* HALLMARKS

Ana Sofia Oliveira, Joana Rolo, Carlos Gaspar, Rita Palmeira-de-Oliveira, João Paulo Teixeira, José Martinez-de-Oliveira, Ana Palmeira-de-Oliveira

2. *Short communication*

MITOCHONDRIAL SELECTION AND PATHOGENICITY IN YEAST: A PHYLOGENETIC ANALYSIS

Mariana Z. Fernandes, Joana Rolo, Emanuel Maldonado, José Martinez-de-Oliveira, Ana Palmeira-de-Oliveira

12h30 – 14h00 – Lunch

Plenary session III

Chairperson: Adriana Santos

14h00 – 14h30 - *THE IMPORTANCE OF BIOINFORMATICS IN INVESTIGATING THE GENETIC CAUSES OF RARE DISEASES*

H&M- Hormones and Metabolism

Emanuel Maldonado, PhD

14h30 – 16h00 - Oral Communications C

1. *Short communication*

NANOCOSMETICS CONTAINING NATURE-DERIVED HYDROPHOBIC COMPOUNDS: NIOSOME DEVELOPMENT AND PHYSICOCHEMICAL AND TOXICITY CHARACTERIZATION

Patrícia C. Pires, Maria Beatriz Pinto, Mafalda Correia, Gabriela Moço, Ricardo C. Calhelha, Ana Rita Silva, Maria João Sousa, Miguel Vilas-Boas, Soraia I. Falcão, Francisco Veiga, Pooyan Makvandi, Ana Cláudia Paiva-Santos

2. *Short communication*

A POTENTIAL EFFECT OF CIRCADIAN RHYTHMS IN THE DELIVERY/THERAPEUTIC PERFORMANCE OF PACLITAXEL-DENDRIMER NANOSYSTEMS

Tânia Albuquerque, Ana Raquel Neves, Milan Paul, Swati Biswas, Elena Vuelta, Ignacio García-Tuñón, Manuel Sánchez-Martin, Telma Quintela, Diana Costa

3. *Short communication*

EXPLORING miR-155 DETECTION USING MOLECULAR BEACONS ON A MICROFLUIDIC DEVICE

David Moreira, Pedro Lourenço, André Miranda, Daniela Alexandre, Pedro Baptista, Cândida Tomaz, Yi Lu, Carla Cruz

4. *Short communication*

HPV16 E6 AND E7 ONCOPROTEINS: ENHANCED PURIFICATION FOR ANTI-CANCER DRUG DEVELOPMENT

Diana Gomes, Nick Vecchiarello, Luís A. Passarinha, Ângela Sousa

5. *Short communication*

AT11-GUIDED LIPOSOMES: POTENTIAL THERAPEUTIC APPROACH FOR ORAL CANCER CELLS

Jéssica Lopes-Nunes, Paula A. Oliveira, Carla Cruz

6. *Short communication*

B-MYB G-QUADRUPLEX TO LIGANDS: STARTING BY BIOPHYSICAL SCREENING TO CELL STUDIES

André Miranda, Beatriz Oliveira, Cyril Esnault, Jean-Christophe Andrau, Paula A. Oliveira, Jean-Louis Mergny, Carla Cruz

16h00 – Group Photo

16h05 – 16h30 – Ice Tea Break

16h30 – 17h30 – Poster Session I – Wine and Cheese

19h30 – Symposium Dinner (separate registration)

17th July – Wednesday

Plenary Session IV

Chairperson: Carla Cruz

09h00 – 09h30 – *BRAIN SENSES: AN UPDATE*

NND- Neurologic and Neurovascular Disorders

Cecília Santos, PhD

09h30 – 11h00 - Oral Communications D

1. *Short communication*

A POOL OF HUMAN NAÏVE CD8αβ T CELLS DOWN-MODULATE THE CD8β CHAIN AND BECOME CD8αα T CELLS UPON IN VITRO CULTURE WITH IL-15

André J. Esgalhado, Débora Reste-Ferreira, Sandra Weinhold, Markus Uhrberg, Elsa M. Cardoso, Fernando A. Arosa

2. *Short communication*

THE EFFECT OF MUSIC ON THE TRANSCRIPTOME OF EVOLUTIONARILY-CONSERVED NEUROPLASTICITY-RELATED GENES ASSOCIATED WITH AUTISM SPECTRUM DISORDER

Ana Luísa Soares Macedo, Luiza Maria Ferreira Lopes Athayde, Maria Antonelle Chaves Gomes, Leonardo Silva Sidney, Lorene M. Gualda, Alessandro Taunay Rodrigues

3. *Short communication*

CAN WE USE HUMAN MIDBRAIN ORGANOIDS TO MODEL PARKINSON´S DISEASE?

Catarina Serra-Almeida, Javier Jarazo, Gemma Gomez-Giro, Isabel Rosety, Alise Zagare, Ana Clara Cristóvão, Liliana Bernardino, Jens Christian Schwamborn

4. *Flash communication*

UNIVARIATE METABOLOMIC ANALYSIS TO UNCOVER URINE METABOLIC MARKERS OF ISCHEMIC STROKE IN ELDERLY

Ana Margarida Carboila, Nádia Oliveira, Ignacio Verde

5. *Short communication*

THE COMBINED EFFECT OF PORTUGUESE NATURAL MINERAL WATERS WITH PLANT EXTRACTS AGAINST SKIN AGING

Carolina P. Gomes, Ana Rita Gama, Ana Sofia Oliveira, Joana Rolo, Rita Palmeira-de- Oliveira, Tayse F.F. da Silveira, Lillian Barros, Ana Palmeira-de-Oliveira

6. *Short communication*

NATURAL ANTI-AGING COSMETICS WITH UNHAIS DA SERRA THERMAL WATER AND *Thymus x citrioddorus* HYDROLATE

Ana Rita Gama, Carolina Gomes, Cátia Caetano, Ana Sofia Oliveira, Joana Rolo, José Martinez de Oliveira, Ana Palmeira de Oliveira, Rita Palmeira de Oliveira

11h00 – 11h30 – Coffee Break

Plenary Session V

Chairperson: Rita Palmeira de Oliveira

11h30 – 12h00 – *GUIDELINES: REACHING BEYOND THE EVIDENCE*

RAD- Respiratory and Allergic Diseases

Olga Lourenço, PhD

12h00 – 12h30 - Oral Communications E

1. *Flash communication*

POTENTIAL ATTENUATION OF *Arcobacter butzleri* VIRULENCE BY PROBIOTICS

Alexandre Vieira, Cristiana Mateus, Fernanda Domingues, Mónica Oleastro, Susana Ferreira

2. *Flash communication*

A PORTRAIT OF THE VIRULENT POTENTIAL OF CANDIDA COLONIZATION: A COMPARATIVE STUDY OF ISOLATES FROM DIFFERENT HUMAN INFECTION NICHES

Filipa Oliveira Castro, Mariana Zagalo Fernandes, José Martinez-de-Oliveira, Ana Oliveira, Ana Palmeira-de-Oliveira, Ana Ferrrão, Paula Pestana, Joana Rolo

3. *Flash communication*

FROM SUBCLINICAL CIPROFLOXACIN CONCENTRATIONS TO INCREASED RESISTANCE: UNDERSTANDING THE EVOLUTIONARY PATHWAYS OF *Arcobacter butzleri*

Inês Martins, Alexandra Nunes, Mónica Oleastro, Susana Ferreira

12h30 – 14h00 – Lunch

14h00 – 15h00 – Poster Session II

15h00 – 16h00 - Oral Communications F

Chairperson: Márcio Rodrigues

1. *Short communication*

TRADITIONAL KNOWLEDGE OF MEDICINAL AND FOOD PLANTS USED IN SERRA DA ESTRELA NATURAL PARK

Radhia Aitfella Lahlou, Filomena Carvalho, Alexandra Varges, Maria João Pereira, João Lopes, Luís R. Silva

2. *Short communication*

REDUCTION OF OXIDATIVE STRESS AND IMPROVEMENT OF THE SURVIVAL RATE OF *Caenorhabditis elegans* BY USING BLACKBERRY EXTRACTS PREVIOUSLY INOCULATED WITH BACTERIAL BIOSTIMULANTS

Rocío Roca-Couso, José D. Flores-Félix, Begoña Ayuda-Durán, Rebeca Ferreras-Charro, Ignacio García-Estévez, Raúl Rivas

3. *Short communication*

PHYTOCHEMICAL COMPOSITION AND BIOACTIVITIES OF POLYPHENOLIC EXTRACTS OF *Prunus avium* L. BY-PRODUCTS

Ana R. Nunes, Ana C. Gonçalves, Amílcar Falcão, Gilberto Alves, Luís R. Silva

4. *Flash communication*

THE VALUE OF *Arbutus unedo* L. AS A BIOACTIVE INGREDIENT FOR SKIN APPLICATION: EXPLORING THE INTERREGIONAL VARIABILITY ACROSS NATIONAL TERRITORY

Pires CP, Oliveira AS, Gomes CP, Marengo, A, Rubiolo, P, Palmeira-de-Oliveira R, Palmeira-de-Oliveira

16h00 – 16h30 – Ice Tea Break

Plenary Session VI

16h30 – 17h00 –*SQUARAINE CYANINE DYES AS PROBES FOR THE DETECTION OF HSA PROTEIN AND AS PHOTOSENSITIZERS FOR PDT*

3DS-Drug Discovery, Development and Safety

Lucinda Reis, PhD

17h00 – 17h30 – Award Session and Closing Session

Oral Communications A

Chairperson:

Cecília Santos

1. EVALUATION OF G4-FORMING MOTIF IN PRE-MIR150

Pedro Lourenço[1(*)], Joana Figueiredo[1], Diogo Dias[1], Carla Cruz[1,2]

[1] Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior (CICS-UBI), Av. Infante D. Henrique, 6201-506 Covilhã, Portugal.
[2] Departamento de Química, Faculdade de Ciências, Universidade da Beira Interior, Rua Marquês de Ávila e Bolama, 6201-001 Covilhã, Portugal.
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ABSTRACT

Elevated levels of microRNA miR150 have been identified in various solid cancers, including lung cancer, highlighting the critical role of microRNAs in cancer biology as oncogenes or tumor suppressors. Precursor microRNAs (pre-miRNAs), intermediate forms of mature microRNAs, are promising biomarkers due to their unique structural properties. Notably, pre-miRNAs with guanine-rich motifs can form stable G-quadruplex structures. These structures are transient and dynamic, influenced by cellular conditions. To study their formation, ligands such as QUMA-1, an RNA G4 live cell probe, have been developed. In this study biophysical analyses to assess G4 formation within pre-miR150 and its interaction with QUMA-1 were conducted. Additionally, confocal microscopy was used in cellular studies to observe G4 formation in cells. Results revealed that pre-miR150 forms a G4 structure, which can be stabilized by QUMA-1. It also resulted in enhanced fluorescence and moderate affinity for the G4. Cellular studies corroborated these findings, showing a significant increase in fluorescence intensity with the ligand and RNA sequence, indicating G-quadruplex RNA formation in cells. This study highlights the potential of G-quadruplex structures in pre-miRNAs as targets for cancer diagnostics and therapeutics.

Acknowledgements: P.L. acknowledges a fellowship grant financed by FCT to CICS-UBI ref. UIDP/00709/2020.

Keywords: pre-miR150; G-Quadruplex; QUMA-1; Lung Cancer.

2. EVALUATION OF SPECIFIC ANTHRAQUINONES AS NEW CATECHOL-*O*-METHYLTRANSFERASE INHIBITORS: VIRTUAL DOCKING, MOLECULAR DYNAMICS AND INHIBITION *IN VITRO* STUDIES

Fábio A. Proença[1(*)], João P. Batista-Silva[1,2,3], Samuel Silvestre[1,5], Luís A. Passarinha[1,2,3,4]

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ABSTRACT

Parkinson´s disease (PD) is the second most prevalent age-related disorder around the world, and until today there is no cure. The disease is caused by the gradual destruction of the brain's dopaminergic neurons. The conventional therapy is the administration of oral levodopa (L-DOPA), a dopamine precursor, and two inhibitors: one for catechol-O-methyl transferase (COMT) and the other for the monoamine oxidase enzyme. In humans, the COMT enzyme is present in two isoforms. The membrane-bonded is physiologically the more relevant isoform because it can methylate catecholamines at their physiological concentrations. COMT inhibitors, like tolcapone, can increase the L-DOPA bioavailability and effectiveness. However, they are often associated with hepatotoxicity and/or incapacity to cross the blood-brain barrier. Here, we analyze the potential of anthraquinones as COMT inhibitors, based on their structural similarity with tolcapone. Anthraquinones are emerging molecules with anti-tumor, anti-inflammatory, and neuroprotective properties. In this work, we evaluated the affinity between the MBCOMT and 9 compounds from the anthraquinones family by *in silico* trials using Autodock Vina. The compounds were ranked based on the binding energy and target residue interactions with the protein active center. The best compounds were assessed with molecular dynamics, tracking their stability and pose dynamics, using tolcapone as a reference. For *in vitro* studies, an inhibition screening based on an analytical method described by Pedro and co-workers was performed. For that, 4 of the 9 compounds exhibited an inhibition capacity close to 60%. Those results suggest these molecules can be new potential COMT inhibitors.

Acknowledgements: The authors acknowledge the support by the Health Sciences Research Centre CICS-UBI (UIDB/00709/2020 and UIDP/00709/2020), the Applied Molecular Biosciences Unit UCIBIO (UIDB/04378/2020 and UIDP/04378/2020) and the Associate Laboratory Institute for Health and Bioeconomy–i4HB (project LA/P/0140/2020) which are financed by National Funds from FCT/MCTES. J.P. Batista-Silva PhD Fellowship (2023.04376.BD) from FCT–Fundação para a Ciência e Tecnologia.

Keywords: Parkinson´s disease; Catechol-O-methyltransferase; Catechol-O-methyltransferase inhibitors; Anthraquinones.

3. OPTIMIZATION OF BSA-COATED PEPTIDE NANOCOMPLEXES USING DESIGN OF EXPERIMENTS FOR ENHANCED CO-DELIVERY OF TEMOZOLOMIDE AND p53 GENE

Inês Afonso[1(*)], Ana R. Neves[1], Dalinda Eusébio[1], Tânia Albuquerque[1], Eric Vivès[2], Prisca Boisguérin[2], Adriana O. Santos[1], Ângela Sousa[1], Diana Costa[1]

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ABSTRACT

Despite significant advancements in the diagnosis and treatment of various human cancers over the past few decades, glioblastoma remains the deadliest brain tumor. Presently, there are no effective treatments or cures available. Gene therapy presents itself as a valuable tool, and when combined with chemotherapy, has shown to offer benefits through a synergistic effect. In this study, bovine serum albumin (BSA) coated temozolomide (TMZ)-peptide (WRAP5)/p53 gene-based plasmid DNA complexes were developed to enable co-delivery of therapeutic agents. A design of experiments (DoE) approach was used to identify the most promising BSA-coated TMZ-WRAP5/p53 nanocomplexes, considering the nitrogen to phosphate groups ratio (N/P) and BSA concentration as inputs, and size, polydispersity index, surface charge, and p53-based plasmid complexation capacity (CC) as outputs. The resulting quadratic models were statistically significant (p-value < 0.05), had adequate coefficients of determination, and the corresponding optimal points were successfully validated. Scanning electron microscopy (SEM) analysis showed the spherical morphology of the developed nanocomplexes. A cytotoxicity assay demonstrated that the BSA-coated TMZ-WRAP5/p53 complexes did not induce toxicity in normal brain cells. Furthermore, the nanocomplexes are hemocompatible. These findings encourage further *in vitro* research to evaluate their potential for co-delivery of therapeutic molecules. The DoE tool proved to be an effective approach for exploring and optimizing the properties of BSA-coated TMZ-WRAP5/p53 complexes, which could significantly impact the advancement of glioblastoma therapy.

Keywords: BSA-coated nanoparticles; Cell-penetrating peptides; Design of experiment; Drug/gene co-delivery.

Oral Communications B

Chairperson:

Susana Ferreira

4. PORTUGUESE NATURAL RESOURCES AS MODULATORS OF *Acne vulgaris* HALLMARKS

Ana Sofia Oliveira[1,2(*)], Joana Rolo[1,2], Carlos Gaspar[1,2,3], Rita Palmeira-de-Oliveira[1,2,3], João Paulo Teixeira[4,5], José Martinez-de-Oliveira[1,2], Ana Palmeira-de-Oliveira[1,2,3(*)]

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ABSTRACT

Acne vulgaris is a multifactorial disease of the pilosebaceous unit that affects millions worldwide, characterized by having different hallmarks associated with its development. As available treatment options are associated with relevant side effects and due to the shift in the consumers' preferences favouring natural alternatives, our aim was to study the anti-acne potential of essential oils (EOs) and hydrolates produced in Portugal from three species with interesting properties for skin application. We based our study design in the hallmarks of disease progression and evaluated the capacity of *Thymus* x *citriodorus* (TC), *Thymus mastichina* and *Cistus ladanifer* (CL) EOs and hydrolates to: modulate lipid production through the quantification of neutral lipids in human sebocytes (SZ95 cell line) with Nile-red staining; to decrease bacteria-induced inflammation in murine macrophages through the quantification of NO production after bacterial infection; along with the direct antimicrobial activity against *C. acnes* strains collected from human volunteers and classified into phylotypes (by Multiplex-Touchdown PCR) and characterized for virulence. Our results showed that TC and CL EOs were the most effective in reducing lipid production (IC_{50}: 0.009% v/v and 0.015% v/v) and in reducing bacterial-induced NO production by approximately 80% in concentrations as low as 0.01% v/v. TC EO also showed a potent antimicrobial activity with minimum inhibitory concentrations ranging from 0.03% to 0.06% v/v in strains with different virulence profiles. The hydrolates, despite showing low antimicrobial activity were not deprived of anti-inflammatory potential. Our results highlight the value of TC EO as a bioactive ingredient for anti-acne application.

Acknowledgements: This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Financial support was provided to ASO: (SFRH//BD/136192/2018) and (CICS-UBI_BI_2024).

Keywords: *Acne vulgaris*; *Cutibacterium acnes*; Inflammation; Sebum production.

5. MITOCHONDRIAL SELECTION AND PATHOGENICITY IN YEAST: A PHYLOGENETIC ANALYSIS

Mariana Z. Fernandes[1(*)], Joana Rolo[1], Emanuel Maldonado[1], José Martinez-de-Oliveira[1], Ana Palmeira-de-Oliveira[1,2]

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ABSTRACT

This study aimed to elucidate the phylogenetic relationships among species closely related to *Candida albicans* and the evolutionary forces acting on their mitochondrial genes, particularly to study the selection pressures acting on these genes and whether they differ between pathogenic and non-pathogenic species. Thirty species were selected based on their genetic proximity to *Candida albicans* and classified as "Potentially Pathogenic", "Non-Pathogenic", or "Rarely Pathogenic/Undefined", according to the bibliography. Mitochondrial genomes were retrieved from NCBI and assembled de novo using NOVOPlasty when necessary. Conserved mitochondrial genes (COX1, COX2, COX3, COB, ATP6, ATP8, ATP9) and tRNAs genes were analysed. Phylogenetic analysis was performed using PhyML with the GTR model, selected through AIC and BIC, and 300 bootstrap replicates. Selection analyses were performed using MEME, BUSTED, and FEL to detect episodic diversifying selection, gene-wide selection, and site-specific selection, respectively. The RELAX test compared selection intensity between pathogenic and non-pathogenic groups. MEME identified codons under episodic diversifying selection in all genes, and BUSTED detected it on COX1, COX2, COB, and ATP6. FEL showed negative selection in most variable codons of all genes except ATP8 (45.7%). RELAX revealed intensified selection in pathogenic species. Additionally, tRNA gene deletion and acquisition were conserved within clusters. Deletion of tRNA genes was only observed in already duplicated or triplicated tRNAs, specifically Arg, Met, and Thr tRNAs. The only acquisition observed was a Sup tRNA in the *Saccharomyces* and *Nakaseomyces* cluster. These findings suggest distinct evolutionary pressures on mitochondrial genes of pathogenic versus non-pathogenic yeast species.

Keywords: Evolution; Mitochondrion; Yeast; Phylogeny.

Oral Communications C

Chairperson:

Adriana Santos

6. NANOCOSMETICS CONTAINING NATURE-DERIVED HYDROPHOBIC COMPOUNDS: NIOSOME DEVELOPMENT AND PHYSICOCHEMICAL AND TOXICITY CHARACTERIZATION

Patrícia C. Pires[1,2,3(*)], Maria Beatriz Pinto[1], Mafalda Correia[1], Gabriela Moço[1], Ricardo C. Calhelha[4,5], Ana Rita Silva[4,5], Maria João Sousa[4,5], Miguel Vilas-Boas[4,5], Soraia I. Falcão[4,5], Francisco Veiga[1,2], Pooyan Makvandi[6,7,8], Ana Cláudia Paiva-Santos[1,2(*)]

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ABSTRACT

Niosomes are self-assembled nanosystems composed of non-ionic surfactants and cholesterol organized into multilamellar vesicles, being highly researched for topical delivery due to their ability to encapsulate both hydrophilic and lipophilic compounds, being able to provide their controlled release, and leading to improved stability. The purpose of this study was to develop niosomes suitable for cosmetic application and able to encapsulate a model nature-derived hydrophobic compound. The vesicles were produced by the thin layer evaporation method, and contained cetyl alcohol, cholesterol, Tween® 20 and/or Kolliphor® RH40. Particle size, PDI, zeta potential, entrapment efficiency, stability and *in vitro* release were characterized. Particle size was below 200 nm and PDI below 0.2, meeting the necessary requirements for topical application. High entrapment efficiency was achieved (above 79%), as well as high zeta potential absolute values (-38 mV), being indicative of relevant stabilization through electrostatic repulsion. A controlled release of the encapsulated bioactive compound was demonstrated, and the developed niosomes had substantial anti-inflammatory potential (RAW 264.7 cell line), not exhibiting relevant cytotoxicity against HaCaT (up to 25 µg/mL) or HFF-1 (up to 12.5 µg/mL) cell lines, and only a slight irritation potential on the HET-CAM test.

Keywords: Cosmetic; Nanosystem; Natural compound; Niosomes.

7. A POTENTIAL EFFECT OF CIRCADIAN RHYTHMS IN THE DELIVERY/THERAPEUTIC PERFORMANCE OF PACLITAXEL-DENDRIMER NANOSYSTEMS

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ABSTRACT

The circadian clock controls behaviour and physiology. Presently, there is clear evidence of a connection between this timing system and cancer development/progression. Moreover, circadian rhythm consideration in the therapeutic action of anticancer drugs can enhance the effectiveness of cancer therapy. Nanosized drug delivery systems (DDS) have been demonstrated to be suitable engineered platforms for drug targeted/sustained release. The investigation of the chronobiology-nanotechnology relationship, i.e., timing DDS performance according to a patient's circadian rhythm, may greatly improve cancer clinical outcomes. In the present work, we synthesized nanosystems based on an octa-arginine (R8)-modified poly(amidoamine) dendrimer conjugated with the anticancer drug paclitaxel (PTX), G4-PTX-R8, and its physicochemical properties were revealed to be appropriate for *in vitro* delivery. The influence of the circadian rhythm on its cellular internalization efficiency and potential therapeutic effect on human cervical cancer cells (HeLa) was studied. Cell-internalized PTX and caspase activity, as a measure of induced apoptosis, were monitored for six time points. Higher levels of PTX and caspase-3/9 were detected at T8, suggesting that the internalization of G4-PTX-R8 into HeLa cells and apoptosis are time-specific/-regulated phenomena. For a deeper understanding, the clock protein Bmal1-the main regulator of rhythmic activity, was silenced by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology. Bmal1 silencing was revealed to have an impact on both PTX release and caspase activity, evidencing a potential role for circadian rhythm on drug delivery/therapeutic effect mediated by G4-PTX-R8.

Keywords: Nano-delivery systems, Cancer therapy, Circadian Rhythms, Bmal1 silencing.

8. EXPLORING miR-155 DETECTION USING MOLECULAR BEACONS ON A MICROFLUIDIC DEVICE

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ABSTRACT

Lung cancer (LC) is the most common deadly cancer worldwide. Therefore, it is imperative to innovate in developing efficient detection methods and treatments. In the diagnostic field, microRNAs (miRNAs) have emerged as potential biomarkers to diagnose several diseases such as LC, and to follow their progression. However, detecting and quantifying miRNAs is time-consuming and frequently expensive when using traditional methods such as PCR. Herein, we explore detecting miR-155-3p overexpressed in LC biological samples through a molecular beacon (MB) bead-based assay immobilized in a microfluidic device. This method is based on the increased fluorescence emitted by the MB when the target bounds it by Watson and Crick complementarity. The hybridization will change the MB conformation from a stem-loop to a linear conformation leading apart the fluorophores that are present on each extremity. MB combination with a microfluidic device allows an easy and quick detection of the miR-155-3p. Herein, miR-155-3p was detected in saline solution with a limit of detection (LOD) on the nanomolar order. Then the method was tested on more complex biological samples (A549 cells', total RNA, and peripheral blood mononuclear cells), spiked with the target miRNA, obtaining a satisfactory recovery, especially in A549 cells' total RNA. This work brings one more step in the development of easy, cheap, and fast tools to detect and quantify potential biomarkers for LC.

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Keywords: Lung cancer; Molecular beacon; Microfluidics; mir-155-3p.

9. HPV16 E6 AND E7 ONCOPROTEINS: ENHANCED PURIFICATION FOR ANTI-CANCER DRUG DEVELOPMENT

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ABSTRACT

Cervical cancer is the fourth leading cause of cancer death in women worldwide, with a substantial impact in developing countries. CC arises from lesions caused by human papillomaviruses (HPVs) and its oncogenic role is mainly linked with two oncoproteins, E6 and E7. These proteins interact and inactivate tumour suppressor proteins p53 and pRb, resulting in apoptosis inhibition and uncontrolled cell cycle progression. For this reason, E6 and E7 have been used as targets to develop anti-HPV drugs. Nevertheless, large amounts of protein with high purity should be obtained to perform biointeraction studies with the selected drugs. Thus, our aim was to recombinantly express E6 and E7 from *Escherichia coli (E. coli)* cells and explore different purification methods to isolate both proteins. The results showed that the target proteins were successfully produced with fusion tags. In the purification strategy, the combination of two affinity chromatographic steps allowed the recovery of a high amount of target proteins with the elimination of host cell proteins. However, the purified sample is composed of monomers and oligomers, so we are exploring salt-tolerant and mixed-mode resins to obtain mostly monomers of both proteins that will be used for stability and biointeraction studies of protein-ligand complexes.

Acknowledgements: This work was funded by national funds for Fundação para a Ciência e Tecnologia (FCT): CICS-UBI Base/Programmatic Funding (DOI: 10.54499/UIDB/00709/2020 and 10.54499/UIDP/00709/2020), DryVac project (2023.00136.RESTART), UCIBIO (UIDB/04378/2020 and UIDP/04378/2020), and the Associate Laboratory–i4HB (project LA/P/0140/2020). D. Gomes acknowledges the doctoral fellowship from FCT ref: 2020.06792.BD and Fulbright Scholarship. Â. Sousa acknowledges FCT and UBI for the research contract CEEC-INST/00016/2021/CP2828/CT0003.

Keywords: Affinity Chromatography; HPV-associated cancers; Mixed-mode resins; Recombinant proteins.

10. AT11-GUIDED LIPOSOMES: POTENTIAL THERAPEUTIC APPROACH FOR ORAL CANCER CELLS

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ABSTRACT

The increasing prevalence of oral cancers, particularly those attributed to human papillomavirus infection, along with the associated drawbacks of conventional therapies, has spurred research into more effective treatment modalities. Liposomes have emerged as a potential strategy to improve the delivery of anticancer molecules. However, existing studies suggest that functionalizing these nanoparticles with targeting aptamers could significantly enhance their selectivity. A prospective approach involves the incorporation of AT11, a nucleolin aptamer, able to fold into G-quadruplex, which could act as a targeting moiety and facilitate drug accumulation within cancer cells. Therefore, AT11 was used for the liposomes functionalization to enhance the selectivity of C_8, a potential anticancer compound, for specifically targeting oral cancer cells. Thus, we produced liposomes (empty or C_8-associated) by ethanol injection method and then, proceeded with their functionalization with AT11-TEG-Cholesteryl. Liposomes with hydrodynamic diameters of ~130 nm were obtained. Additionally, the effect of the produced liposomes on the viability of squamous cell carcinoma of the tongue (UPCI-SCC-154) and healthy (Het1A) cells was determined. The empty liposomes were biocompatible up to 53.6 µg/mL. After treating the cells with C_8-associated liposomes, both cell lines showed a dose-response effect. The AT11-functionalization of the obtained liposomes resulted in an enhancement of the selectivity towards oral cancer cell line, as observed by MTT assay and confocal microscopy. Furthermore, the AT11 C_8-associated liposomes can decrease tongue cancer cell proliferation, migration, and invasion and induce cell death. These findings suggest that AT11 C_8-associated liposomes are promising drug carriers for oral cancer therapy.

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Keywords: Acridine orange derivative; Aptamer; Liposome; Oral cancer.

11. FROM B-MYB G-QUADRUPLEX TO LIGANDS: STARTING BY BIOPHYSICAL SCREENING TO CELL STUDIES

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ABSTRACT

The B-MYB is a proto-oncogene responsible for encoding a transcription factor essential in the regulation of cell cycle and cell differentiation. In tumor cells, these properties are dysregulated and B-MYB aberrant expression, observed in several cancers and a predictor of poor prognosis, leads to gene alterations, tumorigenesis and activation of oncogenic signalling pathways. Bioinformatic analysis showed that gene promoter regions are highly enriched in guanines thus allowing the formation of G-quadruplexes (G4) structures by the self-folding of four guanines in a planar arrangement. Also, it is hypothesized that G4s have a gene regulatory function due to their significant overrepresentation around the transcription start site (TSS), being considered an attractive anticancer target. The G4 stabilization by small molecules may suppress oncogene transcription and inactivate downstream pathways involved in tumorigenesis. Recently we reported the ability of promoter region around TSS to assemble in G4 by several bioinformatic and biophysical methods. To explore the target properties from B-MYB G4, this work focused on the identification of small molecules capable of interacting, stabilising and suppressing B-MYB gene action. We started to access the G4 assemble *in-cell* environment to prove the biological relevance and formation by the G4access method. Then we proceeded to screen molecules using a plethora of biophysical methods to evaluate the affinity and stabilization parameters. Finally, the leading compounds followed by cell studies to explore the cytotoxic and migration properties. The results evidenced the formation in a complex cellular environment and biophysical experiments elucidated the leading compounds to interact with B-MYB G4. Also, the best ligands showed distinct properties and effects in cell studies.

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Keywords: Cancer; B-MYB; G-quadruplex; Nucleic acids biophysics.

Oral Communications D

Chairperson:

Carla Cruz

12. A POOL OF HUMAN NAÏVE CD8αβ T CELLS DOWN-MODULATE THE CD8β CHAIN AND BECOME CD8αα T CELLS UPON *IN VITRO* CULTURE WITH IL-15

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ABSTRACT

Antigen-driven human effector-memory CD8+ T cells expressing low levels of the CD8β chain have been previously described. Nonetheless, the body of knowledge regarding a potential antigen-independent trigger is scarce. In this work, we have assessed the impact that IL-15 has on the expression of CD8β on purified human naïve CD8+ T cells after CFSE labeling and culture with IL-15. Remarkably, IL-15 induced naïve CD8+ T cells to proliferate and differentiate, leading to a cell-cycle dependent down-modulation of CD8β from the cell surface and the generation of $CD8\alpha\beta^{low}$ and $CD8\alpha\beta^{-}$ (i.e., CD8αα) T cells. In contrast, expression of the CD8α chain remained steady or even increased. Interestingly, other members of the gamma common (γc) chain-dependent cytokines, such as IL-2 and IL-7, were unable to mimic the effect of IL-15. Noteworthy, CD8+ T cell blasts obtained after culture of CD8+ T cells with IL-15 showed an increase in the level of the tyrosine kinase Lck, when compared to CD8+ T cells at day 0. This study has shown for the first time that IL-15 generates $CD8\alpha\alpha^{+}\alpha\beta^{low}$ and $CD8\alpha\alpha^{+}\alpha\beta^{-}$ T cells containing high levels of Lck, suggesting that they may be endowed with unique functional features.

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Keywords: IL-15; Effector-Memory CD8+ T cells; CD8β chain; Downregulation.

13. THE EFFECT OF MUSIC ON THE TRANSCRIPTOME OF EVOLUTIONARILY-CONSERVED NEUROPLASTICITY-RELATED GENES ASSOCIATED WITH AUTISM SPECTRUM DISORDER

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ABSTRACT

ASD are defined as potentially severe developmental deficit in communication, socialization, and behavior. Genetics and environmental factors are important to the development of the ASD. Autism treatments include (behavior, speech-language, play-based, physical, nutritional, and music) therapies. Music provokes changes in emotions and pleasure, through its action on the brain's reward centers like the limbic and mesolimbic structures. However, there is currently no neuroscientific evidence supporting music therapy benefits, it has been identified as a strength in people with ASD. The effect of music performance on the genome-wide peripheral blood transcriptome of professional musicians, showed that the up-regulated genes are known to be associated with dopamine signaling, synaptic neurotransmission, learning, memory and cognitive performance, song learning and singing in songbirds and neurogenesis. On the other hand, down-regulated genes are known to cause mammalian neuronal apoptosis, immoderate oxidative phoshorylation and deficits in dopaminergic neurotransmission, which are the characteristics of neurodegeneration. The genes SNCA, RTN4, RGS2 and SLC6A8 are involved in the regulation, secretion, and transport of the neurotransmitter dopamine. The *in-silico* analysis allowed the identification of orthologous genes and inferring their likely roles in dopamine signaling, synaptic neurotransmission and synaptic function in different species. Our analysis discovered highly conserved motifs in these proteins analysed and several genes reported to regulate song perception and production in songbirds displayed similar activities, suggesting a possible evolutionary conservation of sound perception between species. The widely discussed neuroprotective role for music, may provide a working mechanism for the use of music therapy, especially in treating neurodegenerative diseases.

Keywords: Autism; Epigenetic; Neuroplasticity; Dopamine signaling.

14. CAN WE USE HUMAN MIDBRAIN ORGANOIDS TO MODEL PARKINSON´S DISEASE?

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ABSTRACT

Alpha-synuclein (a-syn) aggregation and dopaminergic neuronal loss are the major hallmarks of Parkinson's Disease (PD), for which there is no curative treatment. Several studies showed that reduced autophagy is involved in a-syn accumulation and aggregation. Hence, promoting a-syn clearance by enhancing the autophagy-lysosomal pathway may be a promising therapeutic strategy for PD. Herein, we aim to investigate whether the accumulation of a-syn impairs autophagy and leads to neuronal degeneration by using human midbrain organoids (hMOs). First, hMOs were generated from induced pluripotent stem cells from PD patients with a triplication in the SNCA gene (3xSNCA), along with gender- and age-matched controls to investigate PD-associated phenotype. To monitor autophagy, we used hMOs that genetically encode a Rosella-LC3 system - a dual-colour fluorescent reporter that allows for real-time visualization of autophagic flux - as well as regular hMOs. We observed that hMOs presented a decrease in the autophagic events and in the autophagic flux, as observed after the treatment with bafilomycin A for 24h. At the same time, we observed an increase of the a-syn aggregates and dopaminergic degeneration. In summary, hMOs provide a valuable tool to model the cellular hallmarks of Parkinson's disease and investigate potential therapeutic approaches targeting the autophagy pathway.

Keywords: Parkinson's Disease; Midbrain Organoids; Alpha-synuclein; Autophagy.

15. UNIVARIATE METABOLOMIC ANALYSIS TO UNCOVER URINE METABOLIC MARKERS OF ISCHEMIC STROKE IN ELDERLY

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ABSTRACT

A stroke is a disruption in the blood flow to brain cells, leading to global or focal neurological impairment. It can occur as an ischemic stroke (80-85%) or hemorrhagic stroke (20-25%), and it remains the first and second leading cause of death in Portugal and worldwide, respectively. It is widely recognized that stroke results from the intricate interplay of various risk factors, including age, sex, family history, cardiovascular and metabolic diseases, and lifestyle. However, its physiopathology remains incompletely understood due to its complexity. In this sense, novel biomarkers are currently being studied, trying to highlight new disease pathways. We used Nuclear Magnetic Resonance spectroscopy to study changes in the metabolites present in the urine of individuals living in long-term care facilities of Beira Interior (Covilhã, Fundão, Belmonte), with and without ischemic stroke. After analysis by the Bruker AVANCE III 600 MHz magnetic resonance spectrometer, the univariate statistical analysis showed significant differences between the control group and the stroke group in twenty one out of the forty-three identified metabolites. Of these, eighteen metabolites were decreased (3-hydroxyisovaleric acid, alanine, 2-hydroxyglutaric acid, acetoacetic acid, glutamine, citrate, creatine, methyluric acid, trimethylamine, methanol, total sugar, serine, urea, histidine, phenylalanine, hippuric acid, nudifloramide and trigonelline), while three were increased (threonine, glycolic acid, and acetic acid) in stroke group. Based on these findings, we can consider these metabolites as potential biomarkers for ischemic stroke.

Acknowledgements: The Authors kindly acknowledge all the elders that give their informed consent to participate in this study and all the technicians of the 20 long-term care facilities of Beira Interior. This work is supported by research funds (ICON project; CENTRO-01-0145-FEDER-000013) of "Programa Operacional Centro 2020 (CCDRC, Coimbra, Portugal)", by FEDER funds through the POCI - COMPETE 2020 (Project No. 007491) and by FCT -(Project UID/Multi /00709). Researchers were supported by PhD Fellowships of FCT (SFRH/BD/06028/2020).

Keywords: Ischemic stroke; NMR-based metabolomics; Elders; Urine biomarkers.

16. THE COMBINED EFFECT OF PORTUGUESE NATURAL MINERAL WATERS WITH PLANT EXTRACTS AGAINST SKIN AGING

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ABSTRACT

Natural ingredients have been used in skincare for thousands of years. Natural mineral waters (NMWs) and plant extracts have described anti-aging properties. In this study, we combined 5 NMWs with 5 plant extracts, through several assays, to evaluate their safety and efficacy, thus assessing their potential as active ingredients against skin-aging. Cellular biocompatibility of the mixtures was evaluated in fibroblasts (L929) and macrophages (RAW 264.7) using the MTT assay. The scratch-wound assay evaluated the skin-repairing potential of biocompatible concentrations in L929 cells. Superoxide dismutase (SOD) activity was quantified using a commercial kit, and lipopolysaccharide-induced reactive oxygen species (ROS) produced by macrophages were quantified using a fluorescent probe. The antioxidant capacity of the mixtures was assessed using the (2,2-Diphenyl-1-picrylhydrazyl) DPPH method. An optimal concentration of each mixture was selected based on low cytotoxicity on both cell lines. Various mixtures showed a reduction in ROS, NMW A mixtures being the most promising. The DPPH assay identified that mixtures containing *Rubus idaeus* and *Vaccinium myrtillus* extracts as having significant antioxidant capacity. Combining *Rubus idaeus* with NMW E enhanced cell migration, while mixtures containing NMW B showed promising results in SOD expression. These preliminary results reveal the potential benefits of combining NMWs with plant extracts. Overall, the mixtures promoted wound healing in skin fibroblasts, and increase expression in SOD enzyme in macrophages suggesting an antioxidant effect. Additional research is necessary on skin-aging biomarkers and evaluating more combinations of plant species and NMWs in order to maximize the value of these natural resources.

Acknowledgements: The work was also developed within the scope of the CICS-UBI projects [UIDB/00709/ 2020] and [UIDP/00709/2020], financed by national funds through the Portuguese Foundation for Science and Technology/MCTES (FCT) and through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). This work was also supported by the Projeto Aquae Vitae – ÁguaTermal como Fonte de Vida e Saúde, Project funded under the Promove program of Fundação La Caixa, in partnership with BPI and FCT.

Keywords: Cytotoxicity; DPPH method; Natural ingredients; Reactive oxygen species.

17. NATURAL ANTI-AGING COSMETICS WITH UNHAIS DA SERRA THERMAL WATER AND *Thymus x citrioddorus* HYDROLATE

Ana Rita Gama[1,2(*)], Carolina Gomes[1,2], Cátia Caetano[1,2], Ana Sofia Oliveira[1,2], Joana Rolo[1,2], José Martinez de Oliveira[1,2], Ana Palmeira de Oliveira[1,2,3], Rita Palmeira de Oliveira[1,2,3]

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ABSTRACT

Skin aging is influenced by intrinsic factors such as genetics and extrinsic factors like UV radiation, leading to structural changes in the skin. Current anti-aging cosmetic trends emphasize innovative natural ingredients, including plant extracts, thermal waters, and botanical hydrolates. This work aimed to develop three natural anti-aging cosmetics (a serum, a day cream, and a night cream) with innovative and non-irritating profiles. A rational design was followed based on a market study and the properties of the ingredients. The three products were formulated with Thermal Water from Unhais da Serra and *Thymus x citriodorus* hydrolate. The products were evaluated for stability (4ºC and 40ºC, 4 weeks) and safety (EC Regulation no 1223/2009) and tested for skin irritation *in vitro* (Epiderm model; OECD TG 439). The market study highlighted a gap in anti-aging products combining natural extracts and thermal waters, promoting the development of innovative formulations. All prototypes exhibited the desired textures. Stability tests confirmed the robustness of the formulations under varied conditions. Safety assessments complied with EC Regulation no 1223/2009, and no irritant effects were observed on the Skin Irritation test. The results support the development of natural, effective anti-aging cosmetics rooted in natural resources and the innovative developed products can meet the market demand for natural and sustainable skincare solutions, aligning with current consumer trends.

Acknowledgements: This work was supported by Projeto Aquae Vitae – Água Termal comoFonte de Vida e Saúde, Project funded under the Promove program of Fundação La Caixa, in partnership with BPI and FCT. This work was supported by funds from the CICS-UBI base grant with DOI 10.54499/UIDB/00709/ and the CICS-UBI programmatic grant with DOI 10.54499 /UIDP/00709/2020 with national funds registered in the budget of the Foundation for Science and Technology.

Keywords: Skin-aging; Thermal waters; Hydrolates; Natural resources.

Oral Communications E

Chairperson:

Rita Palmeira de Oliveira

18. POTENTIAL ATTENUATION OF *Arcobacter butzleri* VIRULENCE BY PROBIOTICS

Alexandre Vieira[1(*)], Cristiana Mateus[1], Fernanda Domingues[1], Mónica Oleastro[2], Susana Ferreira[1]
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ABSTRACT

Arcobacter butzleri is a Gram-negative bacterium that can be found in a variety of environments and hosts, for which the main route of transmission is suggested to be the consumption of contaminated food or water. This species is considered an emergent enteropathogen, which to establish gastrointestinal infections, must be able to overcome natural barriers to gut colonization, such as probiotics. Thus, the aim of this study was to evaluate the effect of probiotics, particularly the species of "lactobacilli" (*Lactiplantibacillus plantarum* ATCC8014, *Limosilactobacillus reuteri* ATCC23272 and *Lactobacillus acidophilus* LMG9433) on the survival and virulence of *A. butzleri*. For this purpose, the ability to survive the gastrointestinal tract was first evaluated, considering the survival at acidic pH and the minimum inhibitory concentration of bile salts, both for pathogen and probiotics. Following this, a co-culture assay was performed to assess if there was a growth inhibition of *A. butzleri* in presence of the species of "lactobacilli", and the effect of the culture-free supernatant (CFS) of the "lactobacilli" on the growth and biofilm formation by *A. butzleri*. In addition, the displacement, exclusion and competition effect of the "lactobacilli" against *A. butzleri* was studied by infection of Caco-2 cells. The results indicate that all species under study can survive the gastrointestinal tract. Regarding co-culture, *A. butzleri* growth is inhibited by all species of "lactobacilli", a similar behaviour was observed in the presence of CFS. When examining the impact of CFS from "lactobacilli" on *A. butzleri* biofilm formation, the results varied depending on the specific species studied, showing potentiation, inhibition, or no significant change. Considering the adhesion and invasion, in the displacement and exclusion assays, a decrease in the adhesion of the *A. butzleri* was observed; however, in the competition assay, the probiotic species were unable to affect the adhesion of the *A. butzleri* strain. In short, these results suggest that "lactobacilli" have the potential to limit the growth of *A. butzleri*.

Acknowledgements: This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Cristiana Mateus is recipient of a doctoral fellowship (UI/BD/151023/2021) under the scope of the CICS-UBI Programmatic Funding (UIDP/00709/2020). Susana Ferreira acknowledges UBI and FCT by the contract of Scientific Employment according to DL57/2016.

Keywords: *Arcobacter butzleri*; Probiotics; Survival; Virulence.

19. A PORTRAIT OF THE VIRULENT POTENTIAL OF CANDIDA COLONIZATION: A COMPARATIVE STUDY OF ISOLATES FROM DIFFERENT HUMAN INFECTION NICHES

Filipa Oliveira Castro[1(*)], Mariana Zagalo Fernandes[1], José Martinez-de-Oliveira[1], Ana Oliveira[1], Ana Palmeira-de-Oliveira[2], Ana Ferrrão[3], Paula Pestana[3], Joana Rolo[1]

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ABSTRACT

It is estimated that around 150 million people are affected annually by urinary tract infections (UTIs), with one in five adult women having at least one episode in their lifetime. However, yeasts isolated in urine samples are not routinely characterised and therefore its epidemiology and clinical relevance are elusive. Also, vaginal candidosis is the second most common cause of vaginitis associated most often with the overgrowth of *Candida* species and has a significant impact on women worldwide. However, there is still an incomplete picture of the relationships between genital and urinary infections, preventing a comprehensive understanding of the mycobiome of both niches and their relationship for proper diagnosis and treatment. In this talk, I will address the species distribution and the risk factors from 62 different isolates, 37 from urinary infections (17 male and 20 female) and 25 from vaginal infections. The results of yeasts isolated from urinary samples showed higher susceptibility to both antifungals (fluconazole and clotrimazole), while yeasts from vaginal exudate were on average more resistant. Regarding virulence factors, *C. albicans* isolates from male patients with urinary infections showed a greater capacity for germ tube formation. Finally, yeast isolates from female infections proved to be excellent biofilm formers and demonstrated good adhesion to the surface of HeLa cells. We concluded, in general, that the most virulent yeasts are from elderly users hospitalized, and pregnant patients. Of the different niches, we found that strains from female urocultures had a greater capacity for pathogenicity and infection.

Keywords: Antifungal resistance; Colonization of human host; Candidosis; Virulence factors.

20. FROM SUBCLINICAL CIPROFLOXACIN CONCENTRATIONS TO INCREASED RESISTANCE: UNDERSTANDING THE EVOLUTIONARY PATHWAYS OF *Arcobacter butzleri*

Inês Martins[1(*)], Alexandra Nunes[2], Mónica Oleastro[3], Susana Ferreira[1]

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ABSTRACT

While antimicrobial resistance is typically linked to high therapeutic antibiotic concentrations, recent evidence indicates that subclinical concentrations can also select for multidrug-resistant mutants. However, this is a topic under-researched from a One Health perspective in gastrointestinal pathogens. Exploring this topic in a non-model pathogen like *Arcobacter butzleri*, an emerging enteropathogen with environmental niches and high antibiotic resistance, is particularly relevant since the underlying mechanisms are scarcely known. Hence, following an initial assessment of the contribution of efflux activity to the multidrug resistance phenotypes in *A. butzleri*, ciprofloxacin, a high-priority agent among Critically Important Antimicrobials for Human Medicine by the WHO, was chosen to unveil *A. butzleri'* evolutionary pathways under subclinical antibiotic concentrations. Thus, a food product isolate was phenotypically characterized regarding resistance to ciprofloxacin and submitted to a 12-day adaptive evolution in its presence. Post-evolution, populations' susceptibility to ciprofloxacin and cross-resistance profiles to antibiotics, biocides, heavy metals, and ethidium bromide were evaluated, as well as the underlying resistance mechanisms. Notably, resistant mutants emerged even at concentrations below those found in serum of untreated individuals or in ciprofloxacin-polluted environments, in concentrations between twice and half the MIC of the parental strain (0.0625-0.0153 µg/mL). Furthermore, adapted populations demonstrated decreased susceptibility to ampicillin, cefotaxime, tetracycline, erythromycin and chloramphenicol, acriflavine, and benzalkonium chloride. In addition, decreased susceptibility to ethidium bromide in resistant mutants suggest the mediation by efflux pumps' activity in these phenotypes, reinforced by overexpression of multidrug-resistant efflux pumps with relevance in this species. These findings highlight the impact of subclinical concentrations on antibiotic resistance crisis from a One Health vision.

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Keywords: *Arcobacter butzleri*; Subclinical ciprofloxacin concentrations; Antimicrobial resistance; Efflux pumps.

Oral Communications F

Chairperson:

Márcio Rodrigues

21. TRADITIONAL KNOWLEDGE OF MEDICINAL AND FOOD PLANTS USED IN SERRA DA ESTRELA NATURAL PARK

Radhia Aitfella Lahlou[1,4(*)], Filomena Carvalho[1], Alexandra Varges[1,4], Maria João Pereira[2], João Lopes[3], Luís R. Silva[1,4,5]

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Abstract

Research on medicinal plants including their traditional uses and pharmacological studies, has garnered growing global interest. Portugal's Serra da Estrela Natural Park (PNSE) is renowned for its preservation and biodiversity of medicinal plants, valued for their pharmaceutical applications. Local healthcare relies heavily on traditional medicine, however, contemporary methods endanger this understanding. An ethnobotanical survey was performed using open-ended semi-structured questionnaires with 286 informants across 34 localities in five municipalities (Celorico da Beira, Covilhã, Guarda, Seia, and Gouveia). Data analysis involved Use Reports (UR), Frequency of Citations (FC), Relative Frequency of Citations (RFC), Informant Agreement Ratio (IAR), Fidelity Level (FL), and Informant Consensus Factor (FIC). Plant specimens were collected and bio-authenticated. The informants provided data for 133 species from 49 families and 109 genera, used to treat 105 ailments and as food. Dominant families were Lamiaceae and Asteraceae. Herbs, particularly leaves, were most commonly used and often harvested from the wild. Most applications were infusions taken orally. Frequently cited species included *Melissa officinalis*, *Malva sylvestris*, *Sambucus nigra*, *Genista tridentata*, *Tilia cordata*, *Olea europaea*, *Aloysia citrodora*, and *Hypericum perforatum*. The ailments and food utilisations of the plants were divided into 20 categories. The majority of the plants listed are used to treat conditions related to digestive, respiratory, genitourinary, endocrine, nutritional/metabolic, skin, circulatory systems, and mental, behavioural or neurodevelopmental disorders. This study enhances our understanding of the PNSE traditional medicine and provides a useful resource for future research and the development of new drugs.

Keywords: Serra da Estrela Natural Park; Medicinal plants; Ethnobotanical survey; Traditional medicine.

22. REDUCTION OF OXIDATIVE STRESS AND IMPROVEMENT OF THE SURVIVAL RATE OF *Caenorhabditis elegans* BY USING BLACKBERRY EXTRACTS PREVIOUSLY INOCULATED WITH BACTERIAL BIOSTIMULANTS

Rocío Roca-Couso[1,2(*)], José D. Flores-Félix[1,2], Begoña Ayuda-Durán[3] , Rebeca Ferreras-Charro[3], Ignacio García-Estévez[3], Raúl Rivas[1,2,4]

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ABSTRACT

Bioinoculants are microorganism-based products which are widely used in agriculture to enhance plant growth. Additionally, they can also improve crop nutrition quality. *Caenorhabditis elegans* is a model organism used for medical research, primarily on aging research. The main aim of this work is to evaluate the nutraceutical activity of bioinoculant-enhanced blackberries and evaluate their in vivo impact in the model organism *C. elegans*. For this purpose, blackberry plants were inoculated with plant growth promotion bacteria *Rhizobium laguerreae* CRRU65 and the extract of the fruits were analyzed by HPLC–DAD–MS to determine the composition of phenolic compounds. Bioinoculant-enhanced blackberries showed that the main phenolic compound was cyanidin-3-O-glucoside. C. elegans were exposed to the blackberry extracts and survival rate was measured. Gene expression of several genes which modulate antioxidative process were analyzed by qPCR. Results regarding *C. elegans* in vivo assays showed that those organisms exposed to bioinoculant-enhanced blackberries exhibited a higher survival rate under heat stress. The analysis of several genes expression which modulate antioxidative stress showed the overexpression of the transcriptional factor SKN-1 and the heat shock proteins HSP-16. The results indicated that *R. laguerreae* CRRU65 inoculation in blackberry crops may enhance nutraceutical properties of blackberry fruits, which modifies the expression of key genes involved in the regulation oxidative stress in *C. elegans*, which in turn leads to an increase in the survival rate under stressful conditions.

Acknowledgements: RRC acknowledges the grant PID2019-109960RB-I00 and the FPI grant PRE2020-094762 funded by MCIN/AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe".

Keywords: Antioxidant; Bioinoculant; *Caenorhabditis elegans*; Gene expression.

23. PHYTOCHEMICAL COMPOSITION AND BIOACTIVITIES OF POLYPHENOLIC EXTRACTS OF *Prunus avium* L. BY-PRODUCTS

Ana R. Nunes[1,2(*)], Ana C. Gonçalves[1,3], Amílcar Falcão[3,4], Gilberto Alves[1], Luís R. Silva[1,5,6]

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ABSTRACT

Plants have been used as traditional medicine to sustain and improve human health for thousands of year. Recently, there has been growing interest in the use of their by-products. The production and processing of sweet cherry (*Prunus avium* L.) generate large amounts of bioresidues without commercial and biological value, increasing environmental and management costs. Although some of these by-products are used in folk medicine, little attention has been paid to their phytochemical composition and biological properties. Our research group has been focusing on studying the phytochemical composition and biological properties of cherry by-product extracts (leaves, stems, and flowers). In this work, we assessed the phytochemical profile (phenolic compounds, volatiles, and minerals) and bioactivities of the aqueous infusions and hydroethanolic extracts from cherry by-products. The extracts were chemically characterized, phenolics by HPLC-DAD-ESI/MS^n, minerals by ICP-MS and flame atomic absorption spectrometry, and volatiles by SPME/GC-MS. Their biological activities were evaluated *in vitro*. All extracts are very rich in phenolic compounds, mainly phenolic acids and flavanones, and minerals such as phosphorus and manganese. Moreover, the cherry by-products revealed antioxidant, anti-proliferative, and anti-inflammatory properties. These new findings intended to provide new possibilities of valorization of these by-products and their valuable properties as possible active ingredients for different industries.

Keywords: *Prunus avium* L.; By-products; Bioactive compounds; Biological properties.

24. THE VALUE OF *Arbutus unedo* L. AS A BIOACTIVE INGREDIENT FOR SKIN APPLICATION: EXPLORING THE INTERREGIONAL VARIABILITY ACROSS NATIONAL TERRITORY

Pires, CP[1,2], Oliveira, AS[1,2], Gomes, CP[1,2], Marengo, A[3], Rubiolo, P[3], Palmeira-de-Oliveira, R[1,2,4], Palmeira-de-Oliveira, A[1,2,4(*)]

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ABSTRACT

The strawberry tree (*Arbutus unedo* L.) gained popularity for its antioxidative, anti-inflammatory, and regenerative properties. While the fruit is well-known, the leaves are often considered a waste product, despite being rich in bioactive compounds. This work studied three *A. unedo* aqueous extracts from Proença-a-Nova, Gouveia, and Odemira for their potential be to used as bioactive ingredients in cosmetic formulas. Phytochemical characterization was performed using UHPLC-PDA-MS/MS. Cell biocompatibility was assessed with the MTT assay, anti-inflammatory activity via nitric oxide (NO) production, healing potential through scratch-wound assay, antioxidant capacity with the DPPH radical scavenging assay, and anti-acne potential by measuring lipase activity. Antimicrobial activity was evaluated using a broth microdilution assay against a panel of relevant Gram-positive and negative bacteria, yeast and fungi, conserving a skin application. The main phenolic compounds found were derivatives of gallic acid and flavonoids in their glycosylated form (e.g. quercitrin). A maximum concentration of 0.5 mg/mL was biocompatible with the tested cell lines. The extracts from Odemira and Gouveia exhibited the strongest anti-inflammatory properties. None of the extracts demonstrate wound healing potential. All extracts exhibited very-strong antioxidant capacity (indexes between 4.41 and 6.61), and anti-lipase activity, with the Odemira extract being the most effective in inhibiting lipase. *C. acnes* was the most susceptible strain to all extracts (MIC 0.5 mg/mL). In conclusion, *Arbutus unedo* L. leaf extracts demonstrated promising potential for skin application, particularly considering an anti-acne use, due to their antimicrobial activity against *C. acnes*, anti-lipase, and anti-inflammatory potential.

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Keywords: *Acne vulgaris;* Antimicrobial activity; Antioxidant activity; Strawberry tree.

Poster Session

P1. SURVEY OF NUTRITIONAL HABITS AND EPIDEMIOLOGY OF THE STUDENT COMMUNITY FROM GUARDA CITY (PORTUGAL)
Filomena Carvalho, Sofia Silva Tavares, Radhia Aitfella Lahlou, Alexandra Varges, André R.T.S. Araujo, Cecília Fonseca, Luís R. Silva

P2. ANTIDIABETIC POTENTIAL AND BIOLOGICAL PROPERTIES OF A RED FRUITS FUNCTIONAL BEVERAGE
Alexandra Varges, Filomena Carvalho, Radhia Aitfella Lahlou, Luís Silva

P3. IMIQUIMOD-LOADED LIPIDIC NANOPARTICLE FOR SELECTIVE THERAPY AGAINST HUMAN PAPILLOMAVIRUS-ASSOCIATED DYSPLASIA
I. G. Maocha1, B. Rosado, J. Lopes-Nunes, M. Lopes, J. Rolo, B. Pires, E. Gallardo, A. Palmeira-de-Oliveira, J. Martinez de Oliveira, R. Palmeira-de-Oliveira, R. Medeiros, C. Cruz

P4. IN SILICO INTERACTION BETWEEN EGG LYSOZYME EPITOPES AND PHENOLIC COMPOUNDS
Inês I. Faria1, Cândida T. Tomaz, António G. Mendonça

P5. DEVELOPMENT OF A NATURAL GEL FOR RELIEVING TIRED LEGS IN PREGNANT WOMEN
Rita Silvestre de Andrade, Ana Rita Gama, José Martinez de Oliveira, Ana Palmeira de Oliveira, Rita Palmeira de Oliveira

P6. KETOPROFEN NANOEMULSIONS: DRUG REPURPOSING FOR POTENTIAL APPLICATION IN MEL-ANOMA TREATMENT
Matilde R. Rodrigues, Patrícia C. Pires, Francisco Veiga, Ana Cláudia Paiva-Santos

P7. PLGA-NANOPARTICLES FOR TOPICAL DELIVERY OF CLOBETASOL: FORMULATION DEVELOP-MENT AND PARTICLE SIZE AND PDI CHARACTERIZATION
Matilde S. Marques, Patrícia C. Pires, Antonio J. Guillot, Ana Melero, Francisco Veiga, Ana Cláudia Paiva-Santos

P8. DEVELOPMENT OF MICONAZOLE-LOADED NANOEMULSIONS AND NANOEMULGELS AS INNO-VATIVE THERAPEUTIC SYSTEMS FOR THE TREATMENT OF MELANOMA
Maria Araújo, Patrícia C. Pires, Francisco Veiga, Ana Cláudia Paiva-Santos

P9. NOVEL NANOCOSMETICS: CURCUMIN NANOEMULGEL DEVELOPMENT AND PHYSICO--CHEMICAL CHARACTERIZATION
Isabella Felipe, Patrícia C. Pires, Francisco Veiga, Ana Cláudia Paiva-Santos

P10. ANTHELMINTIC DRUG REPURPOSING IN CANCER THERAPY: FLUBENDAZOLE-LOADED NANOEMULGEL DEVELOPMENT FOR THE TOPICAL TREATMENT OF MELANOMA
Miriana Griesi, Patrícia C. Pires, Francisco Veiga, Ana Cláudia Paiva-Santos

P11. NIOSOMES ENCAPSULATING BEE VENOM AS A NOVEL ANTICANCER TREATMENT: FOR-MULATION DEVELOPMENT AND EVALUATION OF IN VITRO THERAPEUTICAL EFFICACY AN SAFETY
Maria Beatriz Pinto, Patrícia C. Pires, Ricardo C. Calhelha, Ana Rita Silva, Maria João Sousa, Miguel Vilas-Boas, Soraia I. Falcão, Francisco Veiga, Pooyan Makvandi, Ana Cláudia Paiva-Santos

P12. DRUG REPURPOSING AND NANOTECHNOLOGY IN GLIOMA TREATMENT: KETOPROFEN NANOEMULGEL DEVELOPMENT AND CHARACTERIZATION FOR NOSE-TO-BRAIN DELIVERY
Patrícia C. Pires, Francisco Veiga, Ana Cláudia Paiva-Santos

P13. DEVELOPMENT OF A NANOEMULSION FOR INTRAVENOUS ADMINISTRATION OF PARACETAMOL IN COMBINATION WITH KETOPROFEN
Fernando Silva, Francisco Veiga, Patrícia C. Pires, Amélia Vieira, Vera Moura, Ana Cláudia Paiva-Santos1

P14. PH-RESPONSIVE NANOPARTICLES FOR THE PROTECTION AND DELIVERY OF RNA TO LUNG CANCER CELLS
Tiago Graça, Bruno Baptista, Andreia S. R. Oliveira, Patrícia V. Mendonça, Arménio C. Serra, Jorge F. J. Coelho, Fani Sousa

P15. RECOMBINANT pre-miRNA-29B: EXTRACELLULAR PRODUCTION AND PURIFICATION
José Bento, Rita Carapito, Fani Sousa

P16. STUDY OF ULVA LACTUCA EXTRACT PROPERTIES FOR ITS APPLICATION IN ATOPIC DERMATITIS: SAFETY AND EFFICACY
Sofia Louro, Carolina Gomes, Ana Rita Gama, João Cotas, Leonel Pereira, José Martinez de Oliveira, Ana Palmeira de-Oliveira, Rita Palmeira-de-Oliveira

P17. PERIPHERAL PRIMING WITH LPS AND HISTAMINE PREVENTS DOPAMINERGIC DEGENERATION AND MOTOR DEFICITS IN A PARKINSON'S DISEASE MODEL
Ana Margarida Vale, Ana Clara Cristóvão, Liliana Bernardino

P18. NANODIAMONDS SELECTIVITY TOWARDS DNA AND RNA: EXPLOITING A TOOL FOR BIOPHARMACEUTICALS CAPTURE
Maria T. Gomes, Pedro L. Ferreira, Sónia A. C. Carabineiro, Joaquim L. Faria, Cláudia G. Silva, Fani Sousa

P19. OBESOGENIC DYSREGULATION OF PERIPROSTATIC ADIPOSE TISSUE TOWARDS PROSTATE CARCINOGENESIS
Mariana Feijó, César R. Casanova, Lara R.S. Fonseca, Endre Kiss-Toth, Sara Correia, Sílvia Socorro

P20. EXPLORING THE MICROBIAL DIVERSITY OF SWEET CHERRY FRUITS FROM PORTUGAL
Ana C. Gonçalves, José D. Flores-Félix, Amílcar C. Falcão, Gilberto Alves, Luís R. Silva

P21. *IN VITRO* AND *IN SILICO* EVALUATION OF PHARMACOKINETIC PROPERTIES OF PYRIMIDINE DERIVATIVES
Pedro Soeiro, Octávio Ferreira, Mariana Matias, Amílcar Falcão, Samuel Silvestre, Gilberto Alves

P22. COMPARATIVE STUDY OF LEUKOCYTE POPULATIONS OF PATIENTS WITH SARCOIDOSIS AND HYPERSENSITIVITY PNEUMONITIS: CONTRIBUTION OF BRONCHOALVEOLAR LAVAGE
Leonor Fernandes, Ana Mafalda Fonseca, Andreia Monteiro

P23. EXPLORING THE SWIPER MONOLITHIC COLUMN IN THE PURIFICATION OF A DNA VACCINE AGAINST CERVICAL CANCER
Matilde Costa, Ângela Sousa

P24. CHEMICAL SPECIATION AS BASE FOR THE EVALUATION OF 8-HQA AND ITS Ga3+ METAL COMPLEXES ACTION ON MICROBIOTA RADIATION RELATED RESISTANCE
Izabela Ryza, Claudia Granata, Nadia Ribeiro, Edyta Nalewajko-Sieliwoniuk, Beata Godlewska-Żyłkiewicz, Sandra Cabo Verde, Demetrio Milea, Sofia Gama

P25. ELECTROPHYSIOLOGICAL CHANGES IN MYOPIA WITH THETA BURST STIMULATION
Andresa Fernandes, Nuno Pinto, Bruno Ribeiro, Francisco Miguel Brardo, Maria Vaz Pato

P26. ELECTROPHYSIOLOGICAL TESTS IN MYOPIA: A SYSTEMATIC REVIEW OF RETINAL AND VISUAL PATHWAY FUNCTION
Bruno Ribeiro, Andresa Fernandes, Maria Vaz Pato, Francisco Miguel Brardo, Nuno Pinto

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Poster Session I

P1. SURVEY OF NUTRITIONAL HABITS AND EPIDEMIOLOGY OF THE STUDENT COMMUNITY FROM GUARDA CITY (PORTUGAL)

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ABSTRACT

Malnutrition in all its forms, such as undernutrition, micronutrient deficiencies, excess weight, and obesity, remains one of the biggest problems of our day. The most recent data reveals that over 821 million people are dying of hunger, 2 billion have micronutrient deficiencies and over 2 billion are overweight or obese. Although the underlying causes of malnutrition are complex and multifaceted, diets remain one of the main contributors. Our study analysed the nutritional habits and epidemiology of a student community from Guarda, a city in the interior region of Portugal. A survey was conducted among students from primary school to higher education, with questions about sleeping habits, physical exercise, eating patterns, and the use of substances, medicines and dietary supplements. A total of 1,950 students answered, 56.2% female and 43.8% male, aged between 6 and 58 years old: 1,629 from 1st to 12th grade and 321 from higher education. Results reveal that allergic diseases (19.7% of the community), pulmonary diseases (8.4%), and skin diseases (8.2%) are the most prevalent in this community. 86.5% of the students engage in some type of physical activity every week. The Body Mass Index (BMI) was calculated for children (ages 6 to 19) and for adults (from 19 years old). According to its classification, 3.6% of children are underweight, 16.5% are overweight and 13.4% are obese. Among adults, 4.1% are underweight, 17.5% are overweight and 9.7% are obese. These preliminary results will be further analysed to understand the relationship between dietary patterns/life habits and disease incidence.

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Keywords: Student community, Nutritional habits, Epidemiology, Body mass index.

P2. ANTIDIABETIC POTENTIAL AND BIOLOGICAL PROPERTIES OF A RED FRUITS FUNCTIONAL BEVERAGE

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ABSTRACT

Regular consumption of fruits, vegetables, and herbal products promotes health by reducing oxidative stress and inflammation, making them a promising strategy to mitigate chronic diseases like diabetes and boost the immune system. Red fruits are an important component of a healthy diet due to their high content of phenolic acids and flavonoids, especially anthocyanins. Blueberries (*Vaccinium* spp.) and cherries (*Prunus* spp.) are considered one of the five healthy foods certified by the International Food and Agriculture Organization (FAO) because they are rich in phenolic compounds, anthocyanins, and other nutrients. Their consumption has increased in recent years, partially due to the health benefits attributed to their phenolic content. Blueberries are composed of high levels of anthocyanins, flavonols, and flavan-3-ols, as well as benzoic and cinnamic acids [3]. The current study aims to develop a functional beverage and evaluate its antidiabetic and biological potential. A total of 6 beverages from red fruits were prepared and evaluated through various physicochemical tests, such as pH measurement, colour determination, and titratable acidity, as well as biological assays, including α-glucosidase inhibition and sensory analysis. The obtained results revealed that the pH values varied between 3.61 to 3.82, º brix 7.00 to 10.03, and the IC_{50} of the α-glucosidase inhibition varied between 30.10 and 66.43 uL/mL. The preliminary results have indicated that the functional beverage possesses great ability to inhibit α-glucosidase, suggesting a potential antidiabetic effect. Further biological assays and chemical characterization will be conducted to understand the health benefits of beverages from red fruits.

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Keywords: Functional Beverage; Red Fruits; Antidiabetic potential; Anthocyanins.

P3. IMIQUIMOD-LOADED LIPIDIC NANOPARTICLE FOR SELECTIVE THERAPY AGAINST HUMAN PAPILLOMAVIRUS-ASSOCIATED DYSPLASIA

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ABSTRACT

Human papillomavirus (HPV)-associated cervical cancer remains the most prevalent cancer among women globally, with current treatments often leading to increased infertility. Imiquimod (IQ), an imidazoquinoline with proven antiviral effects against persistent HPV infections, activates immune cells via Toll-like receptors 7/8 when formulated in carriers like nanogels for topical application. Nanoparticle drug delivery systems present a promising alternative to conventional therapies. This study explores lipidic nanoparticles loaded with IQ (Lipo IQ) and functionalized with a DNA aptamer, AT11 (Lipo IQ AT11), aimed at enhancing selectivity for cervical cancer cells, combined with the efficacy of essential oils. The formulations showed physicochemical and physiological properties suitable for vaginal drug administration and demonstrated antimicrobial activity at higher concentrations (MIC50 from 0.625%). The final formulations exhibited selective cytotoxicity, significantly reducing cell viability in HeLa cancer cells to less than 10%, while maintaining over 60% viability in normal human dermal fibroblast (NHDF) cells. Essential oils enhanced the cytotoxic effect of Lipo IQ, and the presence of AT11 increased the selectivity towards cervical cancer cells. Permeation assays indicated that these formulations were effectively internalized by cancer cells. The synergistic effect of essential oils and the nanoparticle system potentiated the cytotoxic impact of Lipo IQ, while Lipo IQ AT11 further promoted targeted selectivity towards cancer cells. Overall, these results suggest that Lipo IQ, combined with essential oils and AT11, offers a potent and selective treatment strategy for HPV-associated cervical lesions, potentially reducing the adverse effects associated with conventional therapies.

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Keywords: Human Papillomavirus, DNA-Aptamer, Liposomes, Imiquimod.

P4. IN SILICO INTERACTION BETWEEN EGG LYSOZYME EPITOPES AND PHENOLIC COMPOUNDS

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ABSTRACT

Eggs contain approximately 3% lysozyme (Lyz), a globular and thermolabile protein with high enzymatic and bacteriostatic activities. Around 30% of individuals allergic to hen eggs are sensitized to Lyz. Phenolic compounds (PC), which are abundant in various plant-based foods and beverages, have been shown to interact with proteins, forming soluble or insoluble complexes, potentially altering their native structure and properties. Understanding the interactions between PC and Lyz may provide insights into the potential modulation of Lyz allergenicity. In this work, the interaction of three common PC (quercetin, tannic acid and caffeic acid) with Lyz was studied using *in silico* simulations. The binding interactions were simulated using SwissDock and visualised with ChimeraX to verify whether there was binding to known Lyz epitopes (AA1-18, AA51-61, AA112-129 e AA107-116). The findings suggest that in all cases, the PC can bind non-covalently to Lyz epitopes. These interactions are thermodynamically favoured (dG < 0), as computed by Swissdock, and can contribute to changing Lyz allergenicity, potentially affecting its recognition by IgE antibodies. Understanding the relationship between PCs and Lyz allergenicity could help develop strategies to mitigate egg allergies, such as using PC-rich extracts or modifying food processing methods to enhance PC-protein interactions.

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Keywords: Lysozyme; Egg; Epitopes; Phenolic compounds.

P5. DEVELOPMENT OF A NATURAL GEL FOR RELIEVING TIRED LEGS IN PREGNANT WOMEN

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ABSTRACT

During pregnancy, women experience numerous physiological, hormonal, and cognitive changes. These include significant skin alterations such as hyperpigmentation, stretch marks, and changes in hair and nails. Cosmetic products tailored for pregnancy are important for managing the unique skin and body care challenges during this period. This work aimed to evaluate the Portuguese cosmetic market for pregnant women and based on this information, develop a cosmetic product for this population. A market study was conducted from June to December 2023, analyzing the cosmetic products available on the national market sourced from 32 online websites. A natural gel formulation specifically for tired legs in pregnant women was developed. Gel preliminary stability was evaluated through the centrifugation test (3000 RPM, 30min), and temperature cycle test (alternating 4ºC and 40ºC, daily, for 4 weeks), by monitoring the organoleptic characteristics and pH over time. Rheological characterization was conducted using a cone-plate viscometer, with measurements performed under controlled temperature conditions (T=25 °C ± 2 °C) for 1 minute (>5 cone revolutions). The market study highlights the need for new products for tired legs, specifically designed for pregnant women. Therefore, we developed a natural and minimalist gel formulation that exhibits a characteristic gel texture, is colorless and odorless, and contains 99.2% of natural ingredients. Gel stability was maintained through time. Rheological characterization classifies the gel as a non-Newtonian fluid with pseudoplastic behavior. The development of the gel based on market research was successfully achieved.

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Keywords: Pregnancy; Market study; Cosmetics; Gel.

P6. KETOPROFEN NANOEMULSIONS: DRUG REPURPOSING FOR POTENTIAL APPLICATION IN MELANOMA TREATMENT

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ABSTRACT

Drug repurposing aims to reuse well-known drugs, frequently targeting oncology applications. In melanoma research, non-steroidal anti-inflammatory drugs like ketoprofen (KET) are extensively investigated. However, KET's poor water solubility (0.0213 mg/mL) poses a challenge for formulating it at high strength. To address this issue, the objective of this work was to incorporate KET into an oil-in-water nanoemulsion, for topical administration, for melanoma treatment. Various nanoemulsions were created using Lauroglycol™ 90, Transcutol® HP, Tween® 80, and water. Vehicle formulations were characterized for mean droplet size and polydispersity index (PDI), by dynamic light scattering (Zetasizer Nano ZS), as well as loaded formulations, containing different quantities of KET, to assess the drug strength at which they maintained optimal characteristics (PDI < 0.300). The most promising nanosystem served as a base for creating nanoemulsions with varying oil-to-water ratios. These were further studied for pH, zeta potential (Zetasizer Nano ZS) and *in vitro* drug release (Franz cells). Results indicated that KET's solubility could be increased up to 20 mg/mL, with all formulations having zeta potential values between -9.40 and -6.38 mV, and a pH of 4, confirming their potential compatibility with topical application. Drug release assays showed oil-to-water ratio dependent outcomes, with release percentages ranging from 30% to 95% at the 24-hour mark. Hence, the developed nanoemulsions depicted good characteristics for topical application, being potentially beneficial for enhanced bioavailability in melanoma treatment. Further studies will include long-term and accelerated stability studies, *ex vivo* permeation and the evaluation of *in vitro* cytotoxicity in melanoma cell models.

Keywords: Drug repurposing, Ketoprofen, Melanoma, Nanoemulsion.

P7. PLGA-NANOPARTICLES FOR TOPICAL DELIVERY OF CLOBETASOL: FORMULATION DEVELOPMENT AND PARTICLE SIZE AND PDI CHARACTERIZATION

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ABSTRACT

Inflammatory skin diseases, such as dermatitis, psoriasis, and eczema, can significantly impact patients' quality of life due to symptoms caused by an overactive immune response. Clobetasol, a potent corticosteroid drug, is commonly used to relieve these symptoms by reducing inflammation and suppressing immune responses. However, prolonged use can lead to adverse effects such as skin atrophy, hypopigmentation, Cushing-like syndrome, and steroid acne. To tackle these issues, nanotechnology can be of use, by enhancing drug strength, stability and controlled release, offering higher therapeutic efficacy and safety. Hence, in this work clobetasol-loaded poly lactic-co-glycolic acid (PLGA) nanoparticles were developed for the topical treatment of inflammatory skin diseases. Nanoparticles were prepared by nanoprecipitation, using PLGA (polymer), polyvinyl alcohol (stabilizer), acetone (solvent), and clobetasol (drug). Particle size, polydispersity index (PDI), and zeta potential were determined using a Zetasizer Nano ZS apparatus. The resultant optimized nanoparticles exhibited an average particle size of 232 ± 20 nm, PDI of 0.18 ± 0.06, and a slightly negative surface charge (- 6.94 ± 2.55 mV), with a high drug strength. The current data shows that it was possible to develop small and homogeneous PLGA nanoparticles, containing high amounts of clobetasol, which are properties predictive of an optimal topical drug absorption. Further studies will be conducted to incorporate these nanoparticles into a hydrogel, which will be characterized in terms of *in vitro* drug release, *ex vivo* drug permeation and *in vitro* therapeutic efficacy.

Keywords: PLGA nanoparticles; Clobetasol; Inflammatory skin diseases, Topical delivery.

P8. DEVELOPMENT OF MICONAZOLE-LOADED NANOEMULSIONS AND NANOEMULGELS AS INNOVATIVE THERAPEUTIC SYSTEMS FOR THE TREATMENT OF MELANOMA

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ABSTRACT

Although topical drug delivery is many times the go-to approach for the treatment of skin diseases, conventional therapies' effectiveness and safety can sometimes be inadequate. In this study, nanoemulsions encapsulating miconazole were developed, having Transcutol®, Kolliphor® RH 40 and Plurol® Diisostearique in their composition (oils, surfactants and co-surfactants/co-solvents), as well as water, which were later transformed into nanoemulgels, with the addition of Carbopol® 940 as a gelling agent, for maximized miconazole drug strength, for the topical treatment of melanoma. Emulsification was obtained by the phase inversion method, and each developed formulation's droplet size and polydispersity index (PDI) were measured, using dynamic light scattering (Zetasizer apparatus). Results showed that for the nanoemulsions the PDI increased with increased water phase content (from 0.18 to 0.27), with the same happening for the correspondent droplet size (from 173 nm to 191 nm). On the other hand, the PDI values for the developed nanoemulgels was the lowest for the highest oil phase content (0.24), accompanied by a slight increase in droplet size values (225 nm). A high drug strength was achieved for drug-loaded formulations (9 mg/mL), and, despite the described tendencies, final formulations all depicted good PDI and droplet size values (PDI below 0.3, and droplet size between 100 and 200 nm). Using these promising results as a starting point, more assays will be performed in the future, such as rheology, *in vitro* drug release, and *in vitro* cytotoxicity in melanoma cell models, in order to assess the true potential of the developed nanosystems.

Keywords: Melanoma; Nanoemulsion; Nanoemulgel; Miconazole.

P9. NOVEL NANOCOSMETICS: CURCUMIN NANOEMULGEL DEVELOPMENT AND PHYSICOCHEMICAL CHARACTERIZATION

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ABSTRACT

Curcumin, a natural compound extracted from the rhizome of *Curcuma longa*, has several reported bioactivities, including anti-inflammatory and antioxidant effects. Nevertheless, due to high lipophilicity, it is insoluble in water, which poses a difficulty for formulation aspects. To tackle this issue, in this work curcumin was loaded into an oil-in-water nanoemulsion, for topical application, for cosmetic purposes. Excipients were selected according to their capacity for solubilizing the compound at highest strength, and thus Labrafac PG® (hydrophobic surfactant), Tween® 80 (hydrophilic surfactant) and Transcutol P® (co-surfactant and co-solvent) were chosen. Then, various proportions were tested, and for each obtained nanoemulsion a visual observation was performed, in order to identify possible instability signs (such as immediate or rapid phase separation), and droplet size and polydispersity index (PDI) were measured, by dynamic light scattering, using a Zetasizer apparatus. Being that the recommended average droplet size range for topical application is between 150 and 350 nm, selected formulations had a droplet size bellow 300 nm, and a PDI below 0.3, for adequate size distribution homogeneity, a parameter that can influence not only formulation stability, but also biological performance after formulation application. Additionally, although some formulations presented a change in their visual aspect after storage (from transparent to translucent, and vice-versa), observed phase separation was reversible through new homogenization (flask shaking). Future studies will assess these formulation's stability through objective measurements (instability index and creaming velocity), as well as determine their *in vitro* release and bioactivity in cell models.

Keywords: Cosmetic; Curcumin; Nanoemulgel; Nanocosmetic.

P10. ANTHELMINTIC DRUG REPURPOSING IN CANCER THERAPY: FLUBENDAZOLE-LOADED NANOEMULGEL DEVELOPMENT FOR THE TOPICAL TREATMENT OF MELANOMA

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ABSTRACT

Flubendazole is a broad-spectrum anthelmintic drug that has demonstrated antitumor activity in different types of cancer in recent years, including skin cancer. Nevertheless, its challenging biopharmaceutical properties, such as poor solubility in water and various organic solvents, represents an issue in formulating this drug at high strength. For this reason, in the present work flubendazole was incorporated into an oil-in-water nanoemulgel, for topical administration, for the treatment of melanoma. The first part of the work focused on preformulation studies, namely by performing a screening of excipients according to the drug's solubility. Then, a preliminary oil-in-water nanoemulsion, composed of Imwitor® 988, Labrasol® ALF, Transcutol® HP and water was developed, by spontaneous emulsification. The developed nanoemulsion was characterized for PDI (0.207) and droplet size (193.5 nm), and drug incorporation led to similar values (PDI of 0.209 and droplet size of 204.1 nm), demonstrating that drug loading had no negative impact on these measured parameters. Afterwards, the water phase was replaced with Carbopol® ETD 1% (in acetate buffer, pH 5, for adequate compatibility with the skin), leading to the formation of a nanoemulgel, with the inclusion of this gelling agent meaning to lead to increased viscosity and improved spreadability and skin adherence. The developed nanoemulgel also depicted adequate stability in accelerated and real-time stability studies, proving to be more stable than the preliminary nanoemulsion. Future studies shall be conducted (*in vitro* drug release, *ex vivo* drug permeation, *in vitro* cytotoxicity) to further characterize the developed formulation.

Keywords: Drug repurposing; Melanoma; Nanoemulgel; Topical administration.

P11. NIOSOMES ENCAPSULATING BEE VENOM AS A NOVEL ANTICANCER TREATMENT: FORMULATION DEVELOPMENT AND EVALUATION OF *IN VITRO* THERAPEUTICAL EFFICACY AN SAFETY

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ABSTRACT

Cancer remains a highly incident and lethal disease, with current treatments often lacking efficiency and safety, thus it is imperative to develop new, more efficient, and safer therapies. Bee venom has proven to have significant antitumor effects, but some toxicity issues have been associated with its administration. To tackle these issues, bee venom-loaded niosomes were developed, for cancer treatment, which had a small (150 nm) and homogeneous (PDI of 0.162) particle size, and good therapeutic efficacy in *in vitro* breast, cervical, colorectal, gastric, and lung cancer models (inhibitory concentrations of 12.37 - 14.72 ng/mL), with complementary anti-inflammatory activity (inhibitory concentration of 28.98 ng/mL). Niosome safety was also confirmed, with both *in vitro* (liver, kidney and skin cell lines) and *ex vivo* (hen's egg chorioallantoic membrane) results showing that compound encapsulation increased its safety upon application. Therefore, bee venom-loaded niosomes were successfully developed, with a small and homogeneous size, and relevant anticancer and anti-inflammatory activity, making them potentially promising primary or adjuvant cancer therapies. Future research shall focus on assessing the true potential of the developed nanosystem in *in vivo* models.

Keywords: Anti-inflammatory; Anticancer; Bee venom; Niosomes.

P12. DRUG REPURPOSING AND NANOTECHNOLOGY IN GLIOMA TREATMENT: KETOPROFEN NANOEMULGEL DEVELOPMENT AND CHARACTERIZATION FOR NOSE-TO-BRAIN DELIVERY

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ABSTRACT

In previous studies, the anti-inflammatory drug ketoprofen has shown *in vitro* efficacy against glioma, but the developed formulations have relevant limitations, including being for intravenous administration only, and having achieved low drug strength. To solve these issues, this work aimed to use drug repurposing and nanotechnology to develop a novel high drug-loading and non-invasive therapy, more specifically an intranasal ketoprofen nanoemulgel, containing Capryol® 90, Tween® 80, Transcutol®, and Pluronic® F-127. Droplet size, polydispersity index (PDI), zeta potential, pH, osmolality, viscosity, accelerated stability and *in vitro* drug release were assessed. Results showed that Pluronic addition to lead nanoemulsions led to a significant droplet size and PDI reduction (176 to 22 nm, and 0.3 to 0.1, respectively), and that final formulations had high drug strength (4 mg/mL, more than 50-fold higher than the drug's aqueous solubility). They also had low viscosity under refrigeration, and very high viscosity at mean nasal temperature, due to being in true gel form (mean gelling temperatures between 14 and 22 ºC), making them ideal for nasal instillation after refrigeration, and possibly leading to a longer retention and enhanced drug absorption in the nasal mucosa after administration. High formulation stability (instability indexes between 0.130 and 0.265), and a controlled high cumulative drug release after 24 h (78 to 93%), fitting a Korsmeyer-Peppas kinetic model, was also observed. Hence, a ketoprofen-loaded intranasal nanoemulgel having good target characteristics was successfully prepared. Future studies will assess its true potential for brain cancer treatment in *in vitro* cytotoxicity studies.

Keywords: Drug repurposing; Glioma; Intranasal; Nanoemulgel.

P13. DEVELOPMENT OF A NANOEMULSION FOR INTRAVENOUS ADMINISTRATION OF PARACETAMOL IN COMBINATION WITH KETOPROFEN

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ABSTRACT

Parenteral formulations allow the administration of drugs with poor oral bioavailability or in clinical circumstances where the oral route is not available. Nevertheless, poor solubility of the active pharmaceutical ingredients can be a substantial challenge during pharmaceutical development. Nanoemulsions are considered an advanced pharmaceutical technology for drug delivery in parenteral formulations, since they allow to solubilize poor water-soluble drugs in their oil phase. The combination of paracetamol with nonsteroidal Anti-Inflammatory Drugs (NSAIDs) is well-stablished in clinical practice to avoid the use of opioid drugs. Despite this, in the pharmaceutical market only one parenteral fixed-dose combination containing ibuprofen with paracetamol is available. In this work, we present the development of a new parenteral fixed-dose combination with ketoprofen and paracetamol. Since ketoprofen is practically insoluble in water, we propose the encapsulation of both drugs in a nanoemulsion formulation produced by self-emulsification. The composition of the blank nanoemulsion was optimized by design of experiments (DoE) (Labrafac® WL 1349/Kolliphor® HS 15/Transcutol® HP = 24/52/24% w/w) and the maximum solubility of each drug in this nanoemulsion system was determined (30 mg/mL for paracetamol and 70 mg/mL for ketoprofen). After that, both drugs were combined (25 mg/mL of paracetamol + 5 mg/mL of ketoprofen) and preliminary assays were performed to assess the stability of this nanoemulsion system concerning its droplet size (140 ± 16 nm) and polydispersity index (0.11 ± 0.02). Our results demonstrate that it is possible to encapsulate paracetamol plus ketoprofen in a nanoemulsion stable for at least 1 month.

Keywords: Nanoemulsion; DoE; Fixed-dose combination; Parenteral.

P14. PH-RESPONSIVE NANOPARTICLES FOR THE PROTECTION AND DELIVERY OF RNA TO LUNG CANCER CELLS

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ABSTRACT

Nanoparticles are receiving increased attention due to their significance in delivering biopharmaceuticals for gene-based therapies. At the same time, RNA is being studied to develop new and advanced treatments. However, the successful therapeutic application of these biomolecules is highly dependent on their effectiveness to reach target cells. The development of pH-responsive nanoparticles may improve delivery efficiency in certain diseases that present a microenvironment with deregulated pH, such as lung cancer. In this work, it is explored the pH responsiveness and efficiency of a copolymer composed of poly(2-(diisopropylamino)ethyl methacrylate)) (PDPA) and poly(oligo(ethylene oxide)methyl ether methacrylate) (POEOMA) for encapsulation and release of small RNAs (sRNA). The encapsulation process relies on the electrostatic interaction between the negatively charged sRNA and the protonated tertiary amine groups of the PDPA segment. Three copolymers with different architectures (linear or 4-arm star-shaped) and compositions (4-arm star PDPA-b-POEOMA or 4-arm star POEOMA-b-PDPA) were synthesized by atom transfer radical polymerization. The sRNA encapsulation and release efficiencies were studied by UV spectroscopy and agarose electrophoresis. The best formulations were further characterized regarding the size and surface charge of the polyplexes, by DLS and ELS, respectively. Depending on the copolymer, concentration, and pH of the formulation, the systems achieved complexation efficiencies ranging from 35% to 84%. The polyplexes presenting higher encapsulation efficiencies had sizes ranging from 92 to 104 nm. It was also possible to understand the changes in the nanoparticle's structure at different pH values with TEM. This research demonstrates the potential of pH-sensitive polymers as promising RNA delivery systems.

Keywords: Nanoparticles; pH-responsive polymers; POEOMA-b-PDPA; Small RNAs.

P15. RECOMBINANT pre-miRNA-29B: EXTRACELLULAR PRODUCTION AND PURIFICATION

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ABSTRACT

The field of RNA-based therapeutics has seen rapid and exciting advancements regarding the treatment of various diseases. Pre-miRNA-29b is already known to be involved in one of Alzheimer's disease mechanisms. Large-scale RNA production for biopharmaceutical applications consists of a complex biotechnological process that needs efficient manufacturing strategies and rigorous quality control, to assure RNA purity and biological activity. However, the most used methods have some limitations regarding the quantity and quality of the RNA produced. Given this, recombinant production can be one way to overcome some of these challenges, since it is a more cost-effective method and easier to scale up. *Rhodovulum sulfidophilum*, a marine purple bacterium, offers the advantage of secreting RNAs to the extracellular medium, without secreting RNases. Given this, RNA recovery is significantly simplified compared to standard intracellular extraction protocols, greatly enhancing the further purification process, which is the most critical step. This work focused on the optimization of the extracellular production and subsequent purification of recombinant pre-miRNA-29b. The bacterial culture conditions were optimized to ensure maximum levels of the target molecule, testing different culture times and temperatures. In general, higher pre-miRNA-29b levels were achieved after 36 h of culture at 30 ºC. For pre-miRNA-29b purification, a multimodal chromatographic support was selected, being verified its high performance at selectively interacting with the target RNA. Considering the promising results, it was used Design of Experiments to refine the purification process. The parameters for optimization were NaCl concentration during the equilibration and elution step, as well as buffer pH.

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Keywords: Recombinant production; *Rhodovulum sulfidophilum*; pre-miRNA-29b; Purification.

P16. Study of Ulva lactuca extract properties for its application in Atopic Dermatitis: Safety and Efficacy

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ABSTRACT

Atopic Dermatitis (AD) is a chronic and relapsing inflammatory skin disorder. To fight against the eczema crisis, it is essential to maintain hydration and protection. Plant extracts have demonstrated properties that can be beneficial in managing various skin conditions. Particularly, alga's antioxidant and anti-inflammatory properties can have a preponderant and active role in AD. We evaluated four extracts of the Ulva lactuca (Chlorophyta) species with different solvents for their safety and efficacy. In this way, we use an aqueous extract, one methanolic and two ethanolic extracts, at 40% and 96% concentrations. The cellular biocompatibility was evaluated in the cellular line L929 (fibroblasts) through the MTT assay to determine the biocompatible concentration to be used in the following tests. The antioxidant capacity of the extracts was studied and compared using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method, and the skin-repairing potential of biocompatible concentrations was also evaluated on L929 cells using the scratch-wound assay. Based on the cytotoxicity results, we select a 25% (v/v) concentration of the ethanolic extract and 12.5% (v/v) for the other extracts studied. Regarding the antioxidant activity, all four extracts showed poor antioxidant capacity, but the aqueous extract was the one that showed more activity. In the skin repair potential tests, the methanolic extract demonstrated the ability to promote cell migration. These preliminary results show that the aqueous extract demonstrated more interesting results in our studies. Despite further studies being necessary to corroborate these in vitro results, the studied ingredients present an interesting potential for application in DA products.

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Keywords: Alga Extracts; Antioxidant capacity; Cell biocompatibility; Ulva lactuca.

P17. PERIPHERAL PRIMING WITH LPS AND HISTAMINE PREVENTS DOPAMINERGIC DEGENERATION AND MOTOR DEFICITS IN A PARKINSON'S DISEASE MODEL

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ABSTRACT

Several studies suggest that peripheral inflammatory priming has the ability to induce neuroprotection in various pathologies, such as Alzheimer's Disease and stroke. Among the studied stimuli, lipopolysaccharide (LPS) is the most extensively characterized, with evidence showing that administering low doses for a short period can induce neuroprotection. Additionally, our research group has demonstrated that histamine can act as an inflammatory agent. Therefore, our aim was to investigate the effects of the peripheral priming with LPS and histamine in the ability to induce innate immune response and, consequently, to promote neuroprotection of dopaminergic neurons in the 6-OHDA induced mouse model of Parkinson's Disease (PD). Adult C57BL/6J male mice received 4 consecutive intraperitoneal daily injections with LPS or histamine and, after 3 weeks, were exposed to intrastriatal injury with 6-hydroxydopamine (6-OHDA) for further 2 weeks. Markers of astrocytic response, namely GFAP, and of neuronal plasticity induced by BDNF were evaluated by western blot in the substantia nigra (SN) and striatum (ST). Dopaminergic survival was evaluated by immunohistochemistry for tyrosine hydroxylase (TH), while motor behavior was assessed by the apomorphine test. We found that peripheral priming with LPS and histamine induce astrocytic reactivity in the SN. Additionally, both inflammatory stimuli induced neuroprotection of dopaminergic neurons in a PD model, accompanied by functional motor recovery. Therefore, out data suggest that previous peripheral priming with LPS and histamine can trigger following dopaminergic neuroprotection and motor functional improvement in a 6-OHDA model of PD.

Keywords: Parkinson's disease; Histamine; Lipopolysaccharide; Neuroprotection.

P18. NANODIAMONDS SELECTIVITY TOWARDS DNA AND RNA: EXPLOITING A TOOL FOR BIOPHARMACEUTICALS CAPTURE

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ABSTRACT

The therapeutic potential of RNA has been unveiled since its discovery. Notwithstanding, for RNA to be used for such applications, it has to present high purity, integrity, and biological activity. The production of nucleic acids involves multiple steps, and while improvements have been made in upstream processing, the same is not directly observed for downstream processing. Downstream processing involves the recovery, concentration, and purification of the product, and encompasses three main steps: initial recovery, high-resolution purification, and polishing. The higher the product purity level achieved in the low-resolution purification, the greater the potential for reducing high-resolution purification steps. This is important, as downstream processing accounts for nearly 80% of the total production costs. As such, an optimization of an adsorption method for the pre-purification of RNA, using different carbon materials (CM) as adsorbents was carried out. A simple and efficient method was developed using the most promising CM for the selective capture and recovery of RNA from *E. coli* lysates, containing impurities such as pDNA and proteins. Particularly, oxidised nanodiamonds (ND-ox) were the most promising materials, allowing an adsorption capacity of 86.9 mg of RNA/g of CM, and presenting selectivity towards RNA adsorption in the presence of pDNA. Moreover, an elimination of solubilized proteins of 91.28 % relative to the initial sample was achieved. Globally, the results of this work demonstrated that ND-ox can be used as an adsorbent, being capable of selectively capturing RNA and enabling its complete recovery without contamination.

Keywords: RNA; Solid-phase extraction; Purification; Carbon materials.

P19. OBESOGENIC DYSREGULATION OF PERIPROSTATIC ADIPOSE TISSUE TOWARDS PROSTATE CARCINOGENESIS

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ABSTRACT

The "obese" periprostatic adipose tissue (PPAT) has been implicated in the aggressiveness of prostate cancer (PCa), with the dysregulation of adipokines and chemokines' secretion significantly contributing to a tumour-promoting microenvironment. Evidence also links obesity with environmental influences, namely, by the action of the so-called obesogens, i.e. endocrine-disrupting chemicals capable of dysregulating adipose tissue and promoting fat accumulation. Herein, we hypothesize that obesogens alter the PPAT phenotype, driving prostate carcinogenesis. PPAT isolated from 4-month-old rats was exposed *ex vivo* to the obesogen tributyltin (TBT, 100 nM) for 48 h. The morphological features of PPAT and its secretome were evaluated by histological analysis and colorimetric assays. Non-neoplastic human prostate cells (PNT1A) were exposed to the secretome of TBT-treated PPAT (and control) for 24 h. PNT1A cell fate was evaluated by MTT assays, Ki-67 immunocytochemistry, caspase-3-like activity, scratch assay, and Western blot analyses. TBT promoted adipocyte enlargement, accompanied by alterations in its secretome: increased leptin and C-C motif chemokine ligand 7 and decreased adiponectin levels. TBT-deregulated PPAT secretome increased the viability, proliferation, and migration of PNT1A cells whereas suppressing apoptosis, as evidenced by reduced caspase-3-like activity. These alterations were also underpinned by the increased expression of tribbles pseudokinase 1, phosphorylated extracellular signal-regulated kinase 1/2, and C-C motif chemokine receptor 3. The present study highlighted the role of the environment-obesity-prostate cells triad in fuelling PCa onset. It also brings functional evidence about the capability of TBT as a driving force in prostate carcinogenesis by disrupting the cell fate balance of non-neoplastic cells.

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Keywords: Obesogens; Periprostatic adipose tissue; Prostate cancer; Tributyltin.

P20. EXPLORING THE MICROBIAL DIVERSITY OF SWEET CHERRY FRUITS FROM PORTUGAL

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ABSTRACT

Antibiotic efficacy has declined in recent years. Additionally, as fruits and vegetables are mostly eaten fresh, it is pivotal to consider them as vectors for the transmission of pathogenic microorganisms associated with various disease outbreaks. Thus, it is not surprising that the interest in the microbial community of fruits and vegetables has increased worldwide. Indeed, investigating the microbiome of foods is critical for food safety, consumption, growth control, and for identifying new sources of bioactive compounds and beneficial bacteria. Among fruits, *Prunus avium* fruits, i.e., sweet cherries, have been a focus of several studies, chiefly due to their medicinal and health-promoting properties. Hence, given the absence of data about sweet cherries microbiology, the present study aimed to assess the microbial ecology of Portuguese sweet cherry fruits over almost 3 months. For such, there were cultivated homogenates of sweet cherries on different broths. A total of 125×10^3 UFC/g bacteria and 212×10^2 UFC/g fungi were found on Trypticase soy agar and Sabouraud-Cloramphenicol media, respectively. To deepen the obtained data, the selected isolates were molecularly identified by amplifying the 16S rRNA gene and ITS region. A total of 22 different bacteria and 33 fungi were identified. Genera *Pseudomonas* and *Ralstonia* were the most prevalent bacteria strains, while *Metschnikowia*, *Aureobasidium*, and *Hanseniaspora* were the most abundant fungi genera. The obtained data revealed that fruits ripen showed higher microbial diversity. The present investigation opens doors for further studies to explore interactions between microbial species and the microbiological safety of these fruits.

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Keywords: Sweet cherries; Microorganisms; Microbial diversity; Culturome.

Poster Session II

P21. *IN VITRO* AND *IN SILICO* EVALUATION OF PHARMACOKINETIC PROPERTIES OF PYRIMIDINE DERIVATIVES

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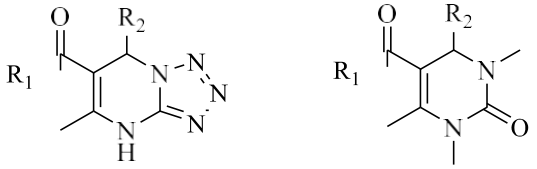
ABSTRACT

Epilepsy is a neurological disorder characterized by recurring seizures that impact the patient's quality of life. Even with the existence of several antiepileptic drugs (AEDs), with different pharmacokinetic and pharmacodynamic profiles, about one-third of patients presents refractory epilepsy. Therefore, the development of new AEDs is crucial to improve the pharmacotherapy ofthe disease. In this context, new anticonvulsant drug candidates with heterocyclic scaffolds have been studied. Among the heterocyclic scaffolds, 48 different pyrimidine derivatives, branched between dihydrotetrazolopyrimidines (DHTTPM) and dimethyldihydropyrimidines (MeDHPM) families, were produced (Figure 1). In order to select the most promising compounds for further *in vivo* studies, *in vitro* evaluation of their pharmacokinetic properties was performed. DHTTPM and MeDHPM's apparent permeability (P_{app}) and interference on the P-glycoprotein (P-gp) drug efflux transporter were determined, through parallel artificial membrane permeability (PAMPA) and cell-based assays, respectively. Overall, 90% of the compounds are expected to have good intestinal permeability ($P_{app} > 1.1\times10^{-6}$ cm/s) and most of them did not modulate P-gp at the tested concentrations of 10 and 50 µM. Additionally, DHTTPM and MeDHPM with 2,3-, 2,4- Cl_2-phenyl, 4-NO_2-phenyl or 3-pyridyl groups presented a low apparent permeability ($P_{app} < 1.1\times10^{-6}$ cm/s). Compounds with 2,3- or 2,4-Cl_2-phenyl also demonstrated a tendency to induce P-gp activity. To obtain a greater information about these pyrimidines and for a better understanding of the obtained results, *in silico* predictions of the pharmacokinetic and toxicity properties were estimated in the online Deep-PK software.



DHTTPM **MeDHPM**

R_1 = OCH_3, OCH_2CH_3; **R_2** = phenyl, 4-CH_3-phenyl, 4-OCH_3-phenyl, 3-OH-phenyl, 2,3-Cl_2-phenyl, 2,4-Cl_2-phenyl, 2,3-F_2-phenyl, 4-NO_2-phenyl, 3-pyridyl, 3-indolyl, 2-furyl, 5-CH_3-2-furyl, 5-Cl-2-furyl, 2-pyrrolyl, 2-thiophenyl, 5-Cl-2-thiophenyl.

Figure 1: General structure of pyrimidine derivatives studied.

Keywords: Epilepsy; Pyrimidine derivatives; Pharmacokinetics; PAMPA.

P22. COMPARATIVE STUDY OF LEUKOCYTE POPULATIONS OF PATIENTS WITH SARCOIDOSIS AND HYPERSENSITIVITY PNEUMONITIS: CONTRIBUTION OF BRONCHOALVEOLAR LAVAGE

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ABSTRACT

Interstitium lung diseases include sarcoidosis and hypersensitivity pneumonitis (HP), and are a group of lung diseases characterised by damage to the cells surrounding the alveoli. In these pathological contexts, the analysis of Bronchoalveolar Fluid (BAL) is important. This study aims to analyse the differences in the cellular profile of patients with sarcoidosis and HP, taking into account various factors such as age, sex and smoking habits. The sample, chosen for convenience, was made up of patients from the Clinical Pathology Service of Centro Hospitalar Universitário da Cova da Beira between 2015 and 2018. The cellular profile present in the BAL fluid of these patients was analysed by flow cytometry, and the SPSS® platform was used for the analysis. It was found that there are statistically significant differences in the cellular profile of total lymphocytes, T lymphocytes, CD8 T lymphocytes, CD4 T lymphocytes, CD4/CD8 ratio, NKT lymphocytes and NK lymphocytes between sarcoidosis and PH patients. It was also found that smoking habits mainly influence the frequency of NK and NKT cells in BALF and that sex is not a statistically significant variable in the pathologies analysed. The results obtained suggest that analysing BAL fluid provide fundamental information for diagnosing lung pathologies.

Keywords: Interstitium lung diseases; Bronchoalveolar lavage; Sarcoidosis; Hypersensitivity Pneumonitis.

23. EXPLORING THE SWIPER MONOLITHIC COLUMN IN THE PURIFICATION OF A DNA VACCINE AGAINST CERVICAL CANCER

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ABSTRACT

Cervical cancer is one of the major cause of death among women and is the fourth most prevalent cancer globally. This cancer is straitly related to the persistent infection of the human papillomavirus (HPV). The virus's E6 and E7 oncoproteins play a crucial role in this connection by promoting the degradation of the p53 and pRb proteins, respectively. Recently, nucleic acid vaccines have shown significant promise in addressing public health threats. DNA vaccines, in particular, offer several advantages: they induce both humoral and cellular immune responses, are more stable compared to other nucleic acids, are free from infectious agents, and can be easily produced in large scale. The main challenge in the manufacturing of a DNA vaccine is the purification step, which can be associated with high cost and low yield. In the last few years, monolithic columns have gained popularity due to its unique characteristics and versatility. This column offers several advantages, including the ability to operate at high flow-rates, resulting in faster separations, and the ability to retain a wide range of molecules. Additionally, their large channel size and convection-based transfer enables flow-independent performance, high sample recovery, and rapid processing times, all while maintaining low shear stress. This work focuses on the purification process of a pDNA vaccine, exploring the Swiper monolithic column already described for mRNA purification. Various chromatographic properties were adjusted, with different concentrations of sodium chloride and pH. These variations allowed for the exploration of multiple binding and elution conditions.

Acknowledgements: This work was developed within the scope of CICS-UBI (UIDB/00709/2020 and UIDP/00709/2020) and DryVac (2023.00136.RESTART) projects, financed by national funds through the Portuguese Foundation for Science and Technology (FCT)/MCTES. Â. Sousa acknowledges FCT and University of Beira Interior for the research contract CEECINST/00016/2021/CP2828/CT0003 under the scope of the CEEC Institutional 2021, funded by FCT. The authors also acknowledge the Swiper monolith, kindly offered by SARTORIUS BIA Separations.

Keywords: DNA vaccine; Ionic chromatography; HPV; Swiper monolith.

P24. CHEMICAL SPECIATION AS BASE FOR THE EVALUATION OF 8-HQA AND ITS Ga^{3+} METAL COMPLEXES ACTION ON MICROBIOTA RADIATION RELATED RESISTANCE

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ABSTRACT

Cancer patients submitted to radiotherapy often suffer from severe side effects, which can arise from imbalance of the normal metabolic pathways. Tryptophan (Trp) is an essential amino acid, with the three major Trp metabolism pathways leading to serotonin, kynurenine, and indole derivatives, directly or indirectly, affected by microbiota. A few gut microbes have been shown to produce kynurenine derivatives and kynurenic acid seems to provide long-term radioprotection *in vivo*. Furthermore, there is evidence that changes in the plasma metabolome and microbial metabolite levels can be associated with disease progression and severity. With this work we aim at understanding the ability of 8-hydroxyquinoline-2-carboxylic acid (8-HQA, an end product of kynurenic Trp metabolic pathway) and the corresponding Ga^{3+} complex in the protection of different human microbiome bacteria against ionizing γ-radiation. This study was preceded by a detailed evaluation of the binding ability of 8-HQA towards Ga^{3+}, investigated by ISE-H^+ (glass electrode) potentiometric and UV/Vis spectrophotometric titrations. The potential effects of 8-HQA and $[Ga(8\text{-}HQA)_2]^-$ complex towards human microbiota (namely *A. viscosus, S. mutans, S. sobrinus, P. putida, P. fluorescens* and *E. coli*) exposed to ionizing radiation were evaluated, as well as their anti-inflammatory properties. A radioprotective effect of $[Ga(8\text{-}HQA)_2]^-$ was observed on *A. viscosus*, while showing a potential radiosensitizing effect against *S. mutans* and *S. sobrinus*. Furthermore, $[Ga(8\text{-}HQA)_2]^-$ presented potential anti-inflammatory properties.

Acknowledgements: The authors would like to thank the financial support from the National Science Centre (NCN), Poland, under the scope of the research project number 2020/39/B/ST4/03060.

Keywords: Stability constants; Human microbiota; Anti-inflammatory action; Ionizing radiation.

P25. ELECTROPHYSIOLOGICAL CHANGES IN MYOPIA WITH THETA BURST STIMULATION

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ABSTRACT

Myopia is an eye disorder due to a refractive error that is characterized by the formation of a blurred image on the retina. Because retinal stimulation in myopia is inadequate, other functional changes may be present in the visual system. This study aimd to further understand the changes that myopia may originate in the function of the visual pathway through Pattern Electroretinography (PERG), Visual Evoked Potentials (VEP), and Theta-Burst Stimulation (TBS). 19 adult volunteers, 10 myopes (mean 22.5 ± 2.95 years), and nine subjects with normal eye (NE) (mean 21.00 ± 2.00 years). Both groups were studied and compared through PERG and VEP testing. After right-hemisphere stimulation with continuous (cTBS) and intermittent (iTBS) protocols, a re-evaluation with VEP and PERG was carried out in both groups. At baseline and after TBS, no significant differences in PERG and VEP in myopic and NE were found. When comparing the dominant and non-dominant eye for each group, significant differences were found in the myopic group: after iTBS, the dominant eye presented a higher N75-P100 amplitude than the contralateral eye ($p=0.028$), while after cTBS the contralateral eye presented a slight increase in P100 latency ($p=0.017$). At baseline, the electrophysiological tests do not seem to be sensitive in detecting potential myopic-driven functional retinal and cortico-retinal impairment. TBS may be a possible neurophysiological tool able to originate a shift in the neuro-visual function of myopic subjects and that VEP responses may be sensitive to detect these changes.

Keywords: Myopia; Pattern electroretinography; Visual evoked potentials; Transcranial magnetic stimulation.

P26. ELECTROPHYSIOLOGICAL TESTS IN MYOPIA: A SYSTEMATIC REVIEW OF RETINAL AND VISUAL PATHWAY FUNCTION

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ABSTRACT

Introduction: Myopia is a condition characterized by abnormal convergence of light rays that enter the eye and focus before the retina. Electrophysiological tests are non-invasive techniques that can provide information about retinal and/or visual pathway cell functionality. The purpose of this systematic review is to evaluate whether electrophysiological tests can be useful to support the hypothesis that relevant impairments may exist in the neurovisual function associated with myopia. Methods: We include observational and interventional studies with myopic subjects and with a control aged-matched group. Searches were conducted in PubMed, Scopus, and Web of Science, using strings with combinations of keywords, expressions, and MeSH terms. The amplitude and latency of the electrophysiological tests were the main outcomes analysed. Results: A total of 1271 articles were found. Of these, 11 articles complied the eligibility criteria and were subsequently included in this review. Among these, six articles focused on multifocal electroretinography (mfERG), three on pattern electroretinography (PERG), one on flash electroretinography (flash ERG) and one on visual evoked potential (VEP). Of those 11 articles, 7 that used mfERG, PERG and flash ERG found an amplitude decrease in myopic eyes. Regarding the latency parameter, 4 articles that used mfERG and PERG, found a delay in myopic eyes. The only study using VEP showed an amplitude decreased and a delay in latency parameters. Conclusions: This review shows promising findings for the use of electrophysiological tests in myopia as complementary exams to better understand the functional impairment of the myopic eye and neuro-visual pathways.

Keywords: Myopia; Electrophysiological tests; Retina; Visual pathways.

P27. TRIBUTYLTIN AT SUBTOXIC LEVELS IMPAIRS ANTIOXIDANT CAPACITY AND ALTERS THE EXPRESSION OF SIRTUINS 1 AND 3 IN RAT SERTOLI CELLS

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ABSTRACT

Obesogens are environmental contaminants that predispose individuals to weight gain. Tributyltin (TBT) has served as an obesogen model due to its adverse effects on male fertility. Within testes, Sertoli cells (SCs) play a critical role in spermatogenesis, and the impact of various compounds on their function has been shown to predict the effects on male fertility potential. In this study, we hypothesized that TBT prompts to oxidative stress in rat SCs, by dysregulating the molecular axis controlled by sirtuin1 (SIRT1) and sirtuin 3 (SIRT3). We exposed SCs to different concentrations of TBT during 24 hours: 0.1 nM, a subtoxic level; 10 nM and the respective control group. We measured the SCs' antioxidant capacity, the protein levels of SIRT1 and SIRT3, as well as protein carbonylation, lipid peroxidation and acetylation by Slot-Blot. The antioxidant capacity of SCs was decreased in both groups of SCs exposed to 10nM and 0.1 nM of TBT. SIRT1 levels were increased in SCs exposed to 0.1nM of TBT, in contrast to the decrease expression of SIRT3. Additionally, SCs exposed to sub-nanomolar concentration of TBT exhibited a decrease in the protein acetylation and protein carbonylation, but no significant alterations were observed in the lipid peroxidation. In summary, sub-nanomolar levels of TBT exert pronounced effects on Sertoli cells, potentially leading to an oxidative environment.

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Keywords: Obesogens; Male fertility; Sertoli cells; Mitochondrial function.

P28. NATURAL COMPOUND-ENCAPSULATED LIPOSOMES FOR NEXT GENERATION WOUND HEALING THERAPY

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ABSTRACT

Chronic skin wounds are an increasingly common and emerging public health problem. Challenges in the healing process usually arise from to the development of infections in the wound bed, which often cannot be controlled with conventional antibiotics and antifungals. This is due to another increasingly common problem today, namely bacterial resistance, which is due to the inappropriate use of conventional antibiotics and antifungals and has contributed to an increase in comorbidities and mortality. In this sense, this work consisted in the development a new nanosystem that allows the encapsulation of mangiferin, a natural compound that has beneficial antioxidant, anti-inflammatory, and antimicrobial properties, to develop a new therapeutic approach for wound healing. We first developed a new liposome formulation prepared by the lipid film hydration method, and then evaluated its physicochemical properties in terms of particle size, polydispersity index, and zeta potential, as well as its storage stability (4 ºC and -20 ºC). Finally, we optimized the mangiferin encapsulation process. We found that the nanosystem preparation process is reproducible and that liposomes have suitable physicochemical properties for topical application. The stability study showed that the nanosystem has high stability. Our results also reveal that the liposomes have good loading capacity and encapsulation efficiency for mangiferin. Considering the results obtained, the nanosystem developed is very promising for topical application in chronic wounds.

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Keywords: Nanotechnology; Liposome; Mangiferin; Wound healing.

P29. NATURAL PRODUCT-BASED BIOMIMETIC-COATED LIPOSOMES FOR CUTANEOUS WOUND HEALING

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ABSTRACT

Wound closure is a very precise and well-coordinated mechanism in healthy individuals. However, due to flaws in this physiological process, there are currently around 40 million patients globally affected by chronic wound, leading to epidemic proportions and a significant financial impact on healthcare systems. Conventional antimicrobial therapies, characterized by prolonged and repeated antimicrobial treatments, have become ineffective due to the emergence of antimicrobial resistance. In this sense, the objective of our work was to develop a new biomimetic nanosystem to create a new and next-generation topical therapeutic approach against chronic wounds. The new biomimetic nanosystem is composed of a mixture the macrophage membrane with liposomes to maximize homotypic targeting of bacteria. In the hydrophilic core of the nanosystem, bromelain is encapsulated, which has antibacterial properties. Our results show that the cell membrane extraction protocol developed by us presents a high extraction yield of macrophage cell membranes. Furthermore, we verified that the cell membranes and liposome formulation present physicochemical properties (size and zeta potential) compatible with the production of our nanosystem. The changes observed in the physicochemical properties of biomimetic nanosystems, in relation to their individual constituents, are indicative of the success our production protocol. Our results also show that the biomimetic nanosystem has a high bromelain loading capacity. Therefore, the obtained results are promising in terms of developing a new topical strategy to combat chronic wounds.

Acknowledgements: This work is financed by FCT - Foundation for Science and Science and Technology, under project with reference: 2022.05270.PTDC.

Keywords: Nanotechnology; Biomimetic nanosystem; Bromelain; Wound healing.

P30. PHENOTYPIC CHARACTERIZATION OF CLINICAL ISOLATES OF *Aliarcobacter butzleri*

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ABSTRACT

Aliarcobacter butzleri is an emergent enteropathogen, isolated from various sources, including humans. Infections caused by this bacterium are often associated with enteritis, but also other diseases such as bacteremia. The pathogenic potential of *A. butzleri* has been recognized, although the underlying virulence mechanisms and specific strain features remain poorly understood. In this work, we aimed to evaluate the phenotypic traits of a group of *A. butzleri* isolates to assess the bacterial virulence and variability among strains. For that, the antimicrobial resistance to five different classes of antibiotics and to bile salts was determined, as well as the susceptibility to oxidative stress. Additionally, the survival profile to acidic pH and human serum, motility, biofilm formation and adhesion and invasion of Caco-2 cells were also evaluated. Most of the isolates presented multidrug-resistance profiles being resistant to at least three antibiotics and all the isolates tested were resistant to bile salts concentrations found in the human gut. The survival profiles showed that all isolates tolerated acidic conditions for at least 20 minutes, and three isolates survived for 60 minutes in the presence of human serum. In terms of virulence, the isolates exhibited high to moderate motility, with five isolates having low motility. Seven isolates had a weak ability to form biofilm, while others presented moderate or high biofilm formation. Lastly, all the isolates adhered to Caco-2 cells, but only 12 were capable of invasion. In conclusion, the results suggest the *A. butzleri* clinical isolates presented phenotypic variability, pointing to strain-specific virulence profile.

Acknowledgements: This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Cristiana Mateus is recipient of a doctoral fellowship (UI/BD/151023/2021) under the scope of the CICS-UBI Programmatic Funding (UIDP/00709/2020). Susana Ferreira acknowledges UBI and FCT by the contract of Scientific Employment according to DL57/2016.

Keywords: *Aliarcobacter butzleri;* Clinical Isolates; Phenotypic; Virulence.

P31. INVESTIGATING THE SERRA DA ESTRELA NATURAL PARK FLORA AS A NATURAL SOURCE OF DIABETES AND OXIDATIVE STRESS

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ABSTRACT

In this study, we conducted a field exploration, including surveys, to collect 38 plants, being collected different plant parts, namely, leaves, flowers, and fruits from Serra da Estrela Natural Park (NPSE) [1]. A total of 52 samples were then subjected to an assessment of their bioactive properties to explore their potential for developing natural treatments for diabetes and oxidative stress-related diseases. The collected plants were dried, ground into fine powders, and prepared as aqueous infusions. The infusions were dried, and the yield of each extract was determined. α-Glucosidase is an enzyme involved in the digestion of carbohydrates and a target for controlling blood sugar levels in people with diabetes. The primary screening aimed to identify plants with significant α-glucosidase inhibitory activity. We identified the most potent extracts and subjected them to further antioxidant assays, including 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), superoxide anion ($O_2^{\bullet-}$) radical scavenging, and nitric oxide ($^{\bullet}NO$) inhibition assays. These tests evaluated the extracts' capabilities to neutralize free radicals and reduce oxidative stress. Additionally, we quantified the polyphenol content of the most active extracts using standard colourimetric methods, as polyphenols are well-known for their health benefits, including potent antioxidant activities. Our results revealed that several plants demonstrated strong α-glucosidase inhibitory effects and significant antioxidant properties, with some extracts showing high concentrations of polyphenols. These findings underscore the potential of Serra da Estrela Park's flora as a valuable source of natural bioactive compounds for therapeutic applications in diabetes management and protection against oxidative stress.

Keywords: Serra da Estrela Natural Park; Plants; Bioactive Compounds; Diabetes.

P32. NMR-BASED STUDY OF DEPRESSION BIOMARKERS IN ELDERLY PEOPLE FROM BEIRA INTERIOR

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ABSTRACT

Depression is the most prevalent health issue in the European Union, affecting approximately 50 million people. Portugal ranks fifth among European countries with the highest number of depression cases. About 8% of the Portuguese population is diagnosed with this condition. In the elderly, the incidence and prevalence of major depression duplicate between the ages of 70 and 85. The factors associated with a higher predisposition to depression include female gender, genetics, social isolation, widowhood or divorce, low socioeconomic conditions, comorbidities, functional impairment, brain changes due to vascular problems, cognitive dysfunction, certain drugs, polypharmacy, and alcohol dependence. The physiopathology of depression is complex and not entirely understood, with many causes primarily linked to various risk factors, thus the discovery of new biomarkers capable of highlighting these mechanisms is crucial. Therefore, in this study, we compared plasma metabolite levels of 251 elderly residents of long-term care facilities of Beira Interior without depression (control group) and those with depression who were taking one (monotherapy), two (double therapy), and three or more (polytherapy) antidepressants. All the samples were acquired on an AVANCE III 600 MHz NMR spectrometer equipped with a quadruple resonance cryoprobe. Were acquired 1D (NOESY, CPMG) and 2D (J-res, HMBC, HSQC) pulse sequences to identify and quantify the metabolites. Trough univariate statistical analysis, we found significant differences between the control group and the intervention study groups for leucine, proline and citrate. The differences found in the metabolic profiles seem to be a promising tool in the clinical diagnosis and outcome of depression.

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Keywords: Depression; Elderly population; Metabolomics; NMR.

P33. COMPARATIVE STUDY OF THE EXPRESSION OF ENZYMES INVOLVED IN ADENOSINE METABOLISM IN HUMAN ASTROCYTES AND GLIOBLASTOMA

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ABSTRACT

Glioblastoma (GBM), derived from astrocytes, is the most aggressive brain tumor in adults. Regions of hypoxia present in GBM are associated with tumor growth and progression. Adenosine (ADO) accumulates during hypoxia, and both pro- and anti-tumor effects of ADO in GBM have been described, depending on whether it acts at the extra- or intracellular level, respectively. This study aimed to understand how the enzymes of the purinergic metabolism influence the tumorigenesis of this cancer. Cell viability, scratch assay and western blot tests were carried out using control conditions, ABT-702 (ADK inhibitor) and a cocktail of antagonists (CA, 4 adenosine receptor antagonists: A1, A2A, A2B and A3) on 3 cancer cell lines and human astrocytes. The Western Blot was done with 3 different antibodies, all 3 being enzymes of adenosine metabolism. In cell lines, the results show that ADK inhibition maintains cell viability of the mutated cells and cells treated with CA demonstrate a lower viability. However, combined CA-ABT lowered the cell viability the most. Given these results, a new understanding has emerged of what happens to adenosine within the cancer environment and how it can be shaped according to the receptors and enzymes.

Keywords: ABT-702; Adenosine; Glioblastoma; Human astrocytes.

P34. NANOEMULSION POTENTIAL, INDEPENDENTLY AND IN COMBINATION WITH ANTIFUNGAL AGENTS AGAINST *Candida albicans*

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ABSTRACT

This study investigated the antifungal potential of a novel nanoemulsion composition (https://doi.org/10.3390/pharmaceutics15071878) against *Candida albicans*, a major fungal pathogen, both independently and in combination with established antifungal agents, such as clotrimazole and fluconazole. The research employed the broth microdilution method to evaluate efficacy across different *Candida albicans* clinical strains. In addition, the antifungal effect was also determined in combination with clotrimazole or fluconazole using the checkerboard method. The nanoemulsion demonstrated noteworthy antifungal activity when administered alone; however, the combination of the nanoemulsion with antifungal agents did not yield significant synergistic effects; instead, it often resulted in a nullification of antifungal activity. This suggests a potential for further exploration and development as a standalone antifungal treatment. The data collected through *in-vitro* analysis underscored the need for continued investigation into the mechanisms underlying the observed interactions between the nanoemulsion and established antifungal agents. While these findings represent preliminary results, they offer valuable insights into the development of novel antifungal therapies and highlight the importance of further research in this area to address the growing challenge of antifungal resistance.

Acknowledgments: The work was part of a project [Nanoemulsão Inovadora para Tratamento Antimicrobiano (NITA)] that was financed through an action of project "INOVC+ Ecossistema de Inovação Inteligente da Região Centro", Co-financed by the Portugal 2020 Program (PT 2020), in the framework of the Regional Operational Program of the Center (CENTRO 2020), and by the European Union through European Regional Development Fund (ERDF, or FEDER).

Keywords: Candidosis; Infection; Antifungal treatment; Synergy.

P35. NEW DELIVERY SYSTEMS BASED ON GELLAN GUM NANOPARTICLES FOR PARKINSON DISEASES THERAPEUTICS

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ABSTRACT

There are up to 10 million people worldwide suffering with Parkinson's disease (PD), a chronic neurodegenerative disorder without cure. Clinically, PD patients show motor and non-motor symptoms, such as resting tremor, rigidity, postural instability, depression and anxiety. Physiologically PD is characterised by the progressive loss of dopaminergic neurons in the *substantia nigra*. To date, the most effective drug combination used as PD treatment is the administration of levodopa (L-DOPA) combined with inhibitors of catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO-B), to restore dopaminergic brain levels. However, the commercially available inhibitors have low capabilities to cross the blood–brain barrier (BBB) and thus low efficacy in the brain. Also, its prolonged use could be associated with high hepatotoxicity, limiting its use. Therefore, the discovery of molecules, with the potential to inhibit COMT, is crucial to improve the efficacy of existing therapies. To fulfil this need, a set of molecules showing around 80% interacting capabilities with ICR mouse brain samples, were selected for further development. The use of polymeric nanoparticles (NPs) as drug carriers presents several advantages, such as the absorption due to its reduced size, compartmentalization of the drug in a restricted environment, and modulation capacity. Also, the application of polysaccharides in the development of NPs has several advantages, such as low cost, biocompatibility and biodegradability. This work aims to improve COMT inhibitors and L-Dopa capability to cross BBB, by its formulation into NPs synthesised through polyelectrolyte complexation, combining the respective drug with gellan gum (GG) and chitosan (CS). GG and CH complexes were tested in several conditions, using chitosans with different molecular weights and loaded with L-Dopa or COMT inhibitor.

Keywords: Parkinson’s disease; COMT inhibitors; Gellan gum; Nanoparticles.

P36. ASSESSMENT OF MIR-155 SILENCING IN HUMAN LUNG ADENOCARCINOMA CELL LINE USING A MOLECULAR BEACON

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ABSTRACT

Lung cancer (LC) is a major cause of cancer-related deaths worldwide, underscoring the need for innovative biomarker detection methods to enhance early diagnosis. Alterations in microRNAs (miRs) are implicated in the initiation and progression of human cancers and serve as potential biomarkers for diagnostics and treatment. In this study, we applied molecular beacon (MB) technology to monitor miR-155-3p expression in human lung adenocarcinoma A549 cells without the need for complementary DNA synthesis, amplification, or costly reagents. Additionally, we produced gold nanoparticles (AuNPs) to deliver antisense oligonucleotides into A549 cells to reduce miR-155-3p expression, which was then detectable using the MB. The MB was designed and structurally characterized using Förster Resonance Energy Transfer (FRET)-melting, Circular Dichroism (CD), Nuclear Magnetic Resonance (NMR), and fluorometric experiments, with optimized hybridization conditions for detecting miR-155-3p in total RNA extracted from the A549 cell line. The expression profile of miR-155-3p was obtained via RT-qPCR. The results demonstrated that the MB was well-designed and effective in targeting miR-155-3p, achieving a detection limit down to nanomolar concentrations and proving the biosensor's specificity. Additionally, the self-assembly of ASOs with AuNPs showed remarkable target specificity, effectively silencing miR-155-3p. Compared to lipid-based transfection agents, AuNPs exhibited superior silencing efficiency. This study highlights the MB's capability to detect changes in target gene expression following gene silencing. Overall, this innovative approach holds promise as a tool for detecting various biomarkers simultaneously, with potential clinical applications.

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Keywords: miR-155-3p; Molecular beacon; Gold nanoparticles; Gene silencing.

P37. LDH ASSAY: OTIMIZATION OF VERSATILE, SENSITIVE AND LINEAR ASSAY

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ABSTRACT

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme responsible for the interconversions that occur during glycolysis between pyruvate and NADH into L-lactate and NAD^+ respectively. When cellular damage occurs, LDH is released by cells into the extracellular environment and the LDH assay has been widely used to quantify cell death . There are several commercial quantification kits [1], but the assay can be easily done with unexpensive self-made solutions. However, procedures found in literature were limited, and we felt the need to better understand and optimize a protocol that we could adapt to our experimental conditions. We followed the consumption of NADH at 340 nm, over time, to determine LDH activity and hence, LDH concentration. By mixing 100 µL of NADH at 1600 µM, 50 µL of pyruvate solution at 10000 µM, and 50 µL of LDH standard or medium from treated cells, the reaction occurred linearly over time until the majority of NADH was consumed. The velocity of the reaction (slope) had a better linear correlation with LDH concentration than absolute absorbance at a given time and allowed for an extended linear range, with great sensitivity. By releasing the full LDH content of control cells with Triton X-100, the results could be normalized to percentual cell death. LDH assay is complementary to viability assays such as the resazurin and MTT reduction assays, can be used over time in the same cells to follow death kinetics, or easily combined with other experiments.

Keywords: Death kinetics; Lactate dehydrogenase; LDH; Viability assays.

P38. IMPROVING THE VIABILITY OF *CAENORHABDITIS ELEGANS* USING PHENOLIC EXTRACTS OF LETTUCE INOCULATED WITH BACTERIAL BIOSTIMULANTS

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ABSTRACT

The consumption of vegetables is essential in a healthy and balanced diet due to their contribution of vegetable fibres, minerals and antioxidant compounds. In other way, the use of bacterial biostimulants can improve plant nutrition and nutraceutical properties of crops. The aim of this work is to determine whether the improvement in the concentration of phenolic compounds induced by bacterial biostimulants can improve the viability of *Caenorhabditis elegans* as *in vivo* model. For this purpose, *Lactuca sativa* var. Romaine inoculated with 4 different bacterial biostimulants, *Pseudomonas* sp. SCPM31, *Priestia* sp. SCPM81 and *Rhizobium* spp. HUTR05 and GPTR29, was used. Different vegetative parameters and phenolic compound composition were analysed by HPLC-DAD-MS. *C. elegans* was exposed to phenolic extracts from each of the treatments. The application of these biostimulants resulted in an improvement in aerial part weight. In addition, the analysis of phenolic compounds showed that all the biostimulants applied were able to improve the total concentration of phenolic compounds, increasing the concentration of chicoric acid between 200% and 540%. Finally, it was observed that the phenolic extracts of the *Rhizobium* sp. GPTR29 treatment improved viability in the first stages of the adult phase, and the *Priestia* sp. SCPM81 and *Rhizobium* sp. HUTR05 treatments improved viability in the last stages of the adult phase, the latter two treatments being the ones that showed the highest concentration of phenolic compounds. These results prove that the application of bacterial biostimulants can produce crops with increased nutraceutical capacities and with an effect on living organisms.

Keywords: Biostimulants; *in vivo* model; Phenolic compounds; Nutraceutical food.

P39. A NEW PURIFICATION APPROACH FOR MEMBRANE BOUND CATECHOL-*O*-METHYLTRANSFERASE BIOSYNTHESIZED WITH A NEW SHUTTLE VECTOR ON *Escherichia coli*

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ABSTRACT

Parkinson´s disease (PD) is one of the most common neurodegenerative disorders in the world, been characterized by the loss of physical functions, due to the death of dopaminergic neurons. Thus, membrane bound catechol-*O*-methyltransferase (MBCOMT), as an enzyme that regulates the metabolization of catecholamine neurotransmitters such as dopamine, is a key protein to be studied, to improve the existent PD therapeutics. However, the production and purification of membrane proteins like MBCOMT can be a challenge due to the low levels of expression and stability. To face these challenges, we proposed a new MBCOMT construct without the first 30 amino acids from the N-terminal and the last 5 amino acids form the C-terminal to increase protein stability. Preliminary studies of *Escherichia coli* BL21 expression cultures were conducted to evaluate the optimal inductor (IPTG) concentration (0.25, 0.5, 0.75 and 1 mM), during a time course between 2 and 8h. The biosynthesis procedure was established based on the highest activity levels obtained, at 0.5 mM of IPTG during 6 hours of induction. Moreover, to facilitate the purification step a dual tag of maltose-binding protein and 6xHis was added at the N-terminal of the protein. Thus, a IMAC methodology, that explores the histidine's affinity to the Ni^{2+} or Co^{2+} ions bound to the resin was explored. The protein elution profile was evaluated under increasing imidazole concentration from 0 to 500 mM in a linear and step mode. All the procedures resulted in the purification of considerable amounts of active MBOCMT.

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Keywords: Catechol-*O*-metyltransferase; Biosynthesis; Affinity tags; Purification.

P40. SYNTHESIS AND BIOPHYSICAL EVALUATION OF BISCYANINE DYES IN THE AFFINITY AND STABILIZATION OF G-QUADRUPLEX STRUCTURES

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ABSTRACT

Cancer remains one of the leading causes of mortality, driving research into novel essential therapies. G-quadruplexes (G4s), non-canonical DNA or RNA structures, have emerged as a promising approach due to their role in regulating cellular processes involved in cancer biogenesis. These structures are formed by the arrangement of planar G-tetrads through Hoogsteen hydrogen bonds between four guanines and are stabilized by monovalent metal cations. Developing G4 ligands with high affinity and selectivity is essential to target specific G4 structures, minimize side effects and enhance antitumor activity. A new approach involves the use of biscyanines, a class of cyanine dyes, as G4 ligands. These dyes consist of a polymethine chain linked to heterocyclic or non-heterocyclic rings and are classified by the polymethine chain length and terminal groups. This study focused on the synthesis and characterization of bisbenzothiazolcyanines, linked by unconjugated three-carbon chains between the benzothiazole nucleic and its precursors. The counterions varied among bromide, iodide, perchlorate, and tosylate. Additionally, the study evaluated their potential as ligands capable of stabilizing G4 structures. Among the bisbenzothiazolcyanines evaluated, the ligands promoted greater stabilization in G4 sequences compared to duplex sequences, with the bromide salt emerging as the most promising counterion, demonstrating significant G4 structure stabilization. These promising results open the opportunity for further biophysical evaluations of different G4 sequences using a larger set of biscyanines, including exploring variations in the methyl chain length and unconjugated carbon chain length, and heterocyclic rings such as indole, benzoxazole, and 2- and 4-quinolines.

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Keywords: Cancer therapy, G-quadruplex (G4), Ligands G4, Biscyanines

P41. Chemical characterization and antioxidant activity of *Piper auritum* Kunth essential oil from Panama.

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ABSTRACT

Piper auritum Kunth (8929) a medicinal plant belonging to the Piperaceae family, and it is traditionally used in Panamanian folk medicine as anti-inflammatory, antibacterial and antifungal remedy. In Panama it is known as "Hinojo sabalero". Therefore, we decided to determine the chemical composition and antioxidant activity of the of essential oil from leaves. To determine the chemical composition, we used the GC-MS technique, using two columns of different polarity (SH-RXI-5HT, Supelcowax); meanwhile for antioxidant activity DPPH, ABTS, $^{\bullet}O_2$, and TBARS assays were performed. Gas chromatographic analysis of the essential oil revealed the presence of 37 compounds (93,4%), the main ones being α-isosafrole (54.0%), δ^3-carene (8.2%), *p*-cymene (4.7%), α-terpinene (3.8%), neomethyl lactate (3.6%), camphor (2.7%), camphene (2,2%), isobornyl acetate (1.7%). The DPPH assay showed a percentage inhibition of 27.4 ± 13.3 at 125 µg/ml compared to the positive control, quercetin (77.7 ± 2.5%). ABTS inhibitory activity was 59.8 ± 1.6 and 93.3 ± 0.3 for the samples and quercetin, respectively. In the non-enzymatic superoxide anion assay, the inhibition of the essential oil was 8.8 ± 7.7%, compared to the most significant activity obtained with quercetin (80.1 ± 0.9%). The TBARS assay revealed an inhibition percentage of 85.9 ± 0.2% for the extract, similar to the standard (84.3 ± 0.1%). Our results indicated that *Piper auritum* Kunth is a plant with biological interest and provide the scientific basis for its traditional use.

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Keywords: *Piper auritum*, Antioxidant, TBARS, Essential oil

Plenary Sessions

I. A DIFFERENT PERSPECTIVE ON BIOMOLECULE ADSORPTION MECHANISM DURING PREPARATIVE CHROMATOGRAPHY

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ABSTRACT

In recent years, the pharmaceutical industry's growth has been largely driven by the production of biopharmaceuticals. However, purification remains a bottleneck in the manufacturing process. Despite competition from non-chromatographic techniques, preparative chromatography continues to be the dominant method for purifying these biomolecules due to its high resolution. Still, the retention and separation mechanisms, which are crucial for predicting and controlling adsorptive behavior, are not yet fully understood. To address this limitation and shed light on the interaction mechanisms between biomolecules and chromatographic resins, it is important to conduct a biophysical characterization of the adsorption process. This presentation aims to provide a state-of-the-art overview of experimental approaches for monitoring biomolecule-surface interactions. The focus will be on flow microcalorimetry (FMC) and small-angle X-ray scattering (SAXS), two non-labeling techniques that simulate a dynamic chromatography system, enabling online and in situ monitoring of the adsorption process. Current applications of these in situ monitoring techniques to better understand the separation of biomolecules of pharmaceutical interest will be discussed.

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Keywords: Biomolecule–Ligand Interactions, Flow Microcalorimetry, Scanning electron microscopy, Small-angle X-ray scattering.

II. UNLOCKING THE HEALTH BENEFITS OF NATURAL PRODUCTS: WHAT ABOUT THE BIOACCESSIBILITY AND BIOAVAILABILITY OF PHYTOCHEMICALS?

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ABSTRACT

Natural products, derived from plants, animals, and minerals, have been used throughout human history to promote health and treat various ailments. Their popularity continues to grow, driven by a desire for holistic and sustainable approaches to health care. Plants have a long history of use in traditional medicine and have greatly contributed to the development of treatments for several high burden diseases. Phytochemicals, molecules from medicinal plants, have high chemical diversity that interact with biological targets and are promising resources for drug development. The successful extraction and isolation of analgesic compounds from opium in nineteenth century, like morphine, marked the beginning of drug discovery. In the last 40 years, approximately 25% of new drugs approved worldwide are natural products and their derivatives and this figure rise to 32% when it comes to only small-molecule approved drugs. (1) On the other hand, the phytochemicals found in fruits, vegetables, etc., when incorporated into one's diet and lifestyle can play a vital role in promoting health and well-being. These compounds can contribute to maintain the overall health, supporting immune function, and preventing chronic diseases.

However, for phytochemicals to exert their beneficial effects, they must be bioaccessible and bioavailable. Bioaccessibility refers to the fraction of a compound that is released from its matrix during digestion and becomes available for absorption into the blood stream in the gastrointestinal system. Bioavailability consists of the fraction of an ingested compound that reaches the systemic circulation and is available to exert its effect or for storage in the body, after absorption, metabolism and distribution by tissues. To maximize the health benefits of phytochemicals, strategies to enhance their bioavailability are often employed, both in its use in the development of new drugs, and in its use to incorporate bioactive factors to strengthen food nutrition for functional food development. Different formulation techniques and delivery systems, such as encapsulation techniques, e.g., the use of liposomes and nanoparticles, are commonly used to protect phytochemicals from degradation and to enhance their absorption.

Understanding the bioaccessibility and bioavailability of phytochemicals is crucial for harnessing their full health potential. Thus, in this presentation it will be discussed the results of the *in vitro* determination of bioaccessibility and bioavailability of the main phytochemicals of two different vegetal matrices, sweet cherries and ayahuasca beverages. The samples were subjected to an *in vitro* digestion process, and the Caco-2 cell line was used as an absorption model. After digestion and cell incubation, the compounds absorbed by the cell monolayer were quantified by high-performance liquid chromatography coupled to a diode array detector, namely phenolic compounds from sweet cherries and psychoactive compounds from plants used in ayahuasca decoctions.

III. THE IMPORTANCE OF BIOINFORMATICS IN INVESTIGATING THE GENETIC CAUSES OF RARE DISEASES

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ABSTRACT

Many diseases are difficult to diagnose, particularly in identifying the causes that are at the heart of the symptoms presented by the patient. Often, the genetic causes are only considered at an advanced stage of the treatment, where its effectiveness is questioned. This is often the case with hereditary diseases, such as diabetes mellitus type MODY (Maturity Onset Diabetes of the Young), Congenital Hypogonadotropic Hypogonadism, Growth hormone deficiency and Pituitary adenomas, which are examples of rare genetic diseases studied in CICS-UBI.

To study such diseases, scientific bioinformatics techniques are frequently applied to identify genetic changes that cause illness. The illness is typically found associated to deleterious genetic changes disrupting the function of bio-molecules. This level of analyses, requires the sample preparation and a sequencing process necessary to reveal the textual form of the complete human genome/exome. Here the bioinformatics techniques are necessary to enable the identification of the patient's genetic causes through final biological and biochemical interpretation of the genomic data. Sanger sequencing, a first-generation sequencing technique, enabled the initial sequencing of the human genome during the years 1990-2003 and nowadays is currently used as a validation step of detected causal variants. Without these initial techniques and the development into second- and third- generation sequencing, it would be very difficult if not impossible to acquire a more complete picture of the human genome and exome. Bioinformatics thus enables the downstream processing of this data and at each step refine the biological information. Five bioinformatics steps are typically involved in the analyses of the exome: 1) Quality control, 2) Pre-processing, 3) Alignment, 4) Post-alignment processing, 5) Variant analyses, including variant calling, annotation and prioritization. Among these processing steps, researchers often face challenges in developing informatics methods and interpreting changes such as small insertions, deletions and mutations within protein-coding and non-protein-coding regions.

Moreover, these processes are time-consuming and subject to errors due to the data complexity and often require manual processing steps. In all these steps, computer science allied to biological and medical sciences plays a fundamental role in the development of methods towards data visualization, improved data analysis, optimization, accuracy and interpretation. Here, we present a typical exome analyses workflow and provide a few insights into how bioinformatics can help in detecting the genetic causes of rare diseases, hereby aiding physicians to administer a more appropriate treatment to their patients.

Keywords: Bioinformatics, WES, Workflow, Genomic Medicine.

IV. BRAIN SENSES: AN UPDATE

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ABSTRACT

The composition of cerebrospinal and brain interstitial fluids is ensured by barriers between the blood and the brain parenchyma (the blood-brain barrier) and between the blood and the cerebrospinal fluid (the blood-cerebrospinal fluid barrier-BCSFB). These barrier systems hold a chemical surveillance system, essential to protect the brain from toxicants, microorganisms, and other harmful compounds but conversely compromises the brain delivery of many pharmacologic agents. Barrier function and this chemical surveillance system result from the combination of tight junctions between cells that impair solute flux via the paracellular pathway, cell membrane transporters that enable selective transcellular solute passage, and intracellular metabolizing enzymes that transform molecules in transit. In recent years, we have identified unique potential players of these chemical surveillance systems in the BCSFB: odorant, vomeronasal and taste receptors that might function as upstream regulators of this surveillance system. These chemosensory receptors are strategically placed to monitor the composition of blood, cerebrospinal and brain interstitial fluids. Some of them are also expressed in the blood-brain-barrier of rodents and humans. Upon ligand-binding, they may deploy the action of transporters and detoxifying enzymes to cope with alterations in the composition of blood and brain cerebrospinal and interstitial fluids, working as guardians of the central nervous system. Interestingly, many of these receptors are also expressed beyond brain barriers in the healthy brain and in brain neoplasms where they can be used as potential therapeutic targets.

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V. GUIDELINES: REACHING BEYOND THE EVIDENCE

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ABSTRACT

Evidence-based medicine (EBM) is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients. This approach integrates clinical expertise with the best available external clinical evidence from systematic research. The types of evidence utilized in EBM range from randomized controlled trials (RCTs) and cohort studies to case-control studies and expert opinions. Each type offers distinct advantages and drawbacks. RCTs, for example, are considered the gold standard due to their ability to minimize bias, but they are often limited by their excessive cost, ethical concerns, and sometimes limited generalizability. Cohort and case-control studies provide valuable insights but can be prone to confounding variables and biases.

The development of clinical guidelines often relies heavily on this hierarchy of evidence, with the GRADE (Grading of Recommendations Assessment, Development and Evaluation) system being a prominent tool for guideline construction. GRADE offers a systematic and transparent framework for rating the quality of evidence and the strength of recommendations, facilitating a balanced consideration of the benefits and risks of interventions. However, an exclusive focus on high-level evidence from RCTs can overlook the nuanced needs and values of individual patients and the insights from real-world data (RWD).

Real-world data, derived from sources such as electronic health records, insurance claims, and patient registries, provide valuable context that is often absent in controlled trial settings. The integration of RWD into guideline development can enhance the applicability and relevance of recommendations by reflecting the diverse patient populations and clinical environments encountered in everyday practice. Nonetheless, the use of RWD must be carefully managed to address potential biases and data quality issues.

The importance of incorporating patient values and preferences into clinical guidelines cannot be overstated. Patient-centred care emphasizes shared decision-making, where healthcare providers and patients work together to make decisions that align clinical evidence with the patient's unique preferences, circumstances, and values. By reaching beyond the traditional confines of evidence and integrating patient values and real-world data into clinical decision-making, guidelines can be more flexible, personalized, and ultimately, more effective.

This comprehensive approach ensures that recommendations are not only scientifically sound but also culturally competent and ethically sensitive, fostering a healthcare environment that respects and responds to the diverse needs of all patients.

VI. Squaraine Cyanine Dyes as Probes for the detection of HSA Protein and as Photosensitizers for PDT

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ABSTRACT

Squaraine dyes are a family of polymethine dyes consisting of a central four-membered ring derived from squaric acid, to which two heterocyclic units are attached. Depending on the structural modifications made to this scaffold, these dyes exhibit unique properties such as strong absorption at near-infrared wavelengths, moderate to high fluorescence quantum yields in organic solvents, and increased fluorescence intensity upon non-covalent binding to specific ligands in aqueous media. Additionally, they have the ability to produce reactive oxygen species (ROS), notably singlet oxygen.

Based on these properties, and given that the quantitative determination of proteins is an essential parameter in biochemistry, biotechnology, and immunodiagnostics, the significance of human serum albumin (HSA) in clinical diagnosis should be highlighted, as alterations in its concentration are associated with hypo- and hyperalbuminemias. This protein has been a target for study for its detection and quantification using these potential fluorescent probes. Additionally, the ability of squaraine dyes to produce ROS and their general stability under light make them "ideal candidates" as photosensitizers in photodynamic therapy (PDT).

Thus, in this presentation, it will be discussed the synthesis of various squaraine dyes developed over the past few years, along with their spectroscopic characterization. Additionally, the *in vitro* evaluation of their potential as fluorescent probes for the detection and quantification of HSA, as well as their potential application as photosensitizers in cancer PDT, will be addressed.

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