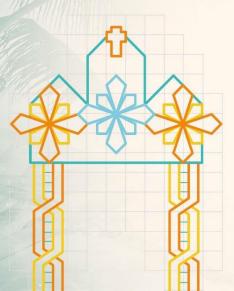
MACEIÓ-ALI **2023** 



# XV REUNIÃO BIENAL DA Sociedade Brasileira De Bioquímica E Biologia Molecular

Bioquímica e Biologia Molecular Aplicada

15 A 18 DE NOVEMBRO

LOCAL **IFAL CAMPUS MACEIÓ** 8h30 às 19h





REALIZAÇÃO:

DATA PARA INSCRIÇÃO E ENVIO DE RESUMO **15/07 a 20/08/2023** 





UNIVERSIDADE FEDERAL DE ALAGOAS

Brazilian Society for Biochemistry and Molecular Biology

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#### PROGRAMAÇÃO DO EVENTO

Data	Horário	Atividade
15/11	16:00 – 18:00h	Inscrição e retirada de material
15/11	18:30 – 19:00h	Sessão de abertura
15/11	19:00 - 20:00h	Palestra de abertura
		Palestrante: Profa. Dra. Helena C.F. de Oliveira (UNICAMP);
		CETP, uma proteína intrigante que desempenha papéis multifacetados no metabolismo
15/11	20:00 - 20:30h	Apresentação cultural
15/11	20:00 - 22:00h	Coquetel
16/11	8:30 – 10:30h	SIMPÓSIO 1: Insetos como um modelo para a pesquisa do metabolismo
		Moderador: Prof. Dr. Luciano Grillo (UFAL)
		<ul> <li>Prof. Dr. Jorge Luiz da Cunha Moraes (UFRJ-Macaé).</li> <li>Desenvolvimento de Compostos Bioativos contra Artrópodes Vetores</li> </ul>
		<ul> <li>Prof. Dr. David Majerowicz (UFRJ) Insetos gordos: o besouro <u>Tribolium castaneum</u> como modelo de obesidade e diabetes</li> </ul>
		<ul> <li>Prof. Dr. Emerson Guedes Pontes (UFRRJ) Mecanismos de tolerância a exposição a amônia em insetos baseado no perfil metabólico</li> </ul>
16/11	10:30 – 10:55h	Coffee break
16/11	11:00– 11:45h	CONFERÊNCIA 1
		Prof. Dr. Vinicius de Andrade Oliveira, (Universidade Federal do ABC).
		Metabolismo celular: um mundo além do sistema imune
16/11	14:00 – 14:45h	CONFERÊNCIA 2
		Prof. Dr. Walter Orlando Beys da Silva (UFRGS).
		Novas implicações patológicas de infecções fúngicas e virais de relevância médica e biotecnológica reveladas pela proteômica

16/11	14:45 – 16:45h	<ul> <li>SIMPÓSIO 2: Agentes antiobesidade: Uma busca por novos agentes terapêuticos</li> <li>Moderador: Prof. Dr. Hugo A.O. Rocha (UFRN) <ul> <li>Profa. Dra. Susana M.Gomes Moreira (Depto. de Biologia celular e Genética - UFRN)</li> <li>Explorando os Polissacarídeos sulfatados de algas marinhas para o tratamento da obesidade</li> </ul> </li> <li>Profa. Dra. Ana Heloneida de Araujo Morais (Depto. de Nutrição - UFRN)</li> <li>Potencial antiadipogênico de Inibidores enzimáticos</li> <li>Prof. Dr. Hugo A.O. Rocha (Depto. de Bioquímica - UFRN)</li> <li>Agarana com ação antiobesidade: caracterização estrutural e farmacológica</li> </ul>
16/11	16:50 – 17:20h	Coffee break
16/11	17:30 – 19:00h	Sessão de cartazes
17/11	8:30 – 10:30h	<ul> <li>SIMPÓSIO 3: Biodiversidade vegetal e aplicabilidades na produção de fármacos, alimentos, fragrâncias, cosméticos e agroquímicos.</li> <li>Moderador: Profa. Dra. Luzimar G. Fernandez (UFBA). <ul> <li>Profa. Dra. Luzimar G. Fernandez (UFBA)</li> <li>Desvendando a biodiversidade vegetal pela metabolômica e suas aplicações</li> </ul> </li> <li>Prof. Dr. Samuel S. da Rocha Pita (UFBA) <ul> <li>Desenvolvimento de fármacos para doenças emergentes e negligenciadas pela perspectiva integrada.</li> </ul> </li> <li>Prof. Dr. Antonio Euzébio Goulart Santana (UFBA) <ul> <li>Plantas Medicinais: propriedades, fitocompostos e inovações biotecnológicas</li> </ul> </li> </ul>
17/11	10:30 – 10:55h	Coffee break
17/11	11:00– 11:45h	CONFERÊNCIA 3: Profa. Dra. <b>Aline Cavalcanti de Queiroz</b> (UFAL-Campus Arapiraca) <i>Bioquímica e microbiologia aplicadas a produtos biotecnólogicos direcionados à saúde (Leishmaniose).</i>
17/11	14:00 – 14:45h	CONFERÊNCIA 4: Prof. Dr. <b>Mateus Matiuzzi Da Costa</b> (Universidade Federal do Vale do São Francisco) Saúde Única e Resistência aos Antimicrobianos: De Alexander Fleming a Nanotecnologia.

17/11	14:45 – 16:45h	<ul> <li>SIMPÓSIO 4: Explorando Propriedades Biológicas de Proteínas de Origem Vegetal.</li> <li>Moderador: Profa. Dra. Patrícia M.G. Paiva (UFPE) <ul> <li>Prof. Dr. Thiago Henrique Napoleão (UFPE)</li> <li>Atividades farmacológicas de proteínas de origem vegetal.</li> </ul> </li> <li>Prof. Dr. Luís Cláudio Nascimento da Silva (CEUMA) <ul> <li>Formulações polissacarídicas contendo lectinas para aplicações biomédicas.</li> <li>Profa. Dra. Adriana Fontes (UFPE)</li> <li>Aplicações glicobiológicas de nanoferramentas fluorescentes constituídas por pontos quânticos e lectinas.</li> </ul> </li> </ul>
17/11	16:50 – 17:20h	Coffee break
17/11	17:30 – 19:00h	Sessão de cartazes
18/11	8:30 – 10:30h	SIMPÓSIO 5: Proteínas com potencial terapêutico de plantas medicinais.
		Moderador: Prof. Dr. Márcio Viana Ramos (UFC)
		<ul> <li>Prof. Dr. Jefferson Soares de Oliveira. Universidade Federal do Delta do Parnaíba (UFDPar)</li> <li>Proteínas do látex de <u>Plumeria pudica</u>: aplicação na produção de nanopartículas de prata</li> </ul>
		<ul> <li>Prof. Dr. Ariclécio Cunha de Oliveira; Universidade Estadual do Ceará (UECE)</li> <li>Efeitos biológicos de proteínas fitomoduladoras isoladas do látex de <u>Calotropis procera</u></li> </ul>
		<ul> <li>Prof. Dr. José Vitor Moreira Lima-Filho; Universidade Federal Rural de Pernambuco (UFRPE)</li> <li>Lectinas vegetais como imunoterapêuticos contra bactérias patogênicas intracelulares</li> </ul>
18/11	10:30 – 10:55h	Coffee break
18/11	11:00– 11:45h	CONFERÊNCIA 5
		Prof. Dr. Pietro Ciancaglini (USP)
		Proteolipossomos: uma abordagem nanobiotecnológica aplicada para a saúde
18/11	14:00 – 14:45h	CONFERÊNCIA 6
		Profa. Dra. Andréa Mara Macedo (UFMG)
		PMBqBM: desafios e potencialidades

18/11	14:45 – 16:45h	SIMPÓSIO 6: Programa Multicêntrico da SBBq: ampliando os horizontes da Bioquímica no Nordeste
		Moderador: Prof. Dr. Francis Soares Gomes (UFAL)
		<ul> <li>Prof. Dr. Hugo Juarez Vieira Pereira (UFAL)</li> <li>Produção, caracterização e aplicação biotecnológica de enzimas fúngicas</li> </ul>
		<ul> <li>Prof. Dr. Heberty di Tarso Fernandes Facundo (UFCA: Universidade Federal do Cariri)</li> <li>Investigação da ativação fisiológica e farmacológica do canal mitocondrial de potássio sensível a ATP: estudos mecanísticos e impactos em isquemia/reperfusão.</li> </ul>
		<ul> <li>Profa. Dra. Michele Dalvina Correia da Silva (UFERSA- Universidade Federal Rural do Semi-Árido)</li> <li>Ação anti-helmíntica de lectinas de plantas</li> </ul>
18/11	16:50 – 17:00h	Coffee break
18/11	17:00 – 18:30h	Sessão de cartazes
18/11	18:45 – 19:30h	SESSÃO DE ENCERRAMENTO E PREMIAÇÕES

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#### A - Bioenergética e Metabolismo

A-01. Involvement of glucose metabolism in thymocyte functionality in vitro

<u>Barros, A.B.B</u>.<sup>1</sup>; Ferreira, E.G.A.<sup>1</sup>; Lins, M.P.<sup>1</sup> <sup>1</sup>Laboratory of Cell Biology, Federal University of Alagoas, Maceió, Brazil

For thymocytes to finalize their differentiation into immunocompetent T cells, they need to carry out the processes of intrathymic adhesion and migration, establishing contacts with thymic epithelial cells (TECs) and extracellular matrix molecules. The participation of glucose metabolism in energy generation for these phenomena has not yet been reported for these cells. Therefore, the aim of this study was to evaluate the repercussions of blocking glycolysis and oxidative phosphorylation on thymocyte adhesion and migration *in vitro*. For this purpose, C57BL/6 male mice, 4-8 weeks aged, provided by the Central Biotherium of Federal University of Alagoas, under the approval of the Ethics Committee in the Use of Animals, nº 19/2019, were used. After euthanasia, fresh thymocytes were obtained by mechanical disruption of the thymus. These cells were treated with 2-deoxyglucose (0.5mM) and rotenone ( $10\mu$ M) during one hour in order to block glycolysis and oxidative phosphorylation, respectively. Doses were established using the MTT assay. Subsequent experiments included survival assessment, cell adhesion assays and transwell migration studies. As results, we observed that the survival of treated thymocytes was not affected at 3 hours, but reduced at 6 hours, mainly in the presence of rotenone. Furthermore, adhesion of thymocytes to TECs or to laminin was not influenced by depletion of glucose metabolism, whereas cell migration was severely decreased. Thus, we list more attributions to glycolysis metabolism in the thymocyte functionality in vitro, mainly to the motility of these cells, impacting the differentiation and generation of mature T cells in vivo.

Keywords: Thymic epithelial cells, glycolysis, oxidative phosphorylation

### A-02. Evaluation of the effects of Tetrahydrolipstatin on lipid metabolism and ACSVL coding genes in *Tribolium castaneum*

<u>Santana, C.C.<sup>1</sup></u>; Nascimento, J.S.<sup>2</sup>; Silva, A.T.<sup>2</sup>; Santos, I.C.<sup>2</sup>; Dornelas C.B.<sup>2</sup>; Grillo, I. A. M.<sup>2</sup> <sup>7</sup>Setor de Fisiologia, Instituto de Ciências Biológicas e da Saúde, UFAL, Alagoas, Brasil; <sup>2</sup>Laboratório de Bioquímica Metabólica, Instituto de Ciências Farmacêuticas, UFAL, Alagoas, Brasil.

Tribolium castaneum is a coleopteran known for its high potential as plague in storage and culture of grains. T. castaneum demonstrates high homology of some proteins sequences of mammalian lipid metabolism, and it's suggested that lipidic metabolism has an important role in insect biological functions, for example in metabolic energy generation and other cell processes. Very long-chain acyl-CoA synthetases (ACSVL) have the sequence conservated between species. These enzymes are important in extracellular fat acid transportation and are also involved in the synthesis of hormones and pheromones. Recent research shows the gene silencing of ACSVLs using RNA interference technique in insects affect fat acid absorption, that enables this insect to be utilised as experimental model to elucidate important metabolic pathway that can be like in mammalian. Due to be easy to incorporate bioactive compounds in their food, this research will evaluate the effects of tetrahydrolipstatin (lipase inhibitor) in lipidic metabolism and in gene expression of ACSVLs in insects. After tetrahydrolipstatin incorporating in larvae food (0.1 mg/ flour mg), we evaluated the effects in larvae weight, triacylglycerol concentrations, intestinal lipasic activity and genic expression of ACSVLs, sulphakinine and genes for insulin. Treatment results with lipase drug inhibitor didn't show effect over average larval weight but shown more than 30 % of reduction on enzymatic activity, it can prove that there is effect in the drug used in this experimental model. According to the qPCR analysed data, ACSVLs genic expression was significantly stimulated after 96 hours of treatment, and it was also observed the expression of 2 insulin similar peptides (In1 and In2) was reduced. Sulphakinines expression and peripherical insulin receptors also reduced. The results of treatment with commercial lipase inhibitor shown flotation in ACSVLs expression. Food intake regulation pathways that involve the express-03. ion of sulphakinines and insulin similar peptides characterized in literature suggest that ACSVLs probably have the expression regulated by food intake. So, it's possible concludes that T. castaneum are an invertebrate model valid to studies of organism- environment interactions, mainly in relation to drugs interference in lipids and carbohydrates metabolism.

KEYWORDS: ACSVL. *Tribolium castaneum*. Tetrahydrolipstatin.

### A-03. Comparative studies of mercury exposure in the brain of hypertensive animals considering related to redox status

Sales, M.V.S.<sup>1</sup>; <u>Moraes, D.R.<sup>1</sup></u>; Queiroz, M.I.C.<sup>1</sup>; Santos J.C.C.<sup>1</sup>; Leite, A.C.R.<sup>1</sup>. <sup>1</sup>Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió, Brazil.

INTRODUCTION: Thimerosal (TM) is a mercury species compound used as a vaccine preservative with side effects in some biological systems. Hypertension is a disease characterized by high pressure levels, and the nervous system has an essential role in its development. Knowing that mercury leads to oxidative stress and the nervous system is the most affected organ by this metal, the lack of studies about the TM effect in the brain from hypertensive animal models is an important point. GOAL: Investigate the impact of acute and chronic exposure to TM in spontaneous hypertensive rats (SHR), focusing on brain antioxidant enzyme activities and secondary markers of oxidative stress. METHODOLOGY: SHR animals were submitted to TM treatment (0.5 mg/kg/day) in two different ways: acute (24 h) and chronic (30 days) exposition in water. The control animals received only water. The homogenate brain assays: antioxidant enzymes activities (Superoxide Dismutase - SOD and Catalase - CAT) and the sulfhydryl content were assessed in a spectrophotometer (Shimadzu UV-1900i). Other secondary oxidative stress markers from the brain, such as GSH/GSSG, were assayed on a plate reader (Tecan Infinite 200 pro). Lactate Dehydrogenase (LDH) from plasma was determined using a commercial kit (Labtest). This study is authorized by CEUA 01/2023. RESULTS: Brain antioxidant enzyme activity increased 100% for SOD and 83% for CAT in the SHR-acute treated group. Similarly, SOD activity showed a 67.5% increase in the chronically treated group, while CAT decreased by 53% compared to the control. The content of the sulfhydryl group in the brains of acutely treated animals showed a reduction of 46%; on the other hand, the chronic group showed no statistical differences ( $\alpha$  = 0.05). Analyzing another redox parameter, the GSH/GSSG ratio, no statistical differences were found in both treatment groups ( $\alpha$  = 0.05). The LDH activity increased by 64% in the treated group, only in the chronic treatment group. CONCLUSION: According to our results, TM treatment collectively (acutely or chronic) leads, in the brain of SHR animals, to an impairment in the redox status and probably a compromise of the nervous system, as seen by LDH activity. These data show that the mercury species could be essential in developing hypertension.

Keywords: Hypertension, Mercury, Oxidative stress. Acknowledgments: FAPEAL, CAPES, UFAL.

#### A-04. Main Mechanisms of Glyphosate Degradation by Edaphic Microorganisms

Souza, K.S.<sup>1</sup>; Silva, M.R.F<sup>1</sup>; Barros, A.V.<sup>1</sup>; Sá, R.A.Q.C.<sup>1</sup>; Dantas, T.F.<sup>1</sup>; Araújo, L.C.A.<sup>1</sup>; Motteran, F.<sup>2</sup>; Silva, K.C.C.<sup>3</sup>; Oliveira, M.B.M.<sup>1</sup>

<sup>1</sup> Department of Biochemistry, Laboratory of Molecular Biology, Federal University of Pernambuco-UFPE, Recife, PE, Brazil; <sup>2</sup>Department of Civil and Environmental Engineering, Federal University of Pernambuco - UFPE, Recife, PE, Brazil; <sup>3</sup> State University of Bahia-UNEB, Paulo Afonso, BA, Brazil

**Introduction:** Glyphosate herbicide is one of the most commonly applied in the agricultural industry, which has been used to remove weeds, mainly in genetically modified plant crops. This defensive presents less toxicity compared to the others, however, it can cause negative effects for the beneficial microorganisms of the soil. Thus, one of the main strategies for remediation of glyphosate in the soil is the use of microorganisms for the development of degradation mechanisms or tolerance to glyphosate. **Objective:** Approach through the scientific literature about the main mechanisms of degradation of glyphosate by soil microorganisms. Materials and Methods: This was a systematic review of the literature, which used the main databases and electronic libraries: Scielo, Scopus, Pubmed and Periódico Capes, using as a search strategy the main keywords and descriptors with operators Booleans: "Biodegradation" AND "Glyphosate" OR "Herbicides" AND "Microbiota" AND "Soil" AND "Bacteria". The studies recommended for this research were in the languages: Portuguese, English and Spanish, from the last 10 years. Results and discussions: According to the delimitation of inclusion and exclusion criteria, there was a total of about 8 studies for the composition of this systematized review. Glyphosate (N-phosphonomethyl) glycine) belongs to the chemical group of pesticide organophosphates. Therefore, this proves to be highly resistant to chemical decomposition, so that it presents a stable bond between carbon and phosphorus (C-P). The edaphic microorganisms are the main representatives for generating the complete degradation of glyphosate, considering that many bacteria use carbon (C), phosphorus (P) and nitrogen (N) in their metabolic pathways. Thus, microorganisms can catabolize glyphosate through the excision of the C-P bond by lyase, which results in the formation of phosphate and sarcosine, in addition to the cleavage pathway of the C-N bond by the enzyme glyphosate oxidoreductase (GOX), forming the acid aminomethylphosphonic acid (AMPA) and glyoxylate. Therefore, these mechanisms are mainly demonstrated by bacteria isolated from soils already contaminated by glyphosate, resulting in them having mechanisms to adapt to the compound. Conclusions: The use of microorganisms for bioremediation of glyphosate in soils is considered a promising tool and adjuvant, however, more studies are needed to be carried out in order to analyze the presence of glyphosate and metabolic degradation genes that are more soluble and present less toxicity.

Keywords: Degradation. Herbicide. Soil microbiota.

**Acknowledgments:** CAPES (Coordination for the Improvement of Higher Education Personnel).

### A-05. Potential for Biodegradation of Microplastics by Microbial Biofilms: An Alternative for Bioremediation

<u>Silva, M.R.F<sup>1</sup></u>; Souza, K.S.<sup>1</sup>; Barros, A.V.<sup>1</sup>; Sá, R.A.Q.C.<sup>1</sup>; Dantas, T.F.<sup>1</sup>; Araújo, L.C.A.<sup>1</sup>; Motteran, F.<sup>2</sup>; Oliveira, M.B.M.<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Laboratory of Molecular Biology, Federal University of Pernambuco-UFPE, Recife, PE, Brazil; <sup>2</sup>Department of Civil and Environmental Engineering, Federal University of Pernambuco - UFPE, Recife, PE, Brazil;

**Introduction:** Due to the growing global production of plastics, mainly polypropylene (PP), polyethylene (PE) and polyethylene terephthalate (PET), the presence of these compounds is increasingly active in the environment, so that, according to weathering processes, they produce whether microplastics. Therefore, microplastics are considered non-biodegradable and have negatively affected the biosphere, especially aquatic environments, which in turn, bioremediation strategies have been addressed to reduce these compounds in nature, including microbial biofilms. Objective: Discuss through the scientific literature about the biodegradation potential of bacterial biofilms in microplastics. Materials and Methods: This was a systematic review of the literature, which used the main databases and electronic libraries: Scopus, Pubmed and Periódico Capes. For the composition of the search strategy, keywords and Boolean operators were used: "Biodegradation, Environmental" AND "Biofilms" AND "Microplastics", with studies in English and Portuguese in the last 5 years. Results and discussions: A total of 12 scientific studies were included in this systematic review, in accordance with the recommended eligibility criteria. The physical and chemical alteration by the action of microorganisms, mainly bacteria, is called biodegradation, so that there are different mechanisms through which they metabolize natural and synthetic compounds, such as microplastics. One of the biodegradation processes of microplastics by bacteria is through biofilm formation, considering that these microorganisms easily have the ability to colonize microplastic surfaces and form the platisphere. This process of biodegradation through the formation of platysphere in microplastics is due to aerobic and anaerobic biodegradation. In aerobic biodegradation the respective organic compounds are transformed into smaller compounds using oxygen as an electron acceptor, resulting in carbon dioxide and water as byproducts. In anaerobic biodegradation, microorganisms use nitrate, iron, sulfate, manganese, and carbon dioxide as an electron acceptor in place of oxygen to break down large organic compounds into smaller compounds. Conclusions: It is concluded that the formation of platysphere in microplastics can influence the metabolic mechanism of direct or indirect degradation of plastic through microbial metabolism.

Keywords: Biodegradation. Microplastics. Bioremediation.

Acknowledgments: CNPQ (National Council for Scientific and Technological Development).

### A-06. Metabolic Modulation in Contrasting Sorghum Varieties Induced by Endoplasmic Reticulum Stress

<u>Cavalcante, F.L.P.</u><sup>1</sup>; Costa, I.R.S.<sup>1</sup>; Lopes, L.S.<sup>1</sup>; Gomes-Filho, E.<sup>1</sup>; Carvalho, H.H.<sup>1</sup> <sup>1</sup> Department of Biochemistry and Molecular Biology, Federal University of Ceará, Fortaleza, Ceará, Brazil

Sorghum (Sorghum bicolor (L.) Moench) is a significant cereal crop native to the African continent and belonging to the Poaceae family. It is considered the fifth most-produced cereal in the world. From the agronomic point of view, this growth is explained by the high potential of grain production and dry matter of the crop. However, like any living organism, sorghum can be affected by abiotic and biotic stresses that compromise its productivity. Several of these responses to unfavorable conditions are modulated by plants, such as morphophysiological and molecular adjustments, in addition to the induction of the unfold protein response pathway occurring in the endoplasmic reticulum (ER). The efficiency of the ER in dealing with the accumulation of poorly folded proteins is fundamental for the survival of the plants in any situation of stress. It is relevant to identify genotypes that present different physiological, biochemical, and molecular responses in relation to the levels of sensitivity to stress. Thus, a study was developed that includes advances in the knowledge of the ER response induced by treatments with tunicamycin (TM) and dithiothreitol (DTT), especially their downstream physiological impacts, in order to help clarify possible mechanisms of adjustment to environmental stresses from adaptation for survival or death. To complement the understanding of the various responses used in the face of stresses, a metabolomic approach was carried out involving metabolomics, multidisciplinary science that offers unique possibilities to decode metabolic modulations through an approach that goes beyond the transcriptome and the proteome. Therefore, the aim of this research was to evaluate changes in the metabolic profiles of two varieties of sorghum seedlings, CSF18 and CSF20, under endoplasmic reticulum (ER) stress-induced by increasing tunicamycin concentrations from 0 to 2.5 ug.ml<sup>-1</sup>. The results showed that stress-induced a reduction in growth, mainly of the root. The lower concentration of TM induced the highest lipid peroxidation of both varieties. The Score plot results indicated a separation of the TM treatments from control. There was a negative modulation of key metabolites in CSF18 and a positive modulation in CSF20 related to a significant increase in carbohydrates and organic acids, which may help to keep the growth of CSF20.

#### Keywords: Metabolomics. Sorghum bicolor. Tunicamycin.

**Acknowledgments:** We would like to thank Instituto Agronômico de Pernambuco (IPA) to provide sorghum seeds. Thanks to National Council for Scientific and Technological Development (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP).

#### B - Biologia Estrutural e Enzimas

B-01. Production and characterization of oxidoreductase from filamentous fungi <u>Sampaio, A.B.C.<sup>1</sup></u>; Silva, M.C.<sup>1</sup>; Souza, C. B.<sup>1</sup>; Santos, M.A.<sup>1</sup>; Gonçalves, A.H.S.<sup>1</sup>; Nicodemus, I.S.<sup>1</sup>; Pereira, H.J.V<sup>1</sup>

<sup>1</sup>Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió, Alagoas, Brazil

Introduction: The production of oxide reductases, which have many applications in biotechnology, involves growing filamentous fungi under controlled conditions, providing nutrients and ideal conditions for expression and secretion. This can be done using different sources of temperature, storage, humidity and number of samples for cultivation. The oxide reductases were obtained by filamentous fungi (Penicillium roqueforti, Pleurotus djamor) through the stress generated in the cultivation with the wheat bran substrate. Objective: the present study has a biotechnological intention for green synthesis, based on the characteristics of the secretion of oxide reductase enzymes, with emphasis on laccase, by filamentous fungi. Materials and methods: the production of oxide reductase enzymes began with the selection of strains of fungi Penicillium roqueforti, Pleurotus djamor. The culture media used were Potato Dextrose (BDA) and Agarose (AGAR). The cultivation conditions were solid fermentation, with wheat bran as substrate. Culture medium samples were collected for a 10day growth curve. After the cultivation period, the enzymes are extracted from the culture medium. Then there was the enzymatic characterization from spectrophotometry with specific substances for its visualization, trying to obtain an absorbance lower than one to identify the presence of the enzyme in that extract. Results and discussions: A growth curve related to the presence of oxido reductase was obtained from the fungus Penicillium roqueforti with the number of 7 mycelia applied to the wheat substrate for cultivation. The day with the highest secretion was day 3, with a spectrophotometric result of 0.817 absorbance. The selection of fungal strains, use of culture media and induction methods such as wheat bran contributed to maximize expression and enzymatic activity. The spectrophotometric analysis of the samples over time provided the growth and secretion profile of the enzymes, revealing a peak of activity on the third day of cultivation. These results indicate a considerable potential for the use of these enzymes in diverse biotechnological applications and contribution to health and innovation in the field of biotechnology. Conclusion: the present study demonstrates a promising approach in the search for biotechnological sources of green synthesis, focus in production of oxide reductase enzymes, especially laccase. Acknowledgments: the authors are grateful to CAPES, CNPq and FAPEAL for funding this research. Keywords: laccase green synthesis, solid fermentation.

#### B-02. PRODUCTION AND CHARACTERIZATION OF A HALOTOLERANT AND THERMASTABLE ENDOGLUCANASE FROM *Penicillium roqueforti* ATCC 10110

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In recent years, there has been a growth in the production of agricultural products, due to the population increase, also generating a large amount of waste that, if not treated and disposed of correctly, can negatively impact the environment. Thus, solid-state fermentation emerges as an alternative for the reuse of waste using fungi for the production of enzymes of biotechnological interest. The present work sought to produce and characterize endoglucanase using the solid state fermentation (SSF) technique by the action of the filamentous fungus Penicillium roqueforti ATCC 10110 on lignocellulosic residues such as pineapple crown (CA), sugarcane bagasse (CA), coconut husk (CC) and wheat bran (FT). The characterization of the enzymatic crude extract containing endoglucanase was carried out regarding thermostability, stability at different pHs, halotolerance, optimal pH and temperature, and stability of the crude extract under frozen (-20°C) and cold (6°C) storage conditions. The fermentation profile of the enzyme showed that the endoglucanase activity was higher in wheat bran (0.879 U mL<sup>-1</sup> after 96 hours), with an optimal temperature of 50°C and maintaining 100% of its activity after 5 hours, pH 5 as optimal and with 80 % of activity after the incubation period. In addition, endoglucanase proved to be halotolerant, as its enzymatic activity increased in all NaCl addition concentrations, obtaining a better result in the 2 M concentration, which had a 35% increase in activity in the presence of salt. The present work concluded that the fungus P. roqueforti is a producer of an endoglucanase with important biochemical characteristics, fast production and low cost. This project was carried out thanks to the support of CNPq, the Federal University of Alagoas, and the Laboratory of Metabolism and Proteomics.

Keywords: Biochemical characterization, Penicillium roqueforti, endoglucanase.

# B-03. Anticancer properties and *in silico* immunogenicity of recombinant L-asparaginase of *Phaseolus Vulgaris*

<u>Gomes, J. G. S.</u><sup>1</sup>; Brandão, L. C.<sup>2, 3</sup>; Ribeiro, D. P.<sup>3</sup>; Furtado, G. P.<sup>3</sup>; Rocha, B. A. M<sup>1</sup>. <sup>1</sup>Department of Biochemistry and Molecular Biology, Federal University of Ceará, Fortaleza, Brazil; <sup>2</sup>Department of Fishing Engineering, Federal University of Ceará, Brazil; <sup>3</sup> Oswaldo Cruz Institute - Ceará, Eusébio, Brazil

INTRODUCTION: Acute Lymphoblastic Leukemia (ALL) is a neoplasia type that primarily affects juveniles and relies on chemotherapy as a first line treatment. Among the medicines used, bacterial L-asparaginase plays a critical role on reducing the malignant cell count, although it frequently causes adverse reactions on patients. Due to its importance on the treatment, the search for alternatives is vital. OBJECTIVES: In this context, this study aims to obtain the recombinant L-asparaginase from Phaseolus vulgaris and evaluate its cytotoxic effect on leukemia cells and its immunogenicity. MATERIALS AND METHODS: The gene of Lasparaginase (Asp-P) was purchased in expression vector pET-28a. The plasmid was transformed into E. coli BL21 (DE) Rosetta. The protein of interest was expressed overnight induced with 0,3 mM IPTG, 200 rpm, 20° C. The cells were lysed through sonication and the protein was partially purified through affinity chromatography. The purification process was monitored by SDS-PAGE analysis. The catalytic activity was assessed through the Nesslerization method. The cytotoxic effect was assessed using Raji and K562 cell lines, which were incubated for 72 h with Asp-P. Afterwards, cell viability was measured by the MTT method. Immunogenicity of Asp-P and the commercial bacterial enzyme was predicted by in silico methods utilizing online tools. RESULTS AND DISCUSSION: Asp-P was successfully expressed in *E. coli*, yielding 4 mg.L<sup>-1</sup> of culture. The partially purified fraction presented a specific activity of 56,69 U.mg<sup>-1</sup> towards L-asparagine at optimal pH 9, 40° C. Only the highest concentration tested of Asp-P (250 µg.mL<sup>-1</sup>) was able to reduce Raji viable cells below 50 %. The same could not be achieved for K562, denotating its higher resistance to this treatment. No IC<sub>50</sub> could be calculated. Lastly, immunogenicity prediction indicated that Asp-P is less immunogenic compared to the bacterial enzyme, since it had fewer predicted epitopes. CONCLUSION: Together, these results show the successful high yield expression of *P. vulgaris* L-asparaginase in bacterial system, with promising cytotoxic activity and lower immunogenicity. Also, these data point to possible improvements on the enzyme by protein engineering in order to enhance its catalytic efficiency and its probability to substitute the commercial enzymes.

Keywords: L-asparaginase; Kidney bean; Anti-neoplastic. Acknowledgements: CAPES; GEPeSS – Fiocruz/CE

# B-04. PRODUCTION AND PURIFICATION OF LIPASE OBTAINED BY LIQUID FERMENTATION (FL) OF YEAST *Moesziomyces aphidis*

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**Introduction:** The enzyme market has gained increasing prominence in industrial production. Enzymes exist and are important in the metabolism of all living beings. Considering the lipases, which are able to catalyze the hydrolysis of lipids that are presented in different sources, making it a promising alternative for the food, pharmaceutical, chemical industry, among others. **Objective:** Therefore, the objective of this work was the production of lipases by the yeast Moesziomyces aphidis in liquid fermentation (FL). The enzymatic production profile of lipase by FL was evaluated in low cost residues: Residual frying oil (OFR), Yeast extract (E) and Green coconut pulp (PC). The yeast Moesziomyces aphidis BRT57 is part of the collection of the Molecular Diversity Laboratory (LDM) at ICBS/UFAL and was isolated from bromeliad leaves, in the Tocaia reserve, in the state of Alagoas. Materials and methods: The yeast was previously tested for the production of lipases through the hydrolysis of the tributyrin substrate in a Petri dish. The liquid fermentation composition for selection of the best yeast and best inducer were residual frying oil, residual frying oil and yeast extract, green coconut pulp and green coconut pulp and yeast extract. Results and discussions: The production time was observed for up to 72 h, with aliquots withdrawn every 24 h to verify the enzymatic activity. The purification of the lipase was performed using saline fractionation, molecular exclusion chromatography and electrophoresis (SDS-PAGE) processes. Conclusion: The study demonstrated the viability of lipase production in liquid fermentation produced by Moesziomyces aphidis yeasts. An unprecedented enzyme was produced with the completion of this work.

**Keywords:** Lipase; purification; liquid fermentation; Moesziomyces aphidis. **Supported by:** CAPES e CNPq

### B-05. IDENTIFICATION OF TWO CATALYTIC SITES IN *SACCHAROMYCES CEREVISIAE* METACASPASE

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Introduction: S. cerevisiae is a yeast that encodes a single metacaspase, called Yeast Caspase 1 (YCA1). The metacaspases depend on calcium ion to self-process and carry out their activity. Thus, the study of the metacaspase YCA1 from S. cerevisiae is important to understand its role in cell death and to find possible therapeutic targets for fungal diseases. Objectives: Production YCA1 in its full form (YCA1) and with the deletion of the N-terminal portion (tYCA1) containing the site-directed mutations of cysteines Cys<sup>155</sup> and Cys<sup>276</sup>. Methodology: Full and truncated YCA1, wild-type and mutants were obtained by the T5 exonuclease DNA assembly (TEDA) method. Expression was in E. coli BL21 (DE3)pLysS cells and induced with 0.25 mM IPTG. Enzymes were purified by affinity chromatography on nickel-sepharose. The importance of cysteines for YCA1 catalysis were evaluated on 15% SDS-PAGE and by Western Blotting using anti-His(6X) antibodies. Results: Was observed that the intact form of YCA1 (YCA1 WT) are quickly degraded after self-processing and assuming the active form and showed low expression yield. With YCA1 C276A it was possible to visualize two fragments formed after incubation with 10 mM Ca<sup>2+</sup>. With YCA1 C155A no self-processing was observed, and with double mutation C155/276A there was no self-processing, proving that Cys<sup>276</sup> is responsible for the catalysis action, exerting proteolytic activity only after the action of Cys<sup>155</sup> that promotes the processing and the formation of the enzyme in its active form. For tYCA1 was observed that this enzyme continued processing. This processing is considered intermolecular and is characterized by the action of active tYCA1 processing other unprocessed tYCA1 enzymes and results in a second processing with fragments of ~31 kDa. Conclusion: Thus, our data demonstrate that the first processing is an self-processing that converts YCA1 to its active form and gives it the ability to perform intermolecular processing of other already processed YCA1, resulting in the fully mature and active form of the enzymes. After self-processing, YCA1 becomes capable of performing intermolecular processing of other YCA1s that have also been processed, thus generating its fully mature and active form.

Acknowledges: FAPESP, FAEP and CAPES.

Keywords: catalysis, mutation, self-processing

# B-06. Production, purification, characterization, and evaluation of the antitumoral activity of the Lectin from the bark of *Genipa americana* L (GaBL)

<u>Costa, R.B.</u><sup>1</sup>; Silva, M.C.<sup>2</sup>; Fraga, C. A. de C.<sup>3</sup>; Santos, M. A. dos<sup>2</sup>; Queiroz, S. F.<sup>4</sup>, Pereira, H. J. V.<sup>2</sup>; Gomes, F. S.<sup>2</sup>;

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**INTRODUCTION:** Plant proteins hold significant economic and pharmacological potential, primarily due to the presence of bioactive compounds. Among these, lectins have been extensively explored for their various biotechnological applications, notably their antifungal, anticoagulant, and antitumor activities. OBJECTIVE: To purify, characterize, and assess the antitumor activity of the lectin from Genipa americana L. (jenipapo) (GaBL). METHODOLOGY: The hemagglutinating activity of the lectin in the crude extract was evaluated against rabbit erythrocytes. Purification was carried out using salt fractionation and molecular exclusion chromatography. Specific assays were conducted to determine the biochemical characteristics of the purified lectin. The antitumor activity of GaBL was assessed in human skin cancer (A431), melanoma (B16), and squamous cell carcinoma of the tongue (SCC9) cell lines. These cell lines were treated with 10  $\mu$ g/ml of GaBL to evaluate cell viability, migration, and invasion. RESULTS AND DISCUSSION: GaBL was successfully purified with 100% yield via molecular exclusion chromatography, with an approximate molecular mass of 242.5 kDa. Hemagglutinating activity was inhibited by lactose and fetuin but unaffected by Ca2+ and Mg2+ ions. GaBL was classified as thermostable, remaining active between temperatures of 30°C and 120°C for 30 minutes and within a pH range of 5.0 to 11.0. GaBL exhibited optimal temperature and pH conditions at 60°C and 5.0, respectively. Treatment with GaBL at a concentration of 10  $\mu$ g/ml showed promising effects on multiple aspects of carcinogenesis. Cell viability was significantly compromised, with a proliferation reduction ranging from 27.5% to 50%. Reduced cell migration was observed in all cell lines, and a decrease in the invasion capacity of SCC9 cells after GaBL treatment was encouraging. CONCLUSION: The results obtained demonstrated that the purified lectin (GaBL) exhibited remarkable recovery and specific activity. GaBL was classified as thermostable due to its stability in a temperature range and pH optimum of 20°C to 120°C and 5.0 to 11.0, respectively. It exerted a strong antitumor effect by inhibiting cell proliferation, reducing migration and invasion. These findings provide a solid foundation for future research aimed at the clinical application of GaBL in in vivo cancer treatment, representing a promising therapeutic alternative.

ACKNOWLEDGMENTS: FAPEAL, CNPq, and CAPES.

**KEYWORDS:** Genipa americana; Plant lectin; antitumor activity.

# B-07. Production, purification, characterization, and evaluation of the antitumoral activity of the Lectin from the bark of *Genipa americana* L (GaBL)

<u>Costa, R.B.</u><sup>1</sup>; Silva, M.C.<sup>2</sup>; Fraga, C. A. de C.<sup>3</sup>; Santos, M. A. dos<sup>2</sup>; Queiroz, S. F.<sup>4</sup>, Pereira, H. J. V.<sup>2</sup>; Gomes, F. S.<sup>2</sup>;

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**INTRODUCTION:** Plant proteins hold significant economic and pharmacological potential, primarily due to the presence of bioactive compounds. Among these, lectins have been extensively explored for their various biotechnological applications, notably their antifungal, anticoagulant, and antitumor activities. OBJECTIVE: To purify, characterize, and assess the antitumor activity of the lectin from Genipa americana L. (jenipapo) (GaBL). METHODOLOGY: The hemagglutinating activity of the lectin in the crude extract was evaluated against rabbit erythrocytes. Purification was carried out using salt fractionation and molecular exclusion chromatography. Specific assays were conducted to determine the biochemical characteristics of the purified lectin. The antitumor activity of GaBL was assessed in human skin cancer (A431), melanoma (B16), and squamous cell carcinoma of the tongue (SCC9) cell lines. These cell lines were treated with 10 µg/ml of GaBL to evaluate cell viability, migration, and invasion. RESULTS AND DISCUSSION: GaBL was successfully purified with 100% yield via molecular exclusion chromatography, with an approximate molecular mass of 242.5 kDa. Hemagglutinating activity was inhibited by lactose and fetuin but unaffected by Ca2+ and Mg2+ ions. GaBL was classified as thermostable, remaining active between temperatures of 30°C and 120°C for 30 minutes and within a pH range of 5.0 to 11.0. GaBL exhibited optimal temperature and pH conditions at 60°C and 5.0, respectively. Treatment with GaBL at a concentration of 10  $\mu$ g/ml showed promising effects on multiple aspects of carcinogenesis. Cell viability was significantly compromised, with a proliferation reduction ranging from 27.5% to 50%. Reduced cell migration was observed in all cell lines, and a decrease in the invasion capacity of SCC9 cells after GaBL treatment was encouraging. CONCLUSION: The results obtained demonstrated that the purified lectin (GaBL) exhibited remarkable recovery and specific activity. GaBL was classified as thermostable due to its stability in a temperature range and pH optimum of 20°C to 120°C and 5.0 to 11.0, respectively. It exerted a strong antitumor effect by inhibiting cell proliferation, reducing migration and invasion. These findings provide a solid foundation for future research aimed at the clinical application of GaBL in in vivo cancer treatment, representing a promising therapeutic alternative.

ACKNOWLEDGMENTS: FAPEAL, CNPq, and CAPES.

KEYWORDS: Genipa americana; Plant lectin; antitumor activity.

# B-08. The coagulant lectin from *Moringa oleifera* seeds (cMoL) inhibited biofilm formation by *Cryptococcus neoformans*, a fungal species with high clinical relevance

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Cryptococcosis, an opportunistic systemic mycosis that mainly affects immunosuppressed patients, is caused by fungi of the genus Cryptococcus. Due to its clinical relevance, C. neoformans was categorized in the critical group of the list of priority fungal pathogens published by the World Health Organization (WHO). In addition, the ability of this species to form biofilms increases resistance and tolerance to antibiotics, impairing the development of new treatments. Lectins are carbohydrate-binding and hemagglutinating proteins with antimicrobial activity. cMoL, a coagulant lectin isolated from Moringa oleifera seeds, recognizes galactose and inhibits the growth of C. neoformans. The present study evaluated the potential of the cMoL to inhibit biofilm formation by C. neoformans. M. oleifera seed proteins (10g) were extracted (6 h) using 0.15 M NaCl (100mL) and after extract treatment with 60% (w/v) ammonium sulfate, the precipitated protein was chromatographed on guar gum column. cMoL was recupered from column with 1M NaCl 1 M. C. neoformans was cultivated in Sabouraud dextrose broth (SDB) for 48 h at 30 °C and cMoL antibiofilm activity was determined in microtiter plates using crystal violet dye. Data were evaluated using oneway analysis of variance (ANOVA) followed by Tukey's test using the Software Prism Graphpad 7.0. cMoL significantly inhibited (80%) biofilm formation at concentrations of 30, 60 and 120  $\mu$ g/mL. The study revealed that cMoL is efficient agent against biofilm formation by C. neoformans and encourages further investigations to define the mechanism involved in this activity. Acknowledgements: The authors express their gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco(FACEPE) for financial support.

Keywords: Antibiofilm. Cryptococcosis. Mycosis.

#### C - Bioquímica e Biologia Computacional

#### C-01. Cellular Architecture of Brain Metastases: A Single-Cell Analysis

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Cancer is a multifactorial disease that involves dysregulations in various cellular and metabolic mechanisms. Among the mechanisms adopted by neoplastic cells to achieve success in tumor dissemination, the architecture of metastatic niches, i.e., microenvironments responsible for receiving neoplastic cells from their primary sites, is noteworthy for bringing together cellular and biochemical modifications in target tissues, aiming to ensure the survival of mutated cells in distant locations. Although some tumor microenvironments have already been described, the architecture of the metastatic microenvironment in the brain remains poorly understood. In light of this, the present study aims to evaluate brain metastases, their microenvironment, and associated molecular events that allow successful cell dissemination, through an approach primarily based on single-cell data (scRNA-seq). The scRNA-seq data for the tumor types analyzed in this work were obtained from the GEO database. Data from lung cancer, breast cancer, melanoma, colon cancer, rectal cancer, kidney cancer, and ovarian cancer were used. The R package 'Seurat' v.2.3.4 was used for the analysis and exploration of scRNA-seq data. This package allowed for quality control of cells and genes, identification of the most variable genes, cluster identification (cell types), clustering, dimension reduction, and identification of marker genes (differential expression). Furthermore, for functional enrichment analysis of cells, the reactome, KEGG, and GO databases were used. We obtained scRNA-seq data from previously published specimens of parenchymal brain metastasis (BrM) from patients diagnosed with melanoma (n = 6), breast cancer (n = 12), lung cancer (n = 14), ovarian cancer (n = 2), colorectal cancer (n = 1), and renal cell carcinoma (n = 1). We observed that there are expression patterns of the genes SDC1, SDC4, MDK, MIF, NCL, and PTN in the different types of metastases analyzed. Additionally, we primarily identified the types of neoplastic, stromal, and immune cells in different types of brain metastases. The metastatic event is related to 90% of deaths involving neoplasms; studies characterizing the developments of this condition can help identify potential therapeutic targets and, consequently, improve the outlook and quality of life for patients.

**Acknowledgments:** We thank the support of our team and funding agencies. **Keywords**: scRNA-seq; Tumor microenvironment; Cancer.

#### C-02. Molecular Docking, Drug Design and *In Silico* Toxicity of Anti-Inflammatory Compounds of *Euphorbia tirucalli* against Colorectal Cancer

<u>Silva, C. U.</u><sup>1</sup>; Lopes, E. D. B<sup>1</sup>; Oliveira, G. B.<sup>1</sup>; Barros, M. R. C.<sup>1</sup>, M. Lima, L. C. G.<sup>1</sup>; Fernandez, L. G.<sup>1</sup>; Da Silva, M. B.<sup>1</sup>

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**INTRODUCTION**: Euphorbia tirucalli Lineu, belonging to the Euphorbiaceae family, originally from Africa and acclimatized in Brazil is a tree that produces latex known for its many popular applications. It has been the subject of research due to its antitumor and anti-inflammatory properties due to the presence of compounds that exhibit the ability to inhibit proinflammatory factors found in various cancers, including colorectal cancer (CRC). This type of cancer is initiated by immune response effector molecules such as COX-2, NFkB, cytokines and chemokines, which are important at different stages of tumor development, including initiation, promotion, malignant conversion, invasion, and metastasis. OBJECTIVES: Perform a virtual screening of the interactions of the proteins responsible for the progression of the colorectal cancer with the bioactive hybrid molecule of Euphorbia tirucalli, which has antiinflammatory effects. MATERIAL AND METHODS: A table of CRC proteins and bioactive compounds from Euphorbia tirucalli were compiled based on bibliographic search of PubMed, Scielo and ScienceDirect. The crystallographic structures of the CRC proteins (STAT3, CDK8, COX-2 and NF-kB) have been downloaded from the PDB database. The 3D ligands structure were obtained from PubChem and LOTUS databases. The generation of the 3D pharmacophore model was determined using the MOE software (https://www.chemcomp.com/Products.htm). When ligands interact with the receptor, the ligand can have many potentially viable conformations. MOE deploys multiple flexible alignment processes for each input ligand separately. Candidate pharmacophores are generated in which one of the ligands serves as a pivot on which the other target ligands are aligned. Site-directed molecular docking was performed by busing AutoDockTools, guided by the amino acids in the active site of each protein. Selective drugs for each molecular target were used. Discovery Studio was employed to analyze the docking results. The in silico evaluation of the toxicity of the hybrid molecule and the drugs was carried out in Pro-TOX II. **RESULTS AND DISCUSSION**: The bibliographic search resulted in 62 molecules involved in CRC and 29 bioactive molecules from Euphorbia tirucalli, all identified with database codes. Docking was carried out by observing the binding energy ranking and the interaction panel, resulting in a more favorable interaction between the hybrid molecule and CCR. In silic toxicity analysis demonstrated lower toxicity of the hybrid molecule in relation to reference drugs..CONCLUSION: This work deduced that the hybrid molecule have good potential antiinflammatory against CRC.ACKNOWLEDGMENTS: UFBA, PMBqBM and FAPESB. KEYWORDS: Euphorbia tirucalli; molecular docking; colorectal cancer.

#### C-03. Immunomodulatory Activity of the Lectin from Aesculus hippocastanum Seeds

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Medicinal plants play a crucial role in the discovery of new compounds with therapeutic potential. The seeds of Aesculus hippocastanum L. (horse chestnut) are used for various medicinal purposes due to their anti-inflammatory, vasoprotective, and analgesic properties. Lectins are proteins that reversibly bind to carbohydrates and have wide biotechnological application. Their interaction with glycan residues in cells can trigger immune responses such as proliferation, differentiation, and cytokine release. In this context, we evaluated the A. hippocastanum seed lectin (AhSL) for immunomodulatory activity on mouse splenocytes. The lectin was purified following a previous established protocol. Powder (10 g) of A. hippocastanum seeds was homogenized in saline solution (100 mL), and the extract was obtained after filtration and centrifugation (12,000 × g, 25 °C, 15 min). For AhSL purification, the extract in Tris buffer (100 mM Tris-HCl pH 8.0) was loaded onto a DEAE-Sephadex column (10 mL bed volume) equilibrated with Tris buffer. AhSL was eluted with Tris buffer containing 1.0 M NaCl. Splenocytes from mice were collected and cultured in RPMI 1640 medium and then treated in the absence and presence of AhSL. Flow cytometry and staining with propidium iodide and annexin V were used to check cell viability. Levels of ROS in the cytosol and mitochondria were measured, along with mitochondrial transmembrane potential (ΔΨm), using specific probes (dihydroethidium; MitoSox Red; and MitoStatus). Cytokine (IL-2, IL-4, IL-6, IL-10, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ ) levels in culture supernatant were quantified by cytokine bead array (CBA) method. AhSL concentrations (3.12 to 50 µg/mL) did not exhibit cytotoxicity while significantly (p < 0.05) induced the release of IL-6, IL-10, and TNF- $\alpha$  by the splenocytes. There were no alterations in  $\Delta \Psi m$  and ROS levels. The findings highlight AhSL as a bioactive protein with promising prospects in Biotechnology, potentially exploitable for immune targeting and antitumor therapy.

Keywords: Horse chestnut. Cytokines. Flow cytometry.

### C-04. Fingerprint Analysis of Interactions of FDA-approved Drugs Toward Chikungunya nsP2 protease – A repurposing *in silico* study

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Chikungunya fever (CHIKF), caused by Chikungunya virus (CHIKV), a neglected tropical disease (NTD). CHIKV is transmitted from human to human by the bite of infected Aedes aegypti mosquitoes. Non-structural proteins are thought to play a crucial role in viral replication, especially nsP2. It plays an important role in cleaving the viral polyprotein. Currently, there are no specific drugs against CHIKV. This study uses in silico methods to repurpose drugs for fighting against CHIKV, gaining an understanding of their main interactions. Initially, 1 µs molecular dynamics (MD) simulations were used to relax the CHIKV nsP2 (4ZTB) to its native form. Then, more than 100 FDA-approved drugs were selected from ZINC drug bank to be investigate as potential inhibitors. Next, docking was performed to evaluate the best orientation of the drugs in the protein and obtain the fingerprint for the most frequent amino acid residues that showed interactions with the target. MD results were analyzed based on RMSD and RMSF plots. For the RMSD plot, it was revealed that nsP2 has good stability throughout the simulation. Moreover, the RMSF plot showed significant variations in the region of the active site (Cys<sup>478</sup>-His<sup>548</sup>-Trp<sup>549</sup>). Among the drugs analyzed, Labetalol, Avobenzone, and Mycophenolic acid showed the highest fitscore values, suggesting that these could be repurposed against CHIKV. Interestingly, Mycophenolic acid has been reported in the literature to have excellent activity against CHIKV-infected cells. From the docking results, a fingerprint was created considering the amino acid residues involved in the interactions with the target. Thus, 23 amino acids are involved in interactions with nsP2, Arg<sup>736</sup>(83%), Cys<sup>739</sup>(59%), Val<sup>740</sup>(53%), Val<sup>737</sup>(43%), and Arg<sup>743</sup>(42%) being the most frequently observed. These residues suggest an important indication of a possible allosteric binding site in relation to that previously reported. The fingerprint generated allowed us to obtain a more precise description of the mode of interaction of various drugs with nsP2, as well as, the nature of these interactions, being van der Waals, H-bond, and  $\pi$ -anion the most relevant. These findings could help with research aimed at developing new drugs to combat CHIKV, acting by inhibiting nsP2. Since amino acid residues other than those known as the active site were observed in this study.

Keywords: CHIKV, Molecular Dynamics (MD), Molecular Docking (MD).

Supported by: CAPES; FAPEAL.

acknowledgments: CAPES, CNPq and FACEPE.

# C-05. Bioactive Compounds Present in *Euphorbia tirucalli* with Inhibitory Potential in Molecules Signaling Pathways in Colorectal Cancer

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**INTRODUCTION:** Colorectal cancer (CRC) is the third most common type of cancer worldwide. For the period from 2023 to 2025, it is estimated that there will be approximately 45,630 new cases, with an estimated rate of 21.10 cases per 100,000 inhabitants. The mTOR, ERK, TGF- $\beta$ , TNF- $\alpha$  and PI3K signaling pathways play a significant role in regulating multiple aspects of cancer progression. Therefore, these pathways have become an important focus for the therapeutic development of antitumor agents. Euphorbia tirucalli, popularly known in Brazil as aveloz, has aroused human interest since antiquity, as it contains several bioactive compounds with the potential with antitumor potential. **OBJECTIVES:** Perform an *in silico* search for bioactive compounds present in the species Euphorbia tirucalli with anticancer properties. MATERIAL AND METHODS: A table of important proteins in the CRC course and bioactive compounds present in Euphorbia tirucalli were created by searching scientific articles in databases such as PubMed, ScienceDirect and Scielo. The proteins were downloaded from the Protein Data Bank (PDB) and the search for the 3D structures of the bioactive compounds presents in Euphorbia tirucalli latex and the reference drugs, were performed using PubChem and Lotus, saved in SDF formats and OpenBabel was used for format conversion. The identification of the pharmacophoric points and the creation of the unique structure of the bioactive compounds was carried in the Molecular Operating Environment (MOE). Anchoring was performed using AutoDockTools at selected active sites. **RESULTS AND DISCUSSION:** The table of bioactive compounds has 29 compounds separated by class and identification number of the bank of origin, while the table of biomolecules has 62 proteins identified with the PDB code. Assessments of anchorage scores showed the following values of binding energy per kcal/mol: mTOR (-8.74), ERK (-8.58), TGF-β (-9.95), PI3K (-7. 48) and TNF- $\alpha$  (-7.69). These values were compared with the results obtained by the reference drug. The drug exhibited similar or lower binding energies than those obtained with the scaffold structure, emphasizing the effectiveness of the scaffold structure as a potential inhibiting agent. CONCLUSIONS: The results of the ligand-receptor interaction showed low binding energy values and the screening of pharmacophores provided structures with bioactive similarity for the inhibitory potential of important proteins in the course of RCC. ACKNOWLEDGMENT: UFBA, PMBqBM and the Young Researcher Project and the financial support bodies FAPESB and Carrefour.

**KEY-WORDS**: *Euphorbia tirucalli*; cancer; molecular docking.

### C-06. *In silico* Identification of Genes for Diagnosis and Correlation with Immune Infiltrate in Non-luminal Breast Cancer (HER2 and Basal) without Metastasis

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Breast cancer is considered the leading malignant neoplasm that affect women in Brazil and worldwide. Molecular subtypes of breast cancer are categorized as luminal and non-luminal. Non-luminal subtypes have an unfavorable prognosis, a high recurrence rate and limited treatment options. Hence, the necessity for prospecting biomarkers to diagnose and understand immune profile in these non-luminal subtypes. The objective proposed in this study is *in silico* prediction of genes for diagnosis and the identification of immune profile in non-luminal subtypes. Gene prediction was obtained from the analysis of 170 RNA expression samples from breast cancer patients with invasive ductal carcinoma, without metastasis and non-luminal subtypes (HER2 and basal), as well as 80 samples of normal breast tissue that was available for download in public databases. The most differentially expressed genes were obtained from these data using the limma package, considering p <0.05 and |logFC| >1 for each subtype. These genes were compared using Venn diagrams. The analysis of enriched pathways for genes unique to each subtype was evaluated in KEGG and Reactome (p < 0.05). The immune profile associated with the most highly expressed genes and enriched pathways involved in the carcinogenesis process was also evaluated using the Timer 2.0 server, considering a positive Spearman's correlation (p <0.05 and  $\rho$ >0). A total of 215 and 205 differentially expressed genes were identified in HER2 and basal, respectively. From these genes, only 80 were more highly expressed in the HER2 subtype, and 70 in the Basal subtype. PSMD1 and PSMB5 were the most highly expressed in HER2 and were associated with enriched pathways regulating apoptosis, programmed cell death, the ROBO receptor signaling pathway, the MAPK1/MAPK3 signaling pathway, and the MAPK6/MAPK4 pathway. LSM2, LSM4, and CHEK2 were the most highly expressed in basal and were related to enriched pathways in RNA processing, cell cycle control, and gene expression regulation, respectively. In HER2, PSMD1 induced an increase in TCD4<sup>+</sup> Th2 cells and a decrease in CD4<sup>+</sup> Th1 cells, indicating an immunosuppressive process, while PSMB5 showed an increase in B cells, indicating an activated humoral response. In basal, LSM2 and CHEK2 induced an increase in TCD4<sup>+</sup> Th2 cells, and LSM4 induced an increase in TCD4+ Th1 cells. Therefore, PSMD1 and PSMB5 in the HER2 subtype, as well as LSM2, LSM4, and CHEK2 in the basal subtype, were identified as candidate genes to be evaluated *in vitro* and in vivo for diagnostic applications. Keywords: in silico, breast cancer, diagnosis.

Acknowledgements: UFAL, IFAL and FAPEAL.

# C-07. *In silico* characterization and phylogenetic analysis of APX genes from sequenced plant genomes of the Poales order

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Ascorbate Peroxidase (APX) is a fundamental element of the ascorbate-glutathione (AsA-GSH) cycle and plays a pivotal role in regulating intracellular reactive oxygen species (ROS) levels. Due to its relevance in maintaining redox homeostasis, APX has been the subject of various biochemical and molecular studies. The aim of this work was to characterize and phylogenetically analyze the multigene family of APX genes in sorghum and other species of the Poales order from available genomes in public databases. An In silico identification of APX genes was carried out using BLAST with homologous APX sequences from Arabidopsis thaliana (A. thaliana) (Brassicales order). Phylogenetic analysis was performed using MEGA 7.0, with A. thaliana as an outgroup. A total of 104 APX genes were identified in the analyzed grass species (Sorghum bicolor, Panicum hallii, Panicum virgatum, Setaria italica, Setaria viridis, Zea mays, Triticum aestivum, Hordeum vulgare and Oryza sativa). Overall, across all analyzed species, APX was found to be encoded by a multigene family, with a variation of 7 to 21 genes, and some species showed variants. In sorghum specifically, the results revealed the presence of 7 APX genes distributed on 6 different chromosomes (1, 2, 4, 6, 7 and 8). Among these genes, those located on chromosome 4 exhibited two variants. The exon/intron structure is conserved among APX genes in sorghum, and a similar pattern was observed in other Poales species. Sequence alignment showed divergent identities, ranging from 44.88% to 99.95% at the nucleotide (cDNA) and from 46.59% to 100% in deduced amino acid sequences. The phylogenetic analysis demonstrated that APX are grouped into four distinct clades of orthologous genes, and are divergent from Arabidopsis genes, which are grouped into two separate clades. Analysis of promoter regions in sorghum revealed different regulatory sequences, suggesting potential differential expression. This same pattern was also observed in other analyzed species. High diversity was observed in the number of APX genes in Poales species, as well as in promoter sequences, underscoring the need for expression studies to identify expression profiles of these genes in different tissues and stress conditions. This opens up the possibility of identifying target genes as potential biotechnological tools, aiming to obtain varieties/genotypes more adapted to stress conditions.

Keywords: APX, Poales, Sorghum.

Acknowledgment: PIBIC/CNPq.

### C-08. Structural Analysis of the interaction between the Recombinant Lectin of *Cucurbita maxima* in complex with chitooligossacharides.

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Lectins are proteins that bind specifically and reversibly to carbohydrates with multiple biological activities. Among the plant lectin classes described, the Cucurbitaceae phloem proteins type 2 (PP2) includes the phloem exudate lectin of Cucurbita maxima (PPL). This protein is a homodimeric protein with 218 amino acids and and molecular weight of 26 kDa. This lectin has extended binding sites to chitooligosaccharides related to chitin. The specific bind to chitin indicates the relation of this protein with the defense mechanisms of plants against pathogens, such as fungi and insects. Despite the primary structure of PPL being wellknown, its carbohydrate binding site is not well characterized due to the lack of threedimensional structures. At this moment, only the structure of the lectin of *Cucumis sativum* has been solved. Thus, the present study aimed to predict the three-dimensional structure of PPL and simulate the binding of chitooligosaccharides. The model of the lectin was obtained using the software Alphafold with an Ab initio approach. The docking simulations were made with the support of two applications: AutoDockVina and AutoDockTools. The ligands used were N-acetylglucosamine (NAG) and the homooligosaccharides (NAG<sub>2</sub>, NAG<sub>3</sub>, NAG<sub>6</sub>), and they were obtained from Protein Data Bank (PDB) and PubChem. The blind docking showed the best affinity for NAG<sub>3</sub> and NAG<sub>6</sub> with the maximum of binding energy of -6,2 and -7,6kcal/mol, respectively. The RMSD (root mean deviation square) for these structures is considered high, with values over 2.0 Å, indicating the need of molecular dynamics assays. On the other side, N-acetylglucosamine and  $NAG_2$  had lower affinity compared to the other carbohydrates, but they had the lowest RMSD, with the minimum of 1.474 and 1.551 Å, respectively. Based on that, the result of docking were similar to that previously obtained through thermodynamics assays, which demonstrated a strong interaction with polymers with more than 3 unities of NAG.

Keywords: Phloem Protein, N-acetylglucosamine, Cucurbitaceae.

We gratefully acknowledge the financial support provided by CNPq, CAPES, FUNCAP.

### C-09. Inhibitory Effects of Bioactive Compounds from *Euphorbia Tirucalli* that Act on Molecules in the Colorectal Cancer Pathway

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**INTRODUCTION:** Colorectal cancer (CRC) is the third most common malignancy in the world and the second most common cause of cancer mortality. *Euphorbia tirucalli* latex is widely used in Brazilian folk medicine, as it has anticancer properties. **OBJECTIVES:** To analyze the ability of single structure flavonoid activity to inhibit GSK-3 $\beta$  and  $\beta$ -catenin related to tumor progression. MATERIALS AND METHODS: The chemical constituents of Euphorbia tirucalli species were obtained from PubChem and Lotus databases. The ligands were downloaded in SDF format by PubChem, then converted to PDB format by OpenBabel. The ProTox-II program was used to predict the level of toxicity of *E. tirucalli* compounds. From these compounds were created a unique structure with pharmacophoric similiraties. The target proteins were searched in the literature through databases such as Pubmed, Scielo. The GSK-3β and βcatetin biomolecules were selected and downloaded from the RCSB Protein Data Bank (PDB) website and prepared using AutoDockTools and DiscoveryStudio software. They were used to remove the water and the ligand coupled to the protein. Subsequently, was add hydrogen molecules in the GSK-3 $\beta$  and  $\beta$ -catetin and the Kollman charges were add, shortly after assign AD4 type was add. Next, the unique structure was added. After that, the docking parameters were defined. It was necessary to run autogrid and then autodock where the necessary files were obtained for the analysis of the binding energy. RESULTS AND DISCUSSION: The best binding energy ranking was observed in AutoDockTools and DiscoveryStudio was the software for analysis of the binding pocket. GSK-3β the most important interaction was with Tyr216, because it activates phosphorylative activity allowing it to act on  $\beta$ -catetin. In  $\beta$ -catetin, the most important interaction is with the amino acid residue Lys242, which is part of the mechanism of action of beta-catenin. Both showed low binding energy, making it promising to invest in vitro studies in the future. CONCLUSION: There was low binding energy of the internal ligand-receptor demonstrating that external connections were optimal with great inhibitory potential and it was observed which amino acid residues interacted between the ligands and the receptors, which provided the importance in the sites where they were housed. ACKNOWLEDGMENT: UFBA, Collegiate of Biotechnology, PMBqBM and the Young Researcher Project and the financial support bodies PIBIC.

**KEY WORDS:** colorectal câncer; *Euphorbia tirucalli*; molecular docking.

#### C-10. OVICIDAL AND LARVICIDAL EFFECTS OF LIQUID FORMULATION CONTAINING LECTIN-RICH FRACTION WSMoL AGAINST EGGS AND LARVAE OF *Aedes aegypti*

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Aedes aegypti is widely known as the mosquito vector of arboviruses, especially dengue, an emerging viral infectious disease with a higher incidence, especially in urban and suburban areas of tropical and subtropical regions. Therefore, controlling the mosquito population is fundamental and is a growing concern of public health agencies. On this basis, knowing the potential larvicides and ovicides of the protein fraction of Moringa oleifera seeds, enriched with WSMoL lectin, against the larvae and eggs of A. aegypti, the current work aimed to develop a liquid formulation, containing WSMoL, and determine its efficiency regarding these effects, under laboratory conditions. The methodology is based on the exposure of 20 larvae, in the most resistant stage (final L3), and 20 mosquito eggs to 1,0 mL of liquid formulation in a system with 19,0 mL of distilled water, with monitoring after 24 and 48 hours. These tests were done in quadruplicate for each treatment and control groups. The formulation developed is in the process of patent submission, however, it is possible to mention, previously, that preservatives, water and WSMoL-rich fraction were used. Regarding larvicidal activity, the liquid formulation was produced in different concentrations, being 3,5 mg/mL, 4,5 mg/mL and 5,5 mg/mL. These concentrations presented a mortality of 30%, 62% and 83%, respectively, of the larvae, after 48h of exposure. In the control performed only with water and with the placebo formulation, there were no mortality. It is likely, among other reasons, that this effect is associated with disruption of the intestinal epithelium of the larvae. Regarding the ovicide test, concentrations of 0,5 mg/mL, 1,0 mg/mL and 2,5 mg/mL were tested, along with the placebo and water formulation. It was observed, after 48h, that at the concentration of 2,5 mg/mL there was no hatching, at the concentration of 1,0 mg/mL there was 32% of hatching and at the concentration of 0,5 mg/mL there was 37% of hatching. At controlling water, 100% of the eggs hatched and in the placebo control 88% of the eggs. Possibly, this is also due to damage caused to the surface of the eggs. It is concluded, therefore, that the larvicidal and ovicidal effects of the developed formulation interfered in the life cycle of A. aegypti, presenting potential for use in the control of this mosquito. Keywords: lectin; WSMoL; formulation.

Acknowledgements: This work was financially subsidized by the Foundation for the Support of Science and Technology of the State of Pernambuco and supported by the Laboratory of Protein Biochemistry and the Federal University of Pernambuco.

#### C-11. Identification of Montelukast as Promising mPGES-1 Inhibitor through Virtual Drug Repurposing Screening

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**INTRODUCTION:** Given the high incidence of side effects of conventional anti-inflammatory drugs and bioinformatics advances, searching for new targets is necessary to overcome gastric and cardiovascular problems. Through drug repurposing, the selective inhibition of prostaglandin  $E_2$  (PGE<sub>2</sub>) has been explored to develop new drugs. **OBJECTIVE:** To perform a virtual drug repositioning screening using molecular docking, molecular dynamics simulations, and MM-PBSA against the mPGES-1 enzyme to identify potential mPGES-1 inhibitors. MATERIALS AND METHODS: A library was built with FDA-approved obtained from the ZINC database. Then, the MARVIN software performed a conformational analysis of the structures, selecting the most stable conformation. At the same time, the crystallized structure of mPGES-1 was obtained from the Protein Data Bank (PDB), and RMSD calculations were performed to choose the most appropriate structure for the screening protocol. Molecular docking was performed using the GOLD software, selecting compounds with *fitscore* greater than or equal to the crystallographic standard (55.00). Discovery Studio software was used to inspect interactions at the active. The best compound was selected for MM-PBSA and molecular dynamics (MD) simulations using GROMACS software. RESULTS AND DISCUSSION: Molecular docking was performed with 1600 drugs, and 264 compounds were selected for the secondary screening. Among the analyzed complexes, montelukast was the most promising drug against mPGES-1 (fitscore 77.99). In addition, studies show that montelukast has an interesting polypharmacological profile and that mPGES-1 activity was suppressed at low micromolar levels (IC<sub>50</sub> between 2 and 4µM) by CysLT<sub>1</sub> receptor (LTRA) antagonists in human cervical carcinoma cell line H. In MD simulation, through analysis of the RMSD plot, the interaction of montelukast at the protein binding site does not produce significant changes compared to the free protein, producing a stable complex. According to the MM-PBSA results, montelukast shows a critical free energy value (-107,607 ± 16,933 kJ. mol<sup>-1</sup>) compared to an inhibitor found in the literature of known activity (-62,550  $\pm$  22,885 kJ. mol<sup>-1</sup>, and IC<sub>50</sub> = 0.13  $\mu$ M), which may indicate mPGES-1 inhibition as a possible anti-inflammatory mechanism for this drug. **CONCLUSION:** Drug repurposing is currently essential in medicinal chemistry, and our findings suggest that Montelukast has the best molecular docking, dynamics, MM-PBSA results, and interactions with critical residues for inhibiting this enzyme, providing new horizons for searching for new promising mPGES-1 inhibitors. However, it is worth noting that in vitro and *in vivo* assays are needed to prove this drug's efficiency further.

KEYWORDS: Anti-inflammatory drugs; Structure-Based Drug Design, Molecular Dynamics Simulations

#### C-12. The microbiome in human semen: a metagenomic shotgun analysis

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Infertility is a prevalent condition that affects around 70 million people worldwide, with male infertility accounting for 50% of cases. Among the main etiological factors responsible for this infertility are infectious and inflammatory conditions. Until now, studies investigating the microbiological diversity of the seminal environment have been restricted to specific analyses of bacteria or viral communities. In this study, the shotgun metagenomic approach was used to identify potential pathogens with DNA genomes in a human seminal pool. For the analysis, we extracted DNA from 50 semen samples donated by participants of a public reproductive health service in Brazil. We observed a high proportion of the Bacteria domain (71.3%), the largest groups of which are Bacillus, Staphylococcus, Mycobacterium and Streptococcus. The eukaryotic domain (27.6%) includes Plasmodium, Trypanosoma and Trichinella. The viruses (1.1%) are made up of Gammaretrovirus, Herv-K and Herv-W.These findings expand the current view of microbial diversity in human semen and point out that the evaluation of uncultured pathogens may be crucial before completing reproductive and prophylactic treatments. In addition, the Herv families identified in seminal samples merit study from a functional and evolutionary perspective. These data contribute to identifying potential pathogens present in semen and their correlations, and open up a new research front in the diagnosis of fertility-related diseases. Finally, it was noted that, as research into the seminal microbiome is an approach of growing scientific interest, the results obtained could be prospected in future studies so that molecular protocols can be developed to facilitate the identification of these pathogens.

Keywords: Male infertility. Assisted reproduction. HERV.

**Acknowledgments:** We want to thank all sample donors for participating in this study in the Januário Cicco Maternity Hospital School of the Federal University of Rio Grande do Norte – MEJC/UFRN. We want to thank the High Performance Processing Center of the Federal University of Rio Grande do Norte - NPAD/UFRN to allow us to access their computer facilities.

# C-13. Elucidation of the physicochemical mechanisms involved in the interaction of *Moringa oleifera* coagulant lectin (cMoL) with galactose.

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cMoL (coagulant Moringa oleifera lectin) is a galactose-binding lectin that has shown insecticidal, anticancer and hemostatic activities. Previous studies have shown that the biological activities reported for cMoL are related to the carbohydrate-recognizing region in this protein. In this sense, this study aimed to elucidate the physicochemical mechanisms of the binding between cMoL and galactose using fluorescence spectroscopy. cMoL was isolated after chromatography of seeds protein fraction on a guar column and elution with NaCl 1 M. Interaction experiments were performed through incubation of the lectin with different concentrations of galactose at the temperatures of 298, 303, and 308 K. Then, the intrinsic fluorescence was measured by excitation at 280 nm and emission spectra of 300 to 400 nm. The Stern-Volmer constants (Ksv), binding constants (Ka), and Gibbs free energies ( $\Delta G$ ) were calculated. From the binding constants it was possible to determine thermodynamic parameters using the Van't Hoff equation. Based on the values obtained for each parameter, the forces involved in the interaction were determined. The data revealed that the formation of cMoL-monosaccharide complex is favored through the static suppression mechanism and negative Gibbs energies, indicating a spontaneous process. Thermodynamic parameters (ΔH > 0 and  $\Delta S < 0$ ) shows that the complex is stabilized by hydrogen bonds and hydrophobic interactions. The study elucidated the interaction of cMoL with galactose and can contribute for definition of the action mechanisms involved in the cMoL biological properties.

Acknowledgements: The authors express their gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for financial support.

Keywords: Protein. Molecular quenching. Fluorescence spectroscopy.

# C-14. Fingerprint Analysis of Phytochemical Drugs Against Enhanced Intracellular Survival (Eis) Protein of *Mycobacterium tuberculosis*: A repurposing study

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Tuberculosis (TB) is an infectious and transmissible disease caused mainly by Mycobacterium tuberculosis (Mtb), which primarily affects the lungs, although it can also affect other organs or systems. Current therapies are hindered by issues of resistance, adherence, and long-term treatment. Consequently, there is an evident need to develop new effective and safe drugs to combat antibiotic resistance. Mtb's enhanced intracellular survival (Eis) protein has a versatile role as an acetyltransferase, in which it performs multi-acetylation of aminoglycoside antibiotics, resulting in their inactivation to bind bacterial ribosome, leading drug resistance. In this study, we analyzed commercially available phytomedicines using in-silico approaches. We utilized the ZINC drug bank to select 100 phytochemical drugs to be investigated toward 3D-crystal structure of *Mtb* Eis (PDB: 6VUR) by using molecular docking. To analyze whether there was an interaction between the molecular target and molecule of interest, we pretreated the biomacromolecule with GOLD v. 5.8.1 software and performed docking. Among the drugs analyzed, Labetalol, Alprazolam, and Ropinirole had the highest fitscore values, indicating their potential against Mtb. After conducting the docking simulations, we generated a fingerprint graph based on the best solutions. The graph's bar heights depict the frequency of each residue with the most interaction with the 6VUR protein. The results reveal that 23 amino acid residues participate in ligand-protein interactions. Trp<sup>36</sup>(91%), Phe<sup>84</sup>(85%), Ala<sup>33</sup>(69%), Ser<sup>83</sup>(47%), and Arg<sup>37</sup>(38%) residues are the most involved in interactions with these phytochemical drugs. This research aims to identify the amino acid residues that may correlate with anti-tuberculosis activity, indicating potentially beneficial targets for enhanced activity. Findings such as these are critical for the development of new drugs. The study further demonstrates how structural data can lead to new inhibitors for the Eis protein. Keywords: Molecular docking; Mycobacterium tuberculosis; Tuberculosis. Supported by: CNPq, CAPES.

#### C-15. Cis-regulatory elements of the aconitase genes in *Ricinus communis* L.

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INTRDUCTION: Ricinus communis L. is an oleaginous plant whose culture is of great importance in Brazil and in the world. It has several properties, including medicinal ones, and its oil is used by the pharmaceutical and fuel industries. Several biomolecules in this species and their mechanisms of action account for its high tolerance to biotic and abiotic stresses. Aconitase, one of the enzymes present in *R. communis*, has a dual role in the Tricarboxylic Acid Cycle and as a regulator of gene expression in the face of Iron deficiency, going against the dogma of one gene => one protein => one function. It can also act as a mediator of oxidative stress and cell death. OBJECTIVES: Based on the above, this study aimed to verify the Cisregulatory elements of the expression of the aconitase (ACO) genes of the R. communis (RcACO). MATERIALS AND METHODS: For this purpose, the National Center for Biotechnology Information (NCBI), Phytozome, the Plant Comparative Genomics (Phytozome), and Simple Modular Architecture Tool (SMART) databases were used to retrieve sequences and confirm the existence of conserved domains of the RcACO, characteristic of the gene members. Then, searches were carried out in the Cis-Acting Regulatory Element (PlantCARE) using the genomic sequences of the RcACO, recovered in the Phytozome using the parameters of 1,000 bp (base pairs) upstream before the beginning of the region coding (ATG) of each sequence. **RESULTS** AND DISCUSSION: R. communis has a small family of aconitase genes with varying amounts of amino acids (aa) and/or nucleotides in their sequences; two to four conserved domains are presented, highlighting the ACO domain. The RcACO genes exhibited a range of regulatory elements throughout their sequences, such as response elements to Light (L) - present in all sequences - to drought (MYC), to low temperatures (LT), to stress (SR), MYB, MeJa and plant hormones (Abscisic acid-AA, and auxin-AX) essential for plant development. These elements are small motifs in the promoter regions and can regulate or initiate gene transcription. For example, AA-ABRE is a positive regulator of response to water and salt stress in the plant. **CONCLUSION**: Aconitase is a multi-function enzyme, which contradicts that a gene leads to only one protein and function. The regulatory elements of the expression are essential in the transcription and/or regulation of aconitase genes in response to the different stresses that R. communis and other plants are subjected to.

Key-words: Computational Biology; Enzymes; Abiotic Stress.

Acknowledgements: CAPES, CNPq, FAPESB, FINEP, PMBqBM/SBBq, UFBA.

**C-16.** Analysis of carrageenan disaccharides with antitumor activity: an in silico approach. <u>Silva, O.L.T.</u>; Rocha, H.A.O.

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Cancer is one of the leading causes of death globally, and although treatments like radiotherapy and chemotherapy are necessary, they can cause significant adverse effects. Natural sources are known to play a major role in the development of approved cancer drugs, accounting for approximately 60% of such drugs. Carrageenans, which are sulfated polysaccharides extracted from red seaweeds (Rhodophyta) is pointed as potential antitumoral drug. In this study, we aimed to assess the potential of iota and kappa carrageenans as antitumor agents through drug-likeness analysis, investigation of their molecular targets, and molecular docking using bioinformatics tools. To begin with, the disaccharide structures of iota and kappa carrageenans, obtained from Pubchem, were subjected to drug-likeness testing using SwissADME (Lipinski's rule of five). Subsequently, the targets of these compounds were predicted using PPB2 and SwissTarget tools, and the common targets were identified. The ligands and the top four ranked targets were optimized and subjected to molecular docking using Autodock Vina software. The main results revealed that carrageenans met 3 out of 4 criteria proposed by Lipinski, which are commonly used in drug screening to predict a molecule's oral activity. Furthermore, seven common targets of the lyase class (Carbonic Anhydrase I, II, IV, VII, IX, XII, and XIV) were identified. The docking simulations showed significant molecular interactions and strong binding energies, with values of -9.7, -9.1, -8.0, and -9.3 kcal/mol for iota carrageenans and -9.2, -8.3, 8.6, and -8.8 kcal/mol for kappa carrageenans with Carbonic Anhydrase I, II, IX, and XII, respectively. Particularly noteworthy was the interaction of iota and kappa carrageenans with the cofactor zinc+2 of anhydrases IX and II, respectively. This cofactor is essential for the activity of these enzymes, which are involved in cancer cell survival. In summary, these molecules possess essential physicochemical properties for orally administered drugs and exhibit interactions that suggest their potential as inhibitors of targets relevant to cancer progression. These findings could contribute to preclinical investigations and the development of alternative therapeutic agents that are more tolerable, have fewer side effects, and offer higher specificity in cancer treatment.

**Acknowledgments:** This work is supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES. I thank the Federal University of Rio Grande do Norte (UFRN) for the opportunity to carry out this study at the BIOPOL Laboratory, and Professor (Ph.D.) Hugo Alexandre de Oliveira Rocha, for his valuable guidance and tireless dedication.

Keywords: Carrageenans, Bioinformatics and Cancer.

### C-17. Partial Purification and Characterization of a Novel Lectin from *Bauhinia cantingae* Seeds

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Lectins are carbohydrate-binding proteins of non-immune origin that reversibly interact with specific sugars or glycoconjugates. This group of proteins are widely found in all realms of life, and for decades have been used as models in the study of the molecular basis of proteincarbohydrate interaction. Despite their wide distribution, plant lectins are the best studied, mainly from the Leguminosae family. Only a few lectins from the Caesalpinioideae subfamily of the legume group have been reported. In this regard, lectins from the Bauhinia genus in comparison with others of the same group are still poorly studied, due to the heterogeneous characteristics of the lectins of this genus. Therefore, this work aimed at the isolation and purification of a lectin present in the seeds of Bauhinia catingae (Harms), termed BCL. Mature seeds from *B. catingae* were ground into a fine powder using a coffee mill. The powder was incubated at a 1:10 ratio (w/v) in different buffers for an extraction screening at room temperature with continuous stirring for 2h before centrifugation at 7500  $\times$  g for 20 min at 4 °C. The crude extract with better specific activity (in 100 mM Tris-HCl buffer, pH 7.6, containing 150 mM NaCl) was then precipitated with ammonium sulfate (25–50% saturation, F25/50) and centrifuged, and the pellet was resuspended and dialyzed in 20 mM Tris-HCl buffer, pH 7.6. The F25/50 was applied to a deae–sephacel column equilibrated with the same solution. After removing the unbound proteins, the lectin was eluted with 100 mM NaCl in an equilibrium buffer. The active fractions were pooled, dialyzed extensively against distilled water, and freeze-dried. Hemagglutination assays were performed in microtitration plates using rabbit erythrocytes to follow lectin activity during purification. The purification process of BCL was monitored by SDS-PAGE, which showed a major band with a molecular mass of approximately 30 kDa in all purification steps, and other minor bands. This result suggests that BCL has apparent molecular mass similar to other lectins of the Bauhinia genus. In conclusion, a single chromatographic step was not enough to purify the lectin present in B. catingae seeds, so it is necessary to use other chromatographic strategies to obtain the lectin in pure form for further structural characterization and biological assays.

Acknowledgements: This study was supported by grants from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) and the Federal University of Ceará (UFC).

Keywords: *Bauhinia catingae*; ion-exchange chromatography; seed lectin.

#### C-18. Biophysical characterization of recombinant frutalin expressed in Pichia pastoris

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INTRODUCTION: Frutalin is a  $\alpha$ -D-galactose and  $\alpha$ -D-manose binding multilectin from Artocarpus incisa L. seeds. It belongs to the 'jacalin lectins related' family and presents around 98% identity with jacalina. Due to the high structural identity between jacalin and frutalin and the use of jacalin for IgA1 purification, a recent study showed the use of immobilized frutalin for human IgA1 purification, like a low-cost alternative to the commercial ImmobilizedJacalin® collumn. One of the problems inherent in the use of immobilized frutalin as an alternative to human IgA1 purification is the amount of biological material necessary for the construction of chromatographic matrices. An alternative is the use of protein heterologously produced in microorganisms. OBJECTIVES: This work aimed to biophysically characterize the recombinant frutalin (rFrutalin). MATERIALS AND METHODS: rFrutalin was expressed in Pichia pastoris KM71H strain transformed and purified in agarose-D-galactose columns (Sigma). For biophysical characterization, circular dichroism (CD) analysis was performed to evaluate the thermal stability, influences of pH variation and prediction of secondary structures, and intrinsic fluorimetric analysis, to monitor conformational changes and interactions with ligands. Dynamic light scattering (DLS), to confirm the oligomeric state of the molecule in solution and scattering electrophoretic light (ELS or Zeta Potential) to analyze the influence of pH on net charge. RESULTS AND DISCUSSION: The rFrutalin CD results showed a negative band with a maximum amplitude 218 and another positive band with a maximum 222 nm profile, like the native frutalin, showing predominantly sheet-β structure. Frutalin is conformationally stable under acidic conditions at temperatures up to 60 °C. The dynamic light scattering method (DLS) confirmed that the oligomerization of rFrutalin does not change with pH change and that its mass corresponds to approximately 59  $\pm$  4 kDa, showing no isoforms. CONCLUSION: By the biophysical point of view, rFrutalina expressed in *P. pastoris* showed no significant change when related to characteristics of the native frutalin, showing itself as a candidate for the use of native lectin for larger-scale production.

Keywords: Frutalin; Protein recombinant; Biophysical characterization. Acknowledgements: CAPES, CNPq, UFC and UFABC.

### C-19. Analysis of Protein Families Involved in Microbial Resistance to Metals: An *In Silico* Analysis.

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Currently, there is a significant increase in interest in metallic nanoparticles, which are highlighted as promising agents due to their remarkable antibacterial properties and their effectiveness in eliminating microorganisms at exceptionally low concentrations. Although the antimicrobial effect of these metallic nanoparticles has been documented, and despite extensive discussions on bacterial resistance to antibiotics in the literature, the potential emergence of resistance to these nanoparticles remains largely unexplored. The aim of this study was to conduct a detailed investigation into the primary bacterial targets of metallic nanoparticles' effects on pathogenic bacteria, utilizing microorganism genomes. In the initial phase, predicted genomes of various species were collected to comprehensively identify genotypes related to the primary effects of metallic nanoparticles. This process involved a thorough analysis of the collected genomes using a Hidden Markov Model developed for families of proteins of interest. Annotation of the relevant proteins in the selected genomes occurred in two distinct stages of identification and annotation. The identification phase employed the "hmmsearch" tool of HMMER 3.3 software, using a Hidden Markov Model (HMM) profile derived from a flatfile database of Pfam protein families. This process was applied to the predicted proteomes of the selected species. Positive results were then subjected to an annotation step, which involved comparison with BLASTP (stand-alone BLAST application - BLAST+2.12.0) against the non-redundant protein sequence database. The predicted proteins were classified into their subfamilies or classes based on the highest similarity values and more significant E-values, generating a distribution pattern. Based on these procedures, a customized database of Hidden Markov Models was created using proteins associated with resistance to metals. These models were applied to identify potential functions in the predicted genomes of bacteria. The results obtained provided a diversified view of the distribution and diversity of protein families related to the effects of nanoparticles in the analyzed genomes, through phylogenetic and structural characteristic analyses. Consequently, this study contributed to a better understanding of the molecular mechanisms involved in metal resistance by pathogenic microorganisms.

Keywords: Metallic nanoparticles; Antimicrobial action; Gene annotation; Sequence alignment; Comparative phylogenetic analysis.

Acknowledgment: CNPQ, CAPES, FAPEAL, UFAL.

#### C-20. Gene Characterization of Gluconeogenesis Enzymes in Ricinus communis L.

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**INTRODUCTION:** Enzymes play an important role on all living beings and one of their main functions is the regulation of metabolic pathways such as gluconeogenesis. This pathway nongenerates energy chemicals from carbohydrate substrates, and the phosphoenolpyruvate carboxykinase (PEPCK) and Fructose 1,6-bisphosphatase (FBPase) regulate irreversible steps of gluconeogenesis. This pathway is crucial in animals and plants as it helps them survive in their environment acting as one of their anti-stress factors especially on species like *Ricinus communis* L. which are know for growing on stressful conditions. **OBJECTIVES**: This work aimed to perform in silico characterization of phosphoenolpyruvate carboxykinase (EC 4.1.1.32, PEPCK) and 1,6- bisphosphatase (EC 3.1.3.11, FBPase) from R. comunis. MATERIAL AND METHODS: FASTA codes genes were searched on databases such as National Center of Biotechnology and Information (NCBI), the Jatropha Genome Database, and the Plant Comparative Genomics (Phytozome). Then, genes had their conserved domains verified using a Simple Modular Architecture Research Tool (SMART) and a phylogenetic tree was constructed on Molecular Evolutionary Genetics Analysis (MEGA11). The subcellular location was predicted by DeepLoc 2.0. The conserved motifs were analyzed by Multiple Em for Motif Elicitation(MEME), and cis-acting regulatory elements were predicted by Cis-Acting Regulatory Element (PlantCARE). GalaxyWEB was used to predict each enzyme's3D Structure and physical-chemical parameters were elucidated by ProtParam. Finally, the functional analysis was made using the Kyoto Encyclopedia of Genes and Genomes (KEGG). RESULTS AND DISCUSSION: The results showed differences between genes that encode these enzymes in *R. communis*, with greater homology with genes from other plants and not between the genes found in R. communis. The FBPase genes that presented two subcellular locations with a similar number of genes (cytoplasmatic and plasmidic). ProtParam analysis showed similarities in hydrophobicity between the genes of the two enzymes. GalaxyWEB analysis confirmed that the aminoacid differences influences each enzyme structure by showing different predicted structures, especially between different-sized genes. A comparison of ciselement regulators between the two enzymes showed that major regulators of each species are common between R. communis genes However, RcFBPase genes presented the main regulator as MYB, and RcPEPCK presented the main as light response. KEGG analysis confirmed that their metabolicpathway is the same. CONCLUSION: This study showed that many points must beworked out on these analyses with more profound studies to understand better why these differences happen and how they influence the Gluconeogenesis regulation and the secondary functions of each enzyme on plants.

Key-words: Bioinformatics; Castor bean; Gluconeogenesis

Acknowledgements: CAPES, CNPq, FAPESB, FINEP, PIBIT-UFBA and PIBIC- UFBA

### D - Biotecnologia (Agricultura)

# D-01. Edible coating for mangaba, umbu and caja containing xanthan gum, sodium alginate and essential oils of clove, cinnamon and/or sage

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Introduction: Tropical agroindustries are looking for new techniques to maintain the quality and extend the storage time of their products, such as the use of non-toxic and biodegradable coatings. Due to its physicochemical characteristics suitable for this and being edible, the combination of xanthan gum (GX), produced by Xanthomonas campestris, with sodium alginate (AS), is a promising biopolymer. In turn, fruits of the cajazeiro (Spondias mombin L.), umbuzeiro (S. tuberosa Arruda) and mangaba (Hancornia speciosa Gomes), from the North and Northeast of Brazil, have nutritional relevance and contribute to the increase in income, being their conservation essential. Objective: This work aimed to evaluate the conservation of these freshly picked fruit species, using coatings based on 0.5% XG (m/v) and 0.5% SA (m/v), with or without 1% essential oil (v/v) of clove (Syzygium aromaticum L.) Merrill & Perry), cinnamon (Cinnamomum zeylanicum Blume) and/or sage (Salvia officinalis L.), ie individually or combined. Material and Methods: The fruits were immediately washed after harvest and immersed again for 2 min in water at 25°C or in a solution of XG (0.5%) + SA (0.5%) (w/v) with or without 1 % (v/v) of one of the three essential oils mentioned (or a combination of them, 1:1:1 v/v/v), dried under ventilation, arranged in polyethylene trays, and stored at 8 ± 2 °C for 15 days. Then, analyzes of mass loss, firmness, color, pH, titratable acidity (TA), total soluble solids (TSS), and fungal deterioration were carried out. At the end of storage, molds and yeasts were also counted. The antioxidant (DPPH test) and antimicrobial (Pseudomonas aeruginosa and Staphylococcus aureus) capacities of the coating solutions were also evaluated. Results and Discussion: No differences were found for color, TSS and sensory testing. Regardless of the coating, there was a reduction in mass loss and pH, and a slight increase in TA during refrigeration, but coatings based on XG + SA were efficient, providing greater fruit firmness, but those also containing essential oils, especially sage or the consortium of the three oils, were the ones that reduced the incidence of fungi. All coatings containing essential oils showed antimicrobial activity and their antioxidant capacity was superior to those that did not contain them. Conclusion: The coating of XG (0.5%) +SA (0.5%) is a promising candidate to increase the shelf life of fruits such as cajá, umbu and mangaba, especially if combined with essential oils of cinnamon, clove or sage.

**Keywords:** Antimicrobial; antioxidant; biopolymer; edible coating; post-harvest quality. **Sponsorship:** FAPEAL and S.A. Usina Coruripe Açúcar e Álcool"

### D-02. EVALUATION OF THE FUNGICIDAL ACTIVITY OF FRACTION FROM *Myracrodruon urundeuva* BARK

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Myracrodruon urundeuva (Aroeira), Anacardiaceae, is a tree species known in folk medicine in Brazil by its anti-inflammatory, healing, antioxidant, antifungal, antibacterial and analgesic properties. M. urundeuva bark preparations are used by the population to treat diseases caused by microorganisms. The medicinal use justifies carrying out studies to determine the presence of compounds and biological activities attributed to the plant and this work investigated the antifungal activity of the protein fraction of the bark of *M. urundeuva*. Bark flour was homogenized with 0.15 M NaCl for 16 hours at 25 °C and after centrifugation (9,000 g, 15 min), the collected supernatant corresponded to saline extract (SE). The SE was treated with ammonium sulfate (20-40% w/v) and after centrifugation (9,000 g, 15 min) the supernatant (SF) was collected and dialyzed against distilled water. SF was evaluated for protein concentration and presence of lectin, hemagglutinating protein which shows biological properties. SF was also evaluated for antifungal activity against Candida (C. albicans, C. krusei, C. parapsilosis) and Cryptococcus (C. neoformans, C. neoformans) species. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were determined. Protein (24.97 mg/mL) and lectin (hemagglutinating activity of 2048<sup>-1</sup>) were detected in SF. The antifungal assay showed that SF inhibited the growth of all fungi tested with MIC of 3.2 mg/mL (C. krusei), 0.8 mg/mL (C. albicans and C. parapsilosis), 0.2 mg/mL (C. neoformans and C. neoformans) but bactericidal action was not detected. The study revealed that M. urundeuva bark contains lectin and bacteriostatic activity against fungal species of clinical interest.

Keywords: Lectin; Candida; Cryptococcus.

#### D-03. Selection of environmental yeasts as potential bioinputs

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The growth and development of plants are mainly influenced by mineral nutrients, hormones and other metabolites secreted by their microbiota, which favors the communication process and responses to external factors. Yeasts and their uses in the food industry, in medical science and in biotechnological research are widely publicized, but few studies in the literature report that yeasts are considered plant growth-promoting microorganisms (PGPM), capable of colonizing parts of the plant, providing benefits to the host, including regulating the production of phytohormones, improving soil fertility, increasing nutrient availability, strengthening resistance to environmental stress and combating phytopathogens. However, research into the potential role of yeast in plant growth and development is limited. The main objective of this study is to investigate and select environmental yeasts, identifying their capabilities for producing biological inputs, including phytohormones, biostimulants and biofertilizers. The methodology involves screening yeasts previously isolated from different biomes. This screening also evaluated the ability to produce the phytohormone auxin and several hydrolytic enzymes relevant to plant growth and development. The tests carried out with 18 yeast isolates involved the evaluation of enzymatic production, solubilization and the ability to produce and fix plant growth-promoting compounds. Previous results indicated that the yeasts tested did not produce amylase, catalase, cellulase, laccase and pectinase. In the protease test, only one of the isolates demonstrated activity, while in the chitinase tests, nine yeasts showed positive results, with variations in the sizes of the internal and external halos. Regarding solubilization and fixation tests, it was observed that some yeasts have the capacity to solubilize potassium, phosphate and zinc, in addition to fixing N2, although these capacities vary between yeasts and the specific tests performed. Additionally, all isolates obtained positive results in the ammonia production test. In conclusion, some isolates have shown promise and have advanced to further testing to determine their capabilities as PGPM. It is hoped that this study will provide the discovery of new species of yeast to be explored and that these discoveries may represent advances in plant biotechnology and the promotion of agricultural sustainability.

**Keywords:** PGPY, Caatinga, biological inputs.

**Acknowledgments:** National Council for Scientific and Technological Development (CNPq); CAPES; INCT Yeasts: Biodiversity, Preservation, and Biotechnological Innovation.

### D-49. Isolation and partial purification of genomic DNA from *Bauhinia ungulata* using the CTAB (hexadecyltrimethylammonium bromide) method.

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Genomic DNA isolated from plants has a multitude of uses and applications favorable to the development of new lines of research, including PCR techniques, construction of genomic libraries and discovery of new molecular markers. The quality of the sample determines whether the material can be used in other experiments. The aim of this work is to present the results of an experiment to extract genomic DNA from the species Bauhinia ungulata, based described Doyle (1987), on the protocol by J using the detergent hexadecyltrimethylammonium bromide (CTAB), since this method helps to separate nucleic acids from polysaccharides. The process seeks to extract DNA without degradation and action of phenolic compounds that would irreversibly oxidize nucleic acids. The leaves of Bauhinia ungulata were macerated with liquid nitrogen using an pestle. The material was weighed at a ratio of 0.1g to 800ul of CTAB (6 replicates were made) and extracted in a water bath for 3 hours. The purification process was carried out by washing with chloroform in a 1:1 ratio, after that, was precipitated with 0.3 volumes of isopropanol and collected as a pellet. It was washed with 1 ml of alcohol and diluted in 10 ul of TE buffer. The results showed that the DNA remained with no signs of degradation, although it was noted that the material still contained RNA. The quantification of the replicates conclude that the samples had a 260/280 ratio of less than 1.6 and a 260/230 ratio of less than 1.4, revealing contamination with proteins and reagents. The medium concentration of nucleic acids was 896,5 ng/ul. Based on the results presented, it can be observed that the samples are not degraded. However, the numbers for 260/230 and 260/280 ratios did not meet the expected quality standards. The method was efficient for extracting genomic DNA with a low final concentration of genomic DNA and RNA contamination. The 260/230 and 260/280 ratios need to be improved, comproving that the samples are still contaminated by proteins and reagents. Therefore, is necessary to optimize this protocol before proceeding with the use of genomic DNA for other assays.

Acknowledges to Universidade Federal do Ceará, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico, Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. Key-words: degradation, contamination, quality.

### D - Biotecnologia (Processo Industrial)

### D-04. PARTIAL CHARACTERIZATION OF CHEESES PRODUCED WITH THE USE OF PEPTIDAZES FROM LATICIFERS

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Introduction: Peptidases are present in all living organisms and participate in a wide range of cellular and extracellular activities. In the food industry, it is common to use enzymes from animals, vegetables, fungi and bacteria. The reduction in production costs, better quality in the appearance and flavor of the products generated are advantages. However, the strategies added to the process, used in the milk coagulation stages; serum separation; weighing and maturation, contribute to the diversity of flavors, textures, presentation and in their organoleptic/sensory properties, which are of paramount importance for the food industry, since they constitute differentials to attract consumers. The sensory properties and quality of coalho-type cheeses are directly related to the peptides generated from enzymatic hydrolysis promoted on milk caseins. The world market is heavily dependent on chymosin, which is obtained from the bovine stomach, and not even its heterologous production, through molecular biology techniques, has reduced this pressure and dependence. Since cheese is an ancient food, it is still challenging to find other alternative enzyme sources to bovine chymosin. For this reason, the scientific literature is full of prospective studies of alternative proteolytic sources capable of inducing clotting in milk, capable of generating cheeses with desirable commercial qualities and circumventing the aforementioned restrictions. **Objective:** To develop, validate and produce a cheese, using vegetable rennet obtained from the latex of Cryptostegia grandiflora. Materials and Methods: The enzyme source will be extracted from the plant Crytpostegia grandiflora, from the latex. Fractionation steps will be performed(centrifugation and dialysis); biochemical characterization (chromatographies, electrophoresis); enzymatic activity tests(colorimetric assays and zymography) to determine total proteolytic activity; Storage conditions; service life; milk clotting tests; laboratory cheese production (5 g; 100 g; 1Kg); characterization of the coagulation process; sensory tests; physical-chemical determination of fat and protein contents; mineral composition; texture, hardness, firmness, among other determinations. In all assays, a commercially available coagulating agent will be used as a reference and positive control. Results and Discussion: The pilot cheese had already been produced and now the research is in the phase of requesting the ethics council to carry out toxicity and allergenicity tests with humans. Conclusions: It is possible to produce rennet cheese from vegetable rennet, keeping its organoleptic properties the same as rennet cheese produced in industry or by hand. Keywords: Peptidases; vegetable rennet; Cryptostegia grandiflora

# D-05. Evaluation of the thermostability and optimal temperature of laccase produced by SSF in a mixture of residues by the fungus *Penicillium roqueforti* ATCC 10110

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The solid state fermentation technique (SSF) is so interesting considering its low cost and high efficiency. Therefore, several biotechnological processes resort the SSF technique as an alternative in enzyme production and in reuse and valorization of agroindustrial waste which would be worthless. The multicopper oxidase enzyme laccase is too important, mainly in dye degradation processes and forest litter decomposition. This enzyme is able to degradate lignin, what is one of the molecule which ridigifies and supports plants. In view of the above, this presente study aimed to evaluate laccase's termostability and optimum temperature produced by SSF of mixture of rice husk, coffee husk and cocoa bean husk as substrate for laccase production by Penicillium roqueforti ATCC 10110 in solid state fermentation. Enzymatic behavior in the face of temperature variations is an important factor considering potential applications and the impact on enzymatic activity. Laccase activities were determined (triplicate) under fixed condition of pH = 5.0 (50 mM sodium acetate buffer), at various temperatures (from 40 to 80 °C). At this stage, the enzymatic behavior in a temperature range of 40 to 80 °C was investigated. The maximum enzymatic activity was obtained at a temperature of 60 °C. In addition to evaluating the optimum temperature, the thermal stability of laccase was studied in the fermentation process for five hours at temperatures ranging from 40 to 80 °C. It was observed that in all temperature ranges, laccase remained stable during the first three hours of incubation, showing an increase in activity at all temperatures within two hours. After four hours of incubation, at a temperature of 80 °C, the enzyme maintained activity close to 52% and 48%, in four and five hours, respectively. These results demonstrate good laccase stability at high temperatures and for long incubation periods. The thermostability of laccase has a great value in the valorization of lignin due to its biocatalysis process and versatility in adapting to industrial conditions.

Acknowledgements: I would like to express my thankfulness to the Coordination of Superior Level Staff Improvement (CAPES) for financial support granted during this work development. I also thank Santa Cruz State University (UESC) and the Laboratory of Biocatalysis and Biotransformation (LaBioCat) for available infrastructure, guidance and collaboration in this project development.

Keywords: Solid state fermentation. Enzyme. Thermostability.

### D-06. Prospecting flocculants and extracellular hydrolases from *Bacillus pumilus* BDL07 and *B. toyonensis* ACO.PR1-Isox

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**INTRODUCTION:** Flocculants derived from living organisms are easily biodegradable and nontoxic. Likewise, enzymes offer advantages over chemical catalysts as they generally act under milder conditions and reduce the generation of toxic by-products. Both can come from microorganisms naturally adapted to environments in which these molecules present their best performance. **OBJECTIVE:** To evaluate the production of bioflocculants, as well as the cellulolytic, lipolytic and proteolytic activities of two Bacillus strains isolated respectively from sediments of a sugarcane washing water lagoon and a yellow oxisol contaminated with the herbicide isoxaflutole. MATERIAL AND METHODS: B. pumilus BDL07 and B. toyonensis ACO.PR1-Isox were submitted both to qualitative/semiquantitative assays (cultures in solid medium containing specific substrates, 72 h at 37 ± 1 °C) and to quantitative ones (submerged cultures also containing specific substrates, monitored at over 144h of incubation at 37 ± 1 °C) for enzymatic activity and production of bioflocculants. RESULTS AND DISCUSSION: In submerged cultures, both strains secreted the enzymes and bioflocculants studied without pH fluctuations over 144 h. B. pumilus BDL 07 showed the best cellulolytic activity in solid medium (IE = 1.40), as well as in the first 48 h of its submerged culture. The maximum lipolytic activity of this strain in submerged culture containing olive oil was 0.450 U.mL<sup>-1</sup> at 72h of incubation, coinciding with its maximum cell growth. On the other hand, the maximum indices of lipolytic and proteolytic activities in solid culture were reached by B. toyonensis ACO.PR1-Isox (IE = 2.41 and 4.55, respectively). In submerged culture, this isolate reached the maximum values of lipolytic and proteolytic activities already at 48 h of incubation. Regarding the production of flocculants, it increased with incubation time, reaching 57% for B. pumilus at 72 h of incubation, while for *B. toyonensis* this was only 21%, but already after 24 h of incubation. **CONCLUSION:** The study established the basis for further exploration of the biotechnological potential of B. toyonensis ACO.PR1-Isox and B. pumilus BDL07, both to produce flocculants and extracellular hydrolases of industrial interest and for environmental remediation, justifying the deepening it under different conditions.

**Keywords:** *Bacillus* strains, enzymatic activity, bioflocculant production **Sponsorship:** "CNPq" and "S.A. Usina Coruripe Açúcar e Álcool"

### D-07. Isolation of an elastase 2-like protease from the fungus *Pleurotus djamur* PLO 13 grown on an alternative medium formed by cacti

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**Introduction:** Microbial proteases are among the most relevant hydrolytic enzymes, as they are the most commercialized group of enzymes and are responsible for approximately 60% of total world sales. Proteases are used extensively in industrial processes, especially in the dairy industry. There is a wide variety of products produced by dairy products, but cheese is one of the most relevant products in this sector. For its production, it is necessary to use rennet, an enzyme that is extracted from young slaughtered calves and used to coagulate milk. Despite the increase in global demand for cheese, there is a shortage in the production of calf rennet and ethical issues have led to the search for an alternative substitute for rennet from microorganisms. The enzymes derived from these beings have a great potential for the production of proteases, since they present a great biochemical diversity and are susceptible to genetic manipulation. One way to obtain these enzymes is through solid state fermentation (SSF), which uses agro-industrial residues as a source of nutrients and substrate, as it is a technique that has many advantages, since it is economical, energy efficient and produces high yields. As cacti are a typical vegetation of the Brazilian caatinga biome, widely used in agriculture to feed ruminants, generating a large volume of waste, rich in various nutrients, including carbon, which is an important macronutrient for the development of various microorganisms, such as the fungi. **Objective:** Isolate the enzyme elastase 2 from the fungus Pleurotus djamor PLO13, through solid state fermentation, using agroindustrial cactus residue as a fermentation medium for application in the food industry as a milk coagulant. Materials and methods: The agro-industrial residue, dehydrated cacti (DC), was acquired from a site located in Coruripe /AL, Brazil and the fungus *Pleurotus djamor* PLO13 was obtained from UFV. The fungus was cultivated for SSF and ethanol precipitation and size exclusion chromatography were used for the isolation of elastase 2. Results and discussion: The fungus demonstrated the ability to grow in an alternative culture medium composed of cacti. Proteolytic activity was tested in milk coagulation assays (caseinolytic) and characterized against specific synthetic substrates for trypsin, chymotrypsin and elastase 2. Conclusions: The agroindustrial waste used proved to be effective for enzyme production. Additionally, the activity demonstrates partial isolation in the proposed purification route. Acknowledgments: the authors are like grateful to CAPES, CNPq and FAPEAL for funding this research. Keywords: protease, filamentous fungus, cacti.

## D-08. Production of proteases by a bacterium from the gut of *Rynchophorus palmarum* for milk coagulation.

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Introduction: Currently, there are several studies in the field of food biotechnology with an interest in identifying and disseminating the use of new materials and alternatives for the production of dairy products, with emphasis on cheese production. As innovative sources, we can highlight the use of proteases in the milk coagulation process. Objective: This work proposed to use a bacterium isolated from the intestine of the insect Rynchophorus palmarum to produce proteases capable of coagulating milk. Methods: we cultivate the bacteria in a culture medium containing agar with 2% casein and incubated at 37°C for 48h. After bacterial growth, the enzymatic extract of the culture medium casein agar was prepared using a Tris-HCl pH 8 50mM buffer, and was named crude extract. The crude extract was then characterized (optimal pH, Optimum Temperature, and inhibition assay) and used for milk coagulation. The bacterium used for the study is undergoing analysis for the genetic identification of the species. Results: The extract proteases showed excellent activity at pH 8 and temperature of 50 °C, in addition to the inhibition assay, which showed a 91.21% inhibition profile for dithiothreitol (DTT). In addition, tests using a solution of skimmed milk powder at 10% plus 10mM of calcium chloride proved the effectiveness of using the extract from the bacteria for milk coagulation. We were able to reach the concentration of 1 ug of protein necessary to coagulate 1 ml of milk solution at 50°C in 1 h. **Conclusion:** With this study, we were able to prove that the use of bacterium from the intestine of insects can be sources of enzymes for biotechnological purposes, in addition to the fact that further studies may improve the production of enzymes to enhance our results. **keywords:** milk coagulation, proteases, bacterium

Supported by, UFAL, CAPES, FAPEAL

### D-09. Selection of Yeasts Producing Extracellular Enzymes from the Alagoas Caatinga

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The Caatinga is an exclusively Brazilian biome, characterized by unique features such as high temperatures, low rainfall, and a high degree of endemic species, including rich vegetation, among which bromeliads stand out. These plants engage in interactions and associations with various organisms, including yeasts. Yeasts have long sparked biotechnological interest due to their high capacity for synthesizing extracellular enzymes. Approximately 80% of enzymes used by industries are produced by microorganisms, and their demand in this global market continues to grow steadily. Thus, the present study aimed to evaluate the biotechnological potential of yeasts previously isolated from the phylloplane of bromeliads in the Alagoas Caatinga and deposited in the culture collection of the Laboratory of Microbial Diversity and Biotechnology - LDBM - Ufal. The yeasts were cultured and tested to produce the following extracellular enzymes: amylase, esterase, lipase, pectinase, and protease. The most produced enzymes were esterase (36.6%), protease (25%), and amylase (15.8%), followed by lipase (15.1%) and pectinase (4.2%). Additionally, 13 isolates (6.7%) showed positivity for three enzymes, 48 isolates (25%) for two enzymes, and 126 isolates (65%) for at least one enzyme. Among the genera frequently produced enzymes, Aureobasidium, Pseudozyma, Cystobasidium, Meira, Vishiacozyma, and Hannaella stood out. The latter exhibited a significant production of 54% esterase. Regarding the most versatile species concerning the evaluated enzymes, Rhodosporidiobolus poonsookiae, Pseudozyma hubeiensis, Symmetrospora vermiculata, and Papiliotrema laurentii displayed positive results in producing four extracellular enzymes. Due to the adaptation of these yeasts to the environment of the Northeastern semiarid region and its various stresses, the isolates from the Caatinga may possess high plasticity to establish themselves in the environment, including enzyme production. Hence, these microorganisms exhibit unique characteristics of significant biotechnological utility. These findings underscore that the phylloplane of bromeliads in the Caatinga offers a substrate rich in yeast diversity and stands out for the considerable biotechnological potential of these microorganisms in producing extracellular enzymes with multiple industrial applications.

#### Keywords: Semiarid; biotechnology; bromeliads.

**Acknowledgments:** National Council for Scientific and Technological Development (CNPQ); Federal University of Alagoas (UFAL); INCT Yeasts: Biodiversity, Preservation, and Biotechnological Innovation.

### D-10. Effect of potentially toxic metal/metalloid salts on the activity of some <u>oxidoreductases</u> expressed by bacteria from agro-industrial residues

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INTRODUCTION: Several microbial enzymes act extracellularly to facilitate the absorption of small molecules or the detoxification of others. In turn, products used in agriculture can be sources of distribution of potentially toxic metals/metalloids (soil, air and water). Among them are different fertilizers (Cd, Cr, Pb, Zn), pesticides (Cu, Pb, Mn, Zn), wood preservatives (Cu, Cr) and residues from intensive animal production (Cu, Mn and Zn). At low concentrations some of these metals/metalloids are essential for metabolic reactions, but others inhibit key enzymes. OBJECTIVE: To evaluate the qualitative effect of exposure to different concentrations of potentially toxic metal/metalloids salts on the extracellular activity of three oxidoreductases from five bacteria from agricultural residues and tolerant to them. MATERIAL AND METHODS: Bacteria isolated from effluent and sludge of a sugaralcohol agroindustry were cultivated (triplicates) in nutrient agar with different concentrations (mM) of the following salts: CuSO<sub>4</sub> (0.02, 0.31, 1.25, 2.51, 5.01, 10.04, 20.05); NiSO<sub>4</sub> 6H<sub>2</sub>O (0.012, 0.023, 0.046, 0.095, 0.19) CdSO<sub>4</sub>. 8/3H<sub>2</sub>O (0.004, 0.032, 0.065, 0.13, 0.52); ZnSO<sub>4</sub>.7H<sub>2</sub>O (0.003, 0.01, 0.022, 0.044, 0.087); PbCl<sub>2</sub> (0.012, 0.022, 0.043, 0.09, 1.44, 5.75, 11.51); CoSO<sub>4</sub>.7H<sub>2</sub>O (0.021, 0.077, 1.29, 2.59, 5.16, 7.74) MnSO<sub>4</sub>. H<sub>2</sub>O (0.021, 0.079, 0.166, 2.65, 5.3, 10.6); K<sub>2</sub>CrO4 (0.016, 0.257, 0.515, 2.06, 4.12, 8.24) and HgCl<sub>2</sub> (0.012, 0.022, 0.044, 0.092, 0.184). After incubation (24h, 30 ± 1°C, dark), the tolerance profile was evaluated (growth on different salts), and the five more tolerant strains (to the two maximum supported doses) were molecularly identified. These were inoculated (colony) in tubes (triplicates) with nutrient broth (NB) and NB + each salt (in the two highest supported doses for each strain) and incubated as above before being evaluated (SILVA et al., 1996) for possible alterations in their expression of catalase (E.C. 1.11.1.6), cytochrome c oxidase (E.C. 1.9.3.1) and nitrate reductase (E.C. 1.7.1.1). RESULTS AND DISCUSSION: While only Bacillus thuringiensis LOIII, Lysinibacillus macroides LOII and Pseudomonas aeruginosa EFI were able to reduce nitrate to nitrite, N2 and subsequent by-products in the presence of the tested salts, L. macroides LOII was the only strain that did not expresscytochrome oxidase(only at 0.04 mM of PbCl<sub>2</sub>). All strains, including Bacillus atrophaeus EFII and Bacillus cereus EFIII, expressed catalase under the conditions studied. CONCLUSION: Of the five bacteria isolated from agro-industrial residues tolerant to different concentrations of potentially toxic metal/metalloid salts, the enzymes catalase, cytochrome oxidase c and nitrate reductase from P. aeruginosa and B. thuringiensis were not affected.

**Keywords:** bioremediation, biotransformation, metal pollution **Sponsorship:** "Capes" and "S.A. Usina Coruripe Açúcar e Álcool"

#### D-11. Emulsifying Activity of Arabinogalactan Extracted from Anacardium ocidentale

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Introduction: Nanoemulsions are nanometer-scale dispersions used as carriers of lipophilic substances in biological systems. The use of nanoemulsions is associated with the increased biological activity and reduced degradation of bioactives sensitive to environmental conditions. Additionally, they can be employed as a controlled drug delivery system. To stabilize these types of emulsions, commercial emulsifiers, such as guar gum, are commonly applied in food and pharmaceutical industry due to their good emulsifying activity and nontoxicity. Other branched polysaccharides, such as the arabinogalactan extracted from the exudate of Anacardium occidentale, may be a viable alternative to replace these commercial gums. Objectives: Characterize emulsions prepared with the arabinogalactan extracted from A. occidentale exudate. Materials and Methods: Five formulations were prepared using magnetic stirring. Initially, the emulsifying coadjutants, Tween 80 (11.25% v/v) and glycerol (3.75% v/v), were homogenized for 5 minutes, then the commercial soybean oil (0.5% v/v)was added as the oily phase and stirred for 15 minutes. Finally, the aqueous phase (84.5% v/v) was added, consisting of 2 and 4 mg/mL of arabinogalactan (A2 and A4, respectively) or the positive control of the commercial guar gum (GG2 and GG4, respectively). A negative control group containing only the emulsifying coadjutants was prepared (C). The formulations were evaluated for their droplet size and polydispersity index (PDI) on the 1<sup>st</sup> and 70<sup>th</sup> days of storage at 4 °C. A4 emulsions showed the smallest droplet size at the end of 70 days (13.2±0.4 nm), followed by GG4 (78.3±6.3 nm), A2 (334.5±12.1 nm), C (561±107.3 nm), and GG2 (1035.5±66.5 nm). The smaller droplet size of emulsion is associated with finer and more stable emulsions. Their reduced size facilitates their diffusion through cell membranes, enhancing the activity of bioactive components added to their formulation. The stability of the emulsions was evaluated using the polydispersity index (PDI). A4 showed the lowest PDI (0.37±0.0) at the end of 70 days, followed by GG4 (0.53±0.0), GG2 (0.79±0.2), A2 (0.83±0.2), and C (1.00±0.0). A lower PDI indicates a more uniform droplet size distribution and better emulsion stability because it hinders coalescing processes. Conclusions: The arabinogalactan extracted from Anacardium occidentale exudate showed a superior emulsifying potential than the commercially guar gum, and at higher concentrations, the arabinogalactan proves to be a viable alternative to guar gum in stabilizing emulsion at the nanometer scale.

Acknowledgements: CNPq, FACEPE and CAPES

Keywords: emulsion stability, emulsifier polysaccharide, exsudate

### D-12. Isolation and Identification of Cellulolytic Microorganisms from *Rhynchophorus palmarum* L. Adult Insects' Intestines

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The intestinal microbiota plays a crucial role in the degradation of complex compounds found in the hosts' diet, including plant components such as cellulose. The insect Rhynchophorus *palmarum* L. is known for feeding on palm trees, consuming cellulose fibers present in its diet. Within the intestinal tract of this beetle, a diverse community of microorganisms is harbored, potentially endowed with cellulolytic activity. This enzymatic activity is of interest both for the species' ecology and for biotechnological applications. Accordingly, this study aimed to isolate and identify microorganisms with cellulolytic activity from the intestinal tract of adult R. palmarum. Intra-intestinal dissections were carried out in a laminar flow chamber on eight adult insects (four males and four females) captured in the field. The intestines were incubated in brain-heart infusion (BHI) broth at 35°C for 24 hours. The resulting cultures were serially diluted (10<sup>-1</sup> to 10<sup>-6</sup>) and plated on nutrient culture media to isolate bacteria and fungi. Microorganism identification was performed using MALDI-TOF mass spectrometry, followed by assessing their ability to degrade cellulose using carboxymethyl cellulose as a carbon source in solid culture medium. The results revealed that six bacterial species (Alcaligenes faecalis, Bacillus cereus, Bacillus megaterium, Citrobacter koseri, Lactococcus lactis, Pseudomonas sp.) and one fungal species (Candida tropicalis) exhibited cellulolytic activity. These species displayed varying levels of activity, as evidenced by the formation of clear zones with Enzymatic Activity Index (IE) ranging from 2.0 to 4.6 (p < 0.05). B. cereus was the most efficient, generating the largest hydrolysis zones (IE = 4.6), followed by B. megaterium (IE = 3.8), C. koseri (IE = 3.2), and L. lactis (IE = 2.8). These findings indicate that the intestinal tract of R. palmarum is an important reservoir of microorganisms with the capacity for enzymatic cellulose degradation, holding promise for future biotechnological applications.

Keywords: Intestinal microbiota, Rhynchophorus palmarum, Cellulolytic activity.

Acknowledgments: This study was supported by the Fundação de Amparo à Pesquisa do Estado de Alagoas and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

#### D-13. Plant serine protease: characterization and biotechnological application

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Introduction: The application of enzymes in biotechnological processes enables a significant cost reduction with a consequent decrease in energy consumption. In this context, proteases are the most produced biomolecules and represent 60% of all enzymes marketed in the world. The importance of using these molecules is due to factors such as high catalytic activity, selectivity, specificity, as well as production variability. Objective: Faced with the demand to produce studies aimed at obtaining enzymes with an industrial bias, we aimed with this work to obtain protease of biotechnological interest from the seeds of Leucaena leucocephala, following its characterization and application. The project's starting point was to investigate the enzymatic activity in the crude extract of L. leucocephala seeds. Through this research, it was possible to describe a new milk coagulant. Optimal enzyme activities were determined at 40°C and pH 9.0. In stability tests, the enzyme remained stable over a wide range of pH (5.0 to 9.0) and temperatures (40°C to 60°C). This highlights the biotechnological potential of this enzyme in various industrial processes. When the enzymatic extract was subjected to different chromogenic substrates conjugated with p-nitroaniline, the hydrolysis showed specificity for BApNA. Furthermore, the enzyme was inhibited by PMSF (95.80%) followed by Benzamidine (84.22%). Therefore, the enzyme belongs to the serine protease class, an endopeptidase that preserves the presence of the amino acids serine, histidine and aspartic acid in its catalytic site. In addition, the enzymatic extract was distributed on a zymography gel where it is possible to verify the hydrolysis of the substrate (casein) incorporated into the polyacrylamide gel. With this, it was possible to verify the ability of the enzyme to be incorporated into a solid matrix with maintenance of its activity, important characteristics in the industrial environment. The aqueous extract of L. leucocephala seeds was submitted to a toxicity test using a bioindicator. It was possible to show that no toxic environment was generated after exposure for 24 hours of the crustaceans, Artemia salina, to different concentrations of the aqueous extract. Conclusion: the biotechnological application study revealed the coagulant capacity of the plant extract; the enzyme showed specificity for BApNA; the protease showed optimal activity at pH 9 and 40°C; in the inhibition tests, the enzyme belongs to the class of serine proteases; the aqueous extract of L. leucocephala seeds showed no toxicity against A. salina.

Keywords: *L. leucocephala*, coagulant, characterization Supported by, CAPES, FAPEAL,

#### D-14. Potential of Yeasts in Brazilian Mangroves: An Emerging Source of Biosurfactants

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Microorganisms from marine environments have the potential to develop new products. Mangroves are coastal ecosystems found between marine and terrestrial environments. These ecosystems present a rich biodiversity and a variety of microorganisms that can adapt to extreme conditions, producing biomolecules with unique properties. In this sense, yeasts are a promising source of biosurfactants with a great interest in industrial processes and bioremediation. This study aimed to evaluate yeasts isolated from mangrove sediments in Alagoas, Brazil, for their ability to produce biosurfactants. The emulsion index (IE<sub>24</sub>%) evaluated the yeasts' emulsifying activity. The biosurfactants were exposed in individual tests to different pH values (pH meter) and temperatures (drying oven) for a defined period of 30 min, then the IE24% was carried out to determine the activity and tolerance profile to pH (2, 4, 6, 8, 10 °C) and temperatures (90, 100, 110, 120, 150 °C), and their ability to reduce the surface tension of water was evaluated using a tensiometer. The results showed that the studied mangrove yeasts had good emulsifying activity in the presence of kerosene, hexane, toluene, lubricating, and motor oil. In addition, the surfactant molecules showed tolerance to high temperatures (90 °C to 150 °C) and different pH (2 to 10). Finally, the results indicated that the yeasts' emulsifying activity was more significant in biomass, biomass supernatant, and biomass supernatant after sonication than in the cell culture-free supernatant. Therefore, these results suggest that the yeasts from mangrove sediments in northeastern Brazil may produce biosurfactants. It offers new alternatives to synthetic surfactants and the possibility of biotechnological applications to mitigate the environmental impacts of hydrocarbon pollution, reinforcing the importance of protecting and sustainably using natural resources. Keywords: Surfactant; Bioemulsifiers; Biotechnology.

**Acknowledgments:** FAPEAL, CNPq, CAPES, Marine Biotechnology Network and INCT Yeasts: Biodiversity, Preservation, and Biotechnological Innovation.

## D-15. Plant peptidases as efficient and eco-compatible tools to achieve enzymatic depilation of goat skin

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**INTRODUCTION:** The implementation of "clean technologies" is essential in the tanning industry to minimize the toxic waste generated. Traditionally, tanneries apply a chemical process based on sodium sulfide (SS) in the dehairing stage of the skins, one of the first in the tanning process. Since SS is hazardous to the environment and human health and is inefficiently recycled, innovative protocols to reduce or eliminate its use in the leather industry are welcome. **OBJECTIVE**: To this end, the use of plant peptidases obtained from the latex of Calotropis procera (0.15 %), a proteolytic preparation that has been previously characterized, was tested for its use in the waxing of goatskin in replacement of SS. METHODS: After waxing, the hides were tanned with chrome salts and processed to the "wet-blue" stage. For comparative purposes, the same process was carried out but using conventional depilation using SS. Finally, all the resulting leathers were dried under controlled conditions and analyzed for their physicochemical and mechanical properties using standardized methods for ash, pH, dichloromethane extractable materials, chromium concentration, tensile strength, N/ mm), percentage elongation at break (elongation at break, %), tear strength (N/mm), distention and bursting (distension at grain crack, mm). RESULTS AND DISCUSSION: The results were similar between both methods in the determination of chemical parameters, while the resistance to the different stresses, measured in the physicalmechanical tests carried out, did not show supremacy in the performance of one or the other product. In addition, the microscopic analysis revealed the presence of totally hair-free follicles in the case of enzymatic depilation. CONCLUSION: In this way, we can conclude that the results obtained suggest that the skins subjected to an eco-compatible process such as enzymatic depilation with C. procera plant peptidases, present physical-chemical and mechanical characteristics like those of the leathers that were obtained, through the conventional process using SS, highly polluting. Latex peptidases are promising eco-friendly alternatives to SS use in tanneries.

Key words: Latex peptidases; leather dehairing Financial Support: CNPq, FUNCAP and CAPES

## D-16. Optimization of saccharification with enzyme produced by *Penicillium roqueforti* ATCC 10110

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Introduction: Cellulases are enzymatic complexes composed of exoglucanase, endoglucanase and  $\beta$ -glucosidase, with endoglucanase being the essential component for the internal hydrolysis of cellulose. Endoglucanases are the third most produced in the world and the demand for them is constantly growing due to their catalytic versatility. Response surface methodologies (RSM), such as the Box-Behnken project (BBD), are valuable tools to optimize the saccharification process, especially because they allow the generation of mathematical models capable of describing the behavior of an experimental response according to variations in the levels of the investigated factors. Objective: The objective of this work is the optimization of saccharification using endoglucanase productized in agro-industrial residues. Materials and methods: The enzymatic extract produced by P. roqueforti ATCC 10110 containing endoglucanase was applied in the saccharification of residues applying multivariable optimization with the variables time (3, 6 and 9 h), substrate (5, 10 and 15%) and enzyme concentration (110, 275 and 440 U/ml). Results and discussion: From the contour graph it was observed that the combined effect of substrate and enzyme concentration was promising in the saccharification process, since a greater amount of lignocellulosic biomass in the saccharification process is favorable as more sugars, like glucose, moreover, increasing the concentration of the biocatalyst increases the amount of active sites available for the reaction. Maximum substrate levels (15%) and enzyme concentration (440 U/mL) were used to validate the model at 6 hours. These conditions allowed the production of 253.19 ± 5.05 mg/g of fermentable sugars and there was no significant difference (t value = 2.89, n = 3, p = 0.05). **Conclusions:** The conditions optimized by BBD resulted in high amounts of fermentable sugars (253.19 mg/g) without the use of any chemical pre-treatment. Our results provide promising perspectives for the application of endoglucanase obtained from *P. roqueforti* ATCC 10110. Acknowledgment: the authors are like grateful to CAPES, CNPq and FAPEAL for funding this research. Keywords: Enzymes, Response surface methodology, Celulase.

### **D-17.** The coating potential of chitosan-alginate in the microencapsulation of probiotics <u>Silva, R.B</u>; Júnior, E.D.S; Cahú, T.B; Bezerra, R.S.

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Chitosan is a pseudonatural, non-toxic and bioabsorbable polysaccharide derived from chitin, an abundant component from crustacean processing industry. Its polycationic nature makes chitosan a suitable surface coating, acting as a protective matrix ideal for cell encapsulation, capable of immobilizing microorganisms such as probiotics and shielding them from harsh environments. Probiotics, when administered in appropriate concentrations, confer health benefits to the host. Therefore, this study aims to establish a microencapsulation delivery system for probiotic strains using chitosan derived from shrimp chitin and alginate and evaluate the loss of viability, zeta potential and morphology. Chitin was extracted from shrimp cephalothorax waste, with the removal of calcium carbonate, proteins, and pigments. Chitosan production was performed by N-deacetylation with 50% (w/v) NaOH and purification. Microcapsules were produced using the extrusion method; Lactobacillus plantarum and Lactobacillus casei strains were coated with 2% alginate and 0.4% chitosan and lyophilized. These microcapsules are resuspended in agar medium for pre- and postlyophilization assessment, vacuum-packed, and stored at different temperatures for viability evaluation. With a 72% extraction yield (from dry weight), chitosan exhibited 80.32% deacetylation degree and positive zeta potential, enabling the formation of a semipermeable membrane around alginate, a negatively charged polymer. Morphologically, microcapsules measure between 2 - 2.5 mm, with swelling above 90% and low solubility. Encapsulation efficiency for L. casei was 90%, and for L. plantarum, 96%. After lyophilization, free strains experienced a log reduction of the colony-forming unit from 12.54 to 7.69 (L. casei) and from 12.03 to 8.95 (L. plantarum), a significant reduction of 38.62% and 25.6%, respectively. For the microencapsulated strains, a slight log decrease could be observed from 12.54 to 9.3 (L. casei) and 12.03 to 10.18 (L. plantarum), corresponding to 25.8% and 15.4%. After 30 days, the coated strains remained viable, once the L. casei CFU decrease were less than 4%. Conversely, free cells decreased by 38.9%. The microencapsulated *L. plantarum* showed a CFU with 0.16% percentage reduction, while non-microencapsulated strains was 15% over the days. The results demonstrate that microencapsulation with alginate and chitosan leads to higher Lactobacillus survival rates under adverse conditions compared to free cells. Therefore, coated strains may exhibit beneficial probiotic effects, and their inclusion in animal feed and human food preparations could potentially result in health benefits. Key words: Food additive. Lactobacillus. Encapsulation.

## D-18. Efficiency of reusing proteolytic enzymes for leather depilation in industrial protocols for Tanneries.

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The use of proteases of Calotropis procera latex as a dehairing agent in leather tanning process has been previously demonstrated. The enzymes were efficient in removing of hairs while preserving integrity necessary of the leather. This study investigated the efficiency of reuse of the enzyme preparation in depilation stage. Pieces of sheep and goat leather (5x5 cm<sup>2</sup>) were used. The proteases, obtained from the soluble protein fraction of the latex, were dissolved in distilled water to a final concentration of 0.15%, added of sodium sulfite 0.6% for a final volume of 150ml. The leathers were immersed in the respective solution in triplicate and kept under orbital shaking (120 rpm) at room temperature (25°C ±2) for 24 hours. After this period, the leather pieces were removed, in the same solutions new pieces were placed and the protocol was repeated for 5 cycles. The leather pieces removed were evaluated about hair removal by visual observation, ease of removal of remaining hairs by microscopy analysis. The solution containing the proteases was able to remove goat and sheep hair for five consecutive cycles. The reduction in efficiency was observed from the third cycle. However, when performed with an increase of 24 hours, the total depilation of the third cycle was achieved. The results showed that the method of enzymatic action had the same or better depilatory efficiency when compared with chemical action method. Thus, the enzymatic source has demonstrated efficiency for reuse which means reduced costs and lower water use in the industrial process. Latex proteases of *Calotropis procera* constitutes a potential input that is ecologically friendly.

Supported by CAPES, CNPq and FUNCAP.

Keyword: clean technology; depilation; enzymes; tannery.

### D-19. Production of chitinases and chitosanases by filamentous fungi isolated from marine invertebrates

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The Earth's surface is covered in 71% by oceanic waters, constituting it as one of the most comprehensive ecosystems. These ecosystems project themselves as important sources in obtaining products for biotechnological applications. Among these, chitinase and chitosanase enzymes stand out, which play essential roles in morphogenesis, cell division, autolysis, and chitin synthesis. In addition, they can be used as biological control instruments due to their antimicrobial and antioxidant properties. One of the most significant applications of chitosanase is in producing chitosan oligosaccharides (COS). COS exhibits biofunctional properties, such as antimicrobial action, and antioxidants, contributing to decreasing blood cholesterol and lowering blood pressure. In addition, they also play an important role in the food industry. Thus, the objective of the present study was to evaluate the biotechnological potential of filamentous fungi isolated from the coastal marine biome in the state of Alagoas concerning the production of chitinase and chitosanase. The filamentous fungi isolates (n =80) were previously isolated from corals and sponges. To evaluate the production of chitinases, a basal medium supplemented with bromocresol purple (BCP), pH 4.7, was used. The isolates were inoculated and incubated at 25±2 °C. The growth and production of chitinase were evaluated with semi-quantitative readings every two days and for up to 10 days through the measurement of the halos generated from the pH change, in addition to the growth of the fungal mycelium. Among the strains tested, 78 were positive for chitinase production. The genera that showed the highest chitinase production were Aspergillus, Penicillium, and Trichoderma. The isolates of the species Aspergillus welwitschiae and Curvularia sp. were the only ones that did not produce chitinase. Of the total fungi tested (n = 80), 12 showed positive results for chitosanase production. The genera Aspergillus and Trichoderma stood out in the chitosanase production, presenting an enzymatic profile superior to the other genera tested. The species with the highest enzymatic indexes were A. flavus and T. harzianum, with 1.8 cm and 1.97 cm, respectively. Within this context, the results are relevant to understanding the production of extracellular enzymes by filamentous fungi in marine environments. They may have significant implications for biotechnology and industrial applications that involve these enzymes. These discoveries are essential to contribute to the identification of promising species in research and biotechnological applications. Keywords: biotechnology, natural products, extracellular enzymes

**Acknowledgments:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Universidade Federal

de Alagoas (UFAL); Rede Brasileira de Biotecnologia Marinha - BiotecMar.

### D - Biotecnologia (Saúde Humana e Animal)

## D-20. Comparative analysis of protein extraction protocols and deparaffinization methods for fresh and fixed tissue from oral mucosa samples.

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Introduction: Oral Mucosa (OM) Biopsies are the gold standard for diagnosing oral cancer, however, samples preserved in formalin undergo changes that make it difficult to detect biomarkers by SDS-PAGE. Objectives: Standardize a protein extraction protocol in fresh and fixed OM samples for SDS-PAGE. Materials and Methods: Project Aproved by the CEP/UFAL: 65956516.1.1001.5013. 10 fragments of human oral mucosa were used (4 fresh and 6 fixed), and three protocols (P1, P2, P3) chosen from the literature and modified as follows for fresh samples: P1 (Triton X-100 at 100 µL/10 mg of tissue - standard for all protocols) with crushing and complete homogenization. The extract was centrifuged at 5000 RPM/30 minutes at 4°C; P2: 100mM DTT, 4% SDS; P3: 200mM DTT, 2% SDS) with centrifugation time of 20 and 10 minutes, respectively. The buffers were applied to the samples and crushed until homogenized and the extracts were incubated for 20 minutes at 100°C and centrifuged at 4°C. Comassie Brilliant Blue R-250 and G-250- based dyes were compared. The best extraction and staining protocol were combined with deparaffinization methods (M1 and M2) as follows for fixed samples, M1: three xylene incubations/05 minutes and four serial ethanol rehydrations/1 minute followed by incubation in distilled water for 30 minutes); M2: shaking and centrifugation for 3 minutes after each incubation with xylene and serial rehydration in ethanol (three/5 minutes). Results were evaluated by microplate reading, with t test for paired samples (p<0.05). Results and Discussion: P1 showed the lowest protein yield (5.75 mg/mL) compared to P2 and P3, (26.15 mg/mL and 27.15 mg/mL, respectively), (p= 0.111). Triton (P1) was a weak denaturant, while P2 and P3 have SDS and DTT, more powerful denaturants. It is worth nothing that P2 and P3 had heating stages, facilitating extraction, however, no significant differences were observed between heating times (20min to 2h). In SDS-PAGE, P2 and P3 showed more and better visible bands. Comassie R-250 stain showed to be more effective and sensitive to abundant proteins. P3 (with higher yield), was then, chosen to be used in fixed samples with the two deparaffinization methods, Method 2 was more effective, allowing better visualization of the bands. Furthermore, the addition of 01 xylene incubation for 03 minutes improved visualization. **Conclusion:** The combined modified P3+M2 protocols proved to be an effective, low cost method for the extraction and analysis of proteins in OM samples through SDS-PAGE.

**Acknowledgements:** UFAL, FAPEAL and CNPq. **Keywords:** Proteins; SDS-PAGE; Formaldehyde.

# D-21. Shelf-life extension of refrigerated shrimp (*Litopenaeus schmitti*) previously subjected to antimicrobial edible coating containing sodium alginate, xanthan gum and sage essential oil

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Introduction: Currently, research on shrimp preservation is mainly focused on refrigeration combined with non-toxic cold sterilization technologies, biological extracts and packaging technologies. Electron beam irradiation, a harmless cold sterilization with enzyme inactivation effects, has been widely used, mainly in the processing of fruits, vegetables and fish such as shrimp and their products, and is able to reduce shrimp allergens. **Objective:** To evaluate the effect of a solution of sodium alginate, xanthan gum and sage essential oil as an edible protective coating for shrimp stored under refrigeration. Material and Methods: A combined polymer of sodium alginate (SA) 1% (w/v), xanthan gum (0.3%) and essential oil of sage (Salvia officinalis L.) (1% (v/v) to coat raw and cooked shrimp (Litopenaeus schmitti) fillets (without exoskeleton and cephalothorax), and stored for 15 days under refrigeration (4°C), evaluating their shelf life through chemical and microbiological parameters (total counts of aerobic mesophilic bacteria and total aerobic psychrotrophic bacteria) as well as the melanosis aspect. The shrimps carotenoids were extracted and their antioxidant capacity (DPPH radical) was evaluated after 15 days of storage. Results and Discussion: The coating had the advantage of reducing the bacterial count by 2 log CFU and reduced the unpleasant taste of the shrimp during the storage period, avoiding the odor of the acetic acid used to dissolve the alginate and xanthan. The shrimps carotenoid content showed excellent antioxidant capacity (DPPH radical) even after 15 days of storage. Conclusion: The coating treatment, with great antioxidant capacity and antimicrobial, prolongs the shelf life of shrimp.

**Keywords:** Antimicrobial; antioxidant; edible coating; film; *Litopenaeus* spp.; *Salvia officinalis* L.

Sponsorship: FAPEAL and S.A. Usina Coruripe Açúcar e Álcool"

# D-22. Healing potential of emulgels based on collagen and gelatin extracted from Nile tilapia in association with *Chlorella vulgaris* extract and silver nitrate for the treatment of burns

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Skin burns represent a global morbidity and mortality problem. Pharmacological agents for topical treatment have limited action on antimicrobial control, which has motivated the search for formulations capable of stimulating healing. This study aimed to develop and characterize emulgels based on collagen (COL) and gelatin (GEL) extracted from tilapia skin in association with the crude extract (CE) of *Chlorella vulgaris* biomass and silver nitrate (AgNO<sub>3</sub>) for the treatment of burns in a murine model. COL and GEL were extracted and characterized using Fourier Transform Infrared Spectroscopy (FTIR), thermogravimetry (TGA), Differential Scanning Calorimetry (DCS), and electrophoresis. CE was characterized by electrophoresis; total protein quantification of CE was performed, and its antioxidant activity was analyzed. Three formulations of emulgels were developed: COL+GEL (E1), COL+GEL+CE (E2), and COL+GEL+CE+AgNO3 (E3), which were characterized by electrophoresis, FTIR, rheology, in vitro cytotoxicity, and healing potential in a full-thickness burn model in rats, using silver sulfadiazine as a positive control (C+) and blood clot as a negative control (C-). COL and GEL showed good physicochemical characteristics, and CE showed a high amount of proteins and antioxidant activity. E1, E2, and E3 presented similar physicochemical and rheological characteristics and varied cytotoxicity profiles. In the in vivo study, there was no significant difference in the percentage of wound contraction between the groups and the controls. However, microscopic analysis showed higher scores for polymorphonuclear cells in E1 and neovascularization and re-epithelialization in E3. The COL+GEL+CE+AgNO<sub>3</sub> emulgel has potential as a formulation for the healing of thermal burns, although its use in a clinical setting requires further studies.

Acknowledgment: CAPES, CNPq e FUNCAP.

Keywords: burn; collagen; microalgae.

## D-23. Bioconjugation of DNA probe in glutamic acid polymer through covalent binding for development of *Mycobacterium tuberculosis* biosensor

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Introduction: In 2020, tuberculosis is still one of the leading causes of death in the world, with an average of 10 million new cases per year. The limiting factor for controlling the epidemic, especially in socioeconomically vulnerable countries is diagnosis. The main diagnostic methods (PCR and bacilloscopy) have specificity problems and are time consuming or are expensive and require skilled labor and sophisticated laboratory structure. Objectives: Therefore, we turned to a quick, cheap and effective diagnostic method: biosensors. The most important part in developing biosensors is the bioconjugation of the bioreceptor on the surface in a stable manner. Methods: After gene prospection through NCBI/GenBank databases, the Rv2341 gene was selected due to being specific for Mycobacterium tuberculosis strains and located in a stable portion of the genome. From this process, the bioreceptor was acquired: an aminated DNA probe with this gene sequence. To build the system, screen printed carbon electrodes were used with a work electrode (WE) and a counter electrode (CE) made of carbon paste and reference electrode (RE) made of Ag/AgCl paste. Results: To create the covalent bond, we generated a film of glutamic acid on the surface through cyclic voltammetry, to provide the carboxylic groups (COOH) necessary for binding with the amine groups (-NH2) present in the DNA probe, enabling the formation of amide bonds. Aiding this process, DMTMM (4-(4,6-Dimethoxy-1,3,5-triazin-2-yl) was used as the bioconjugation agent, since it's capable of enhancing the formation of amide bonds in neutral medium. To infer reaction success and differentiate between modifications, electrochemical impedance spectroscopy was the chosen method. Discussion: Ferricyanide ([Fe(CN)6]-3/-4 was used as redox mediator. Employing Randel's equivalent circuit, values of resistance to charge transfer were obtained and posteriorly represented through the Nyquist plot. Unmodified electrodes show high resistance values (29,5k $\Omega$ ), which decreased after modification with glutamic acid polymer ( $6,2k\Omega$ ). By adding DMTMM, the surface became more electroactive, resulting in the lowest values  $(3,4k\Omega)$ . After forming the covalent bond, the oligonucleotides present and their negative charge reacted with the redox agent, resulting in a repulsion between them that can be observed through the sudden increase of the Rct (11,9k $\Omega$ ). **Conclusion:** We were able to perform bioconjugation of the DNA probe on the surface of the transducer through covalent binding and characterize this process, achieving a vital step in creating a biosensor for the diagnosis of tuberculosis. Acknowledgments: We are thankful for all the members of the Biosensors and BRICS groups, to our friends in the Keizo Asami Institute for their support during the construction of this work and to CNPq (CNPq Process Number 440117/2020-8) and UFPE for providing the necessary resources to allow this work to come to fruition.

Key-words: Biosensors; bioconjugation; tuberculosis.

## D-24. Technological Production Focused on Chagas Disease: A Prospecting of New Drugs and Diagnostic Methods

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Chagas disease, classified as a neglected tropical disease (NTD), is caused by the parasite Trypanosoma cruzi, which is transmitted to animals and people by insect vectors. Annually, it affects thousands of individuals worldwide, particularly in regions marked by significant social and economic vulnerability. For the treatment of the acute phase of the disease, two drugs are used: nifurtimox and benznidazole. Due to the reported adverse effects of these drugs, including nausea, vomiting, and neurological symptoms, there is an urgent need to find safer and more effective treatments. The research aimed to catalog patented technological innovations intended for use in the diagnostic and clinical treatment. In this way, searches were conducted within the databases of the European Patent Office (EPO) and the Collaborative Database for Drug Discovery and Innovation (CDDI). As search descriptors the terms "Trypanosoma cruzi" and "Chagas disease" were used and a specific time period was not established. After screening the results, 31 patents were selected for the analysis, 10 of them were applied for diagnosis, and 21 were applied for drugs. The data were organized in Microsoft Excel (2019) and used for bibliometric analysis via network construction and analysis using Software VOSview version 1.6.15, which focuses on bibliographic data clusters with a distance-based approach. The data gathered reveals a notable concentration of product development in the United States, with a relevant involvement of university research centers as the primary contributors for these innovations. Predominantly, these research efforts focus on investigating promising compounds with the potential to evolve into novel drugs for clinical treatment. However, many of these projects are still in their early stages of development. In the realm of diagnostic technologies, there is a significant emphasis on immunobiological assays. Bibliometric analyses conducted on this subject matter indicate that the research primarily revolves around chemical parameters, the trypanocidal efficacy of many compounds, kinetic and metabolic parameters, and in vivo biological studies. Thus, it was evident that the path to effectively integrating these technologies into healthcare services is prolonged, but the field has potential for the creation of novel products capable of addressing the existing demand. In addition, investments towards new studies are necessary to expand the accessibility of these treatment and diagnostic technologies.

Palavras-chave: Technological production, *Trypanosoma cruzi*, Neglected tropical diseases. Agradecimentos: CNPq, Funcap, CAPES e UFC.

### D-25. Effect of p-coumaric acid on thymic epithelial cells after in vitro cell aging induction with D-galactose

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Thymic involution is characterized by a decrease in the number of T lymphocytes capable of combating new pathogens. Changes in thymic epithelial cells (TECs) may be responsible for this reduction, impairing adaptive immunity. Restoring immunity is crucial, and natural compounds like p-coumaric acid (ApC) have potential as immunomodulators, although their action in thymic aging has not been studied. This work aimed to study the effects of ApC on TECs after in vitro induction of cellular senescence with D-galactose (D-gal). The 2BH4 mouse TEC line was used. Dosages and treatment times were determined in MTT assays. Cell counting with trypan blue vital dye and Acridine Orange/Propidium Iodide staining evaluated proliferation and cell death. Exposure to D-gal significantly reduced cell viability and cell counting, especially at 222 mM for 48 hours. However, ApCcouldn't reverse the effects of Dgal exposure on TECsviability. In another set of experiments, D-gal exposure significantly reduced the expression of the glutathione peroxidase gene and increased reactive oxygen species levels in TECs. Also, ApC (1 and 10 µM) was able to reverse mitochondrial oxidative stress caused by D-gal. These results emphasize the need to further investigate the interactions between compounds and the search for potential molecules to mitigate the effects of aging.

Acknowledgement: UFAL; PROPEP; FAPEAL; CNPq; LBC-ICBS/UFAL; INCT-NIM. Keywords: Thymus; Senescence; p-Coumaric Acid.

#### D-26. Probiotic potential of mangrove yeasts

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The benefits of using microorganisms as probiotics have been studied over the years. When ingested in sufficient amounts, these probiotics confer a health benefit on the host. These benefits include immunostimulating action, balance of intestinal microbiota and treatment of various pathologies. Some criteria are essential to characterize a probiotic as resisting gastrointestinal conditions, adhering to the intestinal mucosa, and not being pathogenic, among others. Among the most studied microorganisms are lactic acid bacteria (LAB) and some yeast species, such as Saccharomyces cerevisiae and Saccharomyces boulardii. Studies with other yeast species have been explored, showing promising potential. In most of these studies, the species are from fermented foods. Thus, this work aimed to investigate the probiotic potential of yeasts isolated from mangroves in Alagoas. Initially, a survey was carried out of 45 species previously isolated from the Santo Antônio and Tatuamunha mangroves and stored in the micoteca of the Microbial Diversity and Biotechnology Laboratory of the Federal University of Alagoas. The exclusion criteria were growth below 37°C and being pathogenic or opportunistic. We selected 24 species. Resistance to gastrointestinal conditions, adhesion ability, antipathogenic activity and safety were tested. Two species showed favorable results for probiotic potential. These yeasts tolerated a temperature of 37°C, pH 2.0, bile salts at different concentrations, and pepsin presence. In addition, they presented a hydrophobic cell surface and self-aggregation capacity. They also showed co-aggregation capacity against the pathogens Escherichia coli, Shigella sp., Salmonella sp. and Staphylococcus aureus. For the enzymatic activity assay, all strains produced lipase and protease, helping in the metabolism of nutrients and improving digestion. None of the species produced gelatinase and had no hemolysis activity. Therefore, it is concluded that the mangrove isolates tested in vitro have a strong probiotic potential.

Keywords: Saccharomyces, fungi, gut microbiome.

**Acknowledgments:** National Council for Scientific and Technological Development (CNPq); CAPES; INCT Yeasts: Biodiversity, Preservation, and Biotechnological Innovation.

### D-27. Protein Profile and Potential Therapeutic Effects of Latex from *Himatanthus drasticus*.

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In Brazil, *H. drasticus* is widely used by locals, primarily for its latex, to treat variety of health problems. Several scientific studies have proved effectiveness of its latex (HdCL) for treatment of ulcers, arthritis, gastritis, inflammations, and diabetes when taken orally and for treatment of wounds and other skin conditions when applied topically. Numerous bioactive substances found in HdCL have been extensively investigated for their therapeutic properties. However, extraordinarily little work has been done on protein fraction (HdLP) of HdCL. This study aims to bridge this gap by analyzing protein profile, biochemical and biological activities of HdLP and its sub-fractions. For this, laticifer proteins from HdCL were purified using DEAE ion exchange chromatography, analyzed by 1D and 2D electrophoresis, and characterized using mass spectrometry. In vitro assays were conducted to assess the antioxidant properties of HdLP and its sub-fractions. Cell viability assays were performed against fibroblast L929 and RAW 264.7 macrophages and in vivo experiments were conducted in Swiss mice to explore the impact of HdLP on immune responses. Results of chromatography showed the presence of two different fractions in HdLP, named Hd-1 and Hd-2, and MS analysis indicated that each fraction has nearby 25 sub-fractions which are comprised of chitinases, proteases, proteins involve in regulation of different metabolic activities and some with unknown functions. Antioxidant assays revealed that HdLP and sub-fractions have high activity for ascorbate peroxidase and superoxide dismutase while low for guaiacol peroxidase and catalase. In viability assays against L929 and RAW 264.7, HdLP seemed to be toxic while Hd-1 and Hd-2 promoted cell proliferation. In vivo experiments indicated a positive influence of HdLP and its sub-fractions on immune responses, as evidenced by increased leukocyte count in blood, when administered intraperitoneally. This research elucidated that HdLP may play a role in combating oxidative stress, a factor implicated in many health conditions. HdLP appears to have toxic effect on cells, while its sub-fractions promote cell proliferation which implies that different components of HdLP may have contrasting effects on various cell types, highlighting the complexity of its composition. It may also possess immunomodulatory properties, which could be significant in various therapeutic applications. This study contributes valuable insights into the potential health benefits of H. drasticus latex, opening avenues for future investigations and the development of novel treatments. Further research is warranted to elucidate the precise mechanisms of action and therapeutic applications of HdLP and its constituent fractions.

**Acknowledgements**: The biochemical and functional studies of latex from *H. drasticus* have been supported by the grant from CNPq. We are in debit with "The Nucleus of Experimental Biology (Nubex)", UNIFOR for providing facilities for mass spectrometry and cell culture experiments.

Keywords: Cell culture; Leukocyte count; Mass spectrometry.

### D-28. ISOELECTRIC ISOLATION AND COUPLING STUDIES OF LACTOFERRIN BUFFALIN WITH QUERCETIN

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Lactoferrin (Lf) is an iron ligand glycoprotein with a molecular weight of about 80 kDa; belonging to the transferrin family, found in several mucous secretion of mammals. Lf is a multifunctional protein, playing several biological roles, such as antibacterial, antiviral, antifungal, anti-inflammatory, anti-tumor, antioxidant, and immunomodulatory activities. Nowadays, spectrofluorimetric and spectrophotometric techniques are largely used to understand the interactions of proteins-drugs. This work aimed to purify buffaloes lactoferrin monitoring the isolation by fluorimetry techniques and further evaluate the lactoferrinflavonoid interactions. Materials and Methods: First buffalo milk was centrifuged for the fat separation. The skimmed milk was acidified with 0.1 M HCL to pH 4.6 (casein pl), acid whey obtained was further titrated in two steps with 0.1 M NaOH for the lactoferrin precipitation by isoelectric pl. First titrated up to pH 5.2 for other proteins removal and later the supernatant was titrated to pH 8.3 to reach the lactoferrin pI. After measured the protein content, samples of the precipitated were spectrophotometric analyzed under conditions of excitation length at 290 nm and emission wavelengths between 300-550 nm to characterized lactoferrin. UV-Vis absorption spectroscopy studies of lactoferrin with quercetin were also performed. Results and Discussion: SDS-PAGE of the pH 8.3 resuspended precipitated showed a band around 75 KDa consistent with the commercial bovine lactoferrin standard. This resuspended sample from pH 8.3 presented fluorescence extinction spectrum with peak in the region of 332 nm (comparable to the fluorescence spectrum of commercial Bovine Lactoferrin). The UV-vis absorption studies of his preparation with quercetin showed the complexation protein-flavonoid, generating blue shift from 268.86 nm to 265 nm, characteristic peak of flavonoids. Conclusion: In this study it was possible to perform buffalo lactoferrin precipitation by isoelectric pl. The UV-vis absorption studies of the lactoferrin preparation coupled with quercetin indicated changes on the protein conformation. Keywords: Lactoferrin; guercetin; spectroscopic studies.

## D-29. Antifungal potential of the *Myracrodruon urundeuva* heartwood lectin (MuHL) against *Cryptococcus* species

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Myracrodruon urundeuva is a plant found in the Brazilian Caatinga and widely used to treat wounds and gynecological diseases. The heartwood of this plant contains lectin, hemagglutinanting protein that bind selectively and reversibly to carbohydrates. The M. urundeuva heartwood lectin (MuHL) binds to chitin and shows insecticidal, antimicrobial, and anticancer properties. Cryptococcus is the etiologic agent of cryptococcosis, a systemic mycosis that affects immunosuppressed or immunocompetent patients. The objective of this study was to evaluate the antifungal activity of MuHL against Cryptococcus. The lectin was isolated after heartwood (10g) protein extraction with 0.15 M NaCl (100 mL) for 16h, precipitation of the protein extract with 40-60% (w/v) ammonium sulfate and chromatography of precipitated protein on chitin column equilibrated with 0.15 M NaCl. MuHL was recupered from column with 1 M acetic acid. Cryptococcus neoformans (B3501) and C. gattii (R265) were cultivated in Sabouraud Dextrose Broth (SDB) for 48 h at 30 °C. Antifungal activity was determined in 96-well microplates, which the first well was the sterility control and the second was growth control (100%). From the third well, MuHL was serially diluted in the culture medium (3.12 - 200 µg/mL). After incubation (30 °C for 48 h), the minimal concentration that inhibited at least 50% of the fungal growth in relation to the control 100% (MIC) was determined. The minimal fungicidal concentration (MFC) was also determined and corresponded to the lowest lectin concentration that reduced the number of colony forming units in 99.9% after inoculation to Sabouraud Dextrose Agar plates (30 °C for 48 h). MuHL inhibited the growth with MIC of 6.25 and 12.5 μg/mL for *C. neoformans* and *C.* gattii, respectively. The lectin was also a fungicide agent with MFC of 12.5 and 25  $\mu$ g/mL for C. neoformans and C. gattii, respectively. Chitin is a polisaccharide present in fungal cell wall, thus the antifungal activity can be linked to interaction of MuHL with Cryptococcus cell wall. Acknowledgements: The authors express their gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de

Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for financial support. Keywords: Aroeira. Cryptococcosis. Mycosis.

### D-30. Selection of biochemical markers for muscle injury and inflammation during resistance exercise in athletes

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**INTRODUCTION:** the body undergoes metabolic responses resulting from exertion, triggering inflammatory signals and other metabolic signals. Monitoring the biochemical profile of biomarkers such as aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH), provide valuable information about muscle response, helping recovery and athletic performance. However, when considering that biochemical and physiological responses may vary between groups of athletes and non-athletes, there is a need to investigate and choose compatible markers for a better analysis of physical performance. **OBJECTIVE:** to determine, through the literature, a pattern of biomarkers for analysis of muscle injury and inflammation triggered by strength exercise in athletes. MATERIALS AND METHODS: this is an integrative review. The search took place in the PUBMED, SciELO and Sports medicine databases, in English and Portuguese, with articles published between 2010 and 2023. The descriptors in Health Sciences (DeCS) were used: Biomarkers; Muscle Strength; Athletes. **RESULTS AND DISCUSSION:** There is a complex interaction between biochemical markers and other clinical, physiological and performance factors. A set of biochemical markers such as CK, AST and LDH have been consistently associated with muscle injury and inflammation in strength training athletes. However, others were also identified, such as cardiac troponin I (cTnI), myoglobin, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- $\alpha$ ). The concentration and temporal pattern of these biomarkers vary according to the intensity of the exercise, the duration of the activity and the individuality of the athlete. By the way, it is also observed that heat can be seen as a predictor of an inflammatory process, due to the increase in local tissue temperature. CONCLUSIONS: intense exercise can cause muscle overload, leading to an increase in plasma AST, CK and LDH enzymes, which can be indicators of muscle damage. However, it is vital to interpret the results with caution, as temporary elevations during or after intense training do not necessarily mean serious injuries, as well-trained athletes can resist these elevations, due to muscular adaptation (increase in skeletal muscle strength). Therefore, integrating these biomarkers with each athlete's history and context is essential for accurate assessments, informing well-informed performance and recovery strategies.

**KEYWORDS:** Biomarkers; Muscle Strength; Athletes.

acknowledgments: Cesmac University Center, CAPES Journal Portal.

### D-31. Analysis of biomarker search strategies for the diagnosis and prognosis of oral neoplasms

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**INTRODUCTION:** The incidence of oral neoplasms, particularly squamous cell carcinoma, has increased due to increased consumption of cigarettes, alcoholic beverages, and the practice of oral sex. Oral neoplasia causes lesions and discoloration in the mouth and on the lips, leading to difficulties in chewing and swallowing and ultimately compromising the eating process. Therefore, to evaluate staging, therapeutic response, prognosis, and recurrence detection in oral neoplasia, researchers have identified different biomarkers. These biomarkers aid in diagnosing oral neoplasia early, making it crucial to define the most effective strategy for biomarker identification to diagnose oral neoplasia assertively and quickly. **OBJECTIVE:** This summary aims to analyze the biomarker identification strategy that can assist in diagnosing and prognosing oral neoplasia. MATERIALS AND METHODS: This integrative literature review utilized a descriptive qualitative approach in August 2023. The bibliographic survey was conducted on the Pubmed, MDPI, and Wiley databases using the following descriptors: Biomarkers, Pharmacological, Mouth Neoplasms, Tumor, and Strategic Research. Articles were limited to the English language and published between 2018 and 2023. RESULTS AND DISCUSSION: Examining biomarkers of ON involves various strategies, including obtaining tissue, serum, and saliva samples, which have unique characteristics. Biomarkers such as p53, Ki67, and the EGF/EGFR protein complex have been identified as crucial in tissue lesions of the oral cavity, using immunohistochemical expression tests. EGF/EGFR is a cell growth and differentiation factor frequently present in the orofacial region in cases of oral neoplasia. Alternatively, non-invasive methods that utilize saliva to detect cytokines are highlighted in other studies. The protein-based pro-inflammatory cytokines IL-6, IL-8 and TNF- $\alpha$  are among the salivary biomarkers that are commonly detected in patients diagnosed with oral neoplasia. Other data indicate significant concentration levels of TSA and LSA in serum as a potential biomarker of deterioration due to oral neoplasia. Recently, salivary metabolites have also been studied as potential biomarkers; however, their accuracy is limited by the interference from various physiological factors in the oral cavity that still require further investigation. CONCLUSIONS: There are potential biomarkers for oral neoplasia in tissue samples, serum, and saliva. This necessitates identifying the most effective strategy for diagnosing oral neoplasia. It is concluded that biopsy of tissues from the orofacial region demonstrated the highest diagnostic accuracy. Orofacial tissue biopsy appears to be the most reliable method of diagnosis, while cytokines and salivary metabolites offer a non-invasive and promising method for monitoring and prognosis, although further research is needed for precise definition.

PALAVRAS-CHAVES: Biomarkers; Mouth Neoplasms; Strategic Research

# D-32. Essential oils of C. tricolor and toxicity tests and nutritional indexes in Tribolium castaneum

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The adaptive capacity of plants in relation to environmental factors highlights the role of secondary metabolites in response to stress situations. Essential oils (EO) are volatile compounds derived from aromatic plants, known for their non-toxic properties and volatility, making them promising for controlling insect pests. For this reason, these compounds are gaining widespread interest from researchers for the control of stored product pests. The study focuses on Tribolium castaneum (stored product pest beetle) and explores the insecticidal potential of essential oils from the Croton tricolor plant. Thus, this study has the general objective of evaluating the insecticidal and repellent potential of essential oils from leaves and branches of Croton tricolor Klotzsch ex Baill against T. castaneum, identifying the concentrations and evaluating the nutritional indexes of the insects. The effects of essential oils on nutritional indices, metabolism, and reproduction of T. castaneum after exposure to LD50 were evaluated. Initially, it carried out the extraction of essential oils from leaves and branches of C. tricolor via hydrodistillation. For the development of the contact toxicity bioassay against T. castaneum, concentrations of 1%, 5%, 10%, 15% and 20% of EO from branches and 1%, 2.5%, 5%, 7.5%, and 10% of EO from leaves, diluted in acetone. The tests were performed in triplicates, in which the insects were transferred to a petri dish, and with the diluted solution, an aliquot of 2.0 µL of this solution was topically applied to the mesothoracic region of the insects, leaving them on display for 2 minutes and then transferred to a container with wheat flour. It evaluated insect mortality after 24, 48 and 72 hours of exposure, along with the calculation of nutritional indices, such as Relative Consumption Rate (RCR), Relative Growth Rate (RGR), and Feed Conversion Efficiency (ECI). The study reveals that the essential oils of Croton tricolor have insecticidal potential against Tribolium castaneum. Essential oils from branches showed greater efficacy, causing significant mortality in insects. The 10% concentrations of essential oils from leaves and stems exhibited the best nutritional indices, with increased feed intake and conversion efficiency. Terpenes present in essential oils may be responsible for the observed insecticidal activity. Therefore, the study highlights the importance of exploring the defensive mechanisms of plants as an alternative to synthetic chemical insecticides, contributing to sustainable conversion control strategies in agricultural production.

Supported by: CNPq, CAPES, FAPEAL.

Keywords: Tribolium castaneum, essential oil, bioinsecticide, biotechnology

#### D-33. Investigation of the *Jatropha gossypiifolia* L. Leaf Extract For Antioxidant Activity, Presence of Lectins and Trypsin Inhibitors and Effect on Trypsin Activity From Gut of *Aedes Aegypti* Larvae

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Introduction: Jatropha gossypiifolia L. (Euphorbiaceae), known as Bellyache bush in English, or "pião roxo" in Portuguese, is native from Central and South America and currently has a pantropical distribution. In folk medicine, it has been used to treat rheumatism, ulcers, dropsy, hypertension and for wound healing. Aim: The saline extract of J. gossypiifolia (JgLE) was characterized for antioxidant potential, as well as presence of lectins and protease inhibitor. Its effect on the activity of gut trypsin from Aedes aegypti (dengue vector) third instar larvae (L<sub>3</sub>) was also described. Material and methods: The leaves were collected in Natal city, Rio Grande do Norte, Brazil. After dried in an oven with air circulation (45°C), The powder (10 g) was homogenized with 0.15 M NaCl (100 mL) under magnetic stirring (16h). After filtration, the resulting solution corresponded to JgLE, which was investigated for antioxidant activity by the DPPH assay, protein content using the Folin reagent, hemagglutinating activity (AH) using rabbit erythrocytes, as well as Trypsin inhibitory activity using bovine trypsin and the substrate  $N\alpha$ -Benzoyl-DL-arginine 4-nitroanilide hydrochloride (BApNA). The effect of JgLE on gut trypsin of A. aegypti L<sub>3</sub> was investigated through the BApNA hydrolysis test using a gut extract in 0.1 M Tris Buffer (pH 8.0) as a source of enzymatic activity. Results and discussion: JgLE (60- $100\mu/mL$ ) caused a significant reduction in DPPH radical scavenging activity, ranging from 61.40% to 67.60%. This data points JgLE as an antioxidant agent. JgLE showed a protein concentration of 87.32 mg/mL and agglutinated the erythrocytes with AH of 4, suggesting the presence of lectins. The extract was not able to reduce BApNA hydrolysis, indicating that trypsin inhibitors were not present. The L<sub>3</sub> gut extract showed trypsin activity of 100 U, which was increased 4.31-fold in the presence of JgLE (2.2 µg). This result suggests that JgLE may cause an imbalance in the activity of proteolytic enzymes from L<sub>3</sub> gut, and stimulates the investigation of its larvicidal activity. conclusion: JgLE is a source of antioxidant and lectin activities, as well as increases the activity of gut trypsin from A. aegypti L<sub>3</sub>. The Investigation of JgLE effect on L<sub>3</sub> survival is in course.

Acknowledgment: CAPES, CNPq, FACEPE.

Keywords: Natural products, hemagglutinating activity, proteases, dengue vector.

#### D-34. Anti-inflammatory Effect of Nanoparticles Containing Crude Carotenoid Extract Extracted From Cantaloupe Melon in an Experimental Model of Diet-induced Obesity Queiroz, J.L.C.<sup>1</sup>; <u>Medeiros, I.</u><sup>1</sup>; Lima, M.S.R.<sup>1</sup>; Carvalho, F.M.C.<sup>2</sup>; Camillo, C.S.<sup>3</sup>; Santos, P.P.A.<sup>3</sup>; Morais, A.H.A.<sup>1,4</sup>; Passos, T.S.<sup>4</sup>

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Carotenoids exhibit anti-inflammatory activity but have low solubility in water, stability, bioaccessibility, and bioavailability. However, nanoencapsulation is a strategy to minimize technological challenges and enhance functional potential. Based on this, this study investigated the effects of gelatin-based nanoparticles (EPG) and carotenoid-rich crude extract (CE) on inflammatory cytokines and adipose tissue. The carotenoid-rich crude extract was obtained from Cantaloupe melon pulp flour through ethanolic maceration (95%, 1:4 w/v), followed by fractionation with PA hexane (1:1 v/v) and sodium chloride (NaCl 10%, 1:10 v/v), rotary evaporation, and freeze-drying. Subsequently, an oil/water emulsification technique was performed using porcine gelatin as the encapsulating agent and Tween 20 as the surfactant under the action of an ultra-disperser at 17000 rpm for 10 minutes. The nanoparticles were characterized using various physical and chemical methods to assess morphology, particle diameter, chemical interactions, and carotenoid incorporation. The in vivo study was approved by the Animal Ethics Committee (CEUA-UnP) (protocol code 019/2017) and conducted at the University Potiguar (UnP) Animal Facility, following ARRIVE guidelines. Twenty adult male Wistar rats fed a high glycemic load and index (HGLI) diet, inducing chronic systemic inflammation for 17 weeks, were divided into four treatments for 11 days: 1) untreated (HGLI diet and gavage with water); 2) conventional diet (nutritionally adequate and water gavage); 3) HGLI diet and gavage with CE (12.5 mg/kg); and 4) HGLI diet and gavage with EPG (50 mg/kg). Inflammatory cytokines (TNF- $\alpha$ , IL-6, and leptin) in plasma and histological sections of adipose tissue were analyzed. CE presented 44.88 (0.10) µg of total carotenoids/g of melon pulp. Smooth spherical particles of 95.2 nm (11.25), 95% (1.66) incorporation efficiency, and chemical interactions between materials were observed. Plasma concentrations of IL-6 and leptin showed a significant reduction (p < 0.05) in the EPG-treated group compared to others. Regarding TNF- $\alpha$ , there was a reduction in the EPG group (p > 0.05). Adipose tissue weight did not exhibit statistical differences (p > 0.05). Still, histological analysis revealed more focal areas with multilocular adipocytes in the EPG-treated group than fibrotic areas and smaller multilocular adipocyte areas in other groups. Therefore, these results suggest that EPG may assist in treating inflammatory diseases related to adipose tissue accumulation. Acknowledgments to the Coordination of Improvement of Higher Education Personnel for financial support (code: 001 - CAPES) and the National Council for Scientific and Technological Development (Process Number: 422405/2016-7–CNPq). Keywords: Cucumis melo L.; nanoencapsulation; adipose tissue.

# D-35. The Chorion Influence on the Teratogenic Potential and Enzymatic Inhibition of New ACMD and QAMD Compounds in Zebrafish Embryos

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The search for new anticancer drugs is growing. Because of this, the screening of new drug candidates has become crucial. In this way, zebrafish embryos have been used for toxicity assessment in preclinical screenings. But few studies estimate the chorion influence during experimental design and results interpretation. Therefore, this study evaluated the influence of the chorion on the teratogenic potential and enzymatic inhibition of the new acridine (ACMD) and quinoline (QAMD) derivative compounds in zebrafish embryos. Fish were separated for breeding, two males and one female, for 12 hours. After reproduction, the embryos are collected for viability analysis. For the chorion-free (C/F) assay, this membrane was removed using 50 mg/mL pronase. The embryos (with and without chorion) were incubated with E3 medium (negative control) and to the compounds ACMD and QAMD at concentrations of 0.5 and 1 µM. In 24-well plates, 20 embryos per well, in duplicate, under semi-static conditions. The embryos were observed under an optical microscope at 24-, 48-, and 72-hours post-fertilization, analyzing the lethal parameters: absence of tail detachment, lack of somite formation, coagulation, and absence of heartbeat. After the exposure, the activity of the enzymes superoxide dismutase (SOD) and catalase (CAT) was evaluated. The data were analyzed using the ANOVA test, and significant differences were considered when p<0.05. The results obtained shoe that the embryo mortality rate remained  $\leq$ 10%, indicating that the compounds were not lethal. In the C/F assay, some embryos exhibited malformations upon exposure to ACMD, at both concentrations. Include sub-lethal alterations. In the assay with Chorion (W/C), embryos exposed to 0.5  $\mu$ M ACMD and 1  $\mu$ M QAMD showed an increase in SOD activity (p=0.0001). In the assay C/F, embryos exposed to ACMD at 0.5 and 1  $\mu$ M exhibited a decrease in SOD activity (p<0.009), while those exposed to QAMD at 0.5 and 1 µM showed an increase (p<0.009). And those exposed to 0.5 and 1  $\mu$ M ACMD showed a decrease in CAT activity (p=0.001). An increase in enzyme activity can indicate positive regulation. While the decrease may be indicative that the enzyme has depleted its function. It has been evidenced that the chorion of zebrafish embryos can influence the exposure to certain compounds. Furthermore, it was observed that the QAMD compound exhibited low toxicity to the embryos.

Acknowledgments to the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

Keywords: Toxicology. Danio rerio. Heterocyclic Compounds.

#### D-36. EXPLORING THE BIOSAFETY OF ULTRASMALL CDSE/CDS QUANTUM DOTS IN Drosophila melanogaster

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CdSe/CdS ultra-small quantum dots (USQDs) are semiconductor nanocrystals composed of a CdSe core and a CdS coating that protects the core and controls its optical and electronic properties. These optical properties allow them to be tailored for specific applications, such as the production of fluorescent biological markers. Despite its many benefits, CdSe toxicity is a concern for its use in biomedical applications. However, the addition of CdS coating can reduce the toxicity and improve the stability of quantum dots, making them a promising option. Thus, in order to investigate the biocompatibility of these USQDs, the in vivo model Drosophila melanogaster was used, a well-established and versatile model organism that, due to its rapid life cycle, allows a rapid assessment of the possible effects of nanomaterials. Our main objective was to investigate the biocompatibility of ultrasmall CdSe/CdS quantum dots in Drosophila melanogaster. We evaluated the effects of CdSe/CdS USPQs on wild type Canton S. The sample, at different concentrations (0.1; 1.0; 10.0; 20.0 and 40.0 micrograms/ml), was mixed in culture of animals, since exposure to the substance occurs orally during the larval stage. Through this methodology, the analyzed parameters were pupation per day, total pupation, larval lethality and enzymatic assays. To investigate mitochondrial redox patterns, we used UAS-mito-roGFP2-Orp1 (H2O2 redox sensor) and UAS-mito-roGFP2-Grx1 (glutathione redox sensor) strains and examined the larval fat body using fluorescence microscopy. Together, the evaluation of these parameters provides us with a better understanding of the effects of exposure of animals to CdSe/CdS. The animals exposed to the analyzed concentrations of CdSe/CdS USPQs did not suffer delay in larval development, in addition to not presenting different larval lethality and total pupation rates compared to the control group. The treatment also did not cause redox imbalance in the fat body of the animals. Enzymatic assays are in progress and should provide us with additional information about cellular mechanisms in vivo. Therefore, our results demonstrate the biocompatibility of CdSe/CdS USPQs at all concentrations analyzed in *Drosophila melanogaster*. Keywords: Toxicity, Quantum dots, Drosophila melanogaster.

# D-37. Effect of Trypsin Inhibitor Isolated from Tamarind Seeds (ITT) and Nanoencapsulated on Weight Change and Satiety

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INTRODUCTION: Obesity is a public health problem that drives a search for new therapeutic agents and long-term treatment strategies. Bioactive proteins with satiety-inducing activity have been the focus of studies due to their action in regulating dietary intake and body weight, such as trypsin inhibitors. OBJECTIVE: Comparing the inhibitory effect of tamarind seeds trypsin inhibitors (TTI) isolated and nanoencapsulated (ECW) in Wistar rats with obesity. MATERIALS AND METHODS: TTI was obtained by Trypsin-Sepharose 4B affinity chromatography, quantified for proteins by the Bradford technique, and its specific activity was monitored by trypsin inhibition assay. The nanoparticles were obtained using the method of nanoprecipitation in absolute ethanol (containing Tween 80) from an aqueous solution containing TTI, purified chitosan, and isolated whey protein (1:2:2 w/w/w). The nanoparticles were characterized by Scanning Electron Microscopy (SEM) and Laser Diffraction (DLS). Wistar rats (n = 25) with obesity induced by a high glycemic index and high glycemic load (HGLI) diet for 17 weeks and were divided into5 groups and evaluated for ten days, namely: Control without treatment (HGLI + water), treatment 1 (nutritionally adequate diet), treatment 2 (nutritionally adequate diet and ECW/12.5 mg/kg), treatment 3 (HGLI diet and ECW/12.5 mg/kg), and treatment 4 (HGLI diet and TTI /25 mg/kg). RESULTS AND DISCUSSION: Obese *Wistar* rats treated with ECW significantly reduced body weight variation (p < 0.05) compared to treatment with TTI. Only TTI treatment significantly changed inflammatory parameters (p < 0.05). The effect of ECW on weight indicates that encapsulation promoted an increase in the impact of TTI with sustained release. CONCLUSION: TTI encapsulation is a promising strategy for this molecule's applicability in treating diseases such as obesity. Acknowledgment: CAPES (001), CNPq (426116/2018-6), UFRN, UnP.

Keywords: Tamarind Trypsin Inhibitor, Nanotechnology, Obesity.

# D-38. Effects of exposure of nile tilapia (*oreochromis niloticus*) to highly toxic inorganic arsenic species

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Introduction: The nile tilapia, Oreochromis niloticus, is a freshwater fish species used as a bioindicator of arsenic contamination through acute toxicity and biochemical tests, in order to assess the quality of an ecosystem and the consequences of its contamination. **Objectives**: The aim was to evaluate the effects of exposure of nile tilapia to different toxic species of arsenic. Materials and Methods: Acute toxicity (LC<sub>50</sub>-96h) and biochemical tests were carried out on gill, brain, liver and muscle tissues: quantification of thiol groups (-SH), glutathione (GSH) and oxidized glutathione (GSSG); catalase (CAT) and superoxide dismutase (SOD) activity, according to pre-established methods in the literature. One-way ANOVA was applied with Dunnett's post-test where  $\alpha$  = 0.05. **Results and Discussion:** As expected, the LC<sub>50</sub> value obtained for As(III), 34.30 mg.L<sup>-1</sup>, was lower than that of As(V), 52.86 mg.L<sup>-1</sup>, demonstrating its prominent toxicity. For the biochemical tests, the percentage (and significant) difference between the exposed and treated groups in the tissues evaluated is shown below. For the quantification of -SH, gills: +182% - As(III) 30 ppm; brain: +257% - As(V) 50 ppm; liver: +150% - As(III) 15 ppm, +134% - As(III) 30 ppm and +151% - As(V) 50 ppm; and muscle: +205% - As(V) 50 ppm. For GSH, gills: -57% - As(III) 30 ppm and -56% - As(V) 50 ppm; brain: -84% - As(III) 30 ppm and -57% - As(V) 50 ppm; liver: +63% - As(V) 50 ppm; and muscle: -53% - As(V) 50 ppm. For GSSG, gills: +98% - As(III) 30 ppm; brain +146% - As(III) 30 ppm; and liver: +118% - As(III) 15 ppm and +215% - As(V) 50 ppm. For CAT, liver: -62% - As(III) 15 ppm, -74% - As(III) 30 ppm and As(V) 50 ppm. For SOD, brain: +14% - As(III) 30 ppm and As(V) 50 ppm; liver: -92% - As(III) 15 ppm and +71% - As(V) 50 ppm; and muscle: +25% - As(V) 50 ppm. The data suggest that there was a cellular dysfunction in the organism of the fish exposed to arsenic. Increased production of reactive oxygen species (ROS) can alter cellular components, including membranes, causing cell death through necrosis or apoptosis. Conclusions: Nile tilapia proved to be sensitive to arsenic, with LC<sub>50</sub>-96h of 34.30 mg.L<sup>-1</sup> As(III) and 52.86 mg.L<sup>-1</sup> As(V). As far as biochemical tests are concerned, it is still necessary to investigate how the fish organism acts to remedy the abnormal ROS variation observed in the tissues.

Acknowledgements: FAPEAL, CNPq, IQB/UFAL, LAQUA, LABIO.

Keywords: Arsenic; Oxidative stress; Oreochromis niloticus.

# D-39. Evaluation of the safety of Hydroxyapatite-based microspheres for bone regeneration

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The methodologies to treat problems related to bone mass loss are still limited, and research to find alternative and highly effective treatments is needed. Research of materials with biocompatible properties capable of regenerating bone tissue are under development. In this context, hydroxyapatite (HA) has been widely studied due to its biocompatible and structural characteristics that resemble the mineral compound present in the inorganic phase of bones. Besides the material's bioactive properties, it is necessary to evaluate its cytotoxic potential, including its genotoxicity, when a clinical application is aimed. In addition, when the objective is to develop a material for bone regeneration, it is also necessary to evaluate its osteogenic potential. Therefore, the objective of this study is to evaluate the cytotoxic, genotoxic and osteogenic potential of microspheres based on nanostructured hydroxyapatite containing different ionic alterations in their structure. The cytotoxicity was assessed by using the 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The genotoxic evaluation was carried out using the cytokinesis-block micronucleus (CBMN) and the bacterial reverse mutation assays. The Alizarin red staining was used to evaluate the osteogenic potential of the HA-based materials, and according to preliminary results, a non-significant increase in mineralization was observed in relation to the negative control; however, this study needs further optimization. The cytotoxicity and genotoxicity results showed that the samples, under the tested conditions, did not cause a reduction in cell viability and also did not cause a significant increase in the occurrence of DNA damage, indicating their safety for biomedical applications.

Acknowledgement: UFRN; PPGBqBM (Graduate Program in Biochemistry and Molecular Biology) CAPES: CNPQ; Instituto REGENERA; LBMG (Laboratory of Molecular Biology and Genomics); LAMA (Laboratory of Environmental Mutagenesis).

Keywords:cytotoxicity. Genotoxicity. Osteogenesis

#### D-40. Chemical Analysis of Polysaccharides in Fermented Food BIONUTRI AR-1®

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According to the National Cancer Institute (NCI), there has been a significant increase in the appearance of cancer cases in Brazil in recent years. One of the reasons severe diseases like this are emerging is mainly malnutrition resulting from poor eating habits. For this reason, consuming "nutraceuticals", which can supply the nutrient deficit and improve the immune system, has become most important. In the academic area, new methods of producing these "nutraceuticals" have been gaining prominence in several countries, for example, fermentation by yeast. Bionutri AR-1<sup>®</sup> is a "nutraceutical", produced by produced by PHARNUTRI P&D Food Industry and Biotechnology LTD, constituted mainly of carbohydrates, proteins, and fiber, among other components, which can improve the immune system of debilitated people. The bioactivity of this product can be directly related to the carbohydrates present in its composition, mainly to glucans that bind to the Dectin-1 receptor. As a result, the chemical analysis of the Bionutri AR-1® was performed to confirm the presence of polysaccharides constituents of that product. First, the product was fractionated according to its solubility in cold water and then analyzed in GC-MS and <sup>13</sup>C NMR. From the fractionations made, it was identified the presence of two main polymers, a linear  $\alpha$ -D-glucan with glycosidic bonds of type  $(1\rightarrow 4)$  and the other branched  $\beta$ -glucan with  $(1\rightarrow 4)$  and  $(1\rightarrow 6)$  linkages. Although the methods of colorimetric are essential as far as analysis techniques are concerned, they are not as specific as the analyses performed by GC-MS and <sup>13</sup>C NMR, since, through them, it is possible to perform the fine structural characterization of polymers.

*Acknowledgments*: To the PHARNUTRI P&D Food Industry and Biotechnology Company for the opportunity and support in developing this research.

*Keywords*: Nutraceuticals, Bionutri AR-1<sup>®</sup>, Structural characterization.

#### D-41. TERATOGENIC SCREENING IN ZEBRAFISH (*Danio rerio*) OF THE CRUDE EXTRACT RICH IN CAROTENOIDS OF CANTALOUPE MELON (*Cucumis melo* L.) AND NANONOENCAPSULATED IN GELATIN

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Cantaloupe melon (Cucumis melo L.) is a fruit rich in carotenoids, a bioactive compound responsible for the orange color of the pulp and which confers antioxidant properties. However, carotenoids are extremely unstable in the presence of oxygen, light, and heat, which can impact their bioactive properties. Nanoencapsulation is a strategy to promote increased water solubility, stability, bioaccessibility, and bioavailability preserving and/or enhancing bioactive effects. However, one of the major current concerns is related to the toxicity effects of these nanoparticles. Thus, this study aimed to perform teratogenic screening of the crude extract rich in carotenoids from Cantaloupe melon (CE) and its nanoparticles (EPG) in an animal model of zebrafish (Danio rerio). The CE was obtained from the pulp of the Cantaloupe melon (*Cucumis Melo* L.) through processes that involved drying, maceration, and partitioning. The nanoparticles were obtained through oil-in-water (O/W) emulsification, using porcine gelatin as an encapsulating agent and Tween 20 as a surfactant. The nanoparticles were evaluated for morphology, chemical interactions, particle size, and encapsulation efficiency by Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Laser Diffraction, and Incorporation Efficiency (IE), respectively. In animal model experiments, the embryos were exposed to 12.5 mg/L and 50 mg/L of CE and EPG for the teratogenicity test. The nanoparticles had a spherical shape, smooth surface, without depressions, with a diameter of 88.7 nm (7.02) and a polydispersion index of 0.41 (0.03). The FTIR showed shifts and the appearance of new bands in EPG, indicating the presence of chemical interactions between the materials in the system. Furthermore, the IE of the extract rich in carotenoids in EPG was 94% (4.04). In experiments in an animal model, after exposure for 96 hours post fertilization (hpf), cardiotoxicity was evaluated where no anomalies were observed in the groups treated with CE and EPG, and the heartbeats remained between 132 and 138 rpm within the expected range for embryos, similar to the negative control and DMSO groups. Groups CE and EPG did not show significant morphological alterations. The level of mortality was below 20%, revealing no teratogenic character of CE and EPG in the concentrations used. Both CE and EPG are safe for future in vivo analysis.

Thanks to Capes, UFRN, Biochemistry and Molecular Biology Postgraduate Program, and the Psychobiology Postgraduate Program.

Keywords:  $\beta$ -carotene, Embryotoxicity, Nanoencapsulation.

#### D-42. Acute Oral Toxicity of Cordia africana Mucilage on Swiss Albino Mice

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Introduction: Soluble fibers are functional ingredients beneficial to human health, in general they can be selectively fermented by probiotic bacteria in the colon, such as Lactobacillus, thus exerting prebiotic effects to maintain the gut microbiota. The fruit of *Cordia africana* has a mucilage rich in soluble fibers, which can be excellent candidates as natural prebiotics. However, before exploring the biological activity of a new drug or natural product, toxicological studies are essential to establish the safety and efficiency of it, this helps to make a decision whether a new drug should be adopted for clinical use or not. The acute toxicity test is the first test to be performed as it provides information about a single dose of drug given in large quantities to determine immediate toxic effect. **Objective:** The present study was carried out to evaluate the safety of novel biomaterial mucilage obtained from the C. africana in Swiss albino mice. Materials and methods: After manually separating the husk and seed, the mucilage was subjected to hot aqueous extraction followed by ethanol precipitation and finally spraying. The acute toxicity analysis followed the 423 guidelines for the Organisation for Economic Co-operation and Development (OECD). The Swiss mice females (n=3/group) were orally treated with *C. africana* mucilage (2000 mg/kg) and control group (10 mL/kg of distilled water). In-vivo acute toxicity (CEUA 0034/2021) effect was assessed by monitoring the survival, behavioral changes, body weight, water and food consumption, effect on hematological and biochemical parameters, weighing and histological analysis of the vital organs. Results and Discussion: After administration of the mucilage in a single dose (2000 mg/kg), the animals didn't show signs of toxicity during the 14 days of the experiment. The standard behavior of all animals were normal, with no signs of sensory or motor alteration. No significant variation was observed in the body and organ weights between the control and the treated group. Furthermore, no renal, hepatic and blood dysfunctions were noted in treated animals compared to control. The organs analyzed didn't show histological changes and didn't observe signs of toxicity between tissues. Conclusion: According to OECD 423, mucilage from *C. africana* can be considered as a substance of low acute toxicity ( $LD_{50} > 2,000$ mg/kg), since no anomalies were observed in the general health status of the treated animals. Proven to be a safe natural polymer for use in pharmaceuticals.

Acknowledgements: UFPE and CAPES.

Keywords: Acute toxicity, Cordia africana, Mucilage.

# D-43. Impedimetric genosensor based on probe immobilization on Poly-L-lysine film through glutaraldehyde for *Mycobacterium tuberculosis detection*

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Introduction: Diagnosis of Mycobacterium tuberculosis is essential considering the high incidence of the disease. 10.6 million cases and more than 1 million deaths in 2021. The tuberculosis epidemic occurs mainly in developing countries, whereas part of the population lives in places seldom reached by medical care. Real-time polymerase chain reaction (PCR) and M. tuberculosis culture are used as diagnostic methods, but they are time-consuming and require well-trained professionals. Objectives: Thus, there is a need for a rapid and straightforward way to identify cases of tuberculosis. Methods: After researching DNA sequences of M. tuberculosis through GenBank-NCBI and analyzing characteristics such as stability and specificity through bioinformatics, only one DNA sequence was chosen as a bioreceptor, the Rv2341 gene. A poly-lysine film was made through cyclic voltammetry using a screen-printed electrode system, with work and auxiliary electrodes made of carbon paste and a reference electrode of Ag/AgCl paste. This film makes (-NH2) groups available on the electrode surface. Glutaraldehyde was used as a crosslinker due to the presence of one aldehyde (-COH) in each extremity. The glutaraldehyde activates the surface by binding an extremity on the amine group to poly-L-lysine and leaves an aldehyde group free to immobilize a modified DNA probe with a -- NH2 terminal. This work used electrochemical impedance spectroscopy to characterize the biosensor. Ferricyanide ([Fe(CN)6]<sup>-3</sup>) and ferrocyanide ([Fe(CN)6]<sup>-4</sup>) were chosen as redox mediators, and through the Nyquist diagram we calculated charge transference resistance (Rct) in each stage of the biosensor. Results: Non-modified electrodes showed high resistance due to carbon paste (29.5kΩ); after polymerization of poly-I-lysine and the addition of glutaraldehyde, the surface becomes more electroactive, reducing the Rct to (4.0k $\Omega$ ) and (3.9k $\Omega$ ) respectively. Due to the negative charge introduced by the oligonucleotides immobilized, resistance increased significantly (10.9k $\Omega$ ), however, when a solution without oligonucleotides was placed on the electrode, creating a false immobilization, the Rct did not increase and remained similar to the previous steps (3.9 $k\Omega$ ). **Conclusion:** This biosensor is the first step to facilitate diagnosis of tuberculosis in vulnerable areas, due to its portability and specificity of DNA probes. We efficiently characterized an immobilized DNA sequence on the surface of a transducer, and were able to differentiate each step of creating a biosensor for Mycobacterium tuberculosis. Acknowledgments: We are thankful to all the members of the Biosensors group, the BRICS group and CNPq for the financial support (CNPg Process Number 440117/2020-8), also to iLIKA and UFPE for the necessary resources to make this work possible. Keywords: Biosensors; DNA-probe; tuberculosis.

#### D-44. DEVELOPMENT OF LIQUID FORMULATION CONTAINING Punica granatum LECTIN AND EVALUATION OF ANTIBACTERIAL ACTIVITY AGAINST Streptococcus mutans

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Dental caries is one of the most prevalent chronic diseases around the world, affecting people of all ages, especially children and pre-adolescents. Caries is the primary cause of oral pain and premature extraction of primary teeth. A diet with a high sugar intake is often related to the development of dental caries, since organic acids resulting from the metabolization of the nutrient by oral microorganisms contribute to the formation of the dental biofilm matrix. Streptococcus mutans is a bacteria that colonizes the tooth surface and causes caries in the presence of fermentable carbohydrates. Lectins are hemagglutinating proteins that can be used in formulations for therapeutic use due to their biological properties. Punica granatum sarcotesta lectin (PgTeL) was isolated at the Protein Biochemistry Laboratory of UFPE and showed antimicrobial activity. The objective of this work was to develop a liquid formulation, like mouthwash, containing protein fraction of pomegranate sarcotesta rich in PgTeL and to determine its efficiency against S. mutans. The production of the protein fraction is simpler and faster than the PgTeL isolation procedure. The protein fraction was obtained from the saline extract of pomegranate sarcotesta using ammonium sulfate at 30% saturation (w/v). Liquid formulations containing the protein fraction or not (placebo formulation) were produced and tested against two generations (4th and 5th) of S. mutans, using the microdilution method in a microplate containing 96 wells. The methodology for producing the mouthwash is in the patent process. The placebo formulation did not show antibacterial activity, while the formulation containing the protein fraction inhibited the growth of S. mutans 4th generation at a minimum inhibitory concentration (MIC) of 56 µg/mL and 5th generation at a MIC of 112  $\mu$ g/mL. Bactericidal activity against the two generations of S. mutans was not detected in the tested formulations. The MIC values determined are in agreement with those presented by preparations containing partially purified lectins. The study showed that the liquid formulation containing the protein fraction of *P. granatum* rich in PgTeL was effective in inhibiting the growth of S. mutans, and therefore has potential for therapeutic use against dental caries.

Keywords: Mouthwash. Plant. Protein.

Acknowledgment: CAPES, CNPq and FACEPE.

#### D-45. Current models for preclinical and clinical drug toxicity screening

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**INTRODUCTION:** Despite the many drugs available on the market, new specific and efficient therapies with the lowest possible toxicity to the human body are required. In initial stages, screening for such agents is done through several in vitro assays, from which a faithful representation of what should occur in vivo is optimistically expected. However, many fully synthetic or partially biologically derived drugs, previously effective in pilot-scale in vitro trials, fail in human in vivo clinical tests at the final stage of screening. Some are launched commercially with warnings of toxicity to certain organs ("black box"), others are removed from the market after several reports of harmful effects, even when initially approved based on strong positive evidence from in vitro tests based on cells and subsequent in vivo preclinical studies. **OBJECTIVE:** To present a review of the scientific literature summarizing the various approaches of *in vitro* and *in vivo* models currently used for screening the action and toxicity of drugs, aiming to analyze their advantages and disadvantages and reduce the boundaries between preclinical and clinical protocols for their effective release. MATERIAL AND **METHODS:** A qualitative/quantitative review of research articles in the Scopus, PubMed and Scielo databases of the scientific literature was carried out, using the descriptors "cell-based models AND toxicity", "clinical and preclinical AND toxicity assays"; "drug toxicity models"; "criteria for drug approval"; "physiological models AND toxicity", considering as inclusion criteria only articles published in English in the last ten years. Thus, in addition to listing and describing such assays and technologies, their advantages in combination with final in vivo clinical studies were raised. RESULTS AND DISCUSSION: Of the 238 articles found, 9 were reviews in which 59% of the selected articles were already cited, being discharged. Most of the 135 research papers emphasize the importance of efficiently combining lower risk to the potential target (using different lineages from pluripotent stem cells, 3D tissue models, advanced microphysiological systems and imaging techniques), in a mechanistically translatable way, with other characteristics specific to the drug studied (physical-chemical properties in addition to absorption, distribution, metabolism and excretion). CONCLUSION: Currently, the preclinical testing protocols of new in vitro drugs strongly depend on the development of physiologically relevant models for humans, with appropriate safety toxicological parameters. The use of organs-on-chips, genomic profiling and high-content imaging technologies are bets that promise to surpass metabolomics, microRNAs and mass spectrometry imaging, quantitative PCR and RNA sequencing.

**KEYWORDS:** cell-based models; clinical and preclinical assays; drug action and toxicity models; drug approval; physiological in vitro models.

# D-46. Therapeutic Effect of Different Chondroitin Sulfates in an Animal Model of Osteoarthritis

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Osteoarthritis (OA) is a disease with no clear and effective treatment that affects more than 237 million people worldwide. Due to the ineffectiveness of current treatments, new and more effective therapeutic alternatives with fewer side effects have been necessary. Chondroitin sulfate (CS) is a sulfated polysaccharide (PS) widely recommended for the treatment of patients with knee OA, although its therapeutic efficacy is not yet well established. Fucosylated chondroitin sulfate (FCS) is a PS extracted from marine organisms that has a structure like the CS of mammals, and due to its excellent anti-inflammatory properties, is a promising candidate for the treatment of OA. Due to the therapeutic potential of these PSs, this work aimed to evaluate the therapeutic effect of CS from bovine trachea (CS-A), shark cartilage (CS-C), and FCS from the body wall of the sea cucumber Holothuria grisea in an in vivo model of OA induced by monosodium iodoacetate in the patellofemoral joint of C57BL/6 mice. After OA induction, mice were randomly divided into groups (n = 5) according to daily oral treatment for 28 days: (1) Sham (sham-operated, saline 0.9% v.o.); (2) negative control (illness, 0.9% saline v.o); (3) CS-A (illness, 100 mg/kg v.o); (4) CS-C (illness, 100 mg/kg v.o); and (5) FCS (illness, 100 mg/kg v.o). The effectiveness of the treatments was evaluated by measuring allodynia (50% of the nociception threshold), motor activity, leukocyte migration, anti-inflammatory activity (IL-10 dosage), morphometric analysis, and histopathological OARSI Score. During 28 days of experimentation, CS-A (100 mg/kg), CS-C (100 mg/kg), and FCS (100 mg/kg) could induce analgesia, increase motor activity, and reduce leukocyte migration in osteoarthritic mice significantly compared to the negative control (saline 0.9%). In addition, morphometry revealed a significant increase in cartilage over the tibia for the animals treated with CS-A and FCS, the results of which were confirmed by OARSI histopathological analysis, showing a reduction in OA severity compared to the negative control. However, only CS-A (100 mg/kg) significantly increased IL-10 levels, suggesting that this molecule has a protective activity against cartilage degeneration by regulating inflammation. Despite being preliminary, the mechanisms involved in FCS treatment need to be further investigated, since its protective effect on OA was similar to that presented for CS-Α.

Acknowledgments: CNPq, Laboratory of Biotechnology and Applied Ethnopharmacology. Keyworsd: fucosylated chondroitin sulfate; inflammation; osteoarthritis.

# D-47. Antibacterial Activity of *Moringa oleifera* Seed Lectin (WSMoL) against *Listeria monocytogenes*

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INTRODUCTION: Moringa oleifera is a plant native to Asia that has several biological properties, such as antimicrobial activity. The *M. oleifera* seeds contain a water-soluble lectin called WSMoL. Lectins are proteins that bind specifically to carbohydrates and have several biological activities, including antibacterial effect. Listeria monocytogenes is a gram-positive bacterium that causes listeriosis in humans, whose main symptoms are fever, muscle aches and gastrointestinal problems. **OBJECTIVE**: This work evaluated the effects of WSMoL on the growth, viability and aggregation capacity of clinical isolates of L. monocytogenes. MATERIALS AND METHODS: WSMoL was obtained as described by Coelho et al. (Chemosphere 77:934-938, 2009) through protein extraction in distilled water (16 h, 28 °C, under agitation) followed by protein precipitation with ammonium sulphate (60% saturation), and chromatography on chitin column. Antibacterial activity was evaluated using the broth microdilution assay to determine the minimum inhibitory (MIC) and minimal bactericidal (MBC) concentrations against the L. monocytogenes strains N53-1 and EDG-e. Growth curves were established in the absence and presence of WSMoL from  $\frac{1}{4}$  MIC to 4 × MIC. The effect of WSMoL on the viability and cell aggregation were determined as well as possible synergistic activity with routinely used antibiotics (by determining the sum of fractional inhibitory concentrations,  $\Sigma$ FIC). RESULTS: WSMoL presented MIC of 250 and 500 µg/mL for N53-1 and EGD-e, respectively. No bactericidal effect was detected. Growth kinetics demonstrated that the control showed exponential growth over 24 hours; bacteria treated with WSMoL at the ¼ MIC, ½ MIC and MIC maintained constant growth. On the other hand, in treatments with 2 x MIC and 4 x MIC, growth was inhibited occurring only after 14 hours. WSMoL showed anti-aggregation effect when tested from ¼ MIC until 4 × MIC. The lectin showed synergistic effect with ciprofloxacin against both clinical isolates ( $\Sigma$ FIC of 0.16 and 0.34 for N53-1 and EGD-e, respectively). **CONCLUSION**: WSMoL was bacteriostatic agent against *L. monocytogenes*, showing potential to be used alone or in combination with antibiotic to treat infections caused by this bacterium. Acknowledgments: FACEPE, CNPq and CAPES.

Keywords: Listeriosis; synergistic action; bacteriostatic effect.

# D-48. Snake Venom-Derived Peptide (pBthTX-I)<sub>2</sub>K, an inhibitor of SARS-CoV-2-Papain-Like Protease Displays Potent Antithrombotic Action

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Background: (pBthTX-I)<sub>2</sub>K, a dimeric peptide derived from the C-terminal region of BthTX-Ilike phospholipase A2 is resistant to plasma proteolysis and exhibits inhibitory activities against SARS-CoV-2 strains with weak cytotoxic effects<sup>(1)</sup>. Vascular problems are among complications of SARS-CoV infection, with evidence of an increased risk of thrombosis. Full anticoagulation for all patients is questionable and has not been recommended by the international community so far, therefore, studies targeting the identification of new drugs that also inhibit thrombosis aimed at minimizing the risk of bleeding are relevant. **Objectives:** To study if the peptide (pBthTX-I)<sub>2</sub>K has also an action on the hemostatic system we investigated the coagulation parameters, platelet function, and its action on an experimental model of arterial thrombosis. Bleeding time was determined in mouse tails immersed in saline at 37 °C after (p-BthTX-I)<sub>2</sub> K (4.0 mg/kg and 8.0 mg/kg) or saline administration. Methods: Human blood was collected in 3.8% sodium citrate (w/v) 1:10, centrifuged at 141 xg/12 min (PRP, platelet-rich plasma) and 350 xg/15 min (PPP, plateletpoor plasma). Platelet aggregation induced by collagen, arachidonic acid, and ADP was performed in a Chronolog Lumi-aggregometer. The extension of coagulation was evaluated by partially activated thromboplastin time (aPTT) and prothrombin time (PT) with (pBthTX-I)<sub>2</sub>K, 37.5-1000 µg) or 0.9% NaCl. Arterial thrombosis was induced by Rose Bengal salt degradation with a 540 nm laser in the carotid artery of male C57BL/6J mice using (pBthTX-I)<sub>2</sub>K, from 3.5 mg.kg<sup>-1</sup> up to 20 mg.kg<sup>-1</sup>. Bleeding time was determined in mice tail immersed in saline at 37 °C after (pBthTX-I)<sub>2</sub>K),4.0 and 8.0 mg.kg<sup>-1</sup>) or saline administration. **Results:** (pBthTX-I)<sub>2</sub>K) enhanced the aPTT and PT by blocking kallikrein and Factor Xa-like activities. It also inhibited ADP-, collagen-, and arachidonic acid-induced platelet aggregation in a dose-dependent manner. Additionally, the peptide at concentrations ranging from 3.5 mg.kg<sup>-1</sup> up to 20 mg.kg<sup>-1</sup> extended the time to artery occlusion. Besides all these effects, it did not prolong bleeding time in mice. **Conclusion**: These results demonstrate the antithrombotic activity of the peptide (pBthTX-I)2K) possibly by kallikrein inhibition, suggesting its strong biotechnological potential.FAPESP (2017/06630-7, 2020/05761-3), CNPq (302658/2021-1, 304739/2021-9) and CAPES (Finance Code: 001) [1] Freire et al., Non-Toxic Dimeric Peptides Derived from the Bothropstoxin-I Are Potent SARS-CoV-2 and Papain-like Protease Inhibitors. Molecules. 2021 Aug 12;26(16):4896. doi: 10.3390/molecules26164896

Agradecimentos: Gostaria de agradecer a todos os participantes desse projeto pelo apoio direto e indireto em todas as suas fases.

Palavras-chaves: Thrombosis, Platelets and Bleeding

#### E - Glicobiologia

#### E-01. Evaluation of the Neuroprotective Effect of Raw Extracts and Polysaccharide Fractions of Macroalgae from the Coast of Rio Grande Do Norte (RN/Brazil)

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As a result of the greater life expectancy of the population, neurodegenerative diseases (NDDs), such as Alzheimer's and Parkinson's, have affected thousands of people around the world, with estimates of a 42% increase in dementia conditions in the global population by 2030 There are several causes that lead to neurodegeneration and among the main mechanisms involved we can mention oxidative stress, inflammation, apoptosis, excitotoxicity and autophagic dysfunction. Despite the great impact of NDDs, current treatments only attenuate the symptoms, failing to prevent or significantly reduce the progression of the disease, in addition to promoting some type of side effect. Thus, the search for new neuroprotective agents has intensified. Within this context, sulfated polysaccharides from macroalgae emerge as possible alternatives, since data in the literature have attested to their antioxidant, anti-inflammatory and neuroprotective activity, whether in vitro or in vivo assays. In addition, it is worth mentioning that algae can produce much more biomass than terrestrial plants and can be cultivated without the use of antibiotics or pesticides, which has led to a growing increase in consumer demand and, consequently, in the economic interest in the last two decades, with emphasis on meeting several sustainable development goals (SDGs) along this productive/economic chain. Thus, aimed availate the neuroprotective potentials of sulfated polysaccharides from crude extracts and fractions of 9 macroalgae in Neuro-2A cells, thus paving the way for the development of safe and economically viable therapeutic alternatives, that can be used in the treatment of DNDs. Cell viability was assessed using MTT assay for all samples, in 4 different concentrations (0.1, 0.25, 0.5 mg/mL and 1 mg/mL). It was observed that almost samples did not produce cytotoxicity effect. The neuroprotection assessment was performed with oxidative damage induced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in a concomitant treatment, for a period of 6 hours. The highest and lowest concentrations that did not cause cytotoxicity were used. In general, it was observed that brown algae showed a greater neuroprotective effect on Neuro-2A cells. From this initial evaluation, it will be possible to carry out future studies that better describe the mechanisms involved, as well as to carry out in vivo tests.

Acknowledgment: CAPES, CNPq e UFRN

Keywords: Neuroprotection. Seaweeds. Sulfated polysaccharides.

# E-02. Development of a blend containing fucoidan and modified dextran using the Factorial Planning tool to enhance antioxidant potential.

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In recent years, the role of oxidative stress in health and physiological homeostasis has garnered interest in scientific research. Mainly because this physiological process is implicated in several diseases, including neurodegenerative ones. As a result, antioxidants, particularly exogenous varieties, have been gaining more attention as potential strategies against oxidative imbalances. Bioactive compounds, such as polysaccharides and their derivatives, are recognized for their antioxidant properties. Within this context, mention can be made of a sulfated fucoidan called Fucan A (FucA) and dextran (Dex). Besides, chemical modifications are consistently being performed on polysaccharides to enhance their pharmacological potential. Building upon this, the purpose of this study was to assess strategies to enhance the in vitro antioxidant activity of FucA and dextran polysaccharides through chemical modification with gallic acid (GA) and the formulation of blends. To achieve this objective, Dex was conjugated with AG (Dex-Gal). Chemical and FT-IR analyses confirmed that Dex-Gal comprises 3% gallic acid in its composition and exhibits an activity 3.2 times greater than Dex in the Total Antioxidant Capacity (TAC) test. Subsequently, Dex-Gal and FucA were employed, guided by factorial planning, to create five blends (BLD1, BLD2, BLD3, BLD4, BLD5). Two blends exhibited low activities in all conducted tests: BLD1 and BLD5. In the CAT test, the three blends that stood out were BLD4 (22 Eq AA), BLD3 (17 Eq AA), and BLD2 (13 Eq AA). In the reducing power test, BLD4 (28% reduction) displayed activity almost twice as high as BLD2 (17%) and BLD3 (15%). Notably, BLD4 also excelled in the hydroxyl radical scavenging test, displaying activity around 100%, while the activity of BLD3 and BLD2 did not exceed 25%. Surface graphs align with the quantitative data, underscoring BLD4 as the optimal blend combination. Based on the results, it can be concluded that the blend formed with Dex:FucA (1:1), referred to as BLD4, is the combination that best enhances the antioxidant activity of its constituent compounds. In the future, the intention is to analyze its in vivo activity. Keywords: sulfated polysaccharides, fucan, polysaccharides, brown seaweed.

Acknowledgements: Capes, CNPq and UFRN.

# E-03. Chemical prospection of the polysaccharides from the wild mushroom of the *Agaricus* genus

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Mushrooms have been used in food, mainly in Eastern countries, for centuries due to their nutritional value and medicinal properties <sup>1</sup>. Among these, the genus Aqaricus is one of the most popularly known, with some species being widely commercialized, such as A. bisporus ("champignon") and A. subrufescens ("mushroom of the sun"). Although approximately 500 species of this genus are known, only about 100 have been studied. Given the diversity of species of this genus that are found in Brazil and that are unexplored as to their potential as a source of bioactive molecules, this research had as its primary objective the chemical study of the polysaccharides of a species of Agaricus that was collected in the city of Tamarana-PR, in plantations of Araucaria angustifolia. To obtain the polysaccharides, the basidiocarps of Agaricus sp. (55.07g) were submitted to aqueous extractions at ~10 and 96°C, successively, for 6 h (3x, each). After each step, the extracts obtained were separated from the residual material by filtration and/or centrifugation, concentrated under reduced pressure, and lyophilized, giving rise to the Ag-AF and Ag-AQ fractions. Then, the aqueous extracts obtained were solubilized in distilled water and precipitated with absolute ethanol in the ratio of 1:3 (v/v; extract: ethanol), which were recovered by centrifugation, dialyzed, and lyophilized. To determine the monosaccharide composition of the extracted polymers, an aliquot of the Ag-PEAF fraction was converted into alditols acetates and analyzed in GC-MS, which were shown to be formed by fucose (21.7%), xylose (4.7%), mannose (9.5%) and galactose (64.3%). Aiming at the fractionation, this extract was subjected to freezing and thawing, separating it into soluble (Ag-SAF) and insoluble fractions in cold water (Ag-IAF), which were shown to be distinct in terms of monosaccharide composition, with Ag-SAF formed mainly by fucose and galactose. In contrast, Ag-IAF contains mainly mannose, galactose, and fucose. Because of heterogalactans in the fractions soluble in cold water, Ag-SAF was precipitated with copper sulfate solution in an alkaline medium for purification. The cupric precipitate formed was separated by centrifugation and submitted to the decomplexation process, resulting in the Ag-PFAF fraction. Additional analyses of homogeneity, monosaccharide composition, <sup>13</sup>C, and <sup>1</sup>H nuclear magnetic resonance are being performed to confirm the structure of this heteropolymer present in the Ag-PFAF fraction. After determining the chemical structure, it will be evaluated, first, against the immunomodulatory and/or antioxidant activities to verify if this wild species can be considered medicinal.

Keywords: Mushroom, *Agaricus* sp., polysaccharides, structural characterization Acknowledgments: This work was carried out with the help of FAPEG and UFCAT.

### E-04. Chemical elucidation of an unusual fucoxylomannan from the medicinal mushroom *Pleurotus eryngii*

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Pleurotus eryngii, popularly known as "king oyster mushroom", "king trumpet", or "cardoncello", is one of the mushrooms cultivated and marketed worldwide, both for gastronomic and medicinal purposes. Despite the numerous studies conducted with P. eryngii, it still needs to be explored because there are few reports about the detailed chemical structure of its polysaccharides. In addition, most reports on the structure of these biomolecules refer to polymers containing high levels of glucose or galactose, which belong, respectively, to the class of glucans and heterogalactans; that is, only 2 of these reports mentioned polymers containing xylose, which suggest the presence of another class of carbohydrates of macrofungi, called heteromannan, little studied, possibly due to the difficulty of obtaining and structural complexity. Given this context, the present research focused mainly on the study of fractions with high levels of xylose and mannose and the alkaline extract obtained by extraction of basidiocarps with 5% KOH (Pe-K5 fraction) chosen to follow the purification and structural characterization steps. From this polysaccharide extract, a heteromannan consisting only of fucose (2.4%), mannose (36.7%), and xylose (60.9%) was purified, which is called fucoxylomamannan. The structural elucidation of this heteropolysaccharide was performed by chemical derivatization methods such as methylation and analytical techniques of <sup>13</sup>C and <sup>1</sup>H NMR, HPLC-RI, and GC-MS. The results obtained suggested the presence of a highly branched structure due to the high levels of non-reducing terminals of xylose (2,3,4-Me<sub>3</sub>Xyl, 22.8%), fucose (2,3,4-Me<sub>3</sub>Fuc, 3.5%) and mannose (2,3,4,6-Me<sub>4</sub>Man, 2.7%), which are proportional to the percentages of Manp 3,4-di-O-substituted (2,6-Me<sub>2</sub>Man, 25.6%) of the main chain. Units of Xylp 2-O- (3,4-Me<sub>2</sub>Xyl, 29.9%) and 3-O-substituted (2,4-Me<sub>2</sub>Xyl, 4.0%) corresponding to the side chains were also observed. These data, together with those obtained by the <sup>13</sup>C and <sup>1</sup>H NMR analyses, confirmed the structure of the purified heteropolysaccharide, which has a main chain of  $\alpha$ -Manp-(1 $\rightarrow$ 3), partially replaced in O-4 by non-reducing terminals of  $\beta$ -Xylp or side chains consisting of units of  $\beta$ -Xylp (1 $\rightarrow$ 2)-linked, mainly. Additional analyses of NMR (HSQC-NOESY and/or ROESY) and molar mass determination are underway to establish the sequence of their monosaccharide units, as well as to prove the suggested structure, which has not yet been described for macrofungi. In addition to structural elucidation, heteromannan is being evaluated, first, against immunomodulation and/or antitumor assays to verify whether this class of molecules may be responsible for part of the therapeutic effects attributed to macrofungi. Keywords: Pleurotus eryngii, Heteromannan, Chemical elucidation.

Acknowledgments: YUKI Cogumelos and UFCAT.

# E-05. Toxicological evaluation and gastroprotective activity of the polysaccharide extracted from *Cenostigma nordestinum* gum in the model of gastric ulcer induced by acidified ethanol

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**Introduction:** Gastric ulcer (GU) is a pathology characterized by injury to the gastric mucosa, which can be triggered by the imbalance between protective factors and aggressors of gastric mucoses. In this context, the use of compounds of natural origin, such as polysaccharides, is one of the alternative therapeutic strategies. Objectives: To evaluate the acute toxicity and the gastroprotective effect of the polysaccharide extracted from *Cenostigma nordestinum* gum (PGCn). Materials and methods: PGCn was extracted by ethanolic precipitation (70%) and its yield, total carbohydrate, uronic acid, protein and phenolic compounds contents were quantified. In the acute toxicity test (CEUA: 0065/2020), female Swiss mice (n=3/group) were treated by gavage with a dose of 2000mg/Kg after 2 hours of fasting. The parameters of body mass, water intake and food consumption were evaluated daily for 14 days. The mass of the animals is organs was also evaluated and hematological and biochemical analyzes were performed. Gastric ulcer induced by Ethanol/HCl was performed after 18 hours of fasting, Swiss mice (n=6/group) were orally pre-treated with PGCn (75; 150 and 300 mg/kg), saline vehicle 0.9 % (negative control; 10 mL/kg) and ranitidine (positive control; 80 mg/kg). All organs of the animals were analyzed histologically. Results: The ethanolic extraction of PGCn provided a yield of 38.4±4.67% relative to the initial dry mass of the gum. Regarding the chemical composition, PGCn exhibited a concentration of 93.61±0.63% total carbohydrates, including 19.0±0.00% uronic acid, low content of proteins (1.70±0.28%) and phenolic compounds (9.16±0.02 mg/EGA). Preliminary assessment of ACn during the acute toxicity test revealed that all animals survived throughout the 14-day experimental period, without presenting motor alterations after the administration of a single dose of PGCn (2000 mg/kg). Furthermore, no significant changes (p > 0.05) were observed between the physiological parameters of the control and PGCn groups, such as water intake, food consumption and body mass of the animals. No changes were observed between hematological and biochemical parameters either. In the ethanol/HCI-induced gastric ulcer model, oral pretreatment with PGCn at doses of 75 mg/kg, 150 mg/kg and 300 mg/kg represented ulcerative inhibition of 65.97±3.11%; 83.07±5.43% and 86.06±3.95%, respectively, while the positive control, ranitidine (80 mg/kg), conferred gastroprotection of 88.85±1.48%. Furthermore, no histopathological alterations were observed in the animals' organs between the experimental groups. **Conclusion:** PGCn is a alternative to be used in gastric ulcer prevention therapy besides not showing toxicity.

Keywords: Polysaccharide; Acute toxicity; Gastric ulcer.

**Acknowledgments:** Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

#### E-06. FUCOIDAN FROM THE SEAWEED *Spatoglossum schröederi*: CHEMICAL CHARACTERIZATION AND POTENTIALIZATION OF ITS ANTIOXIDANT ACTION BY CONJUGATION WITH GALLIC ACID

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During cellular metabolism, important redox processes occur, which are essential for the functioning of living cells. This process can lead to the formation of reactive oxygen species (ROS). These ROS play crucial roles in various cellular processes. However, when there is an imbalance in the production of ROS and/or the action of antioxidant agents, oxidative stress (OxSt) occurs, which, when chronic, contributes to the onset or worsening of diseases such as cancer, and cardiovascular diseases. To reduce complications associated with OxSt, the search for antioxidant molecules has grown. One of these molecules is sulfated polysaccharides (PS) found in seaweeds, such as fucoidans. Within this context, to enhance their antioxidant power, PS has been conjugated with different chemical groups (methyl, acetyl, etc.) or other molecules, such as gallic acid (GA), which is already known for its protective antioxidant properties. During this process, GA transfers its antioxidant properties to PS, and, in turn, PS improves the bioavailability of GA. The objective of this work was to obtain a fucoidan (FucB) from the brown alga Spatoglossum schröederi and produce its conjugated derivative with GA (FucB-GA). Conjugation with GA was confirmed through infrared spectroscopy analysis. The sugar and sulfate content of FucB-GA were similar to those of FucB, as well as its electrophoretic profile. Six experiments were conducted to evaluate the in vitro antioxidant activity of the samples. The samples did not show iron chelating activity under the tested conditions. Additionally, no significant differences were found between FucB and FucB-GA regarding their ability to scavenge superoxide ions (~ 71%) and hydrogen peroxide (~38%), suggesting that the antioxidant activity might be associated with the sulfate groups present in fucoidans rather than the presence of GA. Regarding copper chelation, FucB-GA exhibited significantly higher activity (41.4%) than FucB (32.1%). In the total antioxidant capacity evaluation test, FucB-GA showed an activity 5 times higher than FucB. Moreover, the samples exhibited a reducing activity above 50%, with FucB-GA standing out with a 36% higher activity than FucB. It is likely that the increased antioxidant capacity in these latter tests for FucB-GA is related to the presence of GA in the structure, which is providing greater activity to the molecule. In short, the proposed modification of FucB with gallic acid enhanced its antioxidant potential, which opens new perspectives for the use of this PS in the development of biomaterials for the prevention and treatment of diseases related to OxSt. Keywords: oxidative stress, brown seaweed, fucans, sulfated polysaccharides Acknowledgments: The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Universidade Federal do Rio Grande do Norte for the

financial support.

### E-07. *IN VITRO* AND *IN VIVO* EVALUATION OF XYLAN MODIFIED WITH GALLIC ACID AS ANTIOXIDANT AGENT

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The cellular antioxidant system comprises a series of chemical reactions aimed at maintaining equilibrium between reactive species and antioxidant agents. When this balance is disrupted in favor of reactive species, it leads to cellular oxidative stress. This oxidative stress is associated with the onset of cellular damage, contributing to various degenerative processes such as Parkinson's disease, lung injuries, cardiovascular diseases, and diabetes. Although the endogenous protective system is sometimes insufficient, it necessitates the utilization of exogenous antioxidant agents. Among these, polysaccharides like xylan have been extensively investigated for their antioxidant capabilities. Xylan, a polysaccharide primarily extracted from corn cobs, is recognized for its antioxidant, cytotoxic, anticoagulant, and immunomodulatory activities. Gallic acid, a natural phenolic compound found in various plants, exhibits antiinflammatory, lipid peroxidation inhibition, anticancer, and antioxidant properties with low toxicity and ease of extraction. Therefore, the purpose of this study was to assess the antioxidant potential of gallic acid-conjugated xylan (Xyl-GA) to acquire novel pharmacological attributes and enhance established activities. Initially, a physicochemical characterization was conducted, quantifying sugars, phenolic compounds, and proteins. Xylan presented 62% sugar and 0.9% phenolic compounds, while Xyl-GA showed 59% sugar and 1.4% phenolic compounds. Both samples contained less than 1% protein, which was considered a contaminant for this study. Three antioxidant assays were performed: hydroxyl radical scavenging, where Xyl exhibited approximately 50% and Xyl-GA demonstrated complete scavenging; reducing power, with Xyl at 80% and Xyl-GA at nearly 100%; and copper chelation capacity, where Xyl exhibited no activity, whereas Xyl-GA at 0.5 mg/mL displayed 60% copper ion chelation. In vivo experiments were carried out using a zebrafish model. The survival rate of embryos indicated 50% survival in the positive control group (treated with hydrogen peroxide - H2O2), 80% in the Xyl-treated group, and 85% in the Xyl-GA-treated group. Additionally, when assessing larvae stained with an oxidative stress marker, a 20% reduction in fluorescence intensity was observed in the Xyl-GA and H2O2-treated group compared to the control (larvae exposed only to H2O2). In conclusion, the combination of Xyl-GA demonstrated increased antioxidant capacity compared to Xylan (Xyl), as evidenced by the comprehensive evaluations conducted in this study.

key-words: oxidative stress, corn cobs, polysaccharide, zebrafish

Acknowledgments: To CAPES and CNPq for the financial support to carry out this research.

### E-08. Antioxidant potential of blend formulated with corn cob xylan and garlic and lemon extract

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Antioxidants are substances that can neutralize oxidizing agents or repair the damage caused by them. They are crucial for preventing the emergence and development of a condition known as oxidative stress, which occurs when there is an imbalance between the levels of antioxidants and oxidants, favoring the latter. This condition is associated with various health issues, including cardiovascular disease, diabetes, and obesity. Consequently, organisms both synthesize antioxidants and obtain them from their diet. In this context, there is ongoing research to identify molecules that can be incorporated into the arsenal of antioxidants used in food and medicine. Among these molecules is a polysaccharide rich in xylose called xylan, which is currently under evaluation. It's noteworthy that xylan also possesses mitogenic, comitogenic, immunostimulatory, anticoagulant, antimicrobial, and antiproliferative activities, but it also has potential as an antioxidant. In this study, xylan was extracted from corn cobs, and blends were created by incorporating extracts of lemon and garlic. The objective was to develop a blend with antioxidant properties surpassing those of the individual components. Four distinct blends were produced. Chemical analyses revealed comparable sugar contents across all blends. Protein and phenolic compound levels were identified at approximately 4% and 1%, respectively, in all blends. In vitro antioxidant activity assays demonstrated that all blends exhibited activity equal to or greater than that of xylan, corn, or garlic extracts. Notably, the blend that stood out the most was referred to as "blend 1:1." In the Total Antioxidant Capacity test, this blend achieved 54.7 mg of ascorbic acid per gram of blend, while the reducing power test exhibited 46.2% activity. Additionally, the chelating capacity of copper ions was observed to be around 65.3%. The data clearly indicated that the combination of lemon and garlic extracts with xylan resulted in the production of a blend (1:1) possessing superior antioxidant properties. Future endeavors will involve evaluating the 1:1 blend through in vivo testing.

Keywords: Oxidative stress, polysaccharide, plant extract.

Acknowledgments: The funding agencies CAPES and CNpq, for the financial support to carry out this research.

# E-09. Evaluation Of The antioxidant potential of Chondroitin Sulfate Extracted from Tilapia Viscera (*Oreochromis niloticus*)

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Glycosaminoglycans are polysaccharides present in the cell surface and extracellular matrix of the majority of animals. They perform several biological functions, such as cell growth, differentiation, antioxidant, and others. Among glycosaminoglycans, chondroitin sulfate (CS) stands out for its antioxidant effect; it can also be found in many fish species, such as tilapia whose cultivation represents an important economic activity in Natal (RN / Brazil). eliminating a big quantity of viscera as a waste of its production, daily. Thus, the following study uses the tilapia wastes that have been obtained in free markets in the city of Natal, as raw material for the extraction and purification of CS, in addition to evaluating its antioxidant effect. For this, the residues were exposed to an enzymatic proteolyse process, followed by complexation and decomplexing with Lewatit resin, obtaining a pool of glycosaminoglycans that have been used in purification procedures like acetone fractionation and ion exchange chromatography, accompanied by agarose gel electrophoresis and enzymatic degradation. Subsequently, antioxidant tests were performed to quantify the ability of CS to inhibit reactive species intracellular and in vitro, since the imbalance in their quantities has a role in clinical conditions such as autoimmune and cancer. For this, methods such as inhibition of intracellular ROS (DCFH-DA), chelation of ferrous and cupric ions, inhibition of superoxide and hydroxyl radicals, in addition to inhibition of nitric oxide and lipid peroxidation were used. As a result, it was possible to observe that in each kilogram of tilapia viscera dry mass it is possible to extract approximately 69mg of purified chondroitin sulfate, being 59% composed of chondroitin-4sulfate. By observing the values obtained in the antioxidant tests, it was possible to highlight an inhibition of up to 52% of the reactive intracellular species in culture of RAW 264.7 cells, without demonstration of cytotoxicity up to the concentration of 200mg /mL of CS. The other tests showed that this inhibition occurs due to several factors, ranging from the inhibition of metals and free radicals p<0,05), to the probable participation in secondary pathways, such as the activation of antioxidant proteins. The results obtained in this study strengthen the extraction and use of glycosaminoglycans, especially chondroitin sulfate, helping to understand the biological effects of chondroitins from multiple sources, emphasizing their antioxidant effect and providing a disposal alternative for the biological waste produced by commercial tilapia production, opening perspectives for possible uses of this molecule in research and industry.

Acknowledgments: Universidade Federal do Rio Grande do Norte (UFRN), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES).

Key-words: glycosaminoglycan; Antioxidant; ROS

#### E-10. PRODUCTION, CHARACTERIZATION AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF BLEND CONTAINING ALGINATE AND AQUEOUS EXTRACT OF LEMON AND GARLIC

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Antioxidant molecules play a crucial role in maintaining health and preventing cell damage. They function by combatting free radicals, which are unstable and highly reactive molecules generated by the body's normal metabolism in response to external factors and internal metabolic processes. When an excess of free radicals accumulates, surpassing the capacity of the antioxidant system, it leads to oxidative stress. This stress can result in cellular damage that contributes to premature aging and the onset of various chronic diseases. Prolonged usage of existing medications for these problems can result in harm to organs like the liver and kidneys, necessitating the pursuit of more effective and safer drug treatments. Spices and fruits, like garlic and lemon, are well-explored sources of therapeutic properties due to their potential antioxidant effects. The aqueous extract of garlic and lemon contains substances that exhibit antioxidant effects when administered in combination or alongside other bioactive compounds. Another molecule under investigation for its activities is alginate, a polysaccharide derived from brown algae. Considering the characteristics of the aqueous extract and alginate, a blend was developed with the aim of assessing and comparing its antioxidant activity in comparison to the individual extracts. Initially, the 1:1 blend of alginate and extract was characterized based on sugar, protein, and phenolic compound content. The sugar content was found to be 80.3% for alginate, 82.2% for the extract, and 87.3% for the 1:1 blend. Protein content was less than 1% across all three samples, while phenolic compound content was approximately 5% for each. To evaluate antioxidant capacity, tests were conducted for total antioxidant capacity (TAC), reducing power, and chelation of copper ions on the blend, alginate, and extract. The TAC result was 38.9 mg of ascorbic acid per gram of sample for the 1:1 blend, 1.7 for alginate, and 19.1 for the extract. In the reducing power test, the 1:1 blend exhibited a 26.9% reduction at a concentration of 1 mg/mL, whereas alginate displayed less than 1% reduction and the extract showed 47.4% reduction. In terms of copper chelation, the 1:1 blend at a concentration of 1 mg/mL demonstrated a chelating activity of 72.6%, while alginate and extract displayed less than 1% difference at 72.4% and less than 1%, respectively. In conclusion, the findings suggest that combining extracts in a 1:1 blend enhances antioxidant activity.

Keywords: oxidative stress, acidic polysaccharides, plant extracts

Acknowledgments: To CAPES and CNPq for the financial support to carry out this research.

### E-11. Characterization of Chondroitin-4-Sulfate Obtained from Tilapia Viscera (*Oreochromis niloticus*) and Evaluating Its Effect on Calcium Oxalate Crystallization.

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Glycosaminoglycans (GAGs) are sulfated polysaccharides that are naturally present in urine and have been identified as modulators of urinary stone formation. Among GAGs, chondroitin sulfate (CS) has shown promise as a possible modulator of crystal growth. Studies have revealed that aquatic organisms are alternative sources of CS with therapeutic potential. The fish Oreochromis niloticus, also known as Nile tilapia, was chosen for this study due to its high production and potential for environmental damage. The objective of this study was to isolate CS from O. niloticus viscera and evaluate its modulating effect on calcium oxalate (OxCa) crystal growth. The tilapia culture residues were subjected to an enzymatic proteolysis process, followed by complexation and decomplexation with Lewatit cationic resin to obtain a mixture of GAGs. The GAGs were then purified using fractionation with acetone and ion exchange chromatography. The purified CS was identified as chondroitin-4-sulfate (CSA) and named tilapia chondroitin sulfate (CST). CST (0.01 mg/mL) was found to decrease the size of OxCa crystals and increase the number of crystals formed by 15 times. Fluorescence microscopy assays revealed that CST interacts with the faces of the CaOx monohydrate (COM) crystal, but not with dihydrate crystals. Scanning electron microscopy indicated that CST alters the morphology of COM crystals, making them assume a more elongated shape. Additionally, the study showed that CST does not present cytotoxicity under the evaluated conditions (0.01 - 0.2 mg/mL) in Madin-Darby canine renal cell (MDCK) cells. These findings suggest that CST has potential as an anti-kidney stone agent, but further in vivo studies are needed to confirm its efficacy.

Acknowledgments: Universidade Federal do Rio Grande do Norte (UFRN), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES)

Key-words: glycosaminoglycan; tilapia; urinary stones

#### E-12. EVALUATION OF ANTIOXIDANT ACTIVITIES OF A BLEND CONTAINING SULFATED POLYSACCHARIDES FROM RED SEAWEED AND AQUEOUS EXTRACT OF LEMON AND GARLIC Silva, M. M. C. L<sup>1</sup>; Costa, L.B<sup>1</sup>; Hugo, H. A.O<sup>1</sup>

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Oxidative stress occurs due to an imbalance between the production of reactive species and the action of antioxidant agents. The presence of antioxidants acts to prevent reactive species from causing uncontrolled damage to cells. However, endogenous antioxidant defenses are not always sufficient to effectively neutralize oxidative stress. For this reason, many compounds have been investigated to be potentially indicated as antioxidant agents. Herbs, spices, and fruits, such as garlic and lemon, have been well-explored sources of therapeutic properties. Additionally, marine organisms, like the seaweed Gracilaria birdiae, offer promising bioactive compounds. The objective of this study was to evaluate the antioxidant potential of a blend containing aqueous extracts of lemon (Citrus limon) and garlic (Allium sativum L.) (LG) and agarans from this seaweed (SPGB). To achieve this, the blend was initially analyzed for its sugar, phenolic compound, and protein percentages. LG exhibited 36.8% and 0.3%, SPGB showed 66.5% and 0.7%, and the blend (3:1) had 54.9% and 0.3%, respectively. Notably, no proteins were detected in any of the three samples under the evaluated conditions. Infrared spectroscopy assays identified characteristic bands of the compounds used. The samples were further evaluated for their reducing power and total antioxidant capacity (TAC). The presence of LG was found to be crucial in obtaining better activity in reducing power, as it potentiated the antioxidant activity of the blend by ~20% compared to SPGB alone (p < 0.05). In terms of TAC, the blend showed an activity 15% higher than LG and 30% higher than SPGB (p < 0.05). These results indicated that the blend had significantly higher antioxidant activity than the individual compounds (LG and SPGB) and suggested it as a potential antioxidant agent to be evaluated in in vivo tests.

Keywords: Gracilaria birdiae, agarans, medicinal plants, oxidative stress

Acknowledgments: To CAPES and CNPq for the financial support to carry out this research.

# E-13. SILVER NANOPARTICLES CONTAINING XYLANS: SYNTHESIS, CHARACTERIZATION AND EVALUATION OF THEIR IMMUNOMODULATORY EFFECT

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Nitric oxide (NO) serves as a cytotoxic mediator for activated immune effector cells. However, its high level of toxicity raises the possibility of an optimal concentration limit for this molecule during immune responses. This ensures effective antimicrobial action without causing harm to host cells. Xylan, a polysaccharide extractable from corn cobs, possesses the capability to modulate nitric oxide production. With the aim of enhancing silver's action, silver nanoparticles were synthesized using xylans to aid in their internalization. The objective of this study was to assess the *in vitro* immunomodulatory potential (NO production) of silver nanoparticles synthesized with various xylans. To achieve this, a xylan-rich extract (EBX) and five purified xylans (E0.3, E0.4, E0.8, E1.4, E2.2) were obtained from corn cobs. These were used to synthesize six types of nanoparticles (NANO EBX, NANO E0.3, NANO E0.4, NANO E0.8, NANO E1.4, NANO E2.2). Characterization involved UV-visible spectroscopy analysis, scanning electron microscopy, energy dispersion spectroscopy analysis, dynamic light scattering, and Fourier transform infrared spectroscopy. All nanoparticles exhibited a rounded shape and average size ranging from 79.7 to 105 nm. Moreover, toxicity assessment of these nanoparticles was conducted with murine fibroblast cells (3T3) and macrophages (RAW 264.7), revealing no signs of toxicity. RAW cells exposed to 2  $\mu$ g/mL of bacterial lipopolysaccharide (LPS) along with various xylans and nanoparticles (at 500 and 1000  $\mu$ g/mL) were studied. The resulting nitric oxide produced by these cells was quantified at different time intervals (7, 10, 12, 16, 18, 20, 24 hours). The xylans exhibited no interference with the NO production of RAW cells, except for xylan E1.4. This xylan led to a significant reduction of up to 40% in NO production (p < 0.05) compared to the control. Conversely, all nanoparticles (1000 µg/mL) induced an approximate 80% reduction in NO levels. The time-dependent study revealed that this effect occurred between 10 and 12 hours of nanoparticle exposure. Thus far, the data suggest that nanoparticles have an immunomodulatory effect, decreasing NO production in activated macrophages, with this effect being dependent on time.

Acknowledgments: We express our gratitude to the Federal University of Rio Grande do Norte (UFRN), the National Council for Scientific and Technological Development (CNPq), and the Coordination for the Improvement of Higher Education Personnel (CAPES) for their support. Keywords: Nitric oxide; corn cob; RAW cells.

### E-14. GALLIC ACID GRAFTED FUCOIDAN CONJUGATE VIA REDOX SYSTEM – ANTIOXIDANT ACTIVITY *IN VITRO* AND *IN VIVO*

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Oxidative stress is a phenomenon caused by an imbalance between the emission of oxidants and the natural antioxidant processes within each organism. When there is an excess of oxidants, oxidative stress increases, leading to cellular damage and disruption of the organisms' metabolism. This phenomenon can contribute to various diseases, including cancer, Parkinson's disease, and Alzheimer's disease. Given this, there is a pressing need for studies to identify new molecules that can assist organisms in combatting oxidative agents. One such candidate is fucoidan (FucB) from the brown alga Spatoglossum schröederi. Research also indicates that the chemical modification of polysaccharides can enhance their antioxidant effects. In this context, our main goal was to chemically modify FucB to enhance its antioxidant properties, both in in vitro and in vivo tests (using a zebrafish model). The modification process involved the use of the redox method and gallic acid, resulting in the creation of fucoidan conjugated with gallic acid, known as Fucoidan-GA (FucBG). Confirmation of this compound's conjugation was achieved through Fourier Transform Infrared (FTIR) analysis. Subsequently, tests were conducted to assess its the *in vitro* antioxidant activity, particularly the scavenging of hydroxyl radicals. Additionally, FucBG in vivo antioxidant capabilities were evaluated, including the sample's ability to protect zebrafish embryos from stress induced by hydrogen peroxide. Furthermore, the samples' capacity to reduce levels of ROS (reactive oxygen species) caused by the stressor agent (hydrogen peroxide) was quantified. The FTIR results revealed characteristic bands for polysaccharides (3498 cm<sup>-1</sup>), sulfated polysaccharides (1265, 1038, 854 cm<sup>-1</sup>) in FucB, and bands associated with phenolic compounds (1558 cm<sup>-1</sup>) in FucBG. These findings confirmed the structural changes from the native molecule. In the hydroxyl radical scavenging test, FucB demonstrated a 53% scavenging rate of radicals at a concentration of 0.25 mg/mL, while FucBG exhibited a higher scavenging rate of 91% at the same concentration. This indicated an increased antioxidant potential following conjugation. In the *in vivo* tests, both FucB and FucBG samples exhibited protective effects against hydrogen peroxide-induced damage in zebrafish embryos, resulting in up to a 50% reduction in embryo mortality compared to the positive control. Regarding ROS quantification, FucBG demonstrated approximately 70% lower ROS production compared to FucB, almost halving the observed ROS levels. These results underscore the effectiveness of chemical modification in enhancing the *in vitro* and *in vivo* antioxidant activities of FucB. Keywords: oxidative stress; chemical modification; zebrafish;

#### F - Mecanismos Moleculares de Doenças

# F-01. Establishment of an *in vivo* platform for the screening of new drugs for the treatment of Alzheimer's disease

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Alzheimer's disease, a devastating and untreatable neurodegenerative condition, is closely linked to the accumulation of  $\beta$ -amyloid peptides (A $\beta$ ) in the brain. Introducing modified human Aß peptides into Drosophila fruit flies mirrors Alzheimer's-like symptoms, including age-related decline and memory loss. Our main goal was to establish an in vivo platform for evaluating potential Alzheimer's treatments. We expressed the transgene using the UAS-GAL4 system and fly lineages with human amyloid precursor protein (APP) mutations and the animals were kept under 25°C and 29°C. We assessed the impact of mutated gene expression on lifespan, motor performance, short-term memory, and eye tissue toxicity. The expression of mutated amyloid protein in the brain led to significantly shorter lifespans and severe eye degeneration due to transgene expression during eye development. Memory tests revealed progressive short-term memory impairment, and motor assessments exposed severe locomotor challenges. Our research identified mortality trends in Alzheimer's-affected flies, paralleling human patients' symptoms. Furthermore, we uncovered substantial cellular toxicity and distinct stages of memory loss. Consequently, we established a secure and efficient platform for testing potential drugs targeting amyloid plaque formation in these animals' brains. Currently, we are conducting tests using cannabidiol and lemon essential oil to assess their neuroprotective potential in this Alzheimer's model. Acknowledgements: CnPQ; Fapeal; LAVITOX; LNFI

Keywords: Alzheimer, Drosophila, New drugs

#### F-02. Enzymes as therapeutic targets in cancer treatment: a literature review

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Introduction: Cancer is one of the five diseases that kill the most worldwide, being multicausal, and advancing in the knowledge of the various therapeutic mechanisms is always urgent. In the last decade, the study of enzyme inhibitors gained prominence as an allied therapeutic tool, considering the large number of enzymes involved in the many metabolic pathways that favor this disease. In this review, we seek to emphasize the significant advances in research on the use of different enzymes as potential targets for cancer drugs. **Objective:** To review the scientific literature of the last ten years regarding the main metabolic pathways involved in the survival and multiplication of cancer cells, and the preclinical and clinical trials of enzyme inhibitors of these pathways as a target for the treatment of various types of cancer. Material and Methods: A qualitative/quantitative review of research articles in the Scopus, PubMed and Scielo databases of the scientific literature was carried out, using the descriptors "enzymes AND therapeutic targets AND cancer", "cancer suppressors", "inhibitors of MVA pathway", "ubiquitination process AND cancer", considering as inclusion criteria only articles published in English in the last ten years. Results and Discussion: Twenty articles were selected from the ones found, for having information on ongoing or completed clinical trials. It was seem that HMG-CoA reductase enzyme inhibitors (statins) are safe and effective when used alone or in combination with other drugs against various types of tumors, although for skin and colon cancer there are still no clinical trials enough. Ubiquitinase inhibitors, as they alter the stability of oncoproteins, has also been presented in several studies, some showing efficacy in xenograft models in mice with myeloma and leukemia, but again clinical trials in humans have not yet been sufficiently conducted. Pevonedistat is one that presented promising results, especially in combination with azacitidine against acute myeloid leukemia, but its commercial use has not yet been approved. Many other enzymes have been the target of new specific inhibitor molecules with low toxicity to patients, especially those involving the metabolisms of glutamine (glutaminase or glutamine synthetase isoenzymes), L-arginine and L-aspartate (arginase and asparaginase), and hexosamine synthase. Conclusions: There are several advantages in focusing on the synthesis of inhibitors of key enzymes in the metabolism of neoplastic cells as an alternative therapy against cancer. In general, the combination of more than one inhibitor of greater specificity has been the choice for clinical trials.

**Keywords:** cancer therapy; clinical trials; enzyme markers; metabolic pathways; in vivo assays. **Acknowledgements:** We thank the Institute of Chemistry and Biotechnology of Federal University of Alagoas for support.

### F-03. Human CETP Expression in Female Mice Improves Endothelial Function, Reduces Oxidative Stress and Enhances Estrogen-Mediated Relaxation.

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Introduction: Cardiovascular diseases are a global health concern, with endothelial dysfunction being an early sign. Cholesteryl ester transfer protein (CETP) has been shown to play a role in this process. Previously we found that male mice with human CETP (hCETP) have reduced vascular relaxation due to oxidative stress. Yet, recent data suggests that CETP may have sex-specific effects. Our study explores how CETP affects female mouse endothelial function. Methods: We used transgenic female mice expressing hCETP and their respective non-transgenic (NTg) controls (4-6 months, CEUA 6010-1/2022). We isolated the thoracic aorta and performed dose-response curves to acetylcholine (ACh), phenylephrine (PE) and 17β-estradiol (E2), with or without estrogen receptor alpha (ERα) antagonist methylpiperidinopyrazole (MPP) and heat shock protein (HSP90) inhibitor geldanamycin (GA). Aortic homogenates were used for western blotting analysis, and aortic rings were analyzed for reactive oxygen species (ROS) by DHE and NO by DAF-2DA. Data were analyzed by 2-way ANOVA or Student t-test (P<0.05). Results: We observed a 47% increase in eNOS phosphorylation and 92% in calcium-induced NO levels in CETP aortas, indicating a higher eNOS-NO pro-relaxation pathway. hCETP females exhibited a reduction in alpha-adrenergic vascular contractility in an endothelium-dependent manner (20% decrease in Emax). Aortas from female hCETP mice also presented reduced ROS (15%), and lower expression of the redox enzymes NOX2 (25%) and SOD2 (19%). Moreover, aortas from hCETP females exhibited increased relaxation to E2 (Emax: NTg 71.1±3.9% vs. hCETP 87.5±2.7%) and upregulation of HSP90 (40%) and Caveolin-1 (32%), proteins that stabilize the ER $\alpha$  in the endothelial caveolae, suggesting facilitation of E2-ER $\alpha$  signaling in CETP females. Indeed, the increased E2-mediated relaxation in hCETP aortas was sensitive to the inhibitors of ERa (18% decrease in Emax) and inhibitor of HSP90 (22% decrease in Emax). In the presence of E2, ERa inhibition also impaired the vascular relaxation response to ACh in hCETP females (Emax hCETP: 89.0±3.9% vs. MPP 70.6±4.1%), highlighting the importance of this receptor for the vascular effects of hCETP in females. Conclusions: CETP expression in the vascular system has sex-specific effects. Unlike males, female hCETP mice displayed lower oxidative markers, preserved vascular relaxation, decreased contractility, and enhanced E2-ERa signaling. This involved increased expression and activation of eNOS, Cav-1, ERa, and HSP90. These findings highlight the importance of considering sex differences when studying the impact of CETP on endothelial function. Financial support: FAPESP

Key-words: CETP; Endothelial function; Estrogen-mediated relaxation.

## F-04. *In Vitro* Assessment of Cytotoxicity and antioxidant potential of *Euphorbia tirucalli Lineu* latex in Raw 264.7 macrophage cell lines

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INTRODUCTION: Cancer is responsible for taking thousands of lives in Brazil, with 21,260 deaths in 2021. Colorectal cancer (CRC) is the second most incident for men and women and the third most deadly. Although treatable in early stages, CRC does not present determining symptoms in the beginning, worsening the prognosis and requiring incisive approaches in more advanced stages. The search for alternatives to existing treatments has grown due to the acquisition of chemoresistance by tumors and the side effects of drug therapies. Euphorbia tirucalli, also known as "avelós", is a plant native to Africa and acclimatized in Brazil, which has been widely used by the population, even though it is considered toxic. Despite this, it has several compounds with proven antioxidant action in the literature. To support popular knowledge about the use of E. tirucalli latex, it was necessary to verify, through scientific methodology, the toxicity of latex with Artemia salina, cell viability through the (4,5-Dimethylthiazol-2-yl)2,5-Diphenyl Tetrazolium Bromide (MTT) reduction assay, and antioxidant activity through the capture assay of the organic radical 2,2-diphenyl-1picrylhydrazyl (DPPH) in Raw 264.7 macrophage cell lines,. OBJECTIVE: Evaluate the toxicity of Euphorbia tirucalli Lineu latex on Artemia salina and the antioxidant and cytotoxic activity, in vitro, in RAW 264.7 macrophage cell lines. MATERIALS AND METHODS: The extraction of latex was performed in mangrove areas on the coast of Bahia. As a preliminary assay, the toxicity of the latex was measured using Artemia salina. For viability and antioxidant activity, the MTT and DPPH assays were performed, respectively. RESULTS AND DISCUSSION: As expected, the toxicity assay demonstrated that latex is not toxic at low concentrations, and the cell viability assay indicated high preservation of the cell lineage. Additionally, the DPPH assay verified the high capacity of capturing 2,2-diphenyl-1-picrylhydrazyl radicals, demonstrating the therapeutic potential of latex. CONCLUSION: A toxic effect was evidenced in the Artemia salina assay, obtaining a satisfactory LC50. The joint analysis of the results of the Artemia salina, MTT, and DPPH assays allows to conclude that the latex of Euphorbia tirucalli presented promising biological activities, due to its good antioxidant activity and absence of significant cytotoxicity in RAW 264.7 lineage macrophages and absence of toxicity, when in small concentrations, in Artemia salina. ACKNOWLEDGMENTS: To the Federal University of Bahia for the research incentives. Also to PMBqBM, the biotechnology department at UFBA, and the department that made this project possible. KEYWORDS: Euphorbia tirucalli; RAW 264.7, in vitro.

#### F-05. Establishment of an *in vivo* platform for the screening of new drugs for the treatment of C9orf72 Amyotrophic Lateral Sclerosis.

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with no cure, characterized by the degeneration of motor neurons, resulting in symptoms such as loss of muscle strength and coordination, difficulty with breathing and swallowing, weight loss, and other symptoms that eventually lead to death. Among the genetic causes associated to the disease, studies have indicated that mutations in the human 9th chromosome (C9orf72) account for the majority of familial ALS cases. This mutation is caused by G4C2 hexanucleotides repeats, which after transcription lead to an accumulation of RanGTPases and a pathological nuclear/cytoplasmic gradient, characteristic of the disease. The drug's currently available in the market for the ALS treatment act only by delaying the symptoms, highlighting the need for new research on this pathology. Drosophila melanogaster is an established model for studying neurodegenerative diseases due to its short lifespan, easy maintenance, and complex nervous system. Our goal was to establish an in vivo platform for future drug screening for C9orf72-associated ALS treatment. To simulate patients phenotype, we expressed the transgene containing the repeats in the animals through the UAS-GAL4 system and kept them at 25°C and 29°C to evaluate the transgene overexpression at higher temperatures and their effects on animal longevity, larval and adult motility, and cellular toxicity levels compared to the control. The transgene led to motor neuron degeneration, causing significant reductions in adult and larval motility, eye size, and animal longevity, indicating a high level of toxicity caused by the G4C2 repeats. Therefore, it was possible to establish a safe and reliable in vivo platform to evaluate the effects of those repeats for drug screening for this disease.

**Keywords**: Amyotrophic Lateral Sclerosis; *Drosophila melanogaster*; C9orf72. Thanks to the Federal University of Alagoas, FAPEAL and LAVITOX.

**F-06.** Polymorphism rs2144658 in the *IL23R* gene and Leprosy in a population from Alagoas <u>Freitas, H. A.<sup>1</sup></u>, Santos, L. K. C.<sup>1</sup>, Nogueira, K. R. C.<sup>1</sup>, Santos, B. R. C.<sup>1</sup>, Santos, K. P. <sup>2</sup>, Araújo, N. S.<sup>2</sup>, Duarte, A. K. S. F.<sup>3</sup>, Paz, M. N. V. T.<sup>3</sup>, Souza, J. L. R. A.<sup>4</sup>, Sales-Marques, C.<sup>1,3</sup> <sup>1</sup> Programa de Pós-Graduação em Ciências da Saúde, Instituto de Ciências Biológicas e da Saúde (ICBS), Universidade Federal de Alagoas (UFAL), Laboratório de Biologia Molecular e Expressão Gênica (LABMEG), Laboratório de DNA Forense – Usina Ciência, Alagoas, Brazil. <sup>2</sup>Universidade Federal de Alagoas (UFAL) – Campus de Arapiraca, Alagoas, Brazil. <sup>3</sup>Programa de Pós-Graduação em Bioquímica e Biologia Molecular, Instituto de Ciências Farmacêuticas (ICF), Universidade Federal de Alagoas (UFAL), Alagoas, Brazil. <sup>4</sup>Instituto de Ciências Biológicas e da Saúde (ICBS), Universidade Federal de Alagoas, UFAL), Alagoas, Brazil.

Introduction. Leprosy is a disease caused by the intracellular bacillus Mycobacterium leprae and/or Mycobacterium lepromatosis, which mainly affects the skin and peripheral nerves, and may result in physical disabilities. In the state of Alagoas, in 2022, it presented an incidence rate of new cases of 9.67/100 thousand inhabitants, considered of medium endemicity. Polymorphisms of the interleukin 23 receptor protein (IL23R) gene have been found to be associated with leprosy. Also, IL23R gene SNPs have been shown to affect the profile of inflammatory cytokines in human cells. This gene has already presented its polymorphisms associated with leprosy in the Chinese population, but it had not yet been investigated in the Brazilian population. **Objectives.** The objective of this study was to investigate the association of the SNP rs2144658 in the IL23R gene with leprosy in a sample from Alagoas. Materials and methods. A case-control study was carried out, including cases (patients with leprosy) and controls (individuals without leprosy). Biological samples were collected from the participants (whole blood) and the DNA extraction (salting-out) was performed. From the extracted DNA, genotyping was performed using allelic discrimination assays by real-time PCR (Taqman, Applied Biosystems). Subsequently, estimates of the genetic association between the SNP and leprosy were obtained (OR, P-value and 95% confidence interval). Analyzes were performed using a logistic regression model using OR as a measure of association with leprosy, using the SNPStats platform (dominant, codominant, recessive, overdominant and log additive models). Results and discussion. A total of 189 patients and 264 controls were recruited, the majority being male in both groups (62% cases and 64% controls). The genetic marker in the *IL23R* gene showed a frequency for the A allele (polymorphic allele) of 44% in controls and 47% in cases, in relation to the genotype a frequency of 54% was seen vs. 54% GA in patients and controls, respectively. Genetic analyzes revealed that the marker rs2144658 showed no association with leprosy, according to the genetic models codominant (OR= 1.09, CI= 0.70-1.68, p= 0.73), overdominant (OR= 0.99, p= 0.97), and also no association with leprosy in the other models analyzed with adjustment for the covariate sex. Conclusion. Based on the aspects presented, the rs2144658 polymorphism in the IL23R gene showed no association with leprosy in the population of Alagoas. These results show a new face in relation to the genetic influence on leprosy in the population.

Palavras-chave: IL23R. Case-control. Human and Medical Genetics.

**Agradecimentos:** Esse projeto conta com apoio de fomento do Programa de Pesquisa para o SUS - PPSUS/Fapeal № 06/2020 e está amparado por bolsa de mestrado CAPES/DS (Coordenação de Aperfeiçoamento d/e Pessoal de Nível Superior).

# F-07. Mapping of polymorphisms in the *RAB32* and *IRGM* genes, and leprosy: an integrative review

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Leprosy, a chronic infectious disease caused by Mycobacterium leprae, has been the subject of numerous scientific investigations due to its complexity and clinical variability. In addition, the genetic influence on susceptibility to the disease is highlighted, proving to be a crucial factor in understanding its pathogenesis. The RAB32 gene plays a vital role in the regulation of intracellular traffic, responsible for encoding a protein related to autophagy, while the *IRGM* gene instructs the production of a protein belonging to the GTPases family(M protein), involved in innate immunity, especially against intracellular infections. Polymorphisms in such genes may influence the effectiveness of autophagy, and consequently, the body's ability to control bacillus infection. The aim of this study was to map studies that investigated the relationship between polymorphisms in the RAB32 and IRGM genes and predisposition to leprosy. The study was based on a careful selection that took place between November 2022-August 2023 in the databases PubMed Central NCBI (National Center for Biotechnology Information), Google Scholar and MEDLINE, using the keywords: "Leprosy", "RAB32 " and "IRGM", together with the "AND" or "OR" operators connecting the terms. Articles published between 2009-2021 and with sufficient genetic data for calculating the Odds Ratio (OR) were chosen, discarding: i)Repeated between databases; ii)Abstracts, monographs or theses; iii)Those with deviation in the Hardy-Weinberg equilibrium; iv)With a methodology different from the case-control approach or v)With categorizations that made the analysis unfeasible, thus ensuring a robust set of sources. Four articles were selected, 3 corresponding to the SNP rs2275606/RAB32, of which two demonstrated an association with leprosy in the Chinese population(OR: 1.30, P-value: 3.94x10-14 and OR: 1.41, CI: 1 .03-1.93, P-value: 0.03), while Long et al, 2021 contested (OR: 1.12, CI: 0.98-1.29, P-value: 0.10), pointing to possible ethnic heterogeneity within the Chinese population. One of the articles dealt with the rs13361189 and rs4958842 polymorphisms of the IRGM gene, also in China, resulting in an association with risk(OR: 2.58, 95% CI: 1.65-4.05, P-value: <0.001) and no association , respectively(OR: 0.93, 95% CI: 0.69-1.25, P-value: 0.616). In short, this integrative review highlights the importance of investigating SNPs in the RAB32 and IRGM genes as potential contributors to the susceptibility and clinical course of leprosy, especially in Brazil's population. Advances in studies in the field of genetic susceptibility to leprosy promise to open new perspectives for more effective diagnostic and prevention strategies, in line with the ongoing effort to control and eradicate this debilitating disease.

Keywords: RAB32, IRGM; Leprosy.

Agradecimentos: PPSUS, FAPEAL, CNPQ e UFAL

#### F-08. Chikungunya Virus Infection Impacts Biomechanical Properties and Molecular Components in Human Fibroblast-Like Synoviocytes (HFLS) *in vitro*

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Chikungunya virus (CHIKV) is an Alphavirus transmitted by Aedes sp. This arbovirus causes a disease manifested as severe persistent polyarthralgia. During this infection, the synovial joints become damaged and fibroblast-like synoviocytes (FLS) may be some of the cells affected. FLS can adopts an aggressive profile in diseases with severe polyarthralgia, leading to significant tissue damage during the inflammatory phase. Thus, the aimed of this work was to understand the biomechanical and molecular alterations involved in the human fibroblastlike synoviocytes (HFLS)-CHIKV interaction in vitro. HFLS were infected with CHIKV (MOI 0.5) for 48 hours and the percentage of CHIKV-infected cells was accessed by intracellular flow cytometry by using an anti-CHIKV monoclonal antibody/anti-mouse IgG Alexa Fluor 488. CHIKV-infected HFLS were submitted to Atomic Force Microscopy (AFM) to evaluate morphological changes and the Young's modulus by using a scanning tip. F-actin cytoskeleton was labeled with Alexa Fluor 488 Phalloidin and the nucleus was stained with blue-fluorescent DAPI at 0h, 12h, 24h, and 48h after infection followed by fluorescence microscopy analysis. Biochemical changes were determined by Raman spectroscopy and RT-qPCR was performed to evaluate relative gene expression. Also, the quantification of chemokines was measured in the supernatant using the CBA method (Cytometric Bead Array). Cytopathic effects were observed in HFLS 48h after viral infection and 46,8% of cells were positive to CHIKV. AFM analysis showed CHIKV-infected HFLS with morphological changes and rougher surface. Also, AFM nano-indentation shows an increase of 107.46% in the average of Young's elastic modulus in the CHIKV-infected cells. Significant changes in the F-actin filaments and disruption in the cytoskeleton arrangement were observed after 12h of infection, suggesting the infection can leads to cellular stress with damage in cytoskeleton and cell stiffness. CHIKVinfected HFLS and uninfected cells exhibit a total variation of 65%, according to Raman spectroscopy and PCA analysis. The major alterations detected were related to collagen, with signatures in 1031, 1223, and 1635 cm<sup>-1</sup>. Relative gene expression showed an increase in the expression of MMP1, VEGFA and UGDH in CHIKV-infected cells compared to uninfected cells. Also, an increase in the IP-10 and a reduction in MCP-1 levels were detected. In summary, understand the mechanisms of CHIKV-HFLS interaction and the viral pathogenesis are crucial for the development of novel therapeutic targets for this arbovirus disease.

Key words: Chikungunya virus, human fibroblast-like synoviocytes, biomechanical properties.

#### F-09. Immunobiochemistry and Genetics of LRRK2 and RIPK2 Genes in the Pathogenesis of Leprosy

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Leprosy is a chronic infectious disease caused by the bacterium Mycobacterium leprae, affecting the skin, eyes, peripheral nerves, and mucous membranes, and may result in physical deformities and social stigmatization. Despite being one of the oldest diseases known to humanity, it still represents a significant public health problem. The interaction between the host's immune response, infection, and the effects of genetic variants are pathogenic aspects of leprosy. Immune responses continue to be targets of investigation. Thus, this review aims to discuss the immunobiochemical aspects of Leucine-Rich Repeat Kinase 2 (LRRK2) and Receptor-Interacting Serine/Threonine-Protein Kinase 2 (RIPK2) genes in the pathogenesis of leprosy. To that end, a qualitative literature review was conducted using databases such as Scielo, Portal Capes, and PubMed, using the descriptors "LRRK2 and leprosy" and "RIPK2 and leprosy". Publications from 2013 to 2023 were included in all languages, excluding incomplete publications. After applying the defined criteria, 7 articles were found to support the discussion. As a result, it was observed that large-scale genomic association studies (GWAS) have identified variations in seven different genes (CD13orf31, NOD2, CCDC122, HLA-DR, TNFSF15, RIPK2, and LRRK2) associated with susceptibility to leprosy. The strongest associations were observed in the genes CD13orf31, NOD2, RIPK2, and LRRK2, particularly regarding the multibacillary form of the disease. LRRK2 is an IFN-gamma target gene, involving different immune response signaling pathways, highly expressed, and associated with Type 1 Reactions (T1R), which are immune responses that may lead to complications like neuritis and disabilities. This suggests that this gene could be a therapeutic target in the treatment of this disease. The RIPK2 gene, in turn, is part of the NOD2 signaling pathway, which has been implicated in susceptibility to leprosy and the activation of pro-inflammatory cytokines and NF-kB activity. The interaction between NOD2 and RIPK2 loci supports the involvement of this pathway in susceptibility and cellular immune response. The biochemical profile in cases of virchowian leprosy causes significant changes in biomarkers, such as glucose, serum cholesterol, transaminases, and serum protein electrophoresis. Thus, it is concluded that modulation of signaling pathways may work together to influence susceptibility and progression of leprosy. The genes LRRK2 and RIPK2 are associated both with susceptibility to the disease and with immune responses that may lead to clinical complications in leprosy. Keywords: Leprosy. LRRK2. RIPK2.

# F-10. *In vitro* cytotoxic and antioxidative activity of *Euphorbia tirucalli Lineu* latex in HCT-116 colorectal cancer cells

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**INTRODUCTION:** Colorectal cancer (CRC) ranks as the third most common type of cancer and the second most lethal cancer worldwide. The plant Euphorbia tirucalli Lineu, commonly known as "aveloz," possesses therapeutic properties within its latex that exhibit significant anti-tumor capacity, inducing apoptosis in CRC cells (HCT-116). Consequently, the assessment of antioxidant activity and toxicity profiles marks the inception of the investigation into the studied plant extract. Additionally, the use of Artemia salina was employed to subsequently analyze cells with active metabolic power and high proliferation. Thus, the study of cell viability became indispensable to ensure the benefits and safety of the plant's latex, as well as to determine the dosage yielding significant applications in experimental trials. **OBJECTIVES:** This study aims to evaluate in vitro cell viability, cytotoxicity, and antioxidant potential of the HCT-116 lineage from Euphorbia tirucalli Lineu latex. MATERIAL AND METHODS: The extract of the species was obtained from mangrove areas and coastal vegetation by incising the trunk and branches of mature plants. As an initial testing phase, Artemia salina was utilized to assess sample resistance in lethality studies. Subsequently, in the in vitro model, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was employed to gauge cell viability, along with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method to measure the antioxidant capacity of the extract. **RESULTS AND DISCUSSION**: Analysis using the MTT and DPPH assays demonstrated remarkable efficacy at concentrations that curtailed lineage growth without compromising the survival of healthy cells. Moreover, the latex exhibited significant antioxidant activity and low cytotoxicity. **CONCLUSION:** The findings unveiled promising indications of an anti-tumor response stemming from the plant's latex, showcasing a notable impact on the preservation of cellular integrity and a remarkable ability to neutralize free radicals. These results offer substantial value for innovative therapeutic approaches.

**ACKNOWLEDGEMENTS:** To the Federal University of Bahia for the research incentives and financial support and to the PMBqBM.

**KEY- WORDS:** *Euphorbia tirucalli*; HCT-116; *In vitro* 

#### F-11. EXPRESSION OF THE ENDOGENOUS RETROVIRUS HERVW-1 AND ASSOCIATED WITH THE SEVERITY OF COVID-19

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Introduction: COVID-19, caused by the SARS-CoV-2 virus, is characterized by activation of the inflammatory response that can lead to acute respiratory distress syndrome. Recent studies have shown that reverse transcription and genomic integration of retroviral RNA using a complex of proteins encoded by HERVS may be related to inflammatory diseases and the development of the severe form, such as COVID-19, but their contribution to COVID-19 severity is still under investigation. Objective: To evaluate the expression of human endogenous retroviruses HERVK-10 and HERVW-1 in the peripheral blood of patients with mild and severe forms of COVID-19. Methods: A cross-sectional case study, with 117 patients with a diagnosis confirmed by qRT-PCR, of which 59 participants in the case group and 58 in the control group. We collected whole blood and performed the isolation of mRNA from total leukocytes. The RT-qPCR assay was performed to analyze the relative expression of genes (2<sup>-</sup>  $^{\Delta CT}$ ). The study was approved by the human ethical committee (4.014.165). **Results:** Among the sample studied, 65.5% of the patients were male with a median of 62 years in patients in the severe group (p<0.0001). Considering comorbidities, the patients showed the presence of at least one pre-existing chronic disease, with diabetes being the most common comorbidity present in 27 (46,6%) patients p<0.0001). Compared with mild patients, patients with severe COVID-19 had significantly decreased levels of red blood cells, hemoglobin and percentage of hematocrit (p < 0,001). Patients with the severe form of COVID-19 had lymphopenia and neutrophilia (p< 0,001). Inflammatory markers such as NLR and PLR were significantly higher in severe patients when compared to mild patients (p< 0.001). It was observed that approximately 87.2% of patients in the severe group had NLR  $\geq$  5 and 76.9% had PLR  $\geq$  0.20 (p< 0.001). Severe patients showed a significant increase in HERVW-1 (p<0,05). ROC analysis for HERVW-1 demonstrated area under curve (AUC), sensitivity and p-value (0.67; 90.4%, p=0.037, respectively) resulting in negative diagnostic value. The NLR demonstrated better diagnostic value when compared to the PLR (AUC: 0.978;0.896; p<0.0001, respectively). Final Considerations: Our study demonstrated that the segment most affected by COVID-19 comprises males, the elderly, black people with diabetes, and the high expression of HERVW-1 in patients admitted to the intensive care unit may be a reflection of the exacerbated immune response. Together the findings suggest that HERVW-1 expression, NLR and PLR markers have a potential relationship with predicting the outcome of COVID-19. Keywords: COVID-19, HERVW-1, NLR.

**Support:** FAPESB, CNPq and PIBIC-UFBA.

#### G - Microorganismos e Patógenos

#### G-01. EVALUATION OF THE ANTIBACTERIAL AND ANTI-BIOFILM POTENTIAL OF *Eugenia pohliana* L. (MYRTACEAE) LEAF ESSENTIAL OIL

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The escalating rise in bacterial resistance to conventional antibiotics presents a significant obstacle to modern medicine. The proliferation of resistant strains has underscored the urgency for new and efficacious therapeutic approaches. Essential oils have emerged as a promising avenue for combating bacterial resistance, with *Eugenia pohliana* DC. (Myrtaceae) proving to be a standout among essential oils due to its diverse array of bioactive compounds with antimicrobial properties. The purpose of this study was to investigate the antibiofilm and antibacterial properties of the essential oil extracted from the E. pohliana plant, and to analyze its efficacy against pathogenic bacterial strains. The leaves of E. pohliana were collected from Buíque-PE. The collected E. pohliana leaves underwent hydrodistillation in a Clevenger-type apparatus. The essential oil was analyzed thoroughly utilizing gas chromatography coupled to mass spectrometry (GC-MS), with the aim of identifying the components and their proportions. The oil was assessed for effectiveness against bacterial strains Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 23235). A yield of 0.14% was obtained for the essential oil of *E. pohliana*, where  $\delta$ -Cadinene was predominant (18%). To analyze the antibacterial activity, we implemented the serial dilution method (4096-64 µg/mL) following the modified CLSI protocol (2018) to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the essential oil. The crystal violet fixation method (0.4%) was used to quantify the biomass of the biofilm formed after exposure to different concentrations of essential oil for the antibiofilm activity test. It was observed that the essential oil of *E. pohliana* exhibited antibacterial activity that inhibited the growth of *S.* aureus (2048 µg/mL) and E. coli (4096 µg/mL). Additionally, it showed effective bactericidal action for both bacteria at a concentration of 4096 µg/mL. However, there was no inhibitory effect on either strain tested regarding antibiofilm activity. This study highlights the effectiveness of the essential oil of this plant against S. aureus and E. coli in terms of bacterial inhibition and eradication. Nonetheless, the absence of an antibiofilm effect underscores the intricacy of the interactions. Thus, the essential oil of *E. pohliana* might provide useful insights for the development of antimicrobial methods, expanding the knowledge of the connection between these natural compounds and the issue of bacterial resistance.

Keywords: Bacterial Resistance. Microrganims. Plant.

Acknowledgment: CAPES, CNPq and FACEPE.

#### G-02. Profile of *Senecavirus A* infection in pigs from farrow-to-finish farms after outbreak

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Pig farming is notorious for the world economy, especially for Brazil, the fourth largest pork producer. Nonetheless, emerging diseases related to this activity deserves preventive measures, among these, are the vesicular diseases. Senecavirus A (SVA) infection deserves to be highlighted in pork production by the capacity of vesicular lesions and diarrhea in piglets. Our work investigated the presence of SVA RNA and the serological responses to natural infection in farrow-to-finish farms. Five farms were naturally contaminated with SVA but without clinical manifestation at the time of collection from the Ponte Nova microregion, Minas Gerais. The farms were affected by an outbreak of SVA in 2016. One hundred samples were collected from each farm, totaling 500. PCR real-time was performed through feces and serological assays through serum neutralization. The results showed seropositivity in four farms (55%, 52%, 65% and 80%), with different antibody titers, except one that revealed negative results. Sows, piglets from the farrowing and finishing phase showed high positive antibodies in comparison with pigs from the nursery and growth phases. Through PCR realtime, verifying viral circulation in two farms. Piglets from farrowing were high RNA positive in comparison with other phases. Five strains were partially sequenced from two positive farms. The region of the viral genome is genetically similar to Brazilian strains deposited in GenBank. The SVA-14 and SVA-421 strains showed 99.7% identity with the MZ032152 Brazilian sample, while the SVA-88, SVA-405 and SVA-402 strains showed a similar identity of 99.5%, 99.3%, and 99.2% with the MZ032152 sequence. Considering these results, it was possible to highlight important control points of virus entry routes, which can help elaborate possible vaccine protocols and other preventive measures, just as researchers involving Senecavirus A to the advancement of science.

Acknowledgment: CAPES, CNPq, FAPEMIG and FAPEAL. Key words: Senecavirus, qPCR, serum neutralization.

#### G-03. Antifungal and Antibiofilm Activities of the Pomegranate Sarcotesta Lectin (PgTeL) against *Cryptococcus neoformans* B3501

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Cryptococcus neoformans is an encapsulated yeast that causes cryptococcosis, a systemic opportunistic disease that affects immunocompromised patients and most commonly attacks the lungs. However, in more severe cases, it can progress to meningitis or meningoencephalitis. Currently, the main therapeutic approach for the treatment of cryptococcosis is done through the combination of antifungal drugs, but the increasing levels of resistance to treatments instigate the search for alternatives. PgTeL is a lectin isolated from Punica granatum sarcotesta that has been reported as a potent antimicrobial molecule. This work evaluated the ability of PgTeL in inhibiting the planktonic growth and the biofilm formation as well as in disrupting preformed biofilms of the strain B3501 (serotype D) of C. neoformans. First, broth microdilution assay was performed to detect the minimum inhibitory (MIC) and fungicidal (MFC) concentrations. For the biofilm assays, it was used the crystal violet method for the biomass evaluation and the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl amino)carbonyl]-2H-tetrazolium hydroxide (XTT) method for the evaluation of the metabolic activity and biofilm viability. Three-dimensional images of B3501 biofilms were obtained through scanning electron microscopy (SEM). PgTeL showed fungistatic activity with MIC of 172.0 µg/mL, without fungicidal effect. It inhibited biofilm formation, reducing biomass in 31.0–64.0% (PgTeL at 4.0–256.0 µg/mL) and metabolic activity in 32.0–93.0% (PgTeL at 32.0–256.0 μg/mL). In addition, PgTeL disrupted preformed biofilms, reducing the metabolic activity in 36.0-92.0% when evaluated at 8.0-256.0 µg/mL. SEM images revealed the disappearance of the biofilm matrix. The ability of lectins to interact with fungal cell wall glycans may be linked to their antifungal activity, inhibiting cell multiplication, decreasing ability to absorb nutrients, impairing spore germination, and other modes-of-action. In conclusion, the P. granatum lectin is a potential candidate for anticryptococcal agent, opening doors for further studies on its mechanisms of action and application strategies. Acknowledgment: FACEPE, CNPq and CAPES.

Keywords: Punica granatum, cryptococcosis, antimicrobial activity.

**G-04.** Prospect of the antifungal and modulatory activity of protein fractions and saline extract of *Tephrosia toxicaria* (Sw.) Pers on clinical strains of *Cryptococcus gatii* <u>Silva, M.F.A.<sup>1, 2</sup>; Silva, V.E.<sup>2</sup>; Gomes, R.M.O.S.<sup>2</sup>;Sá, G.C.S.<sup>3</sup>; Theodoro, R.C.<sup>2</sup>; Uchôa, A.F.<sup>2</sup>; <sup>1</sup> Department of Biochemistry, Federal University of Rio Grande do Norte, Rio Grande do Norte, Brazil. <sup>2</sup> Institute of Tropical Medicine of Rio Grande do Norte, Rio Grande do Norte, Brazil. <sup>3</sup> Institute of Studies in Health and Biology, Federal University of South and Southeast Pará, Marapá-PA, Brazil.</u>

In Brazil, cryptococcosis is a compulsory notification disease, in which Cryptococcus gattii affects mainly immunocompetent individuals and has several registered resistance episodes. Botanical formulations have been reported as strong candidates to act as therapeutic adjuvants because they are obtained from renewable sources, present toxic selectivity and ability to concentrate biologically active substances. The present study evaluated the antifungal potential of extracts and protein fractions from *Tephrosia toxicaria* (Sw.) Pers. on clinical strains of *Cryptococcus gatti*, as well as the effects of the combined therapy of these formulations with commercial antifungals. The seed was processed to obtain an extract in Tris-HCl buffer, composing the crude extract ST (Seed in Tris-HCl), also used for fractionation by the Scopes method in four saturation intervals. T. toxicaria formulations were submitted to serial dilutions and associated with C. gatti inoculum for determination of MIC and MIC<sub>50</sub> (Minimum Inhibitory Concentration, capable of inhibiting total and 50% fungal growth, respectively). For the determination of the Minimum Fungicidal Concentration (MFC), aliquots from the MIC determination wells were transferred to Petri dishes. The protein fractions with the best antifungal activity were combined with amphotericin B and fluconazole for evaluation of the Fractional Inhibitory Concentration Index (FICI), using the checkerboard method. The interpretation of the index is given by: synergism ( $\leq 0.5$ ), additivity (> 0.5 and  $\leq 1.0$ ), no interaction (> 1 and < 4.0) or antagonism (> 1 and < 4.0). Among the formulations obtained, the ST extract and the T50-75% and T75-100% fractions stand out with MIC50 of 8, 4 and 16  $\mu$ g/mL, respectively, and MIC of 128  $\mu$ g/mL for ST and 256  $\mu$ g/mL for the fractions, but with no MFC found. After analysis of modulation with commercial antifungals, all tested formulations showed synergism, where ST presented a FICI of 0.28 with amphotericin B and 0.38 with fluconazole and both fractions presented 0.27 for both drugs. All formulations showed MFC when combined with amphotericin B, but only the T75-100 with fluconazole. With the results shown, it is possible to infer that after the protein concentration by the Scopes method, the formulations became more effective in terms of MIC<sub>50</sub> and modulatory. In addition, they indicate the potential of *T. toxicaria* formulations as candidates for the study for the development of antifungal adjuvants, as they act synergistically by decreasing the MIC of commercial drugs that have already reported resistance to the tested strain. keywords: Botanical formulations; Mycoses.

Acknowledgment: Cnpq e CAPES

#### G-05. Group I introns from *Cryptococcus*' Mitogenome and Their Association with Species Complex and Antifungal Susceptibility

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Abstract: Group I introns are small RNAs (250-500 nt) capable of catalyzing their own splicing from precursor RNA through two consecutive transesterification reactions. They are present across all three domains of life, particularly in fungi and algae, but are absent in humans, which makes them valuable molecular markers for diagnosing fungal diseases and potential antifungal targets. It is known that certain medications can inhibit the splicing of these intron. Thus, this study determined the minimum inhibitory concentrations (MICs) of pentamidine, 5flucytosine, fluconazole, and amphotericin-B for 51 Cryptococcus neoformans and 25 Cryptococcus gattii. These species complexes are composed by fungal pathogens that present varying quantities of introns within their mitochondrial genes LSU, cob, and cox1. The objective of this work was to investigate potential associations among the following variables: (i) the quantity of group I introns, (ii) the species complex, and (iii) antifungal susceptibility. Using an ordinal logistic model, it was observed that there was a statistically significant correlation between the minimum inhibitory concentration values of fluconazole, 5flucytosine, and pentamidine, and the species complexes. Notably, C. gattii exhibited elevated MICs for fluconazole, whereas C. neoformans showed higher MICs for 5-flucytosine and pentamidine. The presence of introns was linked to susceptibility to amphotericin-B, for which a higher quantity of introns was associated to lower MIC values. In addition, the presence of introns showed varying influences on susceptibility to 5-flucytosine and pentamidine based on species complexes. Concerning 5-flucytosine, introns were associated with reduced susceptibility in C. neoformans and elevated susceptibility in C. gattii. Conversely, the opposite pattern emerged for pentamidine: introns correlated with increased susceptibility in C. neoformans and decreased susceptibility in C. gattii. Our findings uncover disparities in the manners by which these species interact with these drugs and in the repercussions of autocatalytic introns on their mitogenomes. Subsequent research should explore the drugs' impact on in vitro and in vivo splicing of these genetic elements to gain deeper insights into their autocatalytic mechanisms and potential utilization as therapeutic targets.

Acknowledgements: We thank the Coordination for the Improvement of Higher Education (CAPES) for the fellowship and to National Council for Scientific and Technological Development (CNPq) for the financial support (CNPq 305506/2019-6, and 401513/2016-5). key words: autocatalytic RNAs, ribozymes, medical mycology.

#### G-06. EVALUATION OF THE ANTIFUNGAL ACTIVITY OF *Bixa orellana* L. LEAF EXTRACTS AGAINST SPECIES OF *Trichophyton*

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Introduction: Dermatophytoses are mycotic infections produced by dermatophytes. The treatment of these infections is done using topical and/or systemic antifungals; however, the species have been developing resistance to conventional antifungals, which has stimulated the search for alternative treatments based on medicinal plants. Bixa orellana L. is a shrub belonging to the Bixaceae family, best known for its applicability for body painting and protection against insect bites and solar radiation. Studies have shown that its leaves have antifungal and antibacterial activity. Aim: To evaluate the antifungal activity of aqueous (AE), hydroethanolic (HE) and saline (SE) extracts of leaves of *B. orellana* on the growth and viability of Trichophyton tonsurans, Trichophyton rubrum, and Trichophyton mentagrophytes. Material and Methods: Dry powder of B. orellana leaves was homogenized with distilled water (AE), 0.15 M NaCl (SE) or 1:1 (v/v) ethanol:water (HE) to obtain the extracts. The minimum inhibitory concentration (MIC) was determined for each extract. Cell viability was investigated using flow cytometry: cells stained with propidium iodide were considered necrotic and those stained with annexin V were considered apoptotic. Results and Discussion: Only HE showed antifungal action with MIC of 256, 128 and 128 µg/mL for T. tonsurans, T. mentagrophytes and *T. rubrum*, respectively. Cytometry analysis indicated a significantly higher number (p < 0.05) of cells undergoing necrosis and apoptosis than in the negative control. Conclusion: The hydroethanolic extract of B. orellana leaves is a new candidate for antifungal agent against Trichophyton species.

**Keywords**: dermatophytes; plant extracts; achiote. **Acknowledgment**: CNPq, CAPES and FACEPE. G-07. Impact of phenolic compounds on the growth and biofilm of ESKAPE group bacteria <u>De Sá, R.A.Q.C<sup>1</sup></u>.; Dantas, T.F.<sup>1</sup>; Barros, A.V.<sup>1</sup>; Souza, K.S.<sup>1</sup>; Lima, W.L.R.<sup>1</sup>; Silva, M.R.F.<sup>1</sup>; Oliveira, M.B.M<sup>1</sup>.

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Introduction: Infections caused by multidrug-resistant (MDR) and biofilm-forming bacteria have intensified, especially those of the ESKAPE groups (Enterococcus sp., Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter complex). As a result, the WHO has sought to encourage research aimed at combating or reducing infections caused by these microorganisms. The use of natural compounds from plants is currently being sought. One of these compounds is polyphenols, which come from the secondary metabolism of these plants. Some studies have suggested various health benefits, for example, gastroprotection, and photoprotection, as well as antiinflammatory, antioxidant, antimicrobial, and antibiofilm activities. Objective: To carry out a narrative literature review on phenolic compounds and their antimicrobial and antibiofilm action on the ESKAPE group. Material and Methods: The literature review was carried out using the main scientific article searching platforms, such as Science Direct, PUBMED, Scielo, Google Scholar, and Periódicos CAPES. The articles were selected from 2016 to 2023, in English and Portuguese. Results and Discussion: The fundamental chemical structure of phenolic compounds includes at least one aromatic ring connected to one or more hydroxyl groups, primarily produced from the amino acid phenylalanine that is transformed into cinnamic acid. There are mainly two metabolic pathways for phenolic compound biosynthesis: the mevalonic acid route and the shikimic acid pathway. Phenolic compounds comprise flavonoids, tannins, anthocyanins, phenolic acids, stilbenes, coumarins, lignans, and lignins. These compounds are prevalent in fruits, seeds, and vegetables. Some studies have demonstrated that surfaces coated with B-linked proanthocyanidins can minimize the adhesion of S. aureus and E. faecales, while Quercetin, can inhibit growth and biofilm in P. aeruginosa. Similarly, transferulic and caffeic acids have shown growth and biofilm inhibition uo to 75% in K. pneumoniae. Pyrogallol also inhibited growth and biofilm formation in *P. aeruginosa* and *E. faecales*, as well as, cell adhesion and the cell-to-cell signaling mechanism of A. baumannii. Luteolin inhibited approximately 90% of growth and biofilm. Conclusion: The phenolic compounds acted on both ESKAPE group bacteria's growth and biofilm, indicating their potential use in drug production or as ingredients in existing treatments for MDR bacteria infections.

Keywords: Compostos fenólicos, ESKAPE, Biological activities

**Acknowledgements:** To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for funding the research and to the Department of Biochemistry (UFPE) for the support.

#### G-08. Antibacterial and anti-biofilm properties of plant secondary metabolites: A promising approach to microbial control

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Infections caused by antibiotic-resistant microorganisms currently pose a major public health challenge. Plants produce Secondary Metabolites (MS) for their defense, and these compounds possess antibacterial and antibiofilm properties that could potentially combat infections resistant to conventional antibiotics. The diverse chemical makeup of these compounds provides a broad spectrum of microbial targets. Furthermore, their natural origin suggests reduced likelihood of resistance and toxicity. Investing in further research in this area may yield innovative and potent treatments for infectious diseases. The objective of the study was to assess the antibacterial and antibiofilm properties of MS  $\beta$ -ionone and p-cymene, acquired commercially, against clinically relevant Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 23235) strains. To evaluate the antimicrobial efficacy of the metabolites, a modified version of the CLSI (2018) serial dilution technique (4096-64 µg/mL) was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). In addition, the crystal violet fixation technique (0.4%) was used to measure biofilm biomass after administration of different MS concentrations for antibiofilm activity testing. Based on the results,  $\beta$ -ionone showed MIC values of 1024 µg/mL and 2048 µg/mL against E. coli and S. aureus, respectively. In contrast, p-cymene had an MIC of 4096 µg/mL for both bacteria. However, the compounds did not exhibit any MBC or antibiofilm activity. The data indicates the antibacterial potential of the analyzed secondary metabolites, as demonstrated by the MIC values against E. coli and S. aureus. The lack of a minimum inhibitory concentration suggests that the metabolites can impede bacterial growth, but it cannot eliminate the bacteria entirely. Moreover, the absence of antibiofilm activity prompts an inquiry about whether the metabolites can interfere with the growth or dispersal of bacterial biofilms. These outcomes underscore the need to examine the fundamental mechanisms of secondary metabolites and their potential therapeutic use as antibacterial agents.

Key words: antibiotic-resistant infections; clinical application; phytochemistry.

**Acknowledgements:** To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for funding the research and to the Department of Biochemistry (UFPE) for the support.

## G-09. Biomass of the marine yeast *Yarrowia lipolytica* LMS 24B as a hydrocarbon emulsifying agent

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The unconventional yeast Yarrowia lipolytica has been extensively studied. Its versatility and unique characteristics allow for its utilization in many sectors. Some of its peculiarities that make it so interesting include its tolerance to different physicochemical conditions, such as salt, low temperatures, and the ability to withstand acidic and alkaline pHs. Furthermore, this microorganism has been used as a host for products developed through bioprocesses, such as the production of unique proteins, citric acid, lipids, terpenoids, and different enzymes, primarily including proteases, lipases, esterases, and phosphatases. Another notable aspect is its ability to grow on different hydrophobic substrates and produce biosurfactants and emulsifiers. In the last 20 years, many studies on using biomass for different purposes have been reported. Its Generally Recognized as Safe (GRAS) status allows multiple applications related to food, such as animal feed supplements, additives for flavoring and aroma in meats and dairy products, and the production of nutritional elements like essential amino acids, minerals and carotenoids. Additionally, its biomass has also been reported as a metal adsorbent and raw material for biofuel production. Given the aforementioned, this study aimed to evaluate the use of its biomass as an emulsifying agent for different hydrocarbon sources. For this purpose, the biomass was obtained through growth in YPD broth. Initially, a Microbial Adhesion to Hydrocarbons (MATH) assay was performed using xylene and toluene to investigate the hydrophobicity of its cell surface. Subsequently, the Emulsion Index (IE24%) test was conducted using diesel, kerosene, toluene, crude oil, used motor oil, and synthetic lubricating oil to assess its emulsifying capacity. The results indicated high cellular hydrophobicity, with 92.8% (± 0.4) adhesion to xylene and 85.77% (± 6.9) adhesion to toluene. The IE24% varied among the different hydrocarbons evaluated. The highest values were observed with crude oil, synthetic lubricating oil, and toluene (82.7% ± 3.4, 52.9% ± 9, and  $50.8\% \pm 1.4$ , respectively). The lowest values were found with used motor oil ( $39.1\% \pm 0.1$ ) and diesel, which showed no emulsion. The preliminary results validate the biomass capacity of Y. lipolytica as an emulsifying agent for different hydrocarbons. Further tests are required to examine their tolerance to different physicochemical conditions and the impact of different biomass concentrations on emulsifying capacity.

Keywords: GRAS yeast, Biotechnology, bioremediation.

**Acknowledgments:** National Council for Scientific and Technological Development (CNPq); INCT Yeasts: Biodiversity, Preservation, and Biotechnological Innovation.

#### H - Neuroquímica

# H-01. Investigation of the neuroprotective and anti-neuroinflammatory effects and mechanisms of terpene compounds in Amyotrophic Lateral Sclerosis

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**INTRODUCTION:** Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects nerve cells in the brain and spinal cord, causing gradual loss of muscle function. Glial cells, such as astrocytes and microglia, play an important role in promoting and reversing the inflammatory process in the central nervous system. Therapeutic strategies aimed at reducing inflammation in the nervous system have been investigated in the development of neuroprotective drugs, as well as terpenes, natural substances derived from plants. Lupeol and Ursolic Acid are examples of terpenes that have anti-inflammatory and neuroprotective activities, being able to modulate the inflammatory response in the nervous system, stimulating the production of pro-inflammatory mediators and promoting the expression of neurotrophic and anti-inflammatory factors. OBJECTIVES: To investigate the neuroprotective and anti-neuroinflammatory effects and mechanisms of terpene compounds. METHODS: Cultures of PC12 neuronal cells were treated with Lupeol, Ursolic, Xylopic, Caurenoic, Oleanolic and Methyl Caureonate acids in concentrations from 0.1 to 100 µM, and cell viability was determined after 72 h of treatment by the MTT test. RESULTS AND CONCLUSIONS: Most compounds showed a reduction in viability at the highest concentration adopted (100  $\mu$ M), except for cultures exposed to Lupeol, which obtained a greater number of cells that metabolized MTT at a concentration of 100 µM. PC12 cells treated with Caurenoic Acid, Ursolic Acid, Oleanolic Acid and Methyl Careonate had an increase in viability at concentrations from 0.1 to 50  $\mu$ M when compared to the control culture. PC12 cells treated with Xylopic Acid showed an increase in cell viability only at concentrations of 0.1, 1 and 10 µM when compared to the control. These results encourage the continuation of studies in in vitro terpene models and potential application for the therapy of Amyotrophic Lateral Sclerosis. KEYWORDS: Neuroinflammation, Neuroprotection, Anti-inflammatory, Terpenes

SUPPORT: CAPES, PGNeT, INNT

#### H-02. Evaluation of the Anti-inflammatory Activity of the Apigenin Complex with Beta-Cyclodextrins in an *in vitro* Model of Inflammation in Spinal Cord Cells

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**INTRODUCTION**: Spinal cord injury is severe damage to the central nervous system associated with high morbidity and mortality rates, physical dependence, and financial burdens. Inflammation is the main characteristic of the injury that provides a cascade of cellular and molecular events that increase the area of injury, aggravating neurological deficits and tissue recovery. But current therapies are limited. The literature presents different models of studies in the central nervous system in which apigenin has shown beneficial effects. However, there is little research on the neuroprotective role of apigenin in spinal cord injury. **OBJECTIVE:** To evaluate the anti-inflammatory effect of flavonoid apigenin and conjugates of apigenin with β-cyclodextrins in an in vitro inflammation model in spinal cord cells. MATERIALS AND **METHODS**: In this work,  $\beta$ -cyclodextrin and three other derivatives were used. The cytotoxicity of apigenin and apigenin conjugates with  $\beta$ -cyclodextrins was evaluated by the MTT assay in PC-12 cells in a series of concentrations (0.1-100µM) for 24h. The primary spinal cord culture was submitted to chemical injury by LPS and subsequently treated with the best apigenin conjugates. The molecular analysis of the inflammatory profile was analyzed by nitric oxide dosage. The analysis of cell morphology was performed by phase contrast microscopy, Rosenfeld staining, and immunocytochemistry. Cell migration was evaluated by the scratchwound healing assay, followed by treatment with apigenin's for 48h. RESULTS AND DISCUSSION: No cyclodextrin tested alone was toxic to PC-12 cells. Apigenin and conjugates of apigenin with beta-cyclodextrins also showed no toxicity at the tested concentrations. It was observed that three conjugates increased the viability of PC-12 cells, mainly at a concentration of 10µM. Some morphological changes were observed, including in one of the cyclodextrin derivatives that altered the morphology of PC-12 cells to a more neuronal phenotype. Spinal cord cells were subjected to damage by LPS at five  $\mu g/ml$  for 12h. Apigenin conjugates increased cell viability and reversed chemical damage induced by LPS. Regarding the inflammatory profile, apigenin conjugated with cyclodextrin significantly reduced the concentration of sodium nitrite compared to the control. The apigenin conjugate reduced approximately half the number of spinal cord cells that migrated to the injured area compared to the control. CONCLUSIONS: Apigenin conjugates appear to protect the spinal cord cells from neurochemical dysfunctions caused by inflammation in spinal cord injury but do not induce a strong cell migration to the area of the lesion.

**Keywords**: Spinal Cord Injury; Neuroinflammation; Apigenin. **Acknowledgments:** FAPESB, CAPES, UFBA.

#### H-03. Inhibition of glial reactivity by compounds derived from amburana seeds in an in vitro model of inflammatory damage

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Introduction: Amburana cearensis has been widely studied for its antioxidant, antiinflammatory and neuroprotective activities, which modulate glial reactivity. This effect is attributed to coumarins and methyl-esters present in the plant. Objective: This study aims to evaluate the effect of compounds derived from extracts from A. cearensis seeds on the inflammatory damage induced by LPS in primary cultures of glial cells. Material and methods: Primary cultures of glial cells from the cerebral cortex of newborn Wistar rats were performed. The cells were treated with dichloromethane extract (EDAC) or purified coumarin extracted from A. cearensis seeds, with or without lipopolysaccharide from Escherichia coli (LPS). To assess microglial reactivity, immunofluorescence labeling was performed against Iba-1 and CD68, an indicator of a pro-inflammatory profile. Astrocytic reactivity was evaluated by immunofluorescence and western blot using the astrocytic marker GFAP. Results and discussion: Cells exposed only to LPS showed reduced ramification compared to control. In response to the concomitant exposure to LPS and coumarin, there was an increase in microglial ramification relative to cells exposed to LPS and to the control group. However, there was no change in microglial ramification in response to co-exposure to LPS and EDAC. Cells exposed only to LPS showed increased expression of CD68 and CD68 expression was reduced in response to co-exposure to LPS and coumarin, as well as to co-exposure to LPS and EDAC. There was no change in the number of microglial cells between groups, except for a microglial number reduction in response to co-treatment with LPS and EDAC compared to the other groups. Cells exposed to LPS showed increased astrocytic reactivity and concomitant treatment with EDAC and LPS reduced astrocytic reactivity. Conclusions: These data suggest that EDAC and coumarin reduce LPS-induced microglial pro-inflammatory response and astrocytic reactivity. Its anti-inflammatory action mechanisms should be elucidated in further studies to allow the use of coumarin and other A. cearensis derivatives in the treatment or amelioration of neuroinflammatory and neurodegenerative processes.

Keywords: Amburana cearensis, glia, inflammation.

Support: CNPQ, FAPESB and CAPES.

#### H-04. EFFECT OF NICOTINE ON GLIAL CELLS AND PROTECTION AGAINST AMINOCHROME IN PARKINSON'S DISEASE

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Introduction: Parkinson's Disease (PD) is the second most common neurodegenerative disease in the world. However, there is still no effective therapy for curing or prevention of progressive degeneration in the nigrostriatal system. Conventional treatments are based on promoting physiological levels of dopamine and control of symptoms. Some therapies based on neuroprotective action- such as coenzyme Q10 administration have been successful in preclinical studies, but not in clinical trials. This may be related to the mistaken use of preclinical study models, which are not related to the cause of PD. Among the hypotheses that lead to the discovery of the cause of neuronal loss in PD, the neurotoxic effect generated by a molecule derived from dopamine oxidation, aminochrome, has been considered, and it has been used as a model-inducing neurotoxin. On the other hand, nicotine is a compound derived from Nicotiana tabacum, which has been attributed a neuroprotective action and association with a lower risk of developing PD. **Objectives**: In this study, we evaluated the effect of nicotine on the activation of autophagy in astrocytes and its involvement with neuroprotection. Methodology: In midbrain culture, staining with propidium iodide and rosefeld was performed, under treatment conditions of 0.1 and 1  $\mu$ M nicotine and 25  $\mu$ M aminochrome. We used wild-type and transfected U251 human lineage astrocytes submitted to nicotine treatment, for further investigation of the protective effect against aminochrome treatment. The mtt test was performed to evaluate a viability curve in cells treated with nicotine for 24 hours. In addition, analyzes of LC3 and P62 protein expression were performed by Western blot in cells treated with nicotine at a concentration of 0.1  $\mu$ M for 24 hours, and at concentrations of 0.1 and 10  $\mu$ M for a period of 2 h, 4 a.m. and 6 a.m. A viability test was also performed on U251 cells with  $\alpha$ -synuclein suppression and Wild Type treated with an aminochrome curve (1-100 µM) and nicotine (0.1-50 µM) for 48 hours and at concomitant concentrations of nicotine and aminochrome, nicotine 0.1; 1; 10, and aminochrome 50  $\mu$ M and 75 µM for 48h and 72h. Results in this study we observed that in midbrain culture, nicotine protects cells against damage induced by aminochromes. In wild-type U251 cells, it was observed that nicotine concentrations had no toxic effect on the cells, but no static differences were observed in the expression of LC3 and P62 proteins. On the other hand, at U251 with  $\alpha$ -synuclein suppression, it was observed that the highest concentrations of the aminochrome are toxic to the cells. It was observed that in neuroprotection treatments with nicotine and aminochrome, for 48h and 72h, nicotine exerts a protective effect on transfected U251 cells against damage induced by aminochrome. **Conclusion:** In this sense, further studies will be needed to clarify the effect on the expression of autophagy markers in cells subjected to stress condition by treatment with aminochrome. This study is in progress to assess whether autophagy participates in the mechanisms of the neuroprotective action of nicotine in this experimental model of Parkinson's disease.

**Keywords:** Parkinson's disease; astrocytes; nicotine; neuroprotection. Support: CNPQ; Fapesb

#### H-05. NUTRITION AND NEUROCHEMISTRY: A PARTIAL ANALYSIS OF THE ROLE OF TRYPTOPHAN IN DEPRESSIVE DISORDER

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Introduction: The gut-brain axis establishes a relationship between nutrition and neurochemistry in the understanding of mental disorders such as major depressive disorder (MDD), a multifactorial, complex disorder that occupies the 1st place of disability throughout life. The reduction in serotonin levels has been identified as one of the factors associated with the onset of this disorder, and the deficit of tryptophan (TRP), an essential amino acid precursor of serotonin, has been used to analyze serotonin-dependent behavior. **Objective:** To investigate the neurochemical alterations caused by TRP deficit in MDD. Methodology: An integrative literature review is being conducted, exclusively, on randomized clinical trials (RCTs) obtained from the PubMed database, between 2015 and 2023, using the descriptors depression", "tryptophan depletion "tryptophan depletion AND AND mood", "Neurotransmitters and Dietary Intake". From these combinations, 1309 articles were found, among those studies with potential conflicts of interest and studies of other research nature than RCT were eliminated. Thus, this summary presents a partial review of the articles found. **Results and discussion:** The tryptophan absorbed from the diet is converted into serotonin in serotonergic neurons, and although it is also present in vegetables, fruits and legumes, there are not enough studies that demonstrate the direct influence of serotonin from dietary intake. However, the literature already reports the relationship between TRP and the regulation of mood and anxiety, associated with low levels of brain serotonin. Numerous studies have explored the connection between dietary amino acid absorption, neurochemical depletion and depressive behavior. RCTs indicated that DT led to sadness and depressed mood, with p=0.002 compared to non-depletion. Furthermore, when comparing groups with varied TRP intake, notably higher results (p=0.001) on the Hamilton Depression Scale were observed for those with higher daily TD, implying a potential dose-dependent association between TRP and depressive symptomatology. Imaging studies also suggest that increased levels of 5-HT in the brain decrease the activity of brain regions associated with mood and rationality, emphasizing the role of this neurotransmitter in emotional plasticity. Conclusion: The studies show the relationship between nutrition and depression, as well as the importance of a diet rich in amino acids, especially tryptophan, in maintaining the neurochemical balance of aspects related to mental health. However, to better understand the neuronutrition mechanisms related to depression, further investigation is needed.

Keywords: amino acids, dietary intake, mental health.

### H-06. Antioxidant and anti-inflammatory activity of flavonoids in the Central Nervous System

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Introduction: The imbalance in the nervous system between overproduction of reactive species and antioxidant mechanisms can result in reactive astrogliosis. **Objective**: This work evaluated the antioxidant mechanisms and glial inflammatory response with the use of flavonoids. Materials and methods: Antioxidant activity of flavonoids and synthetic derivatives was verified at different concentrations in a cell-free test, determined by the freeradical scavenging reaction 2,2 Diphenyl 1 Picryl hydrazyl (DPPH); In tests with cells, primary cultures of astrocyte-enriched glia derived from newborn Wistar rats (P0-P2) exposed to lipopolysaccharide (LPS,  $1 \mu g/mL/24 h$ ) and treated with the molecules were used and cell morphology after Rosenfeld staining in primary cultures of glial cells enriched from astrocytes after treatments and The antioxidant effect was analyzed through the GSH depletion test; **Results and discussion:** In the DPPH free-radical scavenging reaction, flavonoids and its diprenylated derivative were obtained with greater antioxidant potential and its other prenylated derivatives showed moderate antioxidant activity; An antioxidant effect was observed through the GSH depletion test with monochlorobimane after treatments and maintenance of cell morphology was observed in primary culture of astrocyte-enriched glia. **Conclusions:** These findings indicate that flavonoids and synthesis derivatives can be strong allies as a neuroprotective, anti-inflammatory and antioxidant adjuvant treatment.

**Acknowledgments** to the research promotion agencies in Brazil, the State of Bahia Research Support Foundation - FAPESB, the Coordination for the Improvement of Higher Education Personnel - CAPES and the National Council for Scientific and Technological Development – CNPq.

Keywords: Flavonoids. Neuroinflammatory. Antioxidant.

## H-07. The flavonoids agathisflavone and apigenin previne oligodendrocytes process loss in a model of ischemic damage

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**Background.** The loss of myelin sheath leads to neuronal death causing demyelinating neurodegenerative diseases. One of the causes of oligodendrocytes death and demyelination is ischemic injury. The protective properties of flavonoids agathisflavone and its monomer apigenin were already shown. Both flavonoids reveal important effects upon controlling the inflammation through modulation of neuroiflammatory response of microglia. In addition, flavonoids can be considered as proactive against oxidative damage in hypoxia events due their antioxidant properties. **Objective.** This work aimed to evaluate the protective effects of flavonoids apigenin and bis-apigenin on oligodendrocyte morphology and viability in an ex vivo model of acute ischemia. Methods. In this study cerebellum slices of p8-p12 mice transgenic EGFP reporter to sox-10 gene was used to identify the oligodendrocyte lineage. The flavonoids were administered in a preventive manner 60 minutes before the ischemic damage, which was induced by deprivation of oxygen and glucose for 60 minutes, and the control group was kept in oxygen-glucose normoxia. Results and discussion. It was observed that one hour of oxygen and glucose deprivation did not cause a significant decrease in oligodendrocytes density in the granulocyte layer. Although there was no reduction in oligodendrocytes due to ischemic damage, their morphology was altered by hypoxia. Ischemic damage decreased the proportion of cells with many processes and increased the proportion of cells with few processes that can impact on myelination capacity. Pre-treatment with agathisflavone (10  $\mu$ M) prevented the loss of processes in hypoxia-induced damage. Moreover, pre-treatment with apigenin (10  $\mu$ M and 15  $\mu$ M) increased the proportion of oligodendrocytes with more processes in the slices subjected to the hypoxia-induced damage. Despite the alteration in the morphology of oligodendrocytes induced by the ischemic damage, evidenced by the reduction of processes per cell, it was observed that one hour of oxygen and glucose deprivation was not enough to reduce the population of cells expressing sox-10 in the white matter region, a highly myelinated region. **Conclusion.** This study shows that the flavonoids induce a different response despite their structural morphology (dimer and the respective monomer). Agathisflavone was able to decrease the effect of hypoxia in branching oligodendrocytes, and apigenin response was found to be more effective in this response after the injury.

Keywords. Ischemia, Demyelination, Flavonoids

**Financial support.** FAPESB Bolsa de Doutorado – Cotas, Processo: 084.0508.2020.0001112-18; CAPES Bolsa Doutorado Sanduiche no Exterior – PROGRAMA GERAL DE COOPERAÇÃO INTERNACIONAL – PGCI, Processo: 88881.117666/2016-01

#### H-08. Molecular mechanisms associated with anti-glioma and immunomodulatory effects of flavonoids related to interaction with AHR

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Conventional treatment for GBM includes surgical resection, radiotherapy, and chemotherapy. However, characteristics of the tumor, such as rapid proliferation and induction of immunosuppression, contribute to its poor prognosis and recurrence. In addition, the transcription factor, aryl hydrocarbon receptor (AhR), constitutively activated in tumor cells, is related to chemo and immunoresistance. AhR activation promotes cell differentiation and increases the expression of resistance genes, placing AhR antagonism as a target in cancer chemotherapy. The demonstrated antitumor and immunomodulatory effects of flavonoids point to the pharmacological potential of these drugs in the treatment of glioblastoma (GBM). Flavonoids may act as AhR antagonists and reduce the viability of tumor cells. The aim of this study is to define the antitumor mechanisms of flavonoid naringenin and the possible association of its anti-glioma activity with AhR antagonism. Naringenin was tested as an AhR antagonist at increasing non-cytotoxic concentrations (5-30µM) using the induction of CYP1A1 mediated EROD activity assay in MCF7 cells, as a marker of AhR- responsiveness. Human U87 GBM cells were exposed to naringenin (30µM) in the presence or not of the AhR agonist Indole-3-carbinol. After 24h treatment cell viability was determined by MTT and SRB essays and cell migration was determined until 48h post-treatment. Our results demonstrated that naringenin has potent inhibitory effects on AhR activity. Furthermore, our data revealed that naringenin reduced cell viability and migration in a dose- and time-dependent manner. Additionally, it was demonstrated that the combination of naringenin and AhR agonist increased cytotoxicity to GBM cells. The characterization of the molecular mechanisms of flavonoid naringenin featuring an antagonistic effect on AhR and its role in chemosensitivity will contribute to sustaining its application as an adjuvant for GBM treatments.

Keywords: glioblastoma, naringenin, aryl hydrocarbon receptor Support; CAPES, CNPq, and FAPESB.

#### I - Plantas

#### I-01. OVICIDAL LECTIN FROM *Myracrodruon urundeuva* LEAVES CAUSES CHANGES IN THE STRUCTURE OF *Aedes aegypti* EGGS

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The mosquito Aedes aegypti is the vector of the main arboviruses of public interest. The resistance of this insect to several commonly used insecticides has encouraged the search for natural products for its control. Lectins are proteins that binds reversibly and specifically to carbohydrates. The chitin-binding lectin isolated from the leaf of Myracroduon urundeuva, called MuLL, showed larvicidal activity against A. aegypti. In this study, was investigated whether MuLL can also act as an ovicide against this insect. Powder from *M. urundeuva* leaves was homogenized in 0.15 M NaCl at a proportion of 10% (w/v) for 16 h at 25 °C and then centrifuged (9,000g, 15 min). The leaf extract was treated with ammonium sulfate (0-60 and 60-80% saturation) and the proteins precipitated in the 60-80% step were collected by centrifugation (9,000g, 15 min). The precipitate was dialyzed and then chromatographed on a chitin column previously equilibrated in 0.15 M NaCl. MuLL was recovered from the column with 1.0 M acetic acid and dialyzed. The hemagglutinating activity (HA) and the protein concentration were determined. A. aegypti eggs were incubated with MuLL for 72 h to determine the concentration at which the hatching rate decreases by 50% (EC<sub>50</sub>). The effects of MuLL on the egg surface structure were evaluated using scanning electron microscopy (SEM). MuLL showed HA of 256 and functioned as an ovicidal agent with an  $EC_{50}$  of 0.88 mg/mL. SEM revealed that eggs treated with MuLL for 24 and 48 h no longer had tubercles and showed damaged exochorionic network. After 72 h, surface deformation and degeneration were observed. In conclusion, MuLL can function as an ovicidal agent against A. *aegypti* through damaging the egg surface.

Keywords: chitin; natural insecticide; dengue mosquito.

Acknowledgment: CAPES, CNPq and FACEPE.

#### I-02. Accumulation of organic solutes in forage sorghum cultivars (*Sorghum bicolor* L. Moech) under water stress

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Drought is one of the most important and prevalent stressors for plants in many parts of the world, especially in arid and semi-arid areas. Sorghum (Sorghum bicolor L. Moench), belonging to the Poaceae family, is the fifth most planted cereal in the world, coming after wheat, rice, corn, and barley, being cultivated in Brazil mainly to produce grains and forage. This work aims to evaluate changes in content of soluble sugars and amino acids in leaves that may help in the osmotic adjustment of the plant during periods of water stress. Four forage cultivars from sorghum at seedling stage were submitted to water stress (30% field capacity) for 17 days. For the quantification of total soluble sugars and amino acids, the Dubois method (Phenol- Sulfuric Acid) and the ninhydrin reaction were used, respectively. The Dubois method was used using 10 µL of ethanolic extract (80%) of fresh leaves diluted in 80% ethanol by mixing the sample dilution in 200 µL of 5% Phenol and then in 1 mL of PA Sulfuric Acid and vortex<sup>®</sup> for 20 s and then leaving to cool. After cooling, the reading was performed in a spectrophotometer at 490 nm. The ninhydrin reaction was performed by placing 100 µL of 80% ethanolic extract in 150 μL of 80% ethanol by mixing 250 μL of 0.2M citrate buffer pH 5.0; 250 μL of 0.03 M Ninhydrin and 250 µL of 8.8 mM stannous chloride II. After adding all the reaction reagents, leave it in a water bath at 100°C for 7 minutes and then let it cool and add 3 mL of 3M NaCl and 1 mL of Butyl Alcohol PA and stir vigorously in vortex<sup>®</sup> and leave for 30 minutes for separating the organic and aqueous phases. The organic phase is read in a spectrophotometer at 570 nm. This indicates that some of the sorghum cultivars tried an osmotic adjustment to adapt to the drought situation, with the Catissorgo cultivar showing less alteration and better adaptation to the stress situation. The results indicate the Catissorgo presents an increase in the accumulation of sugars and amino acids as a form of osmotic regulation and adaptation to water stress. Data were subjected to ANOVA and Fischer LSD at 5% significance. The sorghum cultivars osmotically adjust under drought stress, using soluble sugars. Despite the role of sugars, the impact on plant growth can be negative.

Keywords: amino acids, drought, sugars.

Acknowledgment: UFCA.

# I-03. Prospecting the in vivo and in vitro phytochemical and antioxidant effects of ethanolic and aqueous extracts from the seed of *Geoffroea decorticans*) in the Zebrafish (*Danio rerio*) animal model

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The Geoffroea decorticans, commonly known as Chañar, is a plant native to Chile belonging to the Fabaceae family. Its bark, flowers, fruits, and leaves have uses in traditional medicine with known expectorant activity, including bronchopulmonary disorders and pain relief, as well as performing antioxidant and antinociceptive activities. However, there are no studies reporting such activities with Chañar seed extracts. Therefore, this study aimed to evaluate the phytochemical and antioxidant effects, both in vitro and in vivo, of Ethanol (EE) and Aqueous (EA) extracts from Chañar seeds. The assessment of phytochemical compounds was conducted by quantifying the total phenolic content, total flavonoids, and using HPLC. In vitro assays to evaluate antioxidant potential were carried out through five spectrophotometric tests: total antioxidant capacity (TAC), DPPH radical scavenging, reducing power, and copper chelation. As for the *in vivo* antioxidant aspect using Zebrafish, toxicity assays of the samples and ROS staining with DCFH-DA were employed. In the phytochemical characterization, the total phenolic content of EE and EA was 13.977±0.489 and 6.195±0.599 mg GAE/mg of extract, respectively. For total flavonoid quantification, EE and EA showed 0.014±0.002 and 0.035±0.004 mg QE/mg of extract, respectively. HPLC identified the following compounds: Alpha-tocopherol; Phytol; Vitexin; and Rutin. In the evaluation of *in vitro* antioxidant potential, TAC demonstrated that both extracts had antioxidant capacity, 117.13±15.22 and 83.127±3.760 mg AAE/mg for EE and EA, respectively. However, in the DPPH radical scavenging test, EA showed activity above 70%, and EE exhibited 100% activity. In the reducing power assay, EE displayed around 12% activity, whereas EA only showed 3%. Regarding copper chelation capacity, EA chelated about 40%, while EE showed superior potential at 77%. In the assessment of in vivo antioxidant activity through the toxicity assay, neither extract at concentrations of 100 and 250  $\mu$ g/mL was toxic. In the ROS assay, the extracts demonstrated protective behavior, indicating in vivo antioxidant activity. Thus, the obtained results may suggest that the ethanol and aqueous extracts of Chañar seeds could be a potential source of bioactive compounds with antioxidant properties. These findings hold promise for use in alternative medicine as well as for future phytochemical and pharmacological studies. Keywords: Chañar. Antioxidant activity. Extracts. Zebrafish Supported by CAPES, CNPq, UFRN.

# I-04. Citotoxicity and genotoxicity of the lectins from *Myracrodruon urundeuva* in peripheral blood mononuclear cells (PBMCs)

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Lectins are hemagglutinating proteins that recognize glycidic molecules and showed antimicrobial and anticancer activities. Myracrodruon urundeuva is a tree commonly found in northeastern Brazil and traditionally employed by people to treat inflammation and gynecological diseases. Lectins isolated from *M. urundeuva* bark (MuBL), heartwood (MuHL) and leaf (MuLL) showed antimicrobial activity and the present study aim to evaluate the cytotoxic and genotoxic effect of them using peripheral blood mononuclear cells (PBMCs). The lectins were isolated according procedures already defined in the Department of Biochemistry of Federal University of Pernambuco which includes the steps of protein extraction with 0.15 M NaCl, protein precipitation with ammonium sulfate and lectin isolation by chromatography on chitin column. PBMCs were purified from human blood (Ethics committee number: 60.107.916.8.0000.5208), and the cytotoxicity was determined in cell culture through the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) lectins assay. The concentrations that inhibited 50% of the cell viability ( $IC_{50}$ ) were calculated. Genotoxicity was performed through the comet assay and the micronucleus test using lectins non-toxic concentrations. MuBL, MuHL and MuLL showed  $IC_{50}$  on PBMCs of 58.41, 43.19 and 62.28  $\mu$ g/mL, respectively. Comet assay revealed that MuBL (100  $\mu$ g/mL), MuHL and MuLL (50 µg/mL) showed frequency of damage of 33.5%, 35.67% and 65.2%, respectively. On micronucleus test, the number of micronuclei was 7.91, 7.41 and 8.91 to MuBL (100 μg/mL), MuHL and MuLL (50 µg/mL), respectively, and did not differ from the negative control. In conclusion, M. urundeuva lectins were potentially toxic to human lymphocytes, and showed weak genotoxicity.

Acknowledgements: The authors express their gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for financial support.

Keywords: MTT assay. Comet assay. Micronucleus test.

# I-05. Antimicrobial and toxicological activity of *Laguncularia racemosa* from the North Coast of Bahia

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**INTRODUCTION:** In Brazil, Laguncularia racemosa L. is known as white mangrove, a plant with phenolic compounds with antioxidant effects in its leaves that protect plant cells against oxidative stress. This species manages to develop in different environmental conditions and is used in traditional medicine. Therefore, it is crucial to ethnopharmacological studies and identification of the metabolites responsible for their biological and pharmacological properties. However, detecting the toxicity of extracts or substances present in these plants is essential. The lethality test against Artemia salina is a widely used methodology to evaluate the toxic potential of extracts and isolated substances. It is a quick and inexpensive method due to the practicality of handling and cultivating this microcrustacean that forms dormant cysts. **OBJECTIVE:** This study aimed to evaluate the antimicrobial activity and toxicity of crude leaf extracts of L. racemosa. MATERIALS AND METHODS: The crude extracts were prepared by maceration, using 50% ethanol [hydroethanolic] and ethyl acetate as solvents. The identification of chemical groups was carried out through qualitative phytochemical screening. The antimicrobial activity was determined using the broth microdilution assay to assess minimum inhibitory concentration (MIC) against Escherichia coli (ATCC 94863), Pseudomonas aeruginosa (ATCC 27853), Candida albicans (ATCC 18804) and Candida glabrata (ATCC 0728). The toxicity test was carried out using Artemia salina. RESULTS AND DISCUSSION: Saponins, catechins, and condensed tannins were identified in the extract prepared with ethyl acetate. In contrast, the hydroethanolic extract presented flavanols, free tetracyclic triterpenes, hydrosoluble tannins, and saponins. The extracts are considered moderately toxic when the percentage is 50% at a concentration of 100 µg.mL<sup>-1</sup>. In this concentration, the crude extract in ethyl acetate resulted in 90% and the hydroethanolic extract with 70% lethality. The ethyl acetate extract did not affect the growth of fungi strains C. Albicans and C. glabrata and the gram-negative bacteria E. coli but showed a minimum inhibitory concentration and a minimum bactericidal concentration for P. Aeruginosa. The hydroethanolic extract did not have an effect on the bacteria and fungi analyzed in the concentration tested. CONCLUSION: Ethyl acetate extracts of L. Racemosa leaves present biocompounds that may be responsible for the antimicrobial activity against P. aeruginosa bacteria. However, evaluating the concentration to be used is necessary, considering the toxicity test in Artemia salina.

**Keywords:** White mangrove, Ethnopharmacobotany, Medicinal plants, Active principles **Sponsorship:** CAPES, CNPq, FAPESB, FINEP and PERMANECER/UFBA.

#### I-06. Extraction of gDNA of Bauhinia pentandra's leaves by the CTAB method

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Extracting DNA of plants can be quite the task, due to the high concentration of polysaccharides, the presence of both polyphenols and proteins that can compromise downstream applications. The cetyltrimethylammonium bromide (CTAB) method allows a significant removal of such contaminants, especially proteins and non-acidic polysaccharides, with minimal impact in DNA quantity and quality. The cationic detergent CTAB not only helps removing contaminants, but also improves the renaturation rate of complementary DNAs. Both CTAB and high salt concentration stabilizes the double-stranded DNA, reducing overall damage to its integrity. The CTAB (2%) method was used to obtain the genomic DNA (gDNA) of Bauhinia pentandra's leaves, followed by quantification by spectrophotometry in Nanodrop where we obtained concentration and quality parameters (260/280 and 260/230 ratios), then separated in agarose gel 0.7% by electrophoresis stained with GelRed 1x to infer material integrity. The results (n=6) are as follow: concentration (ng/ul): 574.5-5058; [260/280]: 1.118-1.870 and [260/230]: 1.467-2.252), in electrophoresis an intact band was observed in the gDNA resolution range (high molecular weight), at low molecular weight degraded RNA is visible. The CTAB method was effective for gDNA extraction with both acceptable quantity and integrity, although the purification must be revisited since the quality parameters were not ideal. As future perspective, once the gDNA with high quality and high integrity is isolated, it can be used mainly for genome sequencing, phylogenetic studies contributions and gene amplifications.

Acknowledgements: Federal University of Ceará (UFC); The National Council for Scientific and Technological Development (CNPq); Coordination of Superior Level Staff Improvement (*CAPES*); Cearense Foundation for Scientific and Technological Development Support (FUNCAP)

Keywords: extraction; Bauhinia; gDNA

#### I-07. ACUTE TOXICITY AND GENOTOXICITY OF Moringa oleifera LEAF INFUSION

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People from different countries use plants for therapeutic purposes. Moringa oleifera Lam. is a species of the Moringaceae family and is popularly known as the "drumstick tree" or "miracle tree" due to its medicinal properties. M. oleifera leaf has been used by people to treat inflammation, hyperglycemia as well as to weight control. The infusion of leaves has been consumed by the population, but according to National Health Surveillance Agency (ANVISA) there are no studies that ensure the safe use of this preparation. The aim of this study was to determine the in vivo genotoxicity and acute toxicity of *M. oleifera* leaf infusion. Dry leaves (10 g) were added to distilled water (100 mL) heated at 100°C for 15 min and following, the mixture was filtered and the filtrate corresponded to infusion. The acute toxicity assay used female Swiss albino mice and a single dose of the infusion at 2000 mg/kg orally administered. The animals were observed for 1 and 2 hours after treatment and then once a day for 14 days. Body weight, water and feed intake, biochemical and hematological parameters and histological pattern of the organs were determined. Genotoxicity and mutagenicity of the infusion were determined using the comet assay and micronucleus test, respectively. Treated animals did not show behavioral alteration or altered consumption of water and feed. No changes were detected in the biochemical and hematological parameters or in the histology of the organs. Genotoxicity and mutagenicity were not detected. In conclusion, M. oleifera infusion at a dose of 2000 mg/kg did not promote acute toxicity or damage to genetic material and therefore its consumption is safe for people.

Keywords: Medicinal Plant. Safe use. Miracle tree.

Acknowledgment: CAPES, CNPq and FACEPE.

I-08. Effect of indolacetic acid on proline levels in *Urochloa brizantha* cv. Piatã under saline stress

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Forage grasses of the genus Urochloa have great economic importance and have been used as an alternative to increase livestock exploitation in the semi-arid region. Auxin is a very important phytohormone because it acts directly on the development and growth of plants and stimulate tolerance mechanisms to different environmental stresses. Accumulation of the amino acid proline is a common response in several plant species, which plays an essential role in mitigating the harmful effects of stress, acting as an osmoprotector, maintaining the integrity of membranes and eliminating reactive oxygen species. However, it has been proposed that proline will be an indicator of disturbed physiological conditions caused by a phisical stresses, including salinity. Therefore, the objective of the present study was to evaluate the proline contents in the leaves of Urochloa brizantha cv. Piatã treated with indoleacetic acid (IAA), an auxin, and subjected to salt stress. The experiment was carried out in greenhouse and the Plant Biochemistry and Physiology Laboratory of the Center for Agricultural Sciences and Biodiversity, at the Federal University of Cariri (UFCA), Campus Crato. Four hundred Piatã grass seeds were used in 40 cups containing vermiculite, with 10 seeds per cup, for germination during 10 day. Thereafter, the seedlings were transferred to nutrient solution for 20 days. On the 7th and 9th day of cultivation, foliar applications of 50  $\mu$ M IAA were performed with a sprayer and on the 10th day of cultivation salt stress was applied with NaCl at 75 mM. The plants were harvested after 10 days of exposure to stress. Proline contents were analyzed from leaf extracts obtained in sulfosalicylic acid 3% by the reaction method with acid ninhydrin and read in spectrophotometer at 520 nm. From the results obtained, it is possible to verify the increase of foliar levels of proline when the plants were exposed to salinity, however the application of auxin did not influence the leaf content of proline in Piatã grass. It is concluded from this work that the foliar application of 50  $\mu$ M of AIA in piatã grass is not capable of promoting salt stress tolerance mechanisms based on the alteration of proline levels.

Keywords: Auxin; Proline; Salinity.

Acknowledgment: UFCA; CAPES; Funcap.

#### I-09. Influence of indoleacetic acid on the activity of antioxidant enzymes from *Urochloa brizantha* cv. Piatã under salt stress

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The excess of Na<sup>+</sup> and Cl<sup>-</sup> in the root environment difficult the absortion of water and nutrients, the ionic intracelllar accumulation leads to oxidative damage impairing in plant development. Such adversities may be minimized by tolerance mechanisms which can be constitutive or induced. Vegetable treatment with synthetic compounds such as growth regulators it's a low cost method that search to induce tolerance mechanisms in exposure to salinity. The objective of this study is to present the response of the application of indolacetic acid (IAA), an auxin, in antioxidant enzymatic activity and hydrogen peroxide  $(H_2O_2)$  content in the leaves of Urochloa Brizantha cv. Piatã subjected to salt stress. For this, the grass seeds were germinated in vermiculite for 10 days and later transferred to hydroponic cultivation for 20 days, on the 7th and 9th day of cultivation in nutriente solution, the plant leaves were sprayed with IAA 50  $\mu$ M, in the 10th day of cultivation the plants were submited to salinity with NaCl 75 mM. The harvest were performed after 10 day of salt stress application. To measure the enzymes activities, leaves extracts were made using sodium phosphate buffer 50 mM pH 7, EDTA 5 mM. The activities of guaiacol peroxidase (POD) were evaluated using the production of tetraguaiacol, the superoxide dismutase (SOD) enzyme analyzed by the photochemical reduction of nitroblue tetrazolium, and catalase (CAT) activity determined by  $H_2O_2$  consumption. To quantify the levels of  $H_2O_2$ , was used an extraction with TCA 0,1%, following the method of KI oxidation in acid medium. All the methodologies used were analyzed in a spectrophotometer. The results show that the activity of POD and CAT enzymes were not affected by the IAA application or by salt stress, however there was an increase in SOD activity in the grass leaves exposed to salinity. High levels of  $H_2O_2$  were observed in the IAA treatment and in salinity, although the interaction between the hormone and stress decreased the H<sub>2</sub>O<sub>2</sub> content. In the face of what was presented, it is concluded the application of IAA on the leaves of U. brizantha seems to lower oxidative damage by reducing  $H_2O_2$  levels. However, the absence of significant incremente in activity of enzymes responsible for eliminating the molecule, CAT and POD, suggest that there are other mechanisms responsable for the control of the peroxide levels.

Key words: Auxin; salinity; antioxidants.

Acknowledgements: Fundação Cearense de Amparo ao Desenvolvimento Científico e Tecnologico – Funcap; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES; Universidade Federal do Cariri - UFCA

#### I-10. Antimicrobial Potential of *Ricinus communis* L. Seeds against Bacteria of Clinical Relevance.

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**Introduction:** Hospital infections can be caused by bacteria that have developed the ability to resist multiple types of antibiotics, becoming invulnerable to conventional treatments. This phenomenon arises mainly due to the inappropriate and excessive use of antibiotics in humans and animals and the global spread of these resistant agents. The spread of these bacteria directly affects healthcare systems, requiring more intensive and costly approaches to contain the spread of infections. It represents a growing concern for the World Health Organization (WHO), which has implemented strategies such as using natural resources to control bacterial resistance. Objectives: This work aimed to evaluate the antimicrobial potential of Ricinus communis L. seeds against bacteria of clinical interest. Materials and Methods: Seed husk extracts (SHE) and seed extracts without husks (SWHE) of the MPA34 cultivar of *R. communis* were prepared using the maceration method and different solvents (acetone, dichloromethane, and hexane). The antimicrobial activity of the extracts was evaluated against Pseudomonas aeruginosa (ATCC 27853), Enterobacter cloacae (ATCC 70323), Escherichia coli (ATCC 25922), and Staphylococcus aureus (ATCC 29213), and its minimum inhibitory concentration was determined by microdilution in broth. As a positive control, cephalexin was used. Results and Discussion: There was a variation in the inhibition profile between the extracts and microorganisms tested since microorganisms may have different resistance mechanisms. Extracts produced using seed husks and acetone demonstrated activity against all analyzed bacteria: E. coli (MIC = 3.9 mgmL<sup>-1</sup>) and S. aureus (MIC = 15.6 mgmL<sup>-1</sup>), *P. aeruginosa* and *E. cloacae* (MIC = 62.5 mgmL<sup>-1</sup>), this makes this extract more versatile. On the other hand, SWHE in dichloromethane exhibited inhibitory activity only against *P. aeruginosa* (MIC = 31.2 mgmL<sup>-1</sup>) and *E. cloacae* (MIC = 7.8 mgmL<sup>-1</sup>), consistent with the presence of active principles that only act, like certain antibiotics, on the wall of Gramnegative bacteria. However, the hexane extract of SWH did not show activity against the strains used, which could be due to the extraction solvent used. Conclusion: It is concluded that those from the Seed husks are more efficient among the extracts tested. The extracts have in vitro antibacterial potential concerning the strains analyzed, with the possibility that these extracts can be used to search for promising active principles in the treatment of nosocomial infections and for the development of antibacterials.

Keywords: Bacterial Resistance, Castor bean, Medicinal plants

**Acknowledgements:** CAPES, CNPq, FAPESB, FINEP, UFBA, Laboratório de Microbiologia do Hospital Universitário Professor Edgar Santos (HUPES) e ao Laboratório de Microbiologia Aplicada a Biotecnologia e Imunologia da UFBA.

## I-11. Pectinmethylesterase and polygalacturonase activity of cashew peduncle treated at pre-harvest with gibberellin

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**INTRODUCTION**: The cashew peduncle (Anacardium occidentale L.) is highly perishable, which strongly impacts its marketability and is mainly due to a fast softening process. The activity of enzymes pectinmethylesterase (PME) and polygalacturonase (PG) plays an important role in fruit firmness loss as both are involved in the modification of pectin, akey component of fruit cell wall, which influeces the quality and shelf life of the cashew peduncle. OBJECTIVE - The objective of this work was to evaluate the effect of the pre-harvest application of gibberellin (GA3) on the enzymatic activity of the PME and PG on ripe peduncle of the cashew clone CCP76. MATERIAL AND METHODS - Gibberellin (GA3 at doses of 90, 180, 270 and 360 ppm) was applied to cashew at developmental stage 1 with green nut and peduncle corresponding to 33 days after anthesis. Control corresponded to peduncles without GA3 treatment (0 ppm). Fruits were harvested at the point of commercial maturity with dark orange-colored peduncle, evaluated for enzymes activities using a microplate reader and firmness using a penetrometer and results submitted to analysis of variance (ANOVA) followed by Tukey's test (P<0.050). **RESULTS AND DISCUSSION** - The results showed that there was no significant difference for firmness. However, there was a significant difference for PME in all GA3 treatments when compared with control (91918.629 UA mg-1 mg-1 P), with the highest activity at a dose of 180 ppm (141373.685 UA mg-1 mg-1 P) and the lowest occurred at a dose of 90 ppm (37970.974 UA mg-1 mg-1 P) representing an increase in 53% and a decrease in 58%, respectively. PG activity showed a significant difference with an increase of 140% only at 180 ppm (3.098 nmol AR min-1 mg-1 P) when compared to control (1.268 nmol AR min-1 mg-1 P). CONCLUSION - In view of this, even the application of 90 ppm of GA3 in the pre-harvest can be a possible alternative to improve the quality of the cashew tree peduncle, we did not verify any difference in the firmness of the peduncle in any applied dose. However, PG activity increased, which makes us reflect and open up future research on other pectinases and cellulases that act on fruit firmness. ACKNOWLEDGMENTS - Scholarships by Capes, CNPq/INCT-Frutos Tropicais and Embrapa Agroindustria Tropical.

Keywords: growth regulator; postharvest; cashew apple.

### I-12. Sucrose synthase enzyme activity during ripening of climacteric and non-climacteric melon fruits

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**INTRODUCTION** – Melon (*Cucumis melo* L) is a member of the Cucurbitaceae family and plays a significant role in the food industry. This species has a wide variety of lineages. In Brazil, the inodorus (non-climacteric) and cantaloupensis (climacteric) lineages stand out as having the greatest economic importance. These strains differ in several aspects, with variation in the ripening pattern being a crucial variable to be investigated, due to its direct influence on fruit quality and the economic value associated with them. The activity of the enzyme sucrose synthase (SUS) plays a fundamental role in the metabolism of carbohydrates in plants, as it is involved in the synthesis of sucrose into fructose and glucose, two simple sugars that are used as a source of energy or for the production of other compounds. Therefore, through the analysis of the activity of this enzyme, it is possible to identify differences between the two strains mentioned and understand how these differences influence the quality of its fruits. **OBJECTIVE** - The objective of this work is to evaluate the enzymatic activity of SUS in *inodorus* and cantaloupensis melons. MATERIAL AND METHODS – Six fruits were collected at different stages of ripening, taking into account the days after pollination (DAP), with the *inodorus* being harvested at 24, 26, 28, 30, 32 and 34 DAP and the cantaloupensis harvested at DAP 18, 20, 22, 24, 26 and 28. After harvesting, an enzymatic extract was prepared and enzymatic analyzes were performed using a microplate reader. Data were submitted to analysis of variance (ANOVA) followed by Tukey's test (P<0.050) performed with the aid of RStudio software. **RESULTS AND DISCUSSION** – The results revealed that in the *inodorus* strain, a significant difference can be observed between DAP 24 (9004,0 UAE (nmol/min)/mg protein) and 34 (4379,4 UAE (nmol/min)/mg protein), in which the first one presented greater enzymatic activity when compared to the other DAPs. In the cantaloupensis variety, there was also a difference between the first (2094,0 UAE (nmol/min)/mg protein) and the last DAP (4862,6 UAE (nmol/min)/mg protein). However, contrary to what was found in *inodorus*, there was greater enzymatic activity in DAP 28. CONCLUSION - It is concluded that during the fruit maturation process of the *inodorus* lineage there is a decrease in the enzymatic activity of the SUS, while in the cantaloupensis lineage we can observe an increasing activity of this enzyme during the fruit ripening process. **ACKNOWLEDGMENTS** – CNPq and Embrapa. Keywords: Cucumis melo L.; postharvest; enzymes.

#### I-13. Production and characterization of proteases from yeasts isolated from the Caatinga Nunes, J.A.<sup>1</sup>; Nascimento, B.E.S<sup>2</sup>; Landell, M.F.<sup>2</sup>; Pereira, H.J.V.<sup>1</sup>

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Introduction: The Caatinga, the predominant phytogeographic domain of the Brazilian Semiarid Region, harbors vast plant diversity and a significant number of endemic species. Among the plant groups found in this biome, bromeliads stand out. Their wide morphological variation and functional adaptations create a conducive environment for the colonization of various species, including filamentous fungi and yeasts. Yeasts, in particular, have diverse biotechnological applications, primarily through the production and characterization of enzymes used in industries such as paper, textiles, energy, animal feed, and food production. Specifically, the use of proteases isolated from microorganisms, such as yeast, has been extensively studied, especially in milk coagulation for cheese production within the food industry. Microbial proteases find applications in several corporate sectors and account for approximately 40% of all commercialized enzymes. Objective: Our study aimed to produce proteases from yeast species isolated from bromeliad leaves in the Tocaia Reserve, located in the municipality of Santana do Ipanema, within the Caatinga domain in the state of Alagoas. materials and methods: We initially conducted screening for protease activity, leading to the identification and selection of two yeast strains capable of producing these enzymes. These yeasts were subjected to submerged fermentation, and subsequently, crude protein extracts were obtained, with their proteolytic activities investigated through spectrophotometric methods using specific enzymatic substrates. Results and discussion: The activity was tested against BApNA, N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, and N-succinyl-Ala-Ala-Pro-Leu-pnitroanilide. To further characterize the proteolytic activities, a panel with various serine protease inhibitors was employed. We optimized the enzyme production timeline and, following incubation for the period of maximum protease production, extracted and applied these enzymes in milk coagulation assays to measure caseinolytic activity. This study underscores the potential of these yeast strains as valuable sources for the production of industrially significant proteases. **Conclusions:** It marks the first instance of the production and characterization of proteases in yeasts recently isolated from the Caatinga. Furthermore, this work contributes to advancing our understanding of microbial biodiversity within the Caatinga, particularly in the semi-arid region of Alagoas. Acknowledgments: The authors are grateful to CAPES, CNPq, and FAPEAL for funding this research. Keywords: cheese, milk, serine protease.

#### J - Produtos Naturais

J-01. Secondary metabolites quantification and antioxidant activity of *Cenostigma microphyllum* (Mart. ex G.Don) Gagnon & G.P.Lewis leaves cholform extract fraction <u>Ribas, A.R.</u><sup>1</sup>; Silva, A.P.S. da<sup>1,2;</sup> Barros, M.D. de<sup>1</sup>; Barbosa, F.P.<sup>1</sup>; Silva, M.V. da<sup>1</sup> Lima, V.L. de M.<sup>1</sup>;

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Introduction Oxidative stress is the basis of a diverse group of conditions and diseases, particularly oxygen reactive species plays a major role on it. Cenostigma microphyllum belongs to Fabaceae family, it is a plant popularly known as catingueira rasteira. Brazilian geographical distribution is restricted to the northeast side of the country, occupying caatinga phytogeographic domain. Aim To quantify secondary metabolites and evaluate the antioxidant activity of C. microphyllum leaves chloroform extract fraction. Materials and methods Sample of C. microphyllum was deposited at IPA, under registration 84,888, leaves were collected in the Catimbau National Park, in the city of Buíque, Pernambuco, Brazil. To obtain the chloroform extract, cyclohexane was used (for wax removal), and then chloroform, using the Soxhlet equipment, with subsequent rotary evaporation of the solvent to obtain the crude extract. For phenolic compounds and tannins quantification, a methodology of reaction with Folin-Ciocalteu and sodium carbonate was used, gallic and tannic acid and was used as standard, respectively. Quantification of flavonoids followed the methodology of reaction with ferric chloride, and quercetin was the standard. The antioxidant analysis followed the methods of scavenging radicals DPPH and ABTS+, and the evaluation of total antioxidant capacity (TAC) by the phosphomolybdenum method. Results and discussion Chloroform extract showed a yield of almost 1 %. Then, a phytochemical screening confirmed the presence of flavonoids in the extract, so it was subjected to a column of silica gel 60 eluted with hexane, ethyl acetate and methanol, obtaining thirteen fractions. Then, the fractions were run on thin layer chromatography, which resulted in five final fractions. Of these five fractions derived from chloroform extract, the fraction with the highest yield, called FIII, was subjected to quantification of secondary metabolites and antioxidant activity. FIII presented 70.03 ± 0.02 mg of gallic acid equivalent/g of extract of phenolic compounds, 71.03 ± 0.02 mg of tannic acid equivalent/g of tannins extract, and 62.13 ± 0.08 mg of quercetin equivalent/g of flavonoid extract. FIII showed a reduction of 56.20 ± 3.71 % at the 0.5 mg/mL concentration, and 67.57 % ± 1.7 at the 1 mg/mL concentration in the DPPH radical. TAC was 63.46 % ± 1.74, and the ABTS showed a reduction of 8.61± 0.01% in 1 hour (1mg/ml). Conclusion The FIII of the chloroform extract of C. microphyllum leaves showed a significant number of phenolic compounds and flavonoids, with moderate antioxidant activity by electron scavenging. Acknowledgements: CAPES, FACEPE, CNPq, LAB-DPN, and UFPE Keywords: Cenostigma microphyllum, Secondary metabolites, Antioxidant

#### J-02. Characterization of lectin activity in protein preparations from *Antigonon leptopus* Hook. & Arn.

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Lectins are a class of proteins with the ability to bind to free carbohydrates or to glycoconjugates in a specific and reversible way, without altering the structure of these glycans. The biological effects triggered by interaction with carbohydrates make these proteins excellent biotechnological tools. Antigonon leptopus is a plant species native from Mexico which occurs especially in tropical regions. In Brazil, it is widely used as an ornamental plant, in beekeeping and meliponiculture. Although it has biological properties, its bioactive composition has been little investigated. The aim of this study was to bioprospect lectins in A. leptopus. Crude extracts of seeds (CES) and leaves (CEL) were prepared in 0.15 M NaCl solution. The extracts were subjected to protein fractionation by saturation with ammonium sulfate, followed by centrifugation and dialysis of the obtained protein fractions (F1S and F2S, by saline saturation of EBS at 0-60 and 60-90 %, respectively; F1L, F2L and F3L, by saline saturation of EBL at 30, 30-60 and 60-90 %, respectively). The samples were characterized by haemagglutinating activity (HA) assays with human erythrocytes (ABO system), protein dosage, determination of specific haemagglutinating activity (SHA), HA inhibition assays with carbohydrates, and HA assays under temperature or pH variation. The preparations of both plant tissues showed high titers of HA (with different erythrocytes) and protein content, revealing high SHA, especially F2S and F3L (CES, SHA: 313.23; F1S, SHA: 995.02; F2S, SHA: 1,442.76; CEL, SHA: 107.16; F1L, SHA: 1,356.29; F2L, SHA: 339.52; F3L, SHA: 1,811.18). Therefore, these preparations were selected for further HA characterization assays. F2S and F3L HA titers were partially inhibited by D-glucose, D-mannose and D-galactose. F2S showed high HA in a wide range of temperature (30 to 100 °C) or pH (4.5 to 9), with higher HA between pH 5.5 to 7.5 and a decrease in HA in alkaline pH or at high temperature. F3L maintained a high HA after incubation at 100 °C or at alkaline pH (8, 8.5, 9), with an increase in HA corresponding to an increase in temperature or pH. The results suggest the presence of lectins in A. leptopus, with characteristics of thermostability and stability to pH variation. Acknowledgments: UFERSA.

Keywords: Amor-agarradinho; phytohemagglutinin; lectin activity.

#### J-03. DERMAL TOXICITY AND WOUND HEALING ACTIVITY OF WATER-SOLUBLE LECTIN FROM *Moringa oleifera* SEEDS (WSMoL)

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Lectins are hemagglutinating proteins that reversibly bind to carbohydrates. Moringa oleifera seeds contain a water-soluble lectin (WSMoL) with immunomodulatory and antiinflammatory activities. Natural compounds have been described as healing agents and thus, with potential therapeutic use. The present work evaluated WSMoL for dermal toxicity and healing activity. Female Swiss albino mice were used for the tests.For dermal toxicity assay the dorsal surface of the mice was clean-shaved by 24 h before the start of treatment. After, the animals (N= 5) were kept in separate cages and the shaved areas were treated with 0.15 M NaCl (negative control, NC) or WSMoL at 10, 25 and 50 mg/kg. After the topical application, the animals were observed daily for 14 days for lacrimation, respiratory changes, seizures, behavior and mortality. At the end of the experimental period, biological samples were collected and analized. For wound healing assay, a 1 cm<sup>2</sup> lesion was performed on the dorsal surface of the mice. Following, 0.15 M NaCl (NC), 3 U collagenase (positive control, PC) or WSMoL at concentrations of 10, 25 or 50 mg/kg were topically applied on the wounded area for 10 days. During the experimental period, the lesions were evaluated daily, for the presence of edema, scabies, hyperemia, granulation and scar tissue. Also, the diameter of the lesions was measured daily. WSMoL at all tested concentrations did not show any signs of dermal toxicity. WSMoL at 10, 25 and 50 mg/kg was a healing agent promoting wound contractions of 100%, 87% and 78%, respectively. The rate of wound contractions determined in NC and PC were 55% and 100%, respectively. The data revealed that WSMoL is safe to use topically and has healing activity.

**Keywords**: Protein. Topical application. Tissue injury. **Acknowledgment:** CAPES, CNPq and FACEPE.

### J-04. Evaluation of Acute Toxicity and Genotoxicity of the Lectin from *Schinus terebinthifolia* Raddi. Leaves (SteLL) in Mice

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**Introduction:** Phytotherapy has been utilized as a complementary and alternative approach to conventional treatments of many diseases. However, studies have revealed that certain plant derivatives may induce undesirable adverse reactions, such as carcinogenicity and teratogenicity, as well as be life-threatening. Hence, plant-derived compounds need rigorous testing to assess their toxicity or safety before formulating and marketing them as phytopharmaceuticals. Schinus terebinthifolia Raddi (Anacardiaceae) has been widely used in folk medicine. A lectin (carbohydrate-binding protein) was isolated from S. terebinthifolia leaves and named SteLL. Aim: To assess the acute toxicity and genotoxicity of SteLL in Swiss female mice (*Mus musculus*). Materials and methods: The acute toxicity of SteLL (100 mg/kg) was assessed via a single intraperitoneal (i.p.) or oral (v.o.) administration, followed by daily monitoring of mortality, signs of intoxication, water and feed consumption, and changes in animal weight for 14 days. Also hematological, biochemical, and histological parameters were measured. Genotoxicity was evaluated through comet and micronucleus assays. Approved by the Ethics Committee on Animal Use of UFPE (process no. 0025/2022). Results and discussion: No animal mortality and no signs of toxicity were observed. Additionally, there were no significant changes in food intake or water consumption, animal weight variation, hematological and biochemical parameters. Histological examination revealed normal organ conditions for both routes of administration. Furthermore, the comet assay results were comparable to those of negative control group, with most nucleoids classified in class 0, indicating the absence of damage. Moreover, there was no observed increase in the count of micronucleated polychromatic erythrocytes for treatments with SteLL. These analyses are essential to establish the necessary scientific foundation for the use of SteLL in therapeutic applications and to ensure that the benefits outweigh any potential risks. **Conclusions:** This study demonstrates that SteLL at 100 mg/kg did not induce toxicity by intraperitoneal or oral treatment in mice, as well as did not cause DNA damage.

Keywords: Brazilian pepper tree, lectin, toxicity.

Acknowledgements: CNPq, CAPES and FACEPE.

## J-05. Phytochemistry and antifungal activity evaluation of ethyl acetate extract from Mimosa acutistipula leaves to *Sporothix brasiliensis* and *Sporothix schenckii* strains

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Introduction: Sporotrichosis is a chronic granulomatous disease caused by the dimorphic fungus of the genus Sporothix. Epidemiological data indicated 12,636 cases in the last 10 years, with 87.45 % of occurrence in South America, being an emerging disease in need of new therapeutic alternatives, due to the small therapeutic range of available drugs. Mimosa acutistipula has antioxidant, anti-inflammatory, and analgesic potential, and reported to have phenolic compounds such as tannins and flavonoids, which may act in the treatment of sporotrichosis. Aim: To quantify secondary metabolites and evaluate the antifungal activity of ethyl acetate extract from Mimosa acutistipula leaves against S. brasiliensis and S. schenckii. Materials and methods: Leaves of M. acutistipula were collected at Sítio Serra dos Bois, Taquaritinga do Norte-PE, Brazil, and identified by the Agronomic Institute of Pernambuco, 93061. The leaves were submitted to hot extraction in Soxhlet equipment, with solvents of increasing polarity. For total determination of phenolic compounds and tannins, the reaction to ferric chloride was followed; flavonoids according to the acidified acetate method; The standards corresponded respectively to gallic acid, tannic acid and rutin. The evaluation of antifungal activity followed the guidelines of the Clinical Laboratory Standards Institute. Results and Discussion: Cyclohexane extract showed 11.674 ± 0.02 mg of gallic acid equivalent/g of extract in the total quantification of phenolic compounds (mgEAG/g), with 0.000 ± 0.01 mg of tannic acid equivalent/g of extract (mgEAT/g) and 178.676 ± 0.09 mg of rutin equivalent/g of extract (mgER/g); Chloroform showed 79.452 ± 0.06 mgEAG/g, 15.513 ± 0.02 mgEAT/g and 233.056 ± 0.03 mgER/g; ethyl acetate showed 292.563 ± 0.02 mgEAG/g, 95.535 ± 0.09 mgEAT/g and 95.692 ± 0.02 mgER/g; while the methanolic extract showed 276.748 ± 0.02 mgEAG/g, 81.852 ± 0.20 mgEAT/g and 49.839 ± 0.01 mgER/g. The ethyl acetate extract, due to its higher concentration of phenolic compounds, was followed for evaluation of the atifungal activity. Against S. brasiliensis (URM7969) it showed a MIC of 64 µg/mL and CFM of 128 µg/mL, also for S. brasiliensis (URM8077) MIC corresponding to 16 µg/mL and CFM of 32 µg/mL. Regarding S. schenckii (URM8080) the value of MIC and CFM for both was 8 µg/mL. Conclusion: The ethyl acetate extract showed higher concentrations of phenolic compounds extracted from the leaves of *M. acutistipula* and also presented significant activity against agents that cause sporotrichosis, thus it may become a future alternative for the treatment of this neglected disease.

Acknowledgements: CNPq, CAPES, FACEPE, LABDPN, Laboratório de Micologia médica Sylvio Campos

Keywords: Mimosa acutistipula; Sporotrichosis; Medicinal plants

### J-06. Clinical and Laboratory Changes in COVID-19 Patients and correlation with cytotoxic proteins

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The new coronavirus (SARS-CoV-2) sparked a global respiratory disease outbreak resulting in a 2020 public health emergency declaration by the World Health Organization (WHO). While outcomes varied among individuals, it resulted in a significant global death toll. This study investigates clinical and laboratory changes in COVID-19 patients and their correlation with cytotoxic proteins. It's an observational study with 75 participants: 57 from the University Hospital Professor Alberto Antunes of Federal University of Alagoas (HUPAA/UFAL) (COVID-19 group) and 19 donors (Control group), approved by UFAL's Ethics Committee. Hematological and biochemical analyses were conducted. Cytokines analysis utilized the LEGENDplexTM Human CD8/NK Panel and Spearman correlation tests were applied to assess laboratory parameters and cytotoxic response protein levels. Hematocrit values significantly decreased in the COVID-19 group, including ICU patients ( $p \le 0.05$ ) and those in the ward ( $p \le 0.0001$ ). In the leukogram, COVID-19 patients exhibited leukocytosis, lymphopenia, and neutrophilia, regardless of ICU admission (leukocytes:  $p \le 0.001$ ; lymphocytes:  $p \le 0.001$ ; neutrophils:  $p \le$ 0.0001) or ward admission (leukocytes:  $p \le 0.05$ ; lymphocytes:  $p \le 0.01$ ; neutrophils:  $p \le$ 0.001). Elevated ferritin and C-reactive protein (CRP) levels were observed in COVID-19 patients, both in the ICU (ferritin:  $p \le 0.0001$ ; CRP:  $p \le 0.0001$ ) and the ward (ferritin:  $p \le 0.001$ ; CRP:  $p \le 0.0001$ ). Increased urea and glucose levels were observed exclusively in the ICU COVID-19 group (both  $p \le 0.05$ ), while triglyceride levels increased solely in the ward group (p  $\leq$  0.05). GNLY and GzmA displayed negative correlations with red blood cell count (GNLY: p = 0.002; GzmA: p = 0.008) and hematocrit percentage (GNLY: p = 0.002; GzmA: p = 0.005). PFN and GzmB levels showed a negative correlation with total leukocyte count (PFN: p = 0.025; GzmB: p = 0.021) and neutrophils (PFN: p = 0.019; GzmB: p = 0.007). Additionally, GzmA showed a similar negative correlation with neutrophils (p = 0.038), while sFasL exhibited a directly proportional correlation with platelet count (p = 0.031). Among the biochemical parameters, only PFN, GNLY, and GzmA displayed negative correlations. PFN and GzmA correlated with CPK and total leukocyte levels (PFN: p = 0.003; GzmA: p = 0.016), while GNLY correlated with glucose levels (p = 0.010). Hence, elevated GzmA and GzmB plasma levels in COVID-19 patients, without concurrent PFN and GNLY increases, imply a significant extracellular function. Adittionally GzmA and GzmB may have a role in COVID-19's pathophysiology.

**Agradecimentos**: Cell Biology Laboratory, Funding Support (CNPq, CAPES, and FAPEAL). **Keywords**: COVID-19; Immune System; Granzymes.

#### J-07. INSECTICIDAL ACTIVITY OF THE FIXED OIL FROM *Syagrus coronata* (Mart.) Becc. SEEDS AGAINST *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera, Curculionidae) Bezerra, A.A.<sup>1</sup>; Santos, P.E.M.<sup>1</sup>; Vilarim, Q.H.S.P.A.<sup>1</sup>; Silva, J.N.O.<sup>1</sup>; Santos, E.V.S.<sup>1</sup>; Tavares,

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Sitophilus zeamais, popularly known as maize weevil, is one of the main primary pests of cereals and stored grains in the world. Synthetic insecticides are the main means of controlling this pest. However, their toxicity poses risks to humans, animals, and ecosystems. In addition, the reports on resistant population of S. zeamais are increasing. Thus, the identification of natural insecticides has been welcome. The species Syagrus coronata is a palm tree with high socio-environmental value, being its oil used to treat illnesses such as mycosis, back pain, eye inflammation and wounds. Also, larvicidal activity of the S. coronata oil was reported against Aedes aegypti. The objective of this work was to evaluate the insecticidal action of the fixed oil from S. coronata seeds on S. zeamais through contact toxicity assay. For the contact toxicity test, different volumes (28.8; 36.9; 44.3; 51.7; 59.7; 124 µL) of the oil were placed in Petri dishes containing 20 g of maize grains. Plates were shaken and infested with twenty unsexed S. zeamais adults, being mortality determined after 24 and 48 h. Lethal concentrations required to kill 50% and 90% of the insects ( $LC_{50}$  and  $LC_{90}$ ) were determined by probit analysis. The residual effect was evaluated by simulating the storage conditions of the grain for 1, 5, 10, 15 and 20 days, in Petri dishes infested with the insect. Next, lethal concentration of the oil was added to the dishes. The oil showed contact toxicity with  $LC_{50}$  and  $LC_{90}$  of 59.75  $\mu$ L and 124.15 µL, respectively. For the residual effect, only 24 hours after application of the fixed oil of S. coronata, the insect died, that is, the oil had no residual effect on the grain. The results obtained in this study show that the fixed oil of S. coronata can be studied more deeply as an alternative to synthetic insecticides in the control of *S. zeamais*. Acknowledgments: CAPES; CNPq; FACEPE and MCTI.

Key words: Licuri; botanical insecticides; maize weevil.

#### J-08. Identification, Purification and Characterization of a Lectin from Leaves of *Jatropha multifida* L. (Malpighiales: Euphorbiaceae)

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Introduction: Various tissues of the Jatropha multifida, commonly known by the name 'merthiolate', are utilized in traditional folk medicine. The medicinal properties of this plant could be linked to both secondary and primary metabolites. Among these primary metabolites, lectins are proteins capable of binding to carbohydrates and exhibiting diverse biological activities. The objective of the current study was to identify, isolate, and characterize a lectin extracted from the leaves of J. multifida. Materials and methods: Proteins were extracted from leaf powder through homogenization (16 h, 4 °C) using Tris - HCl 50 mM at pH 8.0. This extraction exhibited a specific hemagglutinating activity (AHE) of 162.44 and a protein concentration of 3.15 mg/mL. The obtained extract underwent treatment with ammonium sulfate at various concentrations to separate proteins into fractions. . Among these, the 0-20% fraction displayed AHE (556.98) activity. This 0-20% fraction was subjected to chromatography on a chitin column equilibrated with 50 mM Tris-HCl at pH 8.0. Fractions of 2 mL were collected and assessed for absorbance at 280 nm and AH. The active protein peak obtained using a 0.5 M acetic acid eluent was pooled and dialyzed against 50 mM Tris HCl at pH 8.0 for 6 hours. To assess lectin purity and determine its apparent molecular mass, the sample was subjected to electrophoresis on a 10% polyacrylamide gel under denaturing conditions, utilizing sodium dodecyl sulfate, both in the presence and absence of a reducing agent. Results and Discussion: A lectin from J. multifida leaves (referred to as JamuLL) was isolated using a single chromatographic step, yielding milligram quantities (2.5 mg; AHE: 10,240). Protein and phenol quantification was conducted at every purification stage, revealing that the purification process removed phenols present in the extract and fractions. Lectin characterization demonstrated its hemagglutinating activity, which was partially inhibited by casein, yet remained unaffected by divalent ions (Ca<sup>2+</sup> and Mg<sup>2+</sup>) as well as EDTA. JamuLL is categorized as a thermostable lectin, retaining activity from 30°C to 100°C over 60 minutes, and within the pH range of 5.0 to 8.0. It exhibited optimal activity at acidic pH levels. Under non-reducing and reducing conditions, a solitary protein band was evident, with an apparent molecular mass of 56 kDa. Conclusion: In this study, a lectin was successfully isolated from a medicinal plant, showcasing promising biotechnological potential.

Financial Support: FAPEAL, CNPq.

Acknowledgement: SEDUC-AL

Keywords: Merthiolate, medicinal plant, protein.

#### J-09. Anxiolytic Activity of *Schinus terebinthifolia* Leaf Lectin

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Introduction: Anxiety disorders have a far-reaching impact on various segments of society, resulting in a substantial economic burden. Current pharmacotherapy, although beneficial for some individuals, lacks efficiency for others and often comes with significant side effects and the potential for resistance due to prolonged use. In the quest for more effective treatments, researchers are exploring new possibilities in drug development daily. In recent years, there has been growing interest in the potential of natural products to address neuropsychological disorders. Plant lectins, which are carbohydrate-binding proteins, have come under scrutiny for their therapeutic potential. One such lectin is found in *Schinus terebinthifolia* leaves and is known as SteLL. This lectin exhibits a range of activities, including antimicrobial, antiangiogenic, and analgesic properties, making it a promising candidate for the treatment of neuropsychological disorders. Objective: In this study, we investigated the anxiolytic effect of SteLL in an in vivo mice model. Materials and Methods: SteLL was isolated by chromatography of saline leaf extract on a chitin column. SteLL (1, 2 and 4 mg/kg, i.p.) was administered to mice and the open field (OF) and elevated plus maze (EPM) tests were performed. It was also evaluated whether SteLL effect was dependent on the carbohydrate recognition domain, in addition to determining the involvement of monoaminergic signaling in the anxiolytic activity. Results and Discussion: In OF test, SteLL (1, 2 and 4 mg/kg) did not interfere with the number of crossings, but significantly reduced the number of rearings. In EPM, SteLL 4 mg/kg and the combination of SteLL 1 mg/kg plus diazepam (1 mg/kg, i.p) significantly increased time spent in open arms and reduced time spent in closed arms. The effect of SteLL does not appear to be dependent on the carbohydrate-binding domain of the lectin since pre-incubation of lectin with casein did not prevent the anxiolytic action. Further, the effect of SteLL on EPM was inhibited by pretreatment with antagonists of the  $\alpha$ 2adrenoceptor, 5-HT2A/2C receptor and dopamine D1 receptor. Conclusion: Our results suggest that SteLL has anxiolytic activity, which is dependent on the monoaminergic signaling cascade.

**KEYWORDS:** anxiety; lectin; animal model.

ACKNOWLEDGMENTS: FACEPE, CAPES and CNPq.

### J-10. Evaluation of the effect of *p*-coumaric acid on thymocytes submitted to *in vitro* senescence with D-galactose

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Introduction: Senescence is a process that directly affects the immune system during aging, contributing to the development of pathologies in different tissues of the body, especially in the elderly. In this sense, products with antioxidant activity such as the p-coumaric acid (pCA) can be useful in combating these alterations. Thus, this work aimed to evaluate the effect of pCA on thymocytes treated with D-galactose (D-gal) in vitro, specifically analyzing the cell viability, the thymocyte subpopulations and adhesion to thymic epithelial cells (TECs). Methods: For this, thymocytes obtained from C57BL/6 mice (CEUAs 47/2016; 19/2019) were treated in vitro with D-gal (0.1, 1, 10 and 20 mg/mL) or pCA (0.1, 1, 5, 10 or 100  $\mu$ M) to test cell viability by the method of capturing propidium iodide (PI). After verification of viability, co-treatment with D-gal (20 mg/mL) and pCA (5 µM) for 24 hours or pre-treatment with pCA (5  $\mu$ M) for one hour was performed to analyze cell viability, immunophenotyping by flow cytometry and heterocellular adhesion using TECs of the 2BH4 cell line. Results and discussion: It was possible to observe that D-gal at the highest concentration decreased the viability of thymocytes, and also decreased mainly the double-positive subpopulation for CD4 and CD8, and pretreatment with pCA significantly reversed this effect. Regarding adhesion, treatment with D-gal for 24 hours diminished the adhesion capacity of thymocytes compared to control and pretreatment with pCA significantly prevented this decrease. Conclusion: Thus, it can be concluded that a high concentration of D-gal affects the survival of thymocytes and the double-positive subpopulation and that pretreatment with p-coumaric acid may have a protective effect on thymocytes exposed to D-gal. It was also observed that treatment with D-galactose impairs thymocyte adhesion and that pCA was shown to have a beneficial effect on adhesion.

Keywords: Senescence, Thymocytes, Natural products.

#### J-11. Metabolic effects of propolis from *Melipona quadrifasciata* and *M. bicolor schenki* on the glycemic and lipid profile, insulin resistance and markers of oxidative stress

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Introduction: There are no studies about the metabolic activities of propolis from native stingless bees (SB). Objectives: to evaluate the metabolic effects of propolis from Melipona quadrifasciata and M. bicolor schenki on the glycemic and lipid profile, insulin resistance and markers of oxidative stress, compared to Apis mellifera propolis. Material and Methods: Propolis extracts from M. quadrifasciata, M. bicolor schenki and Apis mellifera were chemically evaluated for their composition of total phenolics, flavonoids and terpenes and had their identifiable compounds detected by gas chromatography coupled to mass spectrometry (GC -MS). The research was carried out with 94 participants between 20 and 80 years old with or without a diagnosis of previous metabolic disease, with a duration of 90 days. Participants were randomized and divided into a control group (n = 33); two groups that received 200 mg/day of the dry extract of SB propolis, being *M. quadrifasciata* (G1; n = 11), *M. bicolor* (G2; n = 4); and a group of that received 100 mg/day of a commercial *A. mellifera* green propolis dry extract (G3; n = 46). Results and Discussion: Regardless of the type of propolis ingested, we observed in the study population a decrease in total cholesterol and TBARS levels, and an increase in fasting insulin levels, HOMA2-IR, HOMA2-β and total sulfhydryl content. More specifically with propolis from *M. quadrifasciata* there was an increase in the level of total sulfhydryl content. With propolis from M. bicolor there was a decrease in the level of TBARS. With propolis from A. mellifera there was an increase in insulin levels, HOMA2-IR, HOMA2- $\beta$  and total sulfhydryl content. The compounds that most contributed to the variability of the results were guaiol, ferruginol, thunbergol, lupeol acetate, dotriacontane and eicosane. Conclusion: This is the first study on the chemical composition and demonstrating the metabolic activity of *M. bicolor schenki* propolis and the first study on the metabolic activity of propolis from *M. quadrifasciata*.

Key-words: Atherogenesis, Diabetes Mellitus, Stingless Bees.

#### J-12. Phytotoxicity of methanolic extract of leaves of *Cenostigma microphyllum* (Mart. ex G.Don) Gagnon & amp; G.P.Lewis

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Introduction Concern about toxicity and the environmental risk associated with the increased availability of plant-derived products are currently hotly debated. Cenostigma microphyllum is a plant that belongs to the Caatinga domain, but little is known about the ecological interaction possibilities of its products, commonly used in folk medicine. Objective To evaluate the phytotoxicity of the methanolic extract of *Cenostigma microphyllum* leaves. Materials and methods The phytotoxicity study was conducted with 6-well microplates, with filter paper discs. The test consisted of adding 8 seeds of Lactuca sativa L. (Feltrin) per well for each 1 mL of extract, in decreasing dilutions from 1 to 0.125 mg/ mL. The microplates were incubated for 7 days at 25-32°C and a 12-hour photoperiod with yellow light. After the period, the samples were frozen at -22°C for 24 h and the images of the seeds were scanned using ImageJ<sup>®</sup> software, root and hypocotyl length were measured. The germination indices (GI), percentage of germination (PG), relative seed germination (GRS) and relative root growth (CRR) were calculated. The negative control was buffer MES/NaOH with 0.5% DMSO, and the positive control glyphosate herbicide (Citromax<sup>®</sup>). The tests were performed in sextuplicates and analyzed with p<0.05 in one-way ANOVA, followed by Tukey's test. Results and discussion The extract proved to enhance growth in all concentrations. The roots of the seeds of *L. sativa* L. showed the following growth, in buffer solution 2.77 ± 0.23 cm, and in the highest tested concentration of the extract (2 mg/ mL) 3.98± 0.16 cm. In the analysis of the hypocotyl, the growth at the concentration of 2 mg/mL was again higher than the buffer with 1.69 ± 0.12 cm and 1.27± 0.12 cm, respectively. The GI at the highest concentration tested (2 mg/ mL) was 111.80%. Conclusions The methanolic extract of the leaves of Cenostigma microphyllum showed low risk for phytotoxicity, however it indicated stimulation of the growth of L. sativa, which may be of interest for its production, but should be observed for other plant species, given the risk to cause growth of unwanted species.

Acknowledgements: CNPq, FACEPE, CAPES, LAB-DPN, UFPE Keywords: Phytotoxicity; Catingueira; Plant extract

#### J-13. Phytochemical and Nutritional Characterization Moringa oleifera Leaves Powder

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Moringa oleifera, a plant native to the Himalayas and also widely cultivated in Africa and South America, is a highly valued species due to its nutritional and medicinal potential. M. oleifera leaves are recognized as a sustainable food supplement option, especially for people suffering from malnutrition in developing countries. In addition, these leaves have medicinal potentials, such as anti-cancer, anti-inflammatory and antimicrobial activities. The leaves powder is commonly consumed popularly as teas or food supplements. The present study aimed to perform the nutritional and phytochemical characterization of *M. oleifera* leaf powder. *M.* oleifera leaves were washed with distilled water, dried at 28 °C, powdered, and stored at - 20 •C. The nutritional characterization was performed according to the norms of the Adolf Lutz Institute and que following parameters were determined: humidity content, mineral residue (ashes), total lipids, proteins, carbohydrates, crude fiber content and caloric value. The phytochemical composition was determined by high performance liquid chromatography (HPLC). Nutritional characterization revealed that *M. oleifera* leaf powder contained (g/100 g) 9.02 ± 0.06 of humidity, 8.83 ± 0.12 of ashes, 25.51 ± 0.54 of protein, 8.55 ± 0.16 of total lipids, 9.91% ± 0.58 of crude fiber, and 38.19% ± 0.78 of carbohydrates. Total caloric value was in  $371.39 \pm 0.04$  kcal/100 g. The chromatographic profile at 270 nm revealed the presence of eight main compounds. Two peaks were found at retention times of 8.13 min and 12.39 min, whose ultraviolet (UV) spectra indicated the presence the cinnamic derivates caffeic acid and chlorogenic acid. The other six peaks corresponded to flavonoids, in which rutin was identified (18.69 min). The content of total cinnamic derivates was equal to 0.27 g% (0.19%). The total flavonoid content was 2.32 g% (1.43%), and rutin content was 0.66 g% (0.10%). The M. oleifera leaf powder is a rich source of polyphenols and nutrients, which have known health benefits. Keywords: Drumstick tree. Flavonoids. Natural products.

Acknowledgment: CAPES, CNPq and FACEPE.

### J-14. Leishmanicidal activity of Botanical Extracts of *Tephrosia toxicaria* and derived fractions.

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Human Visceral Leishmaniasis (VL) is a systemic parasitic disease caused by the protozoa Leishmania infantum, the most serious form of the disease, and if not treated, can lead to death. Pentavalent antimonial drugs are still the first choice for its treatment. However, known resistive strains, alongside its long treatment protocol and toxic side effects show a sub-optimal therapeutic scenario. With that in mind, is urgent the need for new options to fight this parasite. The goal of this research was to study the activity of the aqueous brute extract (BE) and derived fractions of Tephrosia toxicaria seeds against L. infantum, and to investigate the possible morphological modifications caused by these samples. The BE of seed powder of T. toxicaria was fractionated with  $(NH_4)_2SO_4$  in the following saturation intervals: 0-25%(F1), 25-50%(F2), 50-75%(F3), 75-100% (F4). All samples were diluted in RPMI medium and tested in different concentrations (1000 - 10  $\mu$ g/mL) against the promastigote form of L. *infantum* (10<sup>6</sup> parasites/mL) in 96-well microplates for 24 h. The leishmanicidal activity was verified utilizing resazurin as an indicator of cell viability. Protein quantification was done using the Bradford method. The presence of cysteine proteases inhibitors in the samples was measured using azocasein 1% as a subtract. Additionally, Leishmania cells that were treated with the highest concentration (1000  $\mu$ g/mL) of all samples were studied under light microscopy for evaluation of morphological disturbances. BE promoted inhibition of growth and survival of Leishmania at the tested concentrations, and presented papain protease inhibitors. Papain protease inhibitors were also present in the derived fractions. Fraction F1 presented 61,83% enzyme inhibition, and had 72,6% inhibition of leishmania cells in the higher concentration tested. Fraction F4 had 27,19% protease inhibition, and leishmanicidal activity above 50% in all concentrations tested. Leishmania cell's morphology suffered alterations after incubation with the tested samples, in contrast to the control (no change), with the occurrence of vesicles in cell membrane, and changes in the flagellum morphology. Proteases have important physiological functions inside the leishmanial cell, and the inhibition of papainlike enzymes by specific inhibitors may be one strategy in the fight against this disease. These cell's disturbances can be the result of the cytotoxic activity of the treatment against the parasite. However, more studies are needed to isolate the compounds responsible for the antileishmanial activity and understand its molecular mechanism and its relationship with the morphological changes.

Key-Words: Pentavalent antimonials; Botanical Extracts; Leishmania; Timbó. Acknowledgment: CNPq, Capes, IMT-UFRN.

### J-15. INSECTICIDAL ACTIVITY OF EUCALYPTOL (1,8-CINEOL) FOR THE CONTROL OF *Nasutitermes corniger* MOTS., 1855

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The termite Nasutitermes corniger Mots is a neotropical species that can construct its termite mounds at the base of tree trunks or in isolated areas of buildings. The species primarily feeds on wood, which may include tree branches, moldings, wooden presses, plywood, works of art, fences, and paper. This diverse range of food sources makes the species a significant urban and agricultural pest from an economic standpoint. The insecticides presently employed induce the selection of resistant individuals and harm non-target species. Secondary metabolites which are produced by plants as protection against biotic and abiotic stresses can be alternative way to control insect populations. Eucalyptol (1,8-cineole) is a secondary metabolite used against insects for its repelent and insecticidal effects. The purpose of this study was to investigate the effects of eucalyptol (Sigma-Aldrich) on the two *N. corniger* casts. The ingestion toxicity test used artificial diet consisting of distilled water, avicel, nest meal and eucalyptol at concentrations of 0.5, 1.5, 2.5, 5.0 or 15.0 nL/g. Twenty insects, comprising 16 workers and 4 soldiers, were placed in Petri dishes containing the artificial diet and the experiment was conducted in B. D. climate chambers (28 °C, RH= 70%). Mortality was assessed 48 hours from the onset of the study to determine the lethal concentration for 50% mortality (LC50). The effect of the eucalyptol on digestive enzymes ( $\alpha$ -amylase, exoglucanase, endoglucanase, β-d-xylanase, and protease) was also analyzed. The eucalyptol promoted mortality (CL50 of 13.7 nL/g for workers and soldiers) and was able to modulate enzyme activies since it inhibited  $\beta$ -d-xylanase and stimulated protease. The study revealed that eucalyptol is an insecticidal agent against N. corniger.

Keywords: Essencial oil. Insect. Termite.

Acknowledgment: CAPES, CNPq and FACEPE.

J-16. Immunomodulatory Activity of Brazilian Red Propolis and Its Constituents Isoliquiritigenin and Formononetin in Lipopolysaccharide-Activated Macrophages *In Vitro* <u>Santos, E.C<sup>1</sup></u>; Cavalcante, J.P.M.<sup>1</sup>; Souza, S.J.M.<sup>1</sup>; Coelho, G.L.<sup>1</sup>; Brandão, J.A<sup>1</sup>; Nascimento, T.G.<sup>2</sup>; Câmara, N.O.S.<sup>3</sup>; Anderson, L.A.<sup>4</sup>; Bassi, E.J.<sup>1</sup>

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Brazilian red propolis (RP) is a natural resin with a composition rich in bioactive flavonoids, which are attributed to pharmacological actions, including anti-inflammatory activity. The aim of this work was to evaluate the immunomodulatory potential of Alagoas red propolis (ARP) and its constituents isoliquiritigenin (ISL) and formononetin (FMT) in murine macrophages stimulated with lipopolysaccharide (LPS) in vitro. Initially, the maximum non-toxic concentration (MNTC) of the hydroalcoholic extract (HE) of ARP, ISL, and FMT in murine macrophages of the J774A.1 cell lineage was determined, based on the analysis of cell viability by the MTT assay. The immunomodulatory activities of HE-ARP, ISL, or FMT were assessed by treating LPS-stimulated macrophages under different experimental conditions. The expression of F4/80 and CD86 was evaluated by multiparametric flow cytometry and the quantification of pro- and anti-inflammatory cytokines was assessed by the Cytometric Bead Array (CBA) methodology in cell culture supernatant. The phosphorylation of ERK1/2, p38, and JNK proteins by intracellular flow cytometry was used to assess MAPK (mitogen-activated protein kinase) activation. RT-qPCR was used to assess the expression of the NOS2 and SOCS3 genes. Treatment with HE-ARP decreased the percentage of double-positive cells (F4/80+ CD86+), the expression of the activation molecule CD86, and the levels of pro-inflammatory cytokines IL-6 and TNF. Furthermore, 48 hours after treatment, there was an increase in ERK1/2, p38, and JNK phosphorylation and a decrease in NOS2 and SOCS3 expression. ISL treatment reduced the frequency of F4/80+CD86+ cells, CD86 expression, and phosphorylation of ERK1/2 and p38 proteins but not JNK. Furthermore, IL-6 and TNF levels were reduced, as was the expression of the NOS2 and SOCS3 genes 48h after ISL treatment. On the other hand, FMT treatment did not reduce the percentage of double-positive cells (F4/80+ CD86+) and did not change the phosphorylation of the ERK1/2, p38 and JNK although a reduction in IL-6 and TNF levels was detected. In conclusion, ARP and the ISL were found to have promising immunomodulatory effect on murine macrophages in vitro. These results contribute to a better understanding of the immunomodulatory activity of ARP and its constituents, paving the way for the development of new immunological interventions for the treatment of inflammatory disorders.

**Keywords:** Brazilian red propolis, macrophage, inflammation. **Acknowledgments:** CAPES, CNPq, FAPEAL.

### J-17. Antimicrobial and Antibiofilm Activity of Essential Oil from *Croton urticifolius* Lam. against *Staphylococcus epidermidis* and *Escherichia coli*

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Healthcare-associated infections (HAIs) are one of the main sources of preventable illnesses in hospitalized patients, and cause significant damage to healthcare resources. Antimicrobial resistance can result in the inability to treat infections and control public health threats. Moreover, the pace of development of new antimicrobials is not keeping up with antimicrobial resistance. In addition, some of the resistance strategies that microorganisms acquire are in the communities formation, called biofilms. Biofilms are well-organized structures of microorganisms attached to biotic or abiotic surfaces and whose cells are encased and protected by a self-produced polymeric matrix. In this context, essential oils (EO) emerge as an alternative to mitigate this problem, since their composition presents a variety of chemical compounds with possible antimicrobial activities. The aim of this study was to evaluate the antimicrobial and antibiofilm activity of Croton urticifolius essential oil (CuEO) against Staphylococcus epidermidis ATCC 12228, Staphylococcus epidermidis ATCC 35984 (Methicillinresistant staphylococci - MRS) and Escherichia coli ATCC 11303. The plant material (leaves) was collected in Serra do Lima, in the municipality of Patu-RN. The essential oil was extracted by hydrodistillation method using the Clevenger apparatus. The antimicrobial activity of the oil was evaluated for minimum inhibitory concentration (MIC) test. The antibiofilm activity was evaluated by quantifying the biomass using crystal violet (CV) staining and counting the number of viable cells. The CuEO showed MIC values at concentration of 2.5% against S. epidermidis ATCC 12228 and E. coli ATCC 11303, however, there was no MIC for S. epidermidis ATCC 35984. Regarding biomass quantification, was observed significantly reduction in the S. epidermidis ATCC 12228, S. epidermidis ATCC 35984 and E. coli ATCC 11303 biofilms in all concentrations tested (5 to 0.078%). Moreover, at concentrations of 5 to 2.5% it was possible to observe a reduction of 3 and 4 logs in the number of viable cells in the biofilms of S. epidermidis ATCC 12228 and E. coli ATCC 11303, respectively, however, for S. epidermidis ATCC 35984, the oil was only able to inhibit 1 log at concentrations of 0.625 to 0.078%. Therefore, our results show that the CuEO may be an alternative for the treatment of infections caused by S. epidermidis and E. coli biofilms.

Keywords: biofilm, infections, essential oil

**Acknowledgments:** Thanks to the Federal University of Ceará, specifically to the Graduate Program in Biotechnology of Natural Resources. To the Coordination for the Improvement of Higher Education Personnel (CAPES). National Council for Scientific and Technological Development (CNPq). Cearense Foundation for Supporting Scientific and Technological Development (FUNCAP).

# J-18. Phytochemical analysis and antioxidant activity of *Cenostigma microphyllum* (Mart. ex G.Don) Gagnon & C.P.Lewis leaves chloroform extract flavonoid-enriched fraction <u>Barbosa, F.P.<sup>1</sup></u>; Lima, C. R. F. de<sup>1</sup>; Santos, T. M. dos<sup>1</sup>; Souza, K. C. O.<sup>1</sup>; Ribas, A.R.<sup>1</sup>; Silva, A. P. S. da<sup>1,2</sup>; Silva, M. V. da<sup>1</sup>; Lima, V. L. de M.<sup>1</sup>

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Introduction External antioxidant sources are of great needed lately since it helps to prevent diseases. Particularly, these are in evidence because the commercial ones have showered toxicity. Cenostigma microphyllum is a plant species that occurs in tropical and subtropical regions. It has application in traditional medicine for the treatment of gastrointestinal and respiratory diseases, but fewer studies evaluated its biological potential. Aim To evaluate antioxidant potential of chloroform extract fraction from C. microphyllum leaves. Materials and methods C. microphyllum leaves were collected in Catimbau National Park in Buíque, Pernambuco, Brazil, and identified at IPA, registration number 84,880. Leaves underwent a hot extraction process, with solvents of increasing polarity, in Soxhlet equipment, using cyclohexane, chloroform, ethyl acetate and methanol. For total phenolic and tannins quantification, the methodology of reaction to Folin-Ciocalteau and sodium carbonate was used, with gallic and tannic acid as standard; quantification of flavonoids followed the ferric chloride methodology, and quercetin was the standard. To evaluate the antioxidant activity, activity of scavenging radicals DPPH and ABTS, and total antioxidant capacity (TAC) by phosphomolybdenum assay were tested. Results and discussion Chloroform extract showed a yield of 0.997 % and a greater concentration of total phenols and flavonoids in previous analyses, being subjected to fractionation on a silica gel 60 column to obtain the fraction rich in flavonoids. Then, 10 fractions (F1 to F10) were obtained, and the last fraction (F11) was submitted to a column of Sephadex LH-20 with a total of 45 fractions (F1-F45), these passed through thin layer chromatography for union by similarity, fraction 8 (FVIII) was chosen for secondary metabolites quantification. Fraction presented 54.45 ± 0.07 mg of gallic acid equivalent/g of extract, 52.95  $\pm$  0.06 mg of tannic acid equivalent/g of extract and 118.23  $\pm$ 0.29 mg of quercetin / g of extract for phenols, tannins and flavonoids, respectively. In the antioxidant activity, the highest concentration (1 mg/ mL) showed an inhibition percentage of 46.96 ± 1.99 % in DPPH. In the radical reduction ABTS corresponded to 13.58 ± 0.02 % after 1 hour (1 mg /ml), with CAT corresponding to 42.72 ± 3.02 %. Conclusion FVIII fraction showed a significant concentration of the analyzed metabolites, phenolic compounds and flavonoids, possibly being responsible for the antioxidant activity via electron transferring. Thus, these findings support investigations with this plant product in order to make it a resource in the treatment of disorders associated with oxidative stress. Acknowledgments: CNPq, FACEPE, CAPES, LAB-DPN, and UFPE

Keywords: Medicinal plant; Flavonoids; Antioxidant

### J-19.BIOACTIVITY OF THE ESSENTIAL OIL FROM *Hyptis fruticosa* Salzm. ex Benth. LEAVES ON *Alphitobius diaperinus* Panzer (COLEOPTERA: TENEBRIONIDAE)

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The lesser mealworm (Alphitobius diaperinus Panzer, 1797) holds significant relevance in poultry farming as it is a pest in poultry houses, where it acts as a vector for pathogens such as Escherichia coli, Salmonella sp., Staphylococcus sp., and causes nutritional imbalances in birds. Although synthetic insecticides have traditionally been used to control this pest, excessive use of these compounds can harm the environment. Currently, there is a growing preference for natural compounds, such as essential oils, that act as insecticides and do not promote pest resistance. Thus, the objective of this study was to evaluate the potential of the essential oil from Hyptis fruticosa (Lamiaceae) leaves as an insecticide against A. diaperinus. The leaves of H. fruticosa were collected in Buíque, Pernambuco, and subjected to the hydrodistillation process using a Clevenger-type apparatus for essential oil extraction at the Laboratório de Bioquímica de Proteínas at UFPE. The lesser mealworms were collected in a poultry house in Camaragibe, Pernambuco, and reared in the laboratory according to the methodology of Rice & Lambkin (2009). The conducted assays assessed contact toxicity and the effect of the essential oil from *H. fruticosa* leaves on the acetylcholinesterase (AChE) of *A.* diaperinus. The essential oil solutions were prepared in distilled water and 1% Tween<sup>™</sup> 80 at concentrations of 0, 50, 75, 100, 125 and 150 µL/mL. In Petri dishes, 50 µL of the essential oil at different concentrations and 10 adult insects were added. Mortality was evaluated after 48 hours to determine the lethal concentrations (LC50 and LC90). The effect of the essential oil on AChE was assessed in a 96-well plate; insect extract was incubated with acetylcholine and DTNB for 3 minutes, and then read at 405 nm. The estimated LC50 and LC90 were 67.51 and 149.90  $\mu$ L/mL, respectively. The AChE assay demonstrated that the essential oil from H. fruticosa leaves significantly reduced enzymatic activity at all concentrations (one-way ANOVA; F5,12=11.49; P=0.003). The essential oil from H. fruticosa leaves demonstrated contact insecticidal potential against A. diaperinus, also affecting AChE activity. These results suggest a promising approach for controlling this pest. Therefore, further research is needed to explore the potential of this essential oil as an alternative to synthetic insecticides against A. diaperinus.

Keywords: Lesser mealworm, insecticide, essential oil.

**Acknowledgments:** UFPE (Universidade Federal de Pernambuco), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), BIOPROT (Laboratório de Bioquímica de Proteínas), Nubioma (Núcleo de Biossegurança e Meio Ambiente) and LQPN (Laboratório de Química de Produtos Naturais).

#### J-20.PURIFICATION AND PARTIAL CHEMISTRY CHARACTERIZATION OF LECTIN FROM *Borreria Verticillata* LEAVES.

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**INTRODUCTION**: Borreria verticillata, an herbaceous plant of the Rubiaceae family native to South and Central America, is known as 'vassourinha-de-botão' e 'cordão-de-frade'. Its wide distribution and adaptability for year-round cultivation are advantageous. Multiple pharmacological applications have been confirmed, including antibacterial, anthelmintic, analgesic, anti-inflammatory, antinociceptive, antimalarial, and dermatological activities. However, there are no reports of lectins in its leaves. Lectins are proteins with reversible carbohydrate affinity, exhibiting insecticidal, fungicidal, and bactericidal activities. **OBJECTIVE**: This study aimed to detect, purify, and partially characterize the lectin from B. verticillata leaves. METHODOLOGY: Dried leaves (10 g) were extracted in 0.15 M NaCl (100 mL) for 16 h at 25°C. After filtration and centrifugation, the saline extract (SE16) was assessed for protein concentration and hemagglutinating activity (HA), as well as inhibition by carbohydrates and glycoproteins. The SE16 underwent chromatography on a chitin column with 0.15 M NaCl; BveLL was collected after elution with 1.0 M acetic acid. The HA of BveLL was tested at various pH levels (3.0-12.0) and temperatures (30-100 °C) after 30-min incubation. The activation energy for denaturation ( $\Delta G_{desn}$ ) was calculated using the equation described by Cavada et al. (1998). RESULTS AND DISCUSSION: SE<sub>16</sub> exhibited HA of 2048 and protein content of 26.99 mg/mL, indicating lectins. HA of SE<sub>16</sub> was partially inhibited by carbohydrates (fructose, ribose, xylose, cellobiose, lactose, arabinose, methyl-manopyranoside, and N-Acetylglucosamine), neutralized in the presence of glycoproteins such as casein and thyroglobulin. Inhibition by Nacetylglucosamine motivated the purification of the lectin from *B. verticillata* leaves (BveLL) through chitin chromatography. BveLL maintained thermal stability up to 90°C, losing activity at 100°C. The estimated  $\Delta G_{desn}$  was 29.56 kcal.mol<sup>-1</sup>, similar to other legume lectins, e.g., 26 kcal.mol<sup>-1</sup> for Vatairea macrocarpa lectin (VmL) and 24.87 kcal.mol<sup>-1</sup> for Erythrina velutina (Moraes et al., 1996). BveLL remained stable at pH 5, 6, and 7, decreasing by 50% at pH 8 and completely inhibiting at pH 9. CONCLUSION: B. verticillata contains the lectin (BveLL), which was successfully extracted and purified using the described protocol. BveLL demonstrated thermal stability up to 90°C, and its pH 9 inhibition underscores sensitivity to alkaline conditions. The activation energy for denaturation highlights the lectin's structural robustness. Evaluation of BveLL insecticidal properties against Aedes aegypti and Sitophilus zeamais are in progress.

Keywords: Lectin, *Borreria verticillata*, chemistry *characterization*. Supported by: CNPq, CAPES and FACEPE.

#### J-21. PICEATANNOL AND NARINGENIN EFFECTS IN HUMAN PLACENTAL CELLS INFECTED WITH ZIKA VIRUS

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Zika virus (ZIKV) infection has been associated with several pregnancy deleterious outcomes, such as the Zika Congenital Syndrome, prematurity, and stilbirth. Due the relevant aggravations and limitations of treatment options during pregnancy, the necessity for a protective and safe compound that could be taken during pregnancies to prevent Zika virus placental infection is needed. Piceatannol and naringenin are natural products found in Passiflora edulis seeds ethanolic extract that exhibit several biological properties, especially involving antioxidant and antiviral activity. The aim of this study is to evaluate the effects of the piceatannol and naringenin in vitro administration on Zika virus-infected trophoblast cells. HTR-8/SVneo, a placental cell line derived from first-trimester extravillous trophoblast cells were infected with 1 MOI of ZIKV PE243 strain for 24 h. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was employed for cell viability, and reactive oxygen species (ROS) were analyzed by MitoSOX staining, nitroblue tetrazolium (NBT), and H<sub>2</sub>O<sub>2</sub> production. Antioxidant function was evaluated by Nrf2 staining, superoxide dismutase (SOD) and catalase (CAT) activity. MTT showed viability reduction at concentrations higher than 62.5 ng/mL (p < 0.05), and the chosen concentration for further exeriments was set to 31.25 ng/mL. Cells were 1 h pre-treated with piceatannol or naringenin, followed by 24 h of viral infection. The ZIKVinfected cells pre- treated with piceatannol decreased mitoSOX expression, NBT, and H<sub>2</sub>O<sub>2</sub> production (p < 0.05). The Nrf2 nuclei translocation was also increased in this group, although SOD and CAT production was unchanged. Our preliminary results demonstrate that mainly piceatannol showed a potentially preventive effect against ZIKV infection of placental cells. Financial Support: CNPq/MS/SESAU-AL/FAPEAL-PPSUS Keywords: Zika virus; trophoblast; oxidative stress.

#### J-22. Antioxidant Evaluation of Essential Oils Extracted from Plants of the Genus Eugenia Nascimento, J.W.<sup>1</sup>, Melo, E.C.C<sup>1</sup>, Oliveira, R.G<sup>1</sup>, Silva, M. S.<sup>1</sup>, Soares, P.A.G<sup>1,2</sup>

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Introduction: The genus Eugenia is considered the fourth most important genus of the Myrtaceae family for the production of essential oils. The essential oils obtained from these plants have several biological activities such as antitumor, antimicrobial and antioxidant activity. The species Eugenia brejoensis, and Eugenia pohliana from Caatinga biome, popularly known as "cutia" and "maçã do mato" are applied in several inflammatory diseases as gastrointestinal disorders, diarrhea and inflammation, but so far, no research showed the antioxidant potential of these species. In recent years, the research for biomolecules with antioxidant potential has grown, especially those related to the reduction of free radicals and the disruption of oxidative stress, which are associated with the cause of various pathologies. Objective: Thus, the objective of this work was to evaluate the antioxidant activity of the essential oils extracted from the leaves of E. brejoensis (E.b.) and E. pohliana (E.p.). Materials and methods: The plants were collected in the Catimbau Valley. The essential oils were extracted from the leaves by hydrodistillation process, with the aid of a Clevenger-type extractor. The antioxidant activity was determined through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity assay, using ascorbic acid as standard. The analysis was performed in triplicate and the results expressed as media±stardad deviation from the concentration capable of inhibiting 50% of the DPPH and ABTS radicals ( $IC_{50}$ ). **Results and discussion:** The essential oils E.b. and E.p. were extracted with a good yield presented 0.54 m/m and 0.94 m/m, respectively. Samples of E.b., E.p. and ascorbic acid showed a IC<sub>50</sub> for the DPPH free radical inhibition of 24.68±0.0 mg/mL, 122.98± 0.0 mg/mL and 0.011±0.02 mg/mL, respectively. Regarding to the ABTS free radical reduction, E.b., E.p. and ascorbic acid showed an IC<sub>50</sub> of 14.58±0.01 mg/mL, 7.10±0.0 and 0.21±0.0, respectively. Comparing the species, the essential oil E.b. showed a better antioxidant activity in the DPPH test, while the essential oil E.p. was better in the ABTS method. The difference in the antioxidant activity between the Eugenia species studied in this work could be attributed to the influence of different biotic conditions within the same habitat in the production of their bioactive compounds, which was reflected in its chemical composition and, consequently, its biological activity. Conclusion: The essential oils extracted from the leaves of E. brejoensis and E. pohliana showed antioxidant potential against the DPPH and ABTS free radicals reduction, demonstrating that the biological individuality of each species can influence the production of secondary metabolites. Although preliminary, the results obtained showed the potential application of the essential oils extracted from Eugenia species native from Caatinga to reduce oxidative processes.

Acknowledgements: UFPE, CNPq, FACEPE and CAPES

Keywords: Caatinga, secondary metabolites, volatile compounds.

### J-23. *BORRERIA VERTICILLATA* SALINE EXTRACT : NEW COMPOUNDS WITH LARVICIDAL ACTIVITY ON *AEDES AEGYPTI*

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**INTRODUCTION**: Aedes aegypti is responsible for the transmission of several arboviruses, such as dengue, chikungunya, zika and yellow fever, representing a major challenge for global health. The Borreria verticillata plant, native to South America, has diverse biological activities, including bactericidal, insecticidal and larvicidal action, due to its bioactive compounds, such as lectins, which act as a defense against insects and threats. Lectins are proteins with reversible affinity for carbohydrates, exhibiting insecticidal, deterrent and larvicidal activities. **OBJECTIVE:** This study aimed to evaluate the larvicidal activity and deterrent capacity of oviposition of Borreria verticillata leaf extracts and lectin on Aedes aegypti mosquito larvae. METHODOLOGY: The Dialyzed extract (DE) was assessed for protein concentration and hemagglutinating activity (HA) and phytochemical characterization on high performance liquid chromatography (HPLC) technique. To collect a Lectin (BveLL), the DE underwent chromatography on a chitin column. The BveLL was collected after elution with 1.0 M acetic acid. The leaf extract (LE) were tested for larvicidal activity against the mosquito A. aegypti. The negative control consisted of distilled water, while the positive control used Temephos (0.01 ppm) to kill larvae. Mortality of treated larvae was assessed after 24 and 48 hours, and lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) were calculated. For the oviposition bioassay, ten pregnant females of A. aegypti mosquitoes were placed in cages with test and control solutions. The test solutions had concentrations of 1500 and 2200 ppm, while the control solution was distilled water. The oviposition response was defined by counting the number of eggs deposited on each filter paper. **RESULTS AND DISCUSSION**: The analysis of the DE of B. Verticillata showed HA of 256 and protein content of 11,10 mg/mL, indicating presence of lectins. Performed by the HPLC technique showed the presence of rutin in this plant. The identification of routine in the extract highlights its potential as a larvicide against mosquitoes. The B. verticillata DE showed larvicidal activity against A. aegypti larvae, in projects L3 and L4, demonstrating its potential as a promising source of bioactive compounds to control this vector. The deterrence activity was also promising, resulting in a percentage of 76% and IAO -0.52% for 2,200 ppm and 72% and IAO of -0.44% for 1,500 ppm, majority preference for egg deposition in the control for the test. **CONCLUSION**: The DE of B. verticillata showed larvicidal and oviposition activity against Aedes aegypti. The action may be associated with the presence of rutin. As well as the presence of the lectin BveLL. or even a synergism between these compounds. Assessment of the insecticidal properties and deterrent activity of BveLL against A. aegypti are in progress.

Keywords: Lectin, *Borreria verticillata, Aedes aegypti* Supported by: CNPq, CAPES and FACEPE.

### J-24. BIOACTIVITY OF THE ESSENTIAL OIL OF *Lippia alba* (MILL.) LEAVES AGAINST *Nasutitermes corniger* (BLATTODEA:TERMITIDAE)

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The Nasutitermes corniger termite has an important ecological role in nutrient recycling, but it consume up to a third of annual wood production. Synthetic insecticides have been employed in pest control but these compounds pose risks to the environment and human health. Natural insecticides have emerged as an alternative method to control termite population due to be biodegradable and less deleterious effects on non-target organisms. Lippia alba, commonly called lemon balm, has an essential oil with a highly variable composition. The objective of this study is to investigate the termicidal activity of L. alba essential oil on N. corniger. L. alba was collected at Catimbau National Park (Buíque-PE) and the leaves were hydrodistilled in a Clevenger apparatus to extract the essential oil. An intact nest of N. corniger was collected on the campus of the Universidade Federal Rural de Pernambuco and after, workers and soldiers from it were used in the bioassays. The ingestion toxicity test used artificial diet consisting of distilled water, avicel, nest meal and L. alba oil at concentrations of 0.5, 1.5, 2.5, 5.0 or 15.0 nL/g. Twenty insects, comprising 16 workers and 4 soldiers, were placed in Petri dishes containing the artificial diet and the experiment was conducted in B. D. climate chambers (28 ºC, RH= 70%). Mortality was assessed to determine the lethal concentration for 50% (LC50) using probit analysis. Following this, the effects of the essential oil on the enzymes endoglucanase, exoglucanase,  $\beta$ -d-xylanase,  $\alpha$ -amylase, and total protease was evaluated. The data showed that the essential oil of *L. alba* promoted mortality of *N. corniger* when ingested, with a CL50 of 18.25 nL/g for workers and 8.4 nL/g for soldiers. It also inhibited exoglucanase, xylanase, and protease activity by 26.48%, 78.3%, and 34.81%, respectively, and stimulated endoglucanase activity by 26.17%. The study revealed that essential oil of *L. alba* is a natural insecticide against *N. corniger*. Keywords: Essencial oil. Natural insecticide. Termite.

Acknowledgment: CAPES, CNPq and FACEPE.

#### J-25. Antiglycation and Cytotoxicity Evaluation of Maytenus ilicifolia Mart. ex Reissek

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Advanced glycation end products (AGEs) result from a non-enzymatic reaction between reducing sugars and amino acids, and various factors such as high blood glucose levels, diet, and smoking habits can increase their production. The accumulation of AGEs can modify various biological structures, such as long-lived proteins, including bovine serum albumin. In vitro methods have been developed to evaluate the antiglycation ability of a given compound. This study aimed to assess the antiglycation potential of the ethanolic extract of Maytenus ilicifolia Mart. ex Reissek (EE-Mi) and its organic fractions: hexane (HF-Mi), dichloromethane (DMF-Mi), ethyl acetate (EAF-Mi), n-butanol (BF-Mi), and the hydro methanolic fraction (HMF-Mi), using various glycation models (bovine serum albumin (BSA) and methylglyoxal (MGO); BSA and fructose (FRU); arginine (ARG) and MGO models) and to evaluate their cytotoxic effects on RAW 264.7 macrophages using the MTT assay. The study revealed that EE-Mi and its fractions exhibited varying degrees of antiglycation activity in different models. Notably, BF-Mi and HMF-Mi showed significant inhibitory activity against fructose-induced glycation. However, in BSA/MGO and ARG/MGO models, guercetin outperformed the fractions. Cytotoxicity assays indicated that, at low concentrations, the samples induced minimal toxicity in RAW 264.7 macrophages, with EE-Mi and HF-Mi displaying the lowest cytotoxicity. The ethanolic extract of Maytenus ilicifolia and its organic fractions exhibited variable antiglycation effects in different models, suggesting their potential as antiglycation agents. The study provides valuable insights into the development of natural compounds for combating AGE-related complications. Moreover, the samples demonstrated low cytotoxicity at lower concentrations, highlighting their potential for further investigations as therapeutic agents.

#### Key words: antiglycant; cytotoxicity; Maytenus ilicifolia

Acknowledgments: The authors would like to thank the Institute of Biotechnology of the Federal University of Uberlandia and the UNA University Center.

### J-26. Toxicological evaluation and gastroprotective effect of polysaccharides from *Parkia pendula* exudate on ethanol-induced gastric ulcer in mice

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**Introduction:** Gastric ulcer is a disorder of the digestive system that poses serious public health problems and influences people's quality of life, which makes it necessary to search for new alternatives that prevents and alleviate its symptoms. Objective: Evaluate the acute toxicity and the gastroprotective effect of polysaccharides extracted from Parkia pendula exudate. Materials and methods: The exudate (exsudate n° 92516) was diluted in distilled water (100 mL; 24 h) and the aqueous extract precipitated with 99.9% ethyl alcohol (1:4; 18 h) to obtain the Exudate Polysaccharide of *P. pendula* – PePp, which was chemically analyzed (total carbohydrates -TC, uronic acid -UA, total phenols -FE and total proteins-TP). The acute toxicity (CEUA/UFPE n° 127/2022) was performed using female Swiss mice (n=3/group) that received a single dose of PePp (2000 mg/kg) or 0.9% saline solution and evaluated for 14 days for signs of toxicity, body mass, food consumption, water intake and on the 15th day, hematological, biochemical and histological analyzes were performed. In the gastroprotective analysis (CEUA/UFPE n° 128/2022), female Swiss mice (n=5/group) underwent fasting (18 h) and were pre-treated (60 min) before the application of 99.9% ethanol (0.66 ml/kg) with PePp (10, 25 and 50 mg/kg), saline (10 ml/kg) or the positive control ranitidine (80 mg/kg). Subsequently, the stomachs were removed, opened (greater curvature), washed (saline solution), photographed and the ulcerated areas analyzed. Results: PePp showed a yield of 48.2%, with TC (79%), UA (11%), FE (2.72 mg/g in GAE) and TP (1.20%) in its chemical composition. In terms of toxicity, no signs of morbidity, mortality and changes in body mass, water intake and feed intake were observed between the experimental groups. In addition, the organs (heart, lung, liver, kidney, spleen, thymus and stomach) did not show changes in tissue weight, color or texture. In hematological analyzes (red blood cells, hemoglobin, hematocrit, mean corpuscular volume, leukocytes, typical lymphocytes, monocytes and platelets), biochemistry (glucose, urea, creatinine and total cholesterol) and histopathological (liver, kidneys, lung and spleen) analyzes no significant difference among the groups was observed, no signs of inflammation or other phatological changes in the organs. In the gastric lesion, pre-treatment with PePp showed a significant reduction in the ulcerated area in all doses (51.71%, 70.58% and 83.41%, respectively), as well as ranitidine (95.71%), when compared to saline group (11.01%). Conclusion: PePp is a alternative to be used in gastric ulcer prevention therapy.

**Keywords:** Plant polysaccharides; acute toxicity; gastric ulcer.

**Acknowledgments:** Fundação de Amparo á Ciência e Tecnologia do Estado de Pernambuco (FACEPE); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

### J-27. Phytochemical profile and effect of saline extract of *Punica granatum* L. sarcotesta against *Aedes aegypti* L. larvae

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Aedes aegypti L. is a significant mosquito species in public health due to its transmission of arboviruses like dengue, zika, and chikungunya. The main strategy for reducing these diseases is to control the population of this mosquito. Punica granatum L. (pomegranate) is a species with diverse biological properties widely used in traditional medicine. Therefore, the objective of this study was to assess the impact of the saline extract of *P. granatum* sarcotesta (SSE) on A. aegypti larvae, to observe changes in the activity of digestive enzymes and analyze changes in the midgut of the larvae using histological preparations. The extract was also assessed for secondary metabolites using high-performance liquid chromatography (HPLC). The larvicidal test utilized SSE at various concentrations (0.17 - 1.20 mg/mL of protein) to establish the lethal concentration to kill 50% of larvae (CL<sub>50</sub>). Afterwards, histological examination was conducted to investigate the extract's impact on the treated larvae's intestines. An assay was also implemented to determine modifications in the activity of digestive enzymes (trypsin and amylase) in the midgut of the larvae. HPLC analysis detected ellagic acid (2.3 mg/mL), punicalagin  $\alpha$  and  $\beta$  (2.2 and 2.3 mg/mL), ellagic acid derivatives (2.2 mg/l), and cinnamic acid derivatives (3.9 mg/mL) in SSE. The extract showed larvicidal activity, with  $LC_{50}$  of 0.67 mg/mL. ESS also caused morphological changes in midgut cells, with protrusions in the apical region and thickening of the peritrophic matrix. Furthermore, the extract reduced trypsin and amylase activity. In conclusion, the saline extract of the sarcotesta of P. granatum showed larvicidal activity against A. aegypti, caused conformational changes in the midgut of the larvae, in addition to causing changes in the activities of digestive enzymes, making the pomegranate extract a new tool to control this mosquito.

Keywords: Pomegranate; Natural insecticide; *Aedes aegypti*. Acknowledgment: CAPES, CNPq, FACEPE e UFPE.

#### J-28. ANTIBACTERIAL ACTIVITY OF Moringa oleifera LEAF EXTRACT

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The leaves of Moringa oleifera are consumed by the population of several countries, including Brazil, due to the high content of proteins and vitamins. However, studies report that M. oleifera leaves contain antinutritional proteins such as lectins (hemagglutinating proteins) and trypsin inhibitors. The study investigated M. oleifera leaf extracts obtained under different experimental conditions. The leaf flour was homogenized for 5 min, 4h, 8h or 16h with 0.15 M NaCl or buffer solution with pH 3.0, 6.0, 8.0 and 10.0. The extracts were quantified for protein content and presence of hemagglutinating and trypsin inhibitory activities. The pH 3.0 extract was selected for the assay to evaluate the antibacterial activity against Pectobacterium carotovorum brasiliensis, Pectobacterium carotovorum carotovorum, Pectobacterium sp. (isolated from arugula), Pectobacterium sp. (isolated from lettuce) and Pectobacterium sp. (P. Almeida). The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) were determined. All extracts contain protein and showed trypsin inhibitory activity but hemagglutinating activity was only detected in the extracts pH 3.0 and 10.0 with titers of  $128^{-1}$  and  $2^{-1}$ , respectively. The hemagglutinating activity of the pH 3.0 extract was partially inhibited by the glycoprotein fetuin and this data confirms the presence of lectin in the sample. The pH 3.0 extract inhibited the growth (MIC of 300  $\mu$ g/mL) of all tested bacteria and was bactericidal (MBC 600 µg/mL) against Pectobacterium carotovorum brasiliensis, Pectobacterium carotovorum carotovorum and Pectobacterium sp. (P. Almeida). In conclusion, M. oleifera leaves contain lectin, trypsin inhibitor and antibacterial activity against species that attack crops of economic interest.

**Keywords:** *Pectobacterium* sp; Lectin; Trypsin inhibitor. **Acknowledgment:** CAPES, CNPq and FACEPE.

### J-29. Water-soluble *Moringa oleifera* Seed Lectin Attenuates Anxiety and Depression Symptoms Induced by Unpredictable Chronic Mild Stress in Mice

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Introduction: Phyto-based treatments of anxiety and depression have received significant attention. Moringa oleifera holds a wide range of applications in traditional medicine, including treatment of neurological disorders. Lectins are proteins that possess carbohydraterecognizing domains (CRD), which allows them to bind sugars with high affinity and specificity. These interactions introduce pathways for disease modulation, including neurological disorders such as anxiety and depression. Water-soluble lectin from M. oleifera seeds (WSMoL) already showed anxiolytic and antidepressant-like effects. Aim: To evaluate whether WSMoL has anxiolytic and antidepressant-like effects in mice (Mus musculus) subjected to unpredictable chronic mild stress (UCMS) as a method to induce anxiety and depressive-like behaviors. Materials and methods: This work was approved (protocol 0010/2021) by the Ethics Committee on Animal Use of UFPE. The animals were divided in groups (n=6) and submitted to a 4-week stressor regimen of UCMS, being used seven different stressors once a week. On the day following cessation of the UCMS protocol, the intraperitoneal administration of phosphate buffered saline solution (PBS; negative control), WSMoL (2 and 4 mg/kg), or fluoxetine (10 mg/kg) started and lasted for 21 days. Neurobehavioral analysis was performed at the end of the treatment period and included: the open field test (OFT) and the elevated plus maze test (EPM) for measuring anxiety-like behavior, and the tail suspension test (TST) and sucrose preference test (SPT) for measurement of depression-like behavior. Results and discussion: None of the experimental groups presented impaired locomotion on the OFT. WSMoL was efficient to revert the stress-induced anxiogenic behavior by reducing the number of rearing on OFT (F<sub>4,25</sub>: 8.854, p: 0.0004) as well as by increasing the time spent in the open arms ( $F_{4,25}$ : 5.115, p: 0.0368) and decreasing the time in the closed one ( $F_{4,25}$ : 12.01, p < 0.0001) on EPM. WSMoL was able to reverse the depressogenic effect of UCMS since latency to immobility in TST increased (F<sub>4,25</sub>: 6.128, p: 0.0022), while the number of immobilities (F<sub>4,25</sub>: 9.736, p: 0.0002) and immobility time decreased (F<sub>4,25</sub>: 6.772, p: 0.0013). SPT also confirms the depressogenic effect developed by the UCMS and showed that WSMoL has an antidepressant-like effect, once animals that received the lectin preferred sucrose over water (F<sub>4,25</sub>: 269.4, p<0.0001). Conclusion: WSMoL presented inhibitory effects on the symptoms of anxiety and depression induced by UCMS. Keywords: horseradish tree, lectin, chronic stress.

Acknowledgements: CNPq, CAPES and FACEPE.

#### J-30. The Lectin from *Schinus terebinthifolia* Raddi. Leaves (SteLL) Inhibits Carrageenaninduced Acute inflammation in Mice

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Introduction: Inflammatory diseases are widely known as leading contributors to global morbidity, underscoring the urgent need to explore novel agents endowed with antiinflammatory properties. Natural sources, such as plants, have garnered attention for their potential as safer anti-inflammatory therapies. Plant lectins, proteins that can specific and reversible bind to carbohydrates, can play pivotal roles in various biological processes, including inflammation. The lectin from leaves of Schinus terebinthifolia Raddi (SteLL) has demonstrated a range of potentials, including antimicrobial, antitumor, antinociceptive, anxiolytic, and antidepressive activities. Aim: To evaluate the anti-inflammatory potential of SteLL in two models of acute inflammation in mice (Mus musculus). Approved by the Ethics Committee on Animal Use of UFPE (process no. 0025/2022). Materials and methods: SteLL (1, 5 and 10 mg/kg) were administered intraperitoneally to Swiss female mice 30 minutes before the induction of paw edema or peritonitis by carrageenan. In the paw edema model, paw thickness and morphometry were checked in period between 1 and 4 h after carrageenan injection. In the peritonitis model, the total and differential count of leukocytes that migrated to the peritoneum was performed. Measurement of inflammatory markers (cytokines, nitric oxide, total protein, and myeloperoxidase - MPO) was performed on both models. Results and discussion: SteLL promoted a notable reduction in paw edema after 1 h (42.4-39.4%), 2 h (55.1-52.1%), 3 h (48.4-50.0%) and 4 h (61.1-63.4%). Morphometric analysis showed that SteLL also significantly decreased epidermal edema thickness (30.2-40.7%). Furthermore, SteLL exhibited a regulatory effect on cytokines, reduced nitric oxide levels and attenuated MPO activity in a paw homogenate. SteLL effectively inhibited the leukocyte migration for peritoneum (56%-69%). Neutrophil count decreased by 25-32%, while mononuclear cell count increased by 67-74%. The reduction in leukocyte migration, especially neutrophils, shows the anti-inflammatory effect of SteLL. Also, SteLL treatment resulted in reduced MPO activity, decreased plasma leakage, cytokine modulation, and nitric oxide reduction in the peritoneal fluid. Conclusions: SteLL exhibits a potential role as a modulator of cellular inflammatory events, standing out as a promising candidate in the development of new phytopharmaceuticals with anti-inflammatory action. Keywords: Brazilian pepper tree, lectin, inflammation.

Acknowledgements: CNPq, CAPES and FACEPE.

### J-31. Water-soluble *Moringa oleifera* Seed Lectin Exhibits Anti-depressive-like Effects Mediated via Monoaminergic Signaling in Mice

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**Introduction:** Lectins are proteins that specifically bind to carbohydrates. Compelling insights have introduced a variety of neuroprotective effects of lectins, including antinociceptive, antiepileptic, anxiolytic and anti-depressive-like activities. However, the mechanisms by which lectins exert neurological functions are still unclear. The water-soluble Moringa oleifera lectin (WSMoL) is a protein extracted from *M. oleifera* Lamarck seeds (Moringaceae), a tree widely distributed in tropical regions, including northeastern Brazil. WSMoL has already been described to induce anxiolytic-like effects in mice (Mus musculus). Aim: The present study investigated the acute anti-depressive-like (anti-immobility) effect of WSMoL in mice and the possible pathways involved in its effects. Materials and methods: This work was approved (protocol 0010/2021) by the Ethics Committee on Animal Use of UFPE. The animals were treated with WSMoL (1, 2, and 4 mg/kg, i.p.) 30 min before the tail suspension test (TST). To investigate the involvement of monoaminergic and nitrergic signaling, the mice were pretreated with selective antagonists. The role of the WSMoL carbohydrate-recognizing domain (CRD) was verified by previous incubation of lectin with casein (0.5 mg/mL). The subacute antiimmobility effect was also evaluated by administering WSMoL (1, 2, and 4 mg/kg, i.p.) once a day for 7 days. Finally, the open field test (OFT) was performed to identify possible interferences of WSMoL on animal locomotory behavior. Results and discussion: WSMoL reduced the immobility time of mice in the TST at all doses. Combined treatment with fluoxetine (5 mg/kg, i.p.) and WSMoL (1 mg/kg) was also effective. The CRD appeared to be involved in the anti-immobility effect since WSMoL (4 mg/kg) pre-incubated with casein showed no activity. The lectin effect was prevented by the pre-treatment of mice with ketanserin (5-HT2A/2C serotonin receptor antagonist), yohimbine (antagonist non-selective α2-adrenoceptor), and SCH 23390 (D1 dopamine receptor antagonist), thereby demonstrating the involvement of monoaminergic pathways. In contrast, pre-treatment with L-NAME (nonselective inhibitor of nitric oxide synthase), aminoguanidine (inhibitor of inducible nitric oxide synthase), and L-arginine (precursor of NO) did not interfere with lectin action. WSMoL exhibited a subacute effect in the TST, thereby reducing immobility time even in the seventh day. OFT data revealed that the anti-immobility effect was not caused by interference with locomotor behavior. Conclusion: WSMoL elicits an anti-depressant-like effect that is dependent on monoaminergic signaling.

**Keywords**: horseradish tree, lectin, depression. **Acknowledgements**: CNPq, CAPES and FACEPE.

**J-32.** Toxicity Effect of Bauhinia monandra Fraction on Biomphalaria glabrata Adult Snails, Schistosoma mansoni Cercariae and Ecotoxicological Assay with Artemia salina Aguiar, T.W.A.<sup>1</sup>, Batista, J.J.<sup>1</sup>, Rocha, J.V.R.<sup>2</sup>, Ferreira, S.A.O.<sup>3</sup>, Sampaio, M.V.L.<sup>4</sup>, Pereira,

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**INTRODUCTION:** Biomphalaria glabrata mollusks are the main vector of schistosomiasis in Brazil and plants of the Bauhinia genus are widely found in endemic continents with the disease; leaves of Bauhinia monandra contain a BmoLL lectin with biocidal action. **OBJECTIVES**: To analyze the toxic and genotoxic effect of *B. monandra* leaf fraction (F) on *B.* glabrata cells, cercariae mortality and ecotoxicity assay with Artemia salina. MATERIALS AND METHODS: F was obtained with leaf powder in 10mM citrate-phosphate buffer, pH 6.5, containing 0.15M NaCl. Ammonium sulfate (60%) was added to precipitate proteins. Adult snails were exposed to F (24h) for survival evaluation. After definition of sublethal concentrations, adult snails were exposed for 24h. Surviving mollusks were randomly selected for hemolymph collection, morphological analysis, and comet assay. Hemocytes were analyzed under an optical microscope, with a 100x objective, in triplicate. The slides of comet assay were analyzed using a fluorescence microscope with magnification of 400x. Nuclei (100) were counted for each replica, totaling 400, then classified into 5 categories of DNA damage (0, 1, 2, 3 and 4), depending on the extent of the damage. A suspension of approximately 100 cercariae were kept in watch glass and exposed to F. Lethality was observed at intervals of 15, 30, 60 and 120min of exposure. Finally, encysted eggs of A. salina were placed in a beaker with 500mL of seawater and constant aeration for 48h. After hatching were collected and exposed to F for 24h. RESULTS AND DISCUSSION: F at a concentration of 1.0 mg/mL showed 83.3% mortality and 100% at 2.0 mg/mL. In B. glabrata hemocytes analysis, characteristic cells for apoptosis, micronucleus and binucleation were detected, while for comet analysis, different degrees of nuclear damage were observed. F was able to cause total mortality of cercariae and did not present environmental toxicity. CONCLUSIONS: F was toxic, damaged B. glabrata DNA and was able to interrupt the schistosomiasis cycle, being a good candidate for molluscicide.

Keywords: Schistosomiasis, Genotoxicity, environmental monitoring. Acknowledgment: FACEPE, CNPq.

### J-33. Anti-hemolytic and anti-diabetic activity of *Cenostigma microphyllum* (Mart. ex G.Don) Gagnon & G.P.Lewis ethyl acetate extract of leaves

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Introduction Diabetes is one of the most worrisome metabolic disease due to its high incidence at a global scale, and, because of its prognostic with serious clinical features such as diabetic foot (that can lead to amputation of members) and loss of sight, which compromise the quality of life of millions of people and generate high spendings to the public health systems. Cenostiqma microphyllum, popularly known as catingueira rasteira, is a plant belonging to the phytogeographic domain of caatinga, that has been used in the folk medicine but there are scarce scientific studies that explore its biological activities. Aim To evaluate the anti-diabetic and anti-hemolytic potential of Cenostigma microphyllum ethyl acetate extract of leaves (CmEALE). Materials and methods C. microphyllum was collected in Parque Nacional do Catimbau (Buíque - Pernambuco, Brazil), and deposited in IPA herbarium (nº 84880) and SISGEN (nº A6ACCCB). Leaves were dried (40°C) and the powder (100 g) extracted under heat in Soxhlet apparatus, using organic solvents (cyclohexane, chloroform, ethyl acetate and methanol). Ethyl acetate extract was the one who demonstrated better activity in preliminary experiments, and so was the chosen to perform the anti-hemolytic assay, that consist in evaluated the extract capacity of inhibit erythrocytes lipid peroxidation by H<sub>2</sub>O<sub>2</sub>. For evaluation of the anti-diabetic activity, extract potential of increase intake of glycose by yeast (Saccharomyces cerevisiae) was used. Results were expressed as mean ± SD and statistical analysis of one-way ANOVA followed of Tukey's posttest was done at Graphpad Prism 8.0.2 (p<0.05). Results and discussion CmEALE caused hemolysis inhibition of 74.10 ± 0,02 % with its higher concentration tested (2 mg/ ml), demonstrating its capacity of reacting with H<sub>2</sub>O<sub>2</sub> and not allowing this reactive specie to cause lipid peroxidation of membrane (antioxidant and anti-hemolytic). In vitro evaluation of the glycose intake by yeast demonstrated the highest percentage of 57.66 ± 18.28 % for its higher concentration tested (1 mg/ ml), indicating its potential for reducing the extracellular glycose concentration. Conclusion CmEALE is a hypoglycemic agent by improving glycose intake to cells with the advantage of being also antioxidant by scavenging H<sub>2</sub>O<sub>2</sub> reactive specie, protecting cells from lipid peroxidation. Thus, CmEALE has potential to become a cheaper alternative in diabetes treatment.

Acknowledgment: CAPES, CNPq, FACEPE, LAB-DPN, OrganoMAR, and Laboratório de Patologia Molecular

Keywords: Hypoglycemic, Cantigueira, Phytotherapy

### J-34. *In vitro* antiproliferative activity of lectins from *Myracrodruon urundeuva* Allem. against T47-D human breast tumor cell line

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Myracrodruon urundeuva Allem. (Anacardiaceae) is a native Brazilian plant from Caatinga and Cerrado biomes. This species has proven anti-inflammatory, gastroprotective, antibacterial, antiviral activities against rotavirus and anticancer effect for HCT-116 (colorectal), SF-295 (glioblastoma), HL-60 (leukemia) and RAJI (leukemia) cell strains. However, M. urundeuva lectins are poorly studied in terms of their antineoplastic potential. Lectins are glycoproteins with high specificity for binding to glycans, molecules associated with the capacity for invasion and metastasis of tumoral cells. Here, lectins isolated from *M. urundeuva* MuBL (bark) and MuLL (leaf) were investigated for potential antiproliferative activity against the human breast tumor cell line T47-D. The lectins were purified in Federal University of Pernambuco – UFPE. The T47-D cell line was cultured in RPMI 1640 medium, supplemented with 5% inactivated fetal bovine serum (FBS) at 37°C in a humidified atmosphere with 5% CO2. In the antiproliferative assay, 6 x 10<sup>4</sup> cells were plated in 96-well plates and incubated as mentioned above. After 24 hours, cells were treated with different concentrations (0.25; 2.5; 25 and 250  $\mu$ g/mL) of MuBL and MuLL. After treatment and drying, the plate was stained with sulforhodamine B (SRB), and cell density quantified at 540 nm. Samples were considered active when they showed growth inhibition greater than 50%. The inhibition of The T47-D cells growth was greater than 50% at all concentrations tested with MuLL and MuBL. MuBL showed similar results to the positive control m-AMSA with an inhibition 60.29% and 61.31% at 0.25  $\mu$ g/mL and 2.5  $\mu$ g/mL. These results are promising and show the potential antiproliferative activity of *M. urundeuva* lectins. In this regard, it has been discussed that lectins can induce in vitro programmed cell death by apoptosis or autophagy in tumor chains through three main pathways, including a) direct ribosome inactivation, b) endocytosis-dependent mitochondrial dysfunction and c) binding of receptors containing sugar. MuBL and MuLL lectins from M. urundeuva have antiproliferative activity against the T47-D tumor cell line and may be investigated for the mechanism of action. In addition to the described applications, this study suggests the investigation of lectins obtained from other Caatinga plants.

Acknowledgments to the Grupo de Pesquisa em Oncologia Celular - ONCOCEL da Universidade de Penambuco – UPE; Coordenação de Aperfeiçoamento do Pessoal do Nível Superior – CAPES, Brazil [88887.645986/2021-00].

Keywords: Caatinga. MuLL. MuBL. Cancer.

### J-35. Characterization and Evaluation of the Hemagglutinating Activity of the Extract From The Seeds of Salvia hispanica L.

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Introduction: Lectins are proteins that can perform various biological roles, including antibacterial, antifungal, anti-inflammatory, antitumor, and wound-healing activities. These functions are associated with their ability to bind to carbohydrates present on the cell surface of target organisms, thereby preventing their growth and/or inducing a cellular response. Salvia hispanica L. belongs to the Lamiaceae family, and its seeds are a source of omega-3 fatty acids, fiber, and protein. **Objective:** The objective of this work was to evaluate the presence of lectins in *S. hispânica* seeds. **Methodology:** To achieve this goal, the seeds were soaked in a 50 mM Tris-HCl solution at pH 8.0 during 2 hours. Afterwards, the seeds were dried in an oven at 50°C, and the mucilage formed was then removed using a sieve. Finally, a crude extract (10% w/v) of the triturated seeds was prepared in the same buffer solution for 16 hours under agitation. The extract was subsequentely centrifuged (15,000 G for 15 min at 4 ºC) and filtered. The phenolic compounds in the extract were quantified using the Folin-Ciocalteau method and expressed as milligrams of gallic acid equivalents (GAE). Chemical characterization was performed using High-Performance Liquid Chromatography (HPLC). Subsequently, hemagglutinating activity (HA) tests, protein dosage, and inhibition tests with different carbohydrates were conducted. Protein fractionation was carried out using ammonium sulphate in different concentration ranges, and the fraction with the highest specific activity was subjected to affinity chromatography on a chitin column (7.5 x 1.5 cm). Results and discussions: The crude extract of S. hispânica had an AH of 8192, which was inhibited by arabinose, N-acetylglucosamine, and galactose, confirming its lectin nature. The fraction with the highest specific activity was PF20% (AHE: 162,311), which was subjected to affinity chromatography on a chitin column, showing a single protein peak with hemagglutinating activity. HPLC characterization revealed peaks with high intensity. However, it was not possible to identify them based on secondary metabolites used as standards. The total phenol content indicates that the crude extract has  $4.54 \pm 0.05 \ \mu g EAG/g$ . Conclusion: The seeds of S. hispânica are a source of lectins, and future studies should be conducted to characterize the lectin, define its therapeutic potential, and identify its chemical composition. Acknowledgements: CNPq, UFAL; FAPEAL

Keyword: lectin; plant; extract

## J-36. Effects of Lectin Preparations from *Microgramma vacciniifolia* on *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)

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Introduction: Alphitobius diaperinus is a pest beetle mainly found in poultry houses or associated with stored products, including wheat, herbs, and seeds. In poultry farms, these insects live in litter and feces, feeding on manure and feed. The method of controlling A. diaperinus populations is the use of synthetic insecticides, such as pyrethroids, organophosphates, and cypermethrin. However, these chemicals are often ineffective due to insect resistance and have environmental toxicity. Thus, alternative methods of control are needed. This is the first time that lectin preparations were evaluated for insecticidal activity on this insect. **Objective**: To evaluate the susceptibility of A. diaperinus larvae and adults to saline extract (SE), lectin-rich fraction (FR), and isolated lectin (MvRL) from Microgramma vacciniifolia rhizome. Material and Methods: To determine immediate effects, larvae and adults were exposed to SE (10.5 mg/mL), FR (7.5 mg/mL), or MvRL (1.0 mg/mL) for 48 h. Live insects were collected and evaluated for acetylcholinesterase (AChE) activity. Delayed effects of SE (10.5 mg/mL), FR (7.5 mg/mL), and MvRL (0.2 and 0.4 mg/mL) were checked by incubating the adults for 16 days with a diet containing the preparations. In vitro effects on gut digestive enzymes were of live insects investigated. Results: All preparations showed immediate larvicidal effect (survival lower than 40%) but had no effect on adult survival. Extracts from FR-treated larvae showed higher AChE activity than control insects. In the delayed effect assay, the adults lost biomass after consuming SE and FR. FR was the most effective inhibitory agent of trypsin-like and amylase activities (88% and 65% inhibition, respectively). All preparations inhibited endoglucanase activity in 94-98%, while SE and FR inhibited exoglucanase activity in 93.2 and 94.1%, respectively. Conclusion: M. vacciniifolia rhizomes contain compounds (including MvRL) that affect the survival and physiology of A. *diaperinus*, acting as potential natural insecticides for controlling this pest.

**Keywords**: *Microgramma vacciniifolia*; lesser mealworms; poultry farming; natural insecticide.

# J-37. Sulfated polysaccharides-rich samples from *Caulerpa cupressoides* var *flabellata*: a study of the cytotoxicity and neuroprotective potential against oxidative damage on Neuro-2a cells

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Neurodegenerative diseases (ND), such as Parkinson's and Alzheimer's diseases, are a global health problem affecting millions of people around the world. One of the many causes associated with ND physiopathology is related to the excessive production of reactive oxygen species (ROS). This scenario indicates the urgency for the search for new neuroprotective biomolecules able to mitigate the deleterious effects of ND. In this context, sulfated polysaccharides (SP) extracted from marine seaweed are a promising alternative for treating oxidative stress-induced neuronal damage, due to their antioxidant activity. Therefore, our research aims to evaluate the cytotoxicity and neuroprotective potential of SP-rich samples extracted from C. cupressoides var. flabellata against oxidative damage on murine neuroblastoma cells (Neuro-2a cells). Five SP-rich samples (crude extract, F0.3, F0.5, F1.0, and F2.0) were extracted from C. cupressoides with increasing volumes of organic solvent, using already validated methodology. Thereafter, the cytotoxicity of all samples, at different concentrations (100, 200, 500 µg/mL), on Neuro-2a was evaluated, using the 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. In addition, we assessed the samples' neuroprotective effect against hydrogen peroxide  $(H_2O_2)$  on Neuro-2a cells through different treatment modalities (co-treatment, pre-treatment, and posttreatment), for 24 h, using the MTT method. Results indicated that only the crude extract (500  $\mu$ g/mL) and F0.5 sample (200 and 500  $\mu$ g/mL), showed a cytotoxic effect. All other SP-rich samples showed no cytotoxic effect (cell viability > 80% compared to the control). Additionally, a protective effect against H<sub>2</sub>O<sub>2</sub> damage was observed on Neuro-2a cells exposed to the F2.0 sample, at 500  $\mu$ g/mL, in both the co-treatment and post-treatment modalities  $(141\% \pm 15\% \text{ and } 158\% \pm 5\% \text{ compared to the positive control})$ . These preliminary results suggested a promising neuroprotective effect of the F2.0 sample. In addition, the F2.0 sample did not show cytotoxic effects at any concentration, by MTT assay. Therefore, the present study indicates that SP from C. cupressoides could be a promising new alternative for the treatment of ND and may contribute to the role of SP as a neuroprotective agent. However, more studies, in vitro and in vivo, are necessary to confirm this evidence and elucidate the possible antioxidant potential of the F2.0 sample on oxidative stress-induced neuronal damage.

**Acknowledgments**: This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Chamada Universal, n° 402651/2021-9. **Keywords**: Neurodegenerative diseases; Antioxidant; Neuro-2A cells.

#### J-38. The leishmanidal activity of *Bauhinia forficata* Link aqueous stem extract Figueredo, A.S.; Ferreira, P.F.<sup>2</sup>; Silva, L.A.<sup>1</sup>, da Silva, M.C.P.<sup>1</sup>; <u>Silva-López, R.E<sup>2\*</sup></u>

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Introduction: Bauhinia forficata is an important Brazilian medicinal plant, named "cow's paw", that is employed for different therapeutic purposes, such as, diabetes mellitus, purgative, diuretic, viral and fungal infections, cancers, inflammation, and respiratory, cardiovascular and neurodegenerative diseases. Objectives: to investigate protease inhibitor (PI) activity of aqueous B. forficata leaf, seed and stem extracts using reference and Leishmania amazonensis secreted proteases; to evaluate, in the most active extract, its toxicity against Tenebrio molitor larvae, erythrocytes, macrophages, and L. amazonensis. Material and Methods: B. forficata were collected from the Agroecological Phytomedication Platform (PAF) campus of the Oswaldo Cruz Foundation – FIOCRUZ, Rio de Janeiro, Brazil (S: 22° 56′ 24.10"/ W: 43° 24′ 09.22"), and the plant was deposited in the Botanical Garden of Rio de Janeiro (RB-511.138-JB-RJ). Briefly, fresh leaves, seeds and stems were powdered, and proteins were extracted using aqueous extraction, according to the Gren Chemistry principles. The protein content of extracts was determined by the Bradford method and the PI activities were performed using trypsin, papain, pepsin, and secreted L. amazonensis secreted proteases (FIII). The acute toxicity of the *B. forficata* stem (BF-CA) extract was determined *in vivo* using the larval insect T. molitor, and cytotoxicity was evaluated using human erythrocytes, murine macrophages lineage (RAW 264.7) murine, and L. amazonensis promastigote and amastigote forms (IFLA/BR/67/PH8). Results, discussion and conclusion: B. forficata extracts presented distinctive proteins levels, and the highest concentration was observed in aqueous stem (BF-CA) and tris seed (BF-ST) extracts. Only stem and seed extracts inhibited the reference proteases trypsin, papain and pepsin. All extracts reduced the protease activity of FIII from L. amazonensis, and the greatest inhibition was observed for BF-CA. Thus, BF-CA was elected for the study of toxicity against insect larvae and cytotoxicity to human erythrocytes, murine macrophages and L. amazonensis, since good inhibitors of L. amazonensis proteases induced the parasite dead in vitro. BF-CA were nontoxic to T. molitor larvae, neither cytotoxicity against erythrocytes, and showed low cytotoxicity against RAW macrophages, with 50% cytotoxic <u>concentration</u> (CC<sub>50</sub>) about 166.5  $\pm$  8.61 µg/mL. In relation to *L. amazonensis*, the half maximal inhibitory concentration (IC50) of BF-CA was 54.9  $\pm$  2.86  $\mu$ g/mL for promastigotes and 34.61  $\pm$  3.27 µg/mL for amastigotes. The expressive leishmanicidal effect, low cytotoxicity, and the low cost of BF-CA production, make this extract an attractive and accessible adjuvant agent for leishmaniasis treatment.

**Key words:** *Bauhinia forficata* aqueous extracts; Cytotoxicity; *Leishmania amazonensis*. Acknowledgments: FIOCRUZ (PROEP/CNPq/FARMANGUINHOS number 407849/2017-3) and CAPES.

### J-39. Antioxidant activity of essential oils extracted from the leaves, fruits and seeds of *Eugenia uniflora*

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Introduction: Antioxidant compounds extract from plants reduce the oxidative stress caused by the excess formation of free radicals, and act as a self-defense mechanism. Essential oils can be a source of antioxidant compounds, and species of genus Myrtaceae, such as Eugenia uniflora, known as "pitangueira", are being studied due to this property, and possible application in medical and food areas. However, despite being extracted from different parts of the plant, only the essential oils from the leaves of *E. uniflora* are intensively explored. Objective: Evaluate the antioxidant potential of essential oils extracted from leaves, fruits and seeds of *E. uniflora*. Materials and Methods: The parts of the plants were collected in Recife (PE) and registered in the Herbarium UFP-Geraldo Mariz (voucher 88633). The essential oils were extracted by hydrodistillation using a Clevenger apparatus for 3h and samples were diluted in different concentrations for the antioxidant activity assays through a free radical scavenging of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), using ascorbic acid as standard. The analyzes were performed in triplicate and the results expressed as media±standard deviation of the 50% of the inhibitory concentration (IC<sub>50</sub>). Analysis of variance (ANOVA) was used to compare the results, with a significance level of p<0.05. Results and discussion: The essential oils extracted from the leaves, fruits and seeds of E. uniflora, as well as the ascorbic acid, obtained an IC<sub>50</sub>, by the ABTS reduction, of 16.63±0.0, 130.31±0.0, 40.30±0.0 and 0.211±0.0 mg/mL, respectively; for the DPPH reduction, the samples showed an IC<sub>50</sub> of 39.76±0.0, 32.90±0.0, 48.97±0.0 and 0.011±0.0 mg/mL, respectively. The essential oil extract from the leaves and fruits showed higher antioxidant activity in reduce the DPPH, while the leaves and seeds samples showed better activity in reduce the ABTS. Conclusions: The essential oil extracted from the leaves, fruits and seeds of E. uniflora presented a greater potential reduction against DPPH and ABTS free radicals, being the leaves, the source of the best antioxidant activity. These results showed that the other parts from E. uniflora, as flower and seeds, can be the source of new essential oils for biotechnological applications in the pharmaceutical, cosmetic and food industries.

Acknowledgements: UFPE, CNPq, FACEPE and CAPES Keywords: free radicals, pitangueira, volatile compounds.

#### J-40. ANTIBACTERIAL AND ANTIBIOFILM ACTIVITIES OF Myrciaria tenella ESSENTIAL OIL

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Bacterial resistance to conventional antibiotics is a public health problem and plants have been investigated as sources of essential oils with efficient antibacterial action on resistant bacteria. Myrciaria tenella (DC.) O. Berg (Myrtaceae), commonly called cambuí, is employed in folk medicine by its antimicrobial, anti-inflammatory and antidiabetic properties. This plant contains tannins, flavonoids, monoterpenes, sesquiterpenes, triterpenes and other compounds. The objective of this work was to determine the antibacterial and antibiofilm activities of *M. tenella* essential oil against pathogenic bacterial strains. The leaves of *M.* tenella were collected from Catimbau National Park, Buíque-PE and the fresh leaves were used to essential oil extraction by hydrodistillation. The chemical composition of the essential oil was analyzed by CGMS and the antibacterial activity was evaluated against Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 23235). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil were determined using the serial dilution method (4096 to 64  $\mu$ g/mL) recommended by CLSI (2018). To evaluate antibiofilm activity, we used the crystal violet method. The yield of oil extraction procedure was 0.55% and 1,8-Cineol compound represented 34.5% of the total oil constituents. The essential oil exhibited bacteriostatic activity inhibiting the growth of *S. aureus* (MIC 512 μg/mL) and E. coli (MIC 1024 µg/mL) while the MBC was 2048 µg/mL for both strains. The oil at MICs reduced the biofilm production by E. coli and S. aureus of 60% and 67%, respectively. In conclusion, the *M. tenella* essential oil is an antibacterial agent against human pathogens by its bacteriostac, bactericide and antibiofilm activities.

**Keywords:** Human pathogens. Microrganims. Plant. **Acknowledgment:** CAPES, CNPq and FACEPE.

## J-41. Larvicidal potential and morphophysiological effects of *Amburana cearensis* (Fabaceae) extract on *Aedes albopictus* (Culicidae) larvae

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Dengue, chikungunya and zika are arboviruses transmitted by Aedes albopictus (Skuse, 1894) with recurrent epidemiological outbreaks in tropical countries, such as Brazil, especially in the Northeast region. Population control of vector insects is the main strategy in the arboviruses management, and research on insecticide botanical extracts has gained prominence due to its environmental compatibility and similar or greater effectiveness, compared to chemical insecticides. Thus, this work investigated the larvicidal activity and morphophysiological effects of Amburana cearensis (Allemão) A.C.Sm. seeds extract, a leguminous tree that occurs in Northeastern Brazil, usually known in portuguese language as "Imburana-de-Cheiro" or "Cumaru", against A. albopictus larvae. In quadruplicate, fourth stage A. albopictus larvae (n=20) were exposed to different concentrations (50% to 0%, v/v; 20mL) of aqueous extract of A. cearensis seeds (1:10, w/v; at 80°C) for determination of lethal concentrations capable of killing 50% (LC<sub>50</sub>) of the larvae in 24h and 48h of exposure, using a Probit regression analysis. Dechlorinated water was used as a control. The larvae were registered under a microscope with a camera attached to analyze the morphological alterations. Furthermore, larval intestines were dissected to produce an intestinal homogenate, used in the investigation of the extract effects on the inhibition of insect digestive proteases. Enzymatic activity was determined spectrophotometrically (440 nm) by the presence of peptides from azocasein digestion, a non-specific protease substrate. An inhibition unit (IU) was determined as the ability of the extract to decrease the absorbance by 0.01. The A. cearensis extract showed insecticidal activity against A. albopictus larvae, with LC<sub>50</sub> of 340 ppm and 100 ppm for 24h and 48h of exposure, respectively, suggesting the extract's bioactivity at low concentrations, reducing its active dose as a function of exposure time. Analyzes suggest that after contact with the extract, contractions occur in the abdominal portion of the larvae resulting in shrinkage of the individual, in addition to alterations in pigmentation of the intestines. Regarding enzymatic inhibition, the extract showed specific inhibition of 106 IU/mg of extract protein, suggesting its potential in compromising the larval digestive proteases. These findings indicate the use of A. cearensis extract for the development of botanical insecticides for A. albopictus and suggest that the digestive proteases inhibition represents one of the action modes of extract's larvicidal activity.

Acknowledgment: CAPES, CNPq, BIOPOL-UFRN, DBG-UFRN, DBQ- UFRN, IMT-UFRN, LABENT-UFRN, LQFPB-UFRN.

Keywords: Botanical Insecticides, Arbovirus Vector, Digestive Proteases Inhibition.

# J-42. Insecticidal activity of the essential oil of *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson leaves and its main compound (1,8-cineole) against *Sitophilus zeamais* Mots., 1855 (Coleoptera: Curculionidae)

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Sitophilus zeamais (Coleoptera: Curculionidae), commonly known as the 'maize weevil', is a significant primary pest that causes quantitative and qualitative damage to different stored grains. The excessive use of synthetic insecticides and the emergence of insect-pest populations resistant to them have made it necessary to explore new insecticides effective against pest and least harmful to the environment and non-target species. Essential oils have emerged as eco-friendly alternatives to synthetic insecticides because they are biodegradable and have less or no adverse effects on the environment. Lippia alba is a fragrant shrub found in different parts of Portugal. It is commonly known as 'lemon balm' and has a wide variation in the chemical composition of the essential oils produced in its leaves. This study aims to analyze the chemical composition of the essential oil extracted from leaves of L. alba and evaluate their insecticidal properties. We also aim to assess the insecticidal activity of 1,8cineole (a principal compound of the essential oil) on the species S. zeamais. Leaves of L. alba were collected from Buíque-PE, and their essential oils were extracted through the hydrodistillation process. We identified the constituents of essential oil through gas chromatography coupled with mass spectrometry (GC-MS). We conducted an ingestion bioassay to determine the insecticidal activity of essential oil and 1,8-cineole on S. zeamais adults. The bioassay included an artificial diet containing wheat flour, distilled water, and essential oil or 1,8-cineole at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 µL/g. A fumigation toxicity test was also conducted at concentrations of 25, 62.51, 87.5, and 375 µL/L of air. Nineteen compounds were detected in the essential oil of L. alba using GC-MS analysis. The compound with the highest concentration was 1,8-cineole. In the ingestion test, the essential oil exhibited toxicity at an LC50 concentration of 0.297 µL/g, while 1,8-cineole did not demonstrate any effect. Both the essential oil and the compound exhibited an anti-nutritional effect on the insects. In the fumigation test, the LC50 for L. alba essential oil and 1,8-cineole were 78  $\mu$ L/L and 13.64  $\mu$ L/L, respectively. The results suggest that both *L. alba* essential oil and 1,8-cineole have significant potential as a bioinsecticide for controlling insect pests, including S. zeamais, which infest stored grains.

Keywords: Natural insecticides; Maize; Caatinga.

**Acknowledgements:** UFPE(Universidade Federal de Pernambuco), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), BIOPROT (Laboratório de Bioquímica de Proteínas).

#### J-43. Characterization of aqueous extracts from Arrabidaea chica Verlot

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**Introduction:** Arrabidaea chica is a popular medicinal plant employed as anti-inflammatory, antitumor, and antimicrobial, and have great healing potential in cutaneous wounds. The chemical and pharmacological identification of its secondary metabolites has been widely investigated, but its primary metabolites, such as proteins, are few studied and its pharmacological capacity little explored. **Objectives:** to extract polypeptides and secondary metabolites using aqueous systems; to identify, isolated and characterize polypeptides protease inhibitors (PIs) and polar secondary metabolites. Material and Methods: A. chica (IV morphotype) leaves were collected from the Agroecological Platform of phytomedicines (PAF) campus of the Oswaldo Cruz Foundation – FIOCRUZ, Rio de Janeiro, Brazil (S: 22° 56′ 24.10"/ W: 43° 24′ 09.22″), and the plant was deposited in the PAF (CBPM 668/PAF). Fresh leaves and peduncles were powdered, and proteins were extracted using distilled water and phosphate buffer. Protein amount was determined by Bradford method and protein profile was evaluated by SDS-PAGE, and the PI activities were performed using trypsin, papain and pepsin. These extracts were analyzed by thin lawyer chromatography (TLC) and liquid chromatography-mass spectrometry (LC-MS) analysis to study the secondary metabolites. Results, discussion and conclusion: A. chica extracts presented similar proteins levels, and the highest amount was observed in aqueous peduncle extract obtained using distilled water (AC-CA). The leaf extract obtained with distilled water (AC-A) inhibited only trypsin (67,4%), leaf extract obtained with phosphate buffer (AC-P) reduced only the activity of pepsin (30,5%), and AC-CA inhibited both pepsin (33,6%) and papain (16,7%). In SDS-PAGE, AC-A presented major protein bands about 9 and 15 kDa; AC-P 15, 20 and 25 kDa, and AC-CA 25, 50 and 100 kDa. The TLC analysis revealed flavonoids, terpenoids and anthocyanidins, which were confirmed by LC-MS. The biochemical and chemical characterization is also being performed for comparison with the extracts obtained using only organic solvents. The present study is the first reported on protease inhibitors in aqueous extracts of A. chica and the discovery of new molecules with high pharmacological potential, which may represent an alternative in the production of biological drugs. Our studies were based on the Green Chemistry principles, with the aim of implementing sustainable processes, preserving the environment by using renewable sources of raw materials and generating lesser toxic products.

Key words: Arrabidaea chica aqueous extracts, protease inhibitors, Leishmania proteases. Acknowledgments: FIOCRUZ (PROEP / CNPq / FARMANGUINHOS number 440022/2022-3).

#### J-44. Trypsin protease inhibitor from leaves of *Cajanus cajan* (L.) Millsp.

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Introduction: Pigeonpea (Cajanus cajan L.) Millsp. is protein-rich legume, cultivated in tropical and semitropical regions around the world, and is the main source of protein for billions of habitants in developing countries. Earlier studies showed that C. cajan seed is source of different trypsin and chymotrypsin proteases inhibitors (PIs). Objectives: to purify a serine (PI) from a phosphate C. cajan leave extract (CC-P); characterize the biochemical, kinectic and structural the purified PI; evaluated the inhibitory activity on Leishmania amazonensis extracellular serine proteases (LSPIII). Material and Methods: C. cajan leaves were collected from the Agroecological Phytomedication Platform (PAF) campus of the Oswaldo Cruz Foundation – FIOCRUZ, Rio de Janeiro, Brazil (S: 22° 56′ 24.10"/ W: 43° 24′ 09.22"), and the plant was deposited in the PAF (CBPM 671 / PAF). Fresh leaves were powdered, and proteins were extracted using phosphate buffer, according to the Gren Chemistry principles. The protein content was determined by the Bradford method and PI activities were performed using trypsin, papain, pepsin. The PI, named TIC, was purified using an affinity chromatography in Trypsin-agarose column. The homogeneity and activity of TIC were performed using SDS-PAGE analyses. The TIC kinectic parameters, Ki, IC<sub>50</sub> and stability, were obtained using bovine trypsin and N $\alpha$ -p-Tosyl-L-arginine methyl ester as substrate, and the data analyzed using GraphPad Prism version 6.0. Mass spectrometry were employed to study the primary sequence of TIC. Results, discussion and conclusion: TIC was partially purified by only step of affinity chromatography. SDS-PAGE revealed one band with about 15 kDa with expressive trypsin inhibitor activity by zymography. TIC showed higher affinity for trypsin ( $Ki = 1.617 \mu M$ ) than for chymotrypsin ( $Ki = 6.460^{13} \mu M$ ) and was a competitive inhibitor for both serine proteases, besides it inhibited almost completaly (98%) the activity of LSPIII. TIC The TIC inhibitory activity was maintained after 24h of treatment at 70°C, and after 1h treatments with different pH values, and β-mercaptoethanol increasing concentrations, demonstrating expressive structural stability. However, the activity of TIC was affected in the presence of oxidizing agents. The mass spectrometry analysis identified the protein as a kunitz type trypsin inhibitor. It is the first time that a trypsin inhibitor was isolated and characterized from C. cajan leaves with expressive inhibition of Leishmania serine protease, since good inhibitors of Leishmania proteases killed parasites in vitro. Further studies about the TIC cytotoxicity on Leihmania parasites will be conducted to reinforce about its anti-Leishmania potential. Keywords: Cajanus cajan; trypsin inhibitor; Leishmania serine protease.

Acknowledgments: FIOCRUZ (PROEP CNPq/FARMANGUINHOS-407849/2017-3), FIOCRUZ (INOVA VPPCB-005-FIO-20/2020), and CAPES.

### J-45. Protective effects of ethanolic extract from Passiflora edulis seeds against Zika virus infection in human placental cells

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**Introduction:** Maternal Zika virus (ZIKV) infection during pregnancy still is an ongoing threat, since no therapeutic solutions have been effective to prevent or mitigate the fetal deleterious outcomes. One potential resource for novel therapeutic solutions is natural products, in particular the Passiflora edulis seed extract (PFSE). Previous studies from our group showed absence of toxicity in placental cells and tissues, and can exert an antiviral effect against ZIKV, as it significantly reduced the viral load and infectivity in these models. As such, this study aims to evaluate the effects of PFSE against cell death and oxidative stress caused by the ZIKV infection in human placental cells. Methods: The HTR-8/SVneo placenta cell line was treated for 1 h with PFSE, and further 24 h with the ZIKV PE243 strain at 10<sup>5</sup> PFU. Afterwards, cell death was analyzed by apoptosis, necrosis, and autophagy by flow cytometry. Reactive oxygen species (ROS) were analyzed by MitoSOX staining and by oxidative burst. To assess the antioxidant activity, we analyzed the gene expression of SOD1-3, CAT, HO-1, NQO1, and p62. The Nrf-2 expression and localization was assessed by immunofluorescence. Results: PFSE prevents death by autophagy induced by ZIKV (p < 0.05), and reduced by 25% the production of oxidative stress in trophoblast cells infected by ZIKV (p < 0.01). When evaluating the Nrf-2 expression and localization, it was found that ZIKV utterly reduced Nrf-2 translocation to cell nuclei. This effect was prevented by PSFE pretreatment as well. Regarding mRNA expression, SOD 3 was reduced by ZIKV, and the PFSE prevented the reduction, whereas CAT was also reduced by ZIKV, but the PFSE unchanged CAT expression. The same pattern was also observed in the expression of HO-1 and NQO-1, with the PFSE preventing such changes. **Conclusion:** The PSFE is able to partially prevent cell death of placental cells caused by ZIKV infection, and has potent antioxidant effect, apparently mediated by the NRF2 pathway. Financial Support: CNPq/MS/SESAU-AL/FAPEAL-PPSUS

Keywords: Viral infection; Natural product; Oxidative stress.

### J-46. ANTICANDIDAL POTENTIAL OF THE ESSENTIAL OIL FROM *Croton pluriglandulosus* Carn. (Euphorbiaceae)

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Fungal diseases have caused morbidity and mortality in the population, mainly due to the increase in resistance to conventional antimicrobials, generating a global health problem. The genus Candida is a class of pathogenic yeasts responsible for infections that affect healthy and immunocompromised patients. These yeasts are responsible for causing oral, vaginal, and oropharyngeal candidiasis as well as can invade tissues and organs, causing systemic issues. Besides, these microorganisms have great clinical relevance because some species are resistant to classes of antimicrobials, causing infections that are increasingly worrying for the health system. Given this scenario, essential oils extracted from plants appear as a natural alternative for the control of diseases caused by these pathogens. Thus, they act as promising compounds in the control of microbial resistance. The present study aimed to evaluate the antifungal activity of the essential oil (EO) extracted from Croton pluriglandulosus leaves (EOCp) on human pathogenic microorganisms in planktonic lifestyles. EOCp was extracted through hydrodistillation, using the Clevenger apparatus. The identification of the major chemical compounds in EOCp was performed by gas chromatography analysis coupled to a mass spectrometer (GC/MS) and the antimicrobial activity was evaluated using the microdilution method. Cells were incubated with EOCp at various concentrations to determine the minimum inhibitory concentration. DMSO (5%) and Fluzaconazole were used as controls. The results showed that EOCp has 26 different metabolites, being Elemicin (25.77%), Bicyclogermacrene (9.37%), caryophyllene (8.99%), 1,3,5-trimethoxy-benzene (6.86%) and hedycaryol (6.21%) the major compounds. Many of the components identified in EOCp are present in other species of the genus Croton, but in different concentration. EOCp (50 µg mL<sup>-1</sup>) was able to inhibit the planktonic growth of Candida krusei (89.3%) and C. parapsilosis (80.70%). However, it was not inhibitory for C. albicans. This result is fascinating compared to other oils, due to the low concentration tested. The antimicrobial activity of EOCp may come from compounds such as elimycin, eucalyptol, bicyclogermacrene,  $\alpha$ -terpineol,  $\beta$ -elemene, 4terpineol and  $\delta$ -elemen. These constituents damage the plasma membrane, causing extravasation of intracellular contents and/or interference with ATP synthesis. In addition, several in vitro studies highlight the anticandidal activity of essential oils extracted from plants. Thus, the data obtained bring a future alternative for obtaining drugs and active compounds to be used by the clinical sector for microbiological control purposes.

Keywords: Infections; Natural products; Chemical compounds.

**Acknowledgments:** Thanks to the Federal University of Ceará, specifically to the Graduate Program in Biochemistry. To the Coordination for the Improvement of Higher Education Personnel (CAPES). National Council for Scientific and Technological Development– CNPq. Cearense Foundation for Supporting Scientific and Technological Development – FUNCAP.

## J-47. Toxicity evaluation of *Cenostigma microphyllum* (Mart. ex G.Don) Gagnon & G.P.Lewis methanolic extract of leaves

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Introduction According to the World Health Organization, assays that evaluate natural products safety use are an important part in the establishment of new therapeutics. Toxicity assays in different biological systems should be performed to ensure safety, target specificity, and to evaluate the interactions with its environment. Cenostigma microphyllum, known as catingueira rasteira, belongs to Brazilian caatinga phytogeographic domain, and has been used in popular medicine for treatment of several conditions, but its safety of use has not been deepened evaluated. Aim Evaluate the toxicological effect of C. microphyllum methanol leaves extract (CmMLE) in Artemia salina, and Tenebrio molitor. Materials and methods C. microphyllum was collected in Parque Nacional do Catimbau (Buíque - Pernambuco, Brazil) and registered at SISGEN (nº A6ACCCB) and deposited at IPA herbarium (nº 84880). Leaves were separated, dried (40°C), and pulverized. Powder (100 g) was placed in a Soxhlet apparatus and extracted under heat, with the use of increasing polarity organic solvents, as follow: cyclohexane, chloroform, ethyl acetate, and methanol. Methanolic extract demonstrated to have more bioactive metabolites in preliminary experiments, thus was the chosen to perform the Artemia salina nauplii toxicity, consisting in the evaluation of extract capacity to cause nauplii stage of A. salina death, positive control was potassium dichromate 0.1% and negative, artificial sea water. For evaluation of *T. molitor* larvae toxicity, an assay that allows knowledge of environmental and target specificity was performed. Results were expressed as number of individuals dead/total number of individuals for both toxicity assays. Results and discussion CmMLE did not cause significant toxicity in both organisms tested. In the nauplii, at its highest concentration (1 mg/ ml), CmMLE had a mortality rate of 11/60, but the positive control was 60/60. At T. molitor test, none of the concentrations used (10% - 1.25) %) showed any mortality (0/12), while positive control (commercial insecticide) showed 8/12. Conclusion CmMLE seems to be safe in interactions with A. salina and T. molitor, which indicates its secure target specificity and brings preliminary environmental safety information. Other organisms should be evaluated in future studies in order to confirm all toxicological properties of CmMLE.

Acknowledgment: CAPES, CNPq, FACEPE, and LAB-DPN Keywords: Safety, *Cenostigma*, Nauplii

## J-48. Bioprospecting of larvicides against arboviral vectors in botanical extracts of seeds from Northeast biomes

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Arboviruses such as dengue, zika and chikungunya are tropical diseases of great epidemiological importance, transmitted mainly by Aedes aegypti. Vector management using chemical insecticides is essential, however, due to their synthetic nature, these compounds cause problems in the environment and in non-target populations. Alternatives are explored, such as molecules derived from plants from different biomes. The objective of this study is to evaluate the larvicidal potential of seed extracts of the native plant Bauhinia cheilantha (mororó) against A. aegypti larvae. The seeds were processed to obtain a hot aqueous extract (MO). Groups of twenty larvae at the L4 stage were placed in plastic containers with a final volume of 20 mL. Control group larvae were immersed in dechlorinated tap water, while treated groups were immersed in different concentrations of crude extract (1.56%, 3.12%, 12.5%, 25%, 50%) prepared by dilution in dechlorinated water. Lethal doses (LC50) and (LC90) were defined as the concentrations that decrease the survival of insect larvae by 50% and 90% when compared to the control. The lethal doses of the extracts (LC50) and (LC90) on A. aegypti larvae at 24 and 48 hours were calculated by Probit-type regression analysis (Excel® 2021, Microsoft) with a 95% confidence interval. B. cheilantha extract showed high larval mortality, CL50 of 0.61% (24h) and 0.15% (48 h). The soluble protein content was 0.29 mg/mL. The extract of B. cheilantha (MO) showed lethal doses below 50 ppm, an important parameter for the development of a bioinsecticide on an industrial scale. Thus, MO represents a strong candidate for the development of a low-cost botanical insecticide, as it is an aqueous extract with low lethal doses. Given these results, several studies can be carried out to evolve in the development of a bioinsecticide: insecticidal activities in other stages of A. aegypti life (egg, pupa, adult), investigation of the constituent modes of action of larvicidal activities, analysis of the activity of attractiveness or repellency for oviposition and determination of larvicidal activity on larger scales in the field.

Keywords: *Aedes aegypti*, Arboviruses, insecticide Acknowledgements: CNPq; IMT; LABENT-UFRN; DBG

#### J-49. *Myracrodruon urundeuva* LEAF LECTIN CONJUGATED WITH FLUORESCEIN ISOTHIOCYANATE FOR IDENTIFICATION OF BINDING SITES IN *Aedes aegypti* EGGS

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Lectins are proteins or glycoproteins that binds reversibly and specifically to carbohydrates or glycoproteins. The chitin binding lectin isolated from Myracrodruon urundeuva Leaf, called MuLL, showed larvicidal ( $LC_{50}$  of 0,20 mg/mL) and ovicidal ( $EC_{50}$  of 0.88 mg/mL) activities against A. aegypti. The presente work aimed to identify binding sites of the lectin in A. aegypti eggs using MuLL- fluorescein isothiocyanate (FITC) conjugate and fluorescence microscopy. Powder from *M. urundeuva* leaves was homogenized in 0.15 M NaCl at a proportion of 10% (w/v) for 16 hours at 25 °C and then centrifuged (9,000g, 15 min). The proteins present in the supernatant were precipitated with 60-80% (w/v) ammonium sulfate and after centrifugation (9,000g, 15 min), the precipitated protein was chromatographed on a chitin column previously equilibrated in 0.15 M NaCl. MuLL (20 mg) recupered from column with 1.0 M acetic acid was dialyzed against the buffer 0.1M bicarbonate/carbonate, pH 9.0 containing N-acetyl-Dglucosamine and conjugated with FITC (1.0 mL). The hemagglutinating activity (HA) of MuLL-FITC conjugate was determined and A. aegypti eggs were incubated with MuLL-FITC (0.88 mg/mL) for 24, 48 and 72h. The conjugate showed the same HA of MuLL (256<sup>-1</sup>) and thus, the carbohydrate binding site of MuLL was not blocked after conjugation with FITC. Fluorescence in embryo from eggs incubated with MuLL-FITC was detected in the head (24 h), head and upper portion of the digestive tract (48 h) and head, serous cuticle and throughout digestive tract. Eggs not treated with MuLL-FITC showed low autofluorescence in the serous cuticle and embryo head. The study revealed that MuLL is able to penetrate into the eggs and bind to A. aegypti embryo.

Keywords: MuLL. Chitin. Embryo.

Acknowledgment: CAPES, CNPq and FACEPE.

#### J-50. Characterization of secondary metabolites in an aqueous extract from the stem of bamboo (*Guadua angustifolia*)

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Introduction: Brazil is a country with a vast biological diversity, encompassing a multitude of species and a wide range of variety and complexity within its biomes. From various perspectives, this biological diversity has been extensively studied. One of these perspectives is the interest in the medicinal properties of natural organic compounds derived from plants. In Brazil, you can find the greatest diversity of native bamboo species in the Americas. Among the identified species, Guadua angustifolia stands out. It is a rapidly developing grass with numerous applications, including its use in the medicinal field, where it exhibits various biological activities: antitumor, antidiabetic, anti-inflammatory, antihypertensive, antioxidant, and antimicrobial. Objective: The present work aims at the chemical characterization of the G. angustifolia extract. Materials and methods: To achieve this goal, a crude extract (20% w/v) of the plant stem was prepared in a 50 mM Tris-HCl solution at pH 8.0. The extract underwent High Performance Liquid Chromatography (HPLC) analysis to identify potential chemical compounds that could account for the diverse activities of the bamboo extract. Additionally, the total phenol content (TPC) was evaluated using the classical Folin-Ciocalteu method, expressed as milligrams of gallic acid equivalents (GAE). This analysis aimed to detect phenolic compounds, which have been previously described in the literature for their high antioxidant activity, further supporting their involvement in various biological activities. Results and discussion: Using the HPLC technique, it was observed that the sample displayed highly intense peaks. However, it was not possible to identify them based on secondary metabolites used as standards: standard solutions of gallic acid, catechol, catechin, chlorogenic acid, caffeic acid, (-) epicatechin, syringaldehyde, cumaric acid, coumarin, rutin, myricetin, and quercetin. The analysis of total phenol content revealed that the sample contained a content of 2.07±0.08 µg GAE/g. Conclusion: The aqueous extract of G. angustifolia stem contains significant amounts of phenols, which may be identified in future studies.

Keywords: chromatography; extract; phenols.

Financial supports: FAPEAL; CAPES.

#### *J-51. Crotalaria spectabilis* Roth: the leaf extract activity against *Leishmania amazonensis* <u>Ferreira, P.F.<sup>1</sup></u>; Pacheco, J.S.<sup>1,2</sup>; Torres-Santos, E.C.<sup>2</sup>; Silva-López, R.E<sup>1\*</sup>

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Introduction: Crotalaria spectabilis is a tropical legume found throughout the world and have been used for many purposes, such as, control of soil nematodes, trap plants in endemic regions of dengue, and protection against soil erosion. Plants produce antimicrobial substances, which are the first line of defense, and the most important are protease inhibitors (PIs). These PIs are employed to treat many diseases. Objectives: to investigate protease inhibitor (PI) activity of leaf phosphate C. spectabilis extract (CS-P), using reference and Leishmania amazonensis extracellular serine proteases (LSPIII); to evaluate its cytotoxicity using resident macrophages from the peritoneal cavities of normal BALB/c mice and L. amazonensis (IFLA/BR/67/PH8); and isolate a serine PI. Material and Methods: C. spectabilis were collected from the Agroecological Phytomedication Platform (PAF) campus of the Oswaldo Cruz Foundation - FIOCRUZ, Rio de Janeiro, Brazil (S: 22° 56' 24.10"/ W: 43° 24' 09.22"), and the plant was deposited in the Botanical Garden of Rio de Janeiro (RB- 488.839). Fresh leaves were powdered, and proteins were extracted using phosphate buffer. The protein content of extracts was determined by the Bradford method and the PI activities were performed using trypsin, papain, pepsin, and FIII. The cytotoxicity was evaluated using macrophages and L. amazonensis promastigote and amastigote. Affinity chromatography using a Sepharose-Trypsin column was employed to isolate the serine PI. Results, discussion and conclusion: CS-P showed very low cytotoxicity against macrophages, with 50% cytotoxic concentration (CC<sub>50</sub>) about > 200 µg/mL of protein extract. In relation to *L. amazonensis,* the half-maximal inhibitory concentration (IC<sub>50</sub>) of CS-P was 24.35 ± 2.6 µg/mL of protein extract for promastigotes and 31.74  $\pm$  3.0  $\mu$ g/mL of protein extract for amastigotes. The CSPI, a trypsin-like inhibitor from CS-P, was purified about 6.05-fold with yielded of 61% from 3,2 mg of protein of extract. SDS-PAGE analysis identified three protein bands with 32, 37 and 44 kDa. CSPI inhibited the activity of both trypsin and LSPIII about 67 and 75%, respectively, using Nαp-Tosyl-L-arginine methyl ester as substrate. Trypsin inhibition by CSPI was the highest at 65°C and at pH 7.0. Further biochemical and structural characterization has been performed. Besides, this is the first report of antileishmanial activity of C. spectabilis extracts and the identification of serine polypeptide PI. The expressive leishmanicidal effect, low cytotoxicity, and the low cost of CS-P obtaining, make this extract a possible herbal adjuvant for leishmaniasis treatment.

Key words: *Crotalaria spectabilis* aqueous extracts, protease inhibitors, Leishmania proteases and cytotoxicity.

Acknowledgments: FIOCRUZ, FAPERJ, CNPq and CAPES

#### J-52. Purification, Characterization and Evaluation of the Antimicrobial Activity of the Thermostable Lectin from Bark of *Abarema cochliacarpos* (Gomes, 1803) Barneby & Grimes 1996

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Introduction: Lectins are proteins that can assume several biological roles, including antibacterial and antifungal activities. These activities are related to the ability of lectins to bind to carbohydrates present on the cell surface of target organisms, preventing their development and/or inducing a cellular response. Abarema cochliacarpos is a plant used in folk medicine to treat ulcers, wounds, skin infections, gastritis, among others. This study aims to purify, characterize and evaluate the antimicrobial activity of the lectin from bark of A. cochliacarpos. Materials and methods: The extract was prepared and filtered over activated charcoal. An aliquot of this filtrate was applied into a chitin chromatographic column. Proteins of interest were eluted with 1M acetic acid. Then, the sample was dialyzed and its hemagglutinating activity (AH), protein dosage and characterization tests were evaluated. Antibacterial and antifungal activity were investigated with microorganisms pathogenic to humans by determining the minimum inhibitory concentration (MIC). Results and discussion: It was possible to confirm the purification of the lectin, called AcBL (AH: 512; 0.778 mg of protein), through SDS-PAGE electrophoresis under reducing and non-reducing conditions. Only one protein band in both conditions was observed. AcBL was inhibited by casein, showed thermostability in a wide temperature range, is an acid-neutral protein and had its activity reduced in the presence of EDTA, calcium and magnesium ions. AcBL showed antibacterial activity (MIC from 1,5 to 3 µg/mL) against Acinetobacter baumannii, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Salmonella enteritidis and Streptococcus pyogenes, as well as antifungal activity (MIC of 3 µg/mL) against Cryptococcus neoformans, and can be applied as an alternative in the treatment of diseases caused by pathogenic microorganisms. **Conclusion:** The development of this work contributes to the isolation of new antimicrobial protein, with regional relevance, which may represent a new biomaterial with high biotechnological potential.

Financial Support: FAPEAL, CAPES.

Keywords: Medicinal plant; protein; antipathogenic activity.

### J-53. Antiviral Potential of Brazilian Red Propolis Against Chikungunya Virus: In Vitro and In Silico "Insights"

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Chikungunya virus (CHIKV) causes an acute febrile illness that can progress to a chronic phase characterized by persistent arthralgia. Currently, there are no antivirals or vaccines against this virus. Brazilian Red Propolis (BRP) is a natural resin that bees produce by mixing saliva and exudate from plants, which have antimicrobial activities. Therefore, the aim of this study was to evaluate the antiviral activity of BRP from Alagoas State against CHIKV through in vitro and in silico approaches. The chemical characterization of the hydroalcoholic extract (HE) of BRP was performed using ultra-performance liquid chromatography (UPLC-DAD), and the presence of the liquiritigenin, pinobanksin, daidzein, formononetin, biochanin A and isoliquiritigenin was detected. The BRP cytotoxicity in Vero E6 cells was assessed using the MTT colorimetric assay and CC50 of 200.1 µg/mL was obtained. For the antiviral activity assays, CHIKV adsorption was carried out for 2h on Vero E6 cells, followed by treatment with different concentrations of BRP solutions for 72h. Cell viability was then analyzed using MTT assay. Promising antiviral activity was detected, with IC50 and selectivity index (SI) values of 22.80  $\mu$ g/mL and 8.7, respectively. To confirm the antiviral activity, intracellular virus labeling was performed and the percentage of CHIKV-positive cells was assessed by flow cytometry 48h after infection. BRP treatment resulted in a reduction effect over 50% on the percentage of infected cells. In the inactivation assay, CHIKV suspensions were initially incubated with BRP for 2h, diluted, and then incubated for 48h with Vero E6 cells. Plaque-forming units (PFU) were detected, revealing a significant reduction from 31,000 PFU/mL (untreated control) to 4,867 PFU/ mL (BRP). Additionally, a time-of-drug addition assay was performed, and cells were treated with BRP for 2h before CHIKV infection, simultaneously with infection (0h), or at different times post-infection (2h, 4h and 6h). The inhibitory activity of BRP treatment was observed 2h, 4h and 6h after infection. To analyze the potential targets of the compounds detected by UPLC-DAD against CHIKV proteins, an in silico molecular docking analysis was conducted. The molecular docking results revealed that the compounds biochanin A, daidzein, formononetin, isoliquiritigenin, liquiritigenin, pinobanksin exhibited a high affinity for the E3-E2-E1 CHIKV complex. In conclusion, BRP demonstrated promising antiviral activity against CHIKV in vitro, contributing to the development of new therapeutic agents for this arbovirus. Keywords: Brazilian red propolis, Chikungunya virus, antiviral. Acknowledgments: CAPES, CNPq, FAPEAL.

#### J-54. NEUROPROTECTIVE EFFECT OF SULFATED POLYSACCHARIDES FROM MARINE ALGAE Asparagopsis armata Harvey IN A RAT MODEL OF PARKINSON'S DISEASE

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Parkinson's Disease is a neurodegenerative condition characterized by a range of motor and non-motor symptoms, being one of the leading causes of death in individuals over 65 years of age. In this context, marine algae have been extensively explored for their potential therapeutic properties, owing to the abundance of their metabolites, such as sulfated polysaccharides. These, in turn, exhibit multiple biological activities, including antiinflammatory, antioxidant, and neuroprotective properties. In this study, the sulfated polysaccharides from the red algae Asparagopsis armata Harvey (TPS-Aa) were investigated for their structural characterization and neuroprotective effects in a 6-hydroxydopamine (6-OHDA)-induced rat model of Parkinson's disease. Structural characterizations, conducted using Fourier Transform Infrared Spectroscopy (FT-IR) and Nuclear Magnetic Resonance (NMR) techniques, revealed that TPS-Aa exhibited hybrid structures composed of 3-β-Dgalactopyranose- $(1\rightarrow 4)$ - $\alpha$ -D/L-galactopyranose. To evaluate the neuroprotective effect, male Wistar albino rats (250-270 g, 8-9 weeks old) were anesthetized and subjected to stereotaxic surgery. In this surgery, intracerebral administration (i.c.) of the neurotoxin 6-OHDA or 0.9% saline solution was performed in the right striatum. The treatment was carried out over a span of 14 days, through oral administration once a day, with doses of TPS-Aa at 0.3 mg/kg and 3.0 mg/kg. At the end of the treatment period, the animals underwent behavioral tests for evaluation. In the open field test, doses of TPS-Aa at 0.3 mg/kg and 3.0 mg/kg demonstrated a significant increase of 32.12% and 21.45%, respectively, in locomotor activity of the animals compared to the positive control group treated with 6-OHDA. In the cylinder test, an increase in activity of both forelimbs was observed, measuring (21.73 ± 2.86 total touches) and (32.34  $\pm$  2.47 total touches) for animals treated with doses of 0.3 mg/Kg and 3.0 mg/Kg, respectively. In the context of the apomorphine test, the dose of 3.0 mg/kg TPS-Aa exhibited the ability to reduce contralateral rotations by 61% compared to the 6-OHDA group. On the 15<sup>th</sup> day, the animals were euthanized, and their brains (ipsilateral and contralateral striatum, hippocampus, and prefrontal cortex) were extracted for neurochemical analyses, including measurements of nitrite/nitrate levels, lipid peroxidation, and reduced glutathione. It was observed that TPS-Aa (0.3 mg/Kg and 3.0 mg/Kg) exerted anti-nitrosative effects, reduced lipid peroxidation, and exhibited antioxidant activity in the different brain regions analyzed. Thus, the results of the effects of TPS-Aa present themselves as new therapeutic strategies in the treatment of neurodegenerative diseases.

Keywords: Algae; Marine Natural Products; Neurodegenerative Diseases.

**Acknowledgements:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 and by the Portuguese Foundation for Science and Technology (FCT) through the Strategic Projects granted to MARE-Marine, Environmental Sciences Centre (UIDP/04292/2020 and UIDB/04292/2020), Associate Laboratory ARNET (LA/P/0069/2020).

### J-55. Bioactive Compounds, Antimicrobial, Cytotoxic and Neuroprotective Potential of Green Propolis

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Introduction: Different types of Propolis are widely used in different regions of Brazil. Studies show that Brazilian Propolis has several medicinal properties and metabolic activities. It contains complex chemical components, mainly flavonoids and polyphenols, varying in geographic location, plant species, and season of the year in which they are produced. Green Propolis is known for its color, and it is produced by Apis mellifera bees that use Baccharis dracunculifolia, a common species found in the Brazilian cerrado, as the main plant source. **OBJECTIVES:** This work aimed to use a multidisciplinary and integrated approach to evaluate the "in natura" and "commercial" Brazilian Green Propolis extract for its antimicrobial, cytotoxic, and neuroprotective potential. MATERIALS AND METHODS: The crude extract from the "in natura" sample was obtained through maceration using ethyl acetate as solvent. The commercial ethanolic and aqueous extracts were obtained from a partner company of the laboratory. Phytochemical screening was carried out to evaluate the chemical groups expressed in greater quantity and evidenced in the extracts. The PC12 cells from the spinal cord of Wistar rats were used to evaluate the cytotoxic and neuroprotective activity. The cells were cultured and plated with DMEM medium and fetal bovine serum. Antimicrobial activities were performed against Staplylococcus aureus (ATCC6538), Bacillus subtilis (ATCC 6633), Escherichia coli (ATCC 94863), Pseudomonas aeruginosa (CCT=0090; ATCC 27853), Candidas albicans (ATCC 18804), and Candida glabrata (CCT 0728), and were evaluated using broth microdilution in 96-well plates. **RESULTS AND DISCUSSION:** The main secondary metabolites found in the extracts, through the phytochemical screening, were condensed tannins, free steroids, flavones, xanthones, and triterpenes. The antimicrobial activity showed better inhibitory results for the crude extract in Gram-positive bactéria. In contrast, the commercial aqueous extract inhibited Gram-negative strains, while the commercial ethanolic extract showed no activity. There was no inhibition of fungal growth by any of the evaluated extracts. In the cytotoxic activity in PC12 cells, there were better results for the working dilutions in the concentrations of the extracts between 0.5 <sup>[]</sup>LmL<sup>-1</sup> and 2.0 <sup>[]</sup>LmL<sup>-1</sup>. These dilutions did not induce cytotoxicity in the cells. CONCLUSION: These results will be contributed to a better characterization of "in natura" and commercial Green Propolis extracts regarding active principles, in vitro pharmacological properties, and biotechnological potential of Propolis, which are essential for developing of new phytopharmaceuticals. Keywords: Flavonoids; Metabolic profile; Traditional medicine.

Acknowledgments: CAPES, CNPq, FAPESB, FINEP and UFBA.

### J-56. Biochemical characterization and analysis of antioxidant activities of *Bovista* sp. Mushroom from Parque das Dunas, Natal-RN

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The current demand for new sources of bioactive compounds with medical/pharmacological applications is driving interest in research on less explored organisms, such as fungi. Macrofungi or mushrooms, was historically used in ethnopharmacology in Eastern populations and by ancestral indigenous in Brazil. In current scientific research, some of these fungi have an unexplored biomolecular repertoire and biological applications. In this context, the study aimed to analyze the biomolecules present in the aqueous extract of Bovista sp., such as proteins, carbohydrates, phenolic compounds, and flavonoids, with an emphasis on biological activities. The mushroom was collected from Parque Estadual Dunas do Natal, Rio Grande do Norte, Brazil, and was identified following traditional taxonomic methods. The methodology involved obtaining the crude extract (CE) from the mushroom, which was triturated and solubilized in distilled water. Biomolecule quantification was performed using methods such as the Bradford assay for proteins, Dubois method for carbohydrates, Folin-Ciocalteu method for phenolic compounds, and aluminum chloride method for flavonoid content. The results revealed significant amounts of proteins and carbohydrates in the CE, along with phenolic compounds and flavonoids in lower concentrations - a consequence of the aqueous extraction method and the higher affinity of secondary metabolites to organic solvents. Thus, 0.813 mg/mL of proteins, 0.171 mg/mL of carbohydrates, 0.002 µg of gallic acid equivalent per µg dry weight of CE, and 0.020 µg of quercetin equivalent per µg dry weight of CE. The hemolytic activity of the extract was absent in human erythrocytes, indicating the non-toxicity of the CE in these cell. Additionally, the Total Antioxidant Capacity (TAC) was quantified at 0.003 µg of ascorbic acid equivalent per µg dry weight of CE. To better understand the antioxidant activity, the DPPH scavenging ability was evaluated at different CE concentrations (0.25, 0.5, 1, 2, and 5 mg/mL). The best result was observed at 2 mg/ml, showing 93.95% DPPH scavenging. In conclusion, this study provides a comprehensive overview of the biochemical characterization and prior antioxidant action of the *Bovista* sp. mushroom. The identified compounds and assessed activities pave the way for future applications in biotechnology and therapy. However, further research is needed to fully comprehend the potential and action of these molecules, providing a solid foundation for future exploratory and application-focused studies.

Acknowledgments: Federal University of Rio Grande do Norte (UFRN), National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES).

Keywords: proteins, carbohydrates, dpph, biological activities, biotechnology.

### J-57. Antioxidant Potential of *Maytenus ilicifolia* Mart. ex Reissek in Inhibiting LDL Oxidation and Peroxidation

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The oxidation of low-density lipoprotein (LDL) is a critical event in the pathogenesis of cardiovascular diseases, including atherosclerosis. Natural compounds such as polyphenols have shown antioxidant potential against LDL oxidation, which can aid in mitigating these complications. This study aimed to assess the antioxidant potential of the ethanolic extract of Maytenus ilicifolia Mart. ex Reissek (EE-Mi) and its organic fractions: hexane (HF-Mi), dichloromethane (DMF-Mi), ethyl acetate (EAF-Mi), n-butanol (BF-Mi), and the hydro methanolic fraction (HMF-Mi) in vitro for inhibiting the oxidation and lipid peroxidation of LDL. The LDL was isolated from healthy human donors (in accordance with the approval by the Human Research Ethics Committee of the UNA University Center, located in Uberlandia -Minas Gerais, Brazil (protocol: 5.671.038; National Health Council Resolution CNS 466/12) and oxidized using copper ions (Cu<sup>2+</sup>). LDL oxidation was induced using 5 µM copper (II) sulfate, and the process was monitored for 2 hours with readings taken every 2 minutes to determine the intercept of tangents between the slow and fast absorption of conjugated dienes. After the monitoring period, the samples were taken directly from the plate and incubated with 10% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA) for 2 hours in a water bath at 100°C. After incubation, 400 µL of butanol was added to extract malondialdehyde (MDA), a product of lipid peroxidation. EE-Mi demonstrated the highest inhibitory capacity, followed by HF-Mi and BF-Mi, all comparable to non-oxidized LDL. Quercetin, a known polyphenol, also exhibited a significant inhibitory effect. All M. ilicifolia samples inhibited 100% of the lipid peroxidation induced by LDL oxidation. The results suggest that EE-Mi and its organic fractions possess antioxidant properties that protect LDL from oxidation. This effect can be attributed to the ability of these molecules to interact with metal ions such as Cu<sup>2+</sup> and donate electrons to neutralize free radicals. Additionally, the compounds may interact with lipid components of the membrane, preserving its integrity. The study demonstrated the remarkable potential of the ethanolic extract of *M. ilicifolia* in preventing LDL oxidation and peroxidation *in vitro*. Its antioxidant properties, possibly mediated by interactions with metal ions and lipid membrane components, could contribute to protection against atherosclerosis and other cardiovascular diseases. These findings underscore the relevance of natural compounds, such as those found in *M. ilicifolia*, in the pursuit of preventive and therapeutic strategies for these conditions.

Key words: LDL; antioxidant; lipid peroxidation;

Acknowledgments: The authors would like to thank the Institute of Biotechnology of the Federal University of Uberlandia and the UNA University Center.

#### J-58. DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS AND EVALUATION OF HEMAGGLUTINATING AND INSECTICIDAL ACTIVITIES FROM *Bixa orellana* L. FRUIT EXOCARP

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Introduction: Bixa orellana L. is a bushy plant native to tropical America. It is known as "urucuzeiro" in Portuguese and achiote in English. Its main application is concentrated in the food industry, where it is used as a dye source. In addition, extracts from this plant showed distinct biological activities. Currently, studies with natural products derived from plants have shown extracts and essential oils as promising tools for pest management. Lectins are carbohydrate-binding proteins that exhibit various biological activities, including insecticidal action. Objective: This study investigated the insecticidal potential of aqueous (AE) and hydroethanolic (HE) extracts from the fruit exocarp of B. orellana against S. zeamais. In addition, it was evaluated the phytochemical profile of both extracts and the presence of lectin in the aqueous extract. Material and Methods: Dry powder of the fruit exocarp was homogenized with distilled water or ethanol: water (1:1) to obtain the extracts. For phytochemical analyses, thin layer chromatography (TLC) was performed. To investigate the presence of lectin, the hemagglutinating activity (HA) assay was done and protein concentration was determined according to Lowry's method. AE was also loaded onto a chitin column and adsorbed proteins were eluted with 1.0 M acetic acid and, after dialysis, evaluated for HA and protein concentration. Insecticidal activity was evaluated through the contact toxicity assay against adults of S. zeamais, being mortality evaluated after 48 h. Results and **Discussion**: TLC showed positive results for flavonoids, hydrolysable tannins, condensable tannins, terpenes/steroids, saponins and sugars in both extracts. AE contained lectin, as evidenced by a HA of 2048. The protein concentration was 33.23 mg/mL. Protein from this extract adsorbed on chitin and, when recovered, showed HA of 1024 and protein concentration of 0.037 mg/mL. AE and HE did not show insecticidal activity against *S. zeamais*. Conclusion: AE and HE were found to be a source of different classes of secondary metabolites and AE also contains lectins with chitin-binding ability.

Keywords: achiote; maize weevil; chitin-binding lectin.

Financial Support: CNPq, CAPES and FACEPE.

#### J-59. Bioprospecting of lectins in seeds from *Microdesmia rigida* (Benth.) Sothers & Prance Barbalho Neto, F. C.<sup>1</sup>; <u>Silva, M. D. C.<sup>1</sup></u>

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Lectins are proteins that bind reversibly and specifically to carbohydrates and have many biological activities with biotechnological applications, such as antimicrobial, insecticidal and antitumor action. *Microdesmia rigida* is known and exploited for its high content of fixed oils in the seeds, which are widely used in the production of paints and varnishes. The species is popularly used to treat, for example, diabetes, high cholesterol and mycosis. However, there are no studies on the biological properties or on the biotechnological potential that are related to its protein constitution. This research aimed to detect and characterize the activity of lectins in *M. rigida* seeds. Crude seed extract (CE) was prepared in 100 mM citrate-phosphate buffer, pH 6.5. Then, the CE was subjected to protein precipitation by fractionation using two saturations with ammonium sulfate (0-60 and 60-90 %), followed by centrifugation and dialysis of the precipitates, called protein fractions 1 and 2 (F1 and F2), respectively. All preparations were submitted to hemagglutinating activity (HA) assays with human erythrocytes (ABO system), protein quantification, determination of specific HA (SHA) and the characterization of HA under temperature and pH variation. The protein fractions were submitted to HA inhibition assays in the presence of carbohydrates. All preparations showed high HA titers with different erythrocytes, high protein content and high SHA (CE, SHA: 15,787; F1, SHA: 5,004; F2, SHA: 26,301) that indicate preparations rich in lectins. The preparations kept HA high after incubation at temperatures between 20 and 100 °C, with a small and gradual reduction in HA titers after incubation at gradually higher temperatures. When exposed to different pH values (4.5 to 9.0), the preparations also maintained high HA, with optimal HA at pH 7.0 and 7.5, but presenting a small and gradual reduction in HA titers after incubation in pH values gradually more acidic or more alkaline. The data suggest that the seeds have thermostable lectins that are also stable to pH variations. The HA titers of the seed protein fractions were not inhibited by D-glucose, D-mannose or D-galactose. It is possible that the preparations have lectins with specificity to other untested monosaccharides, or with affinity only to glycoconjugates. The results indicate that *M. rigida* is a promising source for the isolation of thermostable lectins, active in a wide pH range, with affinity to glycoconjugates of the human erythrocyte membrane.

Acknowledgments: CNPq; UFERSA.

Keywords: Oiticica; phytohemagglutinin; lectin activity.

#### J-60. Hypoglycemic activity of the essential oil from the leaves of Eugenia uniflora

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**Introduction:** Hyperglycemia is a state in which blood sugar levels are high. Due to the scarcity of insulin in the blood, patients with diabetes are more prone to hyperglycemic crises, which involve numbness, pain and blurred vision. These crises occur when eating foods rich in carbohydrates, which are converted to glucose by intestinal enzymes such as  $\alpha$ -amylase. Certain plants, such as Eugenia uniflora, produce compounds capable of inhibiting the enzymes responsible for hyperglycemia. The hydroalcoholic extract of E. uniflora was identified as an  $\alpha$ -amylase inhibitor, however, until so far, no research showed this activity applied for the essential oil extracted from its leaves. **Objectives:** Evaluate the hypoglycemic activity of the essential oil of the leaves of E. uniflora. Material and Methods: The leaves of the plant were collected in Recife (PE), the material was registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) (registration number A6ABAE1). The extraction of E. uniflora essential oil (EuEO) was performed by hydrodistillation in a Clevenger apparatus for 3 h. The chemical composition and quantification of the compounds present in EuEO were performed by gas chromatography coupled to mass spectrometry (GC-MS) and gas chromatography with a flame ionization detector (GC-FID), respectively, and the results were expressed in % of the media±standard deviation of the peak area of the mass spectrum. The sample was diluted in different concentrations and the hypoglycemic activity was evaluated through the inhibition of  $\alpha$ amylase by the dinitrosalicylic acid (DNS) method for reducing sugars, using acarbose as a positive control. The results were expressed as the media±standard deviation of the 50% inhibitory concentration (IC<sub>50</sub>) of the enzyme activity using the t test for the statistical analysis, with a significance level of p<0.05. Results and discussion: The major components of EuEO were selin-1,3,7(11)-trien-8-one (41.76±0.85%) and caryophyllene (14.49±1.0%). EuEO and acarbose showed IC<sub>50</sub> of 10.38±0.73 and 15.04±3.0 µg/mL, respectively, without statistical difference. The essential oil rich in selin-1,3,7(11)-trien-8-one was as efficient in enzymatic inhibition as acarbose, an oligosaccharide drug used to control glycemia. Conclusions: The essential oil from the Eugenia uniflora leaves showed hypoglycemic potential in the inhibition of the enzymatic activity of  $\alpha$ -amylase. These results show that in addition to the alcoholic extract, the essential oil of this plant has phytochemical compounds capable of being used in the pharmaceutical and food industries to reduce the glycemia.

**Agradecimentos:** FACEPE, CNPq, Capes.

Keywords:  $\alpha$ -amylase inhibition, pitangueira, secondary metabolites

#### J-61. Methodologies to isolate metalloprotease activities of legume *Canavalia ensiformis*

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Introduction: Protease activities are essential for all organisms. In seeds of the tropical legume Canavalia ensiformis were identified a cysteine protease and two different serine proteases. Our group isolated and characterized a cucumisin-like serine protease from leaf of C. ensiformis, with distinct features of the previous serine proteases obtained from seeds of this legume. Objectives: to isolate metalloproteases from leaf water C. ensiformis extract (CE-A), using three different methodologies that included three distinctive affinity chromatography collums and different buffers; to prepare the Gelatin-Sepharose affinity chromatography collum; to analyzed the yield (%) and purification of metalloproteases using the three columns; to study the activities of all metalloproteases fractions using peptide and protein substrates; to analyze the influence of pH, temperature and proteases inhibitors on the protease activity; and to perform electrophoresis profile of different metalloprotease fraction. Material and Methods: C. ensiformis were collected from the Agroecological Phytomedication Platform (PAF) campus of the Oswaldo Cruz Foundation – FIOCRUZ, Rio de Janeiro, Brazil (S: 22° 56' 24.10"/ W: 43° 24' 09.22"), and the plant was deposited in the Botanical Garden of Rio de Janeiro, under number RB-550.352. Fresh leaves were powdered, and proteins were extracted using destilled water. The protein content of extracts and fractions was determined by the Bradford method and the protease activities were performed using hemoglobin, bovine serum albumin, casein, gelatin, N-Benzoyl-L-tyrosine ethyl ester (BTEE) Nα-p-Tosyl-L-arginine methyl ester (L-TAME) as substrates. The extract was dialyzed against water and buffers, centrifuged (10,000 g/ 30 min/4<sup>o</sup> C), and the clear supernatant was applied to three distinctive affinity chromatography collums that were: Gelatin-Agarose®, Colagen-Agarose® and Gelatin-Sepharose, the agarose collumns were obtained from Sigma-Aldrich, and the Gelatin-Sepharose was prepared using Sepharose-CNBr. Results, discussion and conclusion: The best yield (86 to 105%) and the higher purification (40 to 60-fold) were observed using Gelatin-Agarose and Gelatin-Sepharose columns and was obtained the fractions CE-AGelA and CE-AGelS, respectively. The yield obtained with Colagen-Agarose<sup>®</sup> was the lower than gelatin columns. The maximal protease activities of both CE-AGel metalloproteases were at pH 9. The best substrates for both fractions were gelatin, as expected, and BTEE and L-TAME. The major protein has about 63 kDa by SDS-PAGE under non-reducing conditions. Our results indicated a successful purification of metalloproteases from CE-A. However, further experiments are necessary to characterize these proteases. This is the first report about metalloproteases in C. ensiformis.

Key words: *Canavalia ensiformis;* affinity chromatography; metalloprotease activity. **Acknowledgments: FIOCRUZ and CAPES** 

#### J-62. Phytochemical prospecting of the methanolic extract obtained from leaves of *Costus* genus.

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Medicinal plants have diverse biological and therapeutic properties due to a wide variety of secondary metabolites in their composition. Among them, we have plants of the genus Costus, which are native to Brazil and can be found in different biomes. The leaves of plants of this genus are widely used by folk medicine in the treatment of various diseases, especially those of the urinary system, due to their diuretic activity. Given the high medical and therapeutic value presented by species of this genus, the present work aimed to evaluate the phytochemical profile of the hexane, chloroform, ethyl acetate, ethanolic and methanolic fractions extracted from the leaves of Costus arabicus and Costus spitacus. The C. arabicus and C. spitacus plants were collected in the Cariri region, municipality of Crato, CE-Brazil. The leaves were macerated in liquid nitrogen and subjected to methanol extraction for 72 hours to obtain crude extract (EB). The supernatant was filtered and the solvent was removed by evaporation. The EB was fractionated by silica gel chromatography, using solvents in increasing order of polarity (hexane (FH), chloroform (FC), ethyl acetate (FAE), ethanol (FE) and methanol (FM)) and the fractions were submitted to qualitative tests to detect the presence of flavonoids, saponins, tannins, triterpenes and steroids according to the methodology of MATTOS (1998). To evaluate the profile of secondary metabolites present in each fraction, silica thin-layer chromatography (TLC) was performed. The yield of methanolic EB obtained was 4 grams (8%) for each species. After fractionating 1 gram of EB, the fraction yields were 29.8% (FH), 25.6% (FC), 26.5% (FAE), 31.2% (FE), 10.5 % (FM) for C. arabicus and 4.1% (FH), 11.0% (FC), 53.2% (FAE), 30.7% (FE), 10.7% (FM) for C. spitacus. Phytochemical prospecting revealed the presence of flavonoids FE and FM from both species and saponins in FE and FM from *C. arabicus*. The appearance of tannins was positive in the FAE, FE and FM of C. arabicus and in the FE of C. spitacus. The presence of steroids was confirmed in all fractions of both species and of pentacyclic triterpenoids only in the FM of C. arabicus. The revelation of the TLC plates shows an abundance and diversity of secondary metabolites in all fractions. The results obtained so far demonstrate the potential for obtaining new molecules with therapeutic and pharmacological purposes from the leaves of Costus genus.

Keywords: Secondary metabolites; Medicinal plants; Thin-layer chromatography.

#### Supported by: CNPQ, CAPES and FUNCAP.

## J-63. *Moringa oleifera* SEED LECTIN (WSMoL) PROMOTES WOUND HEALING IN HYPERGLYCEMIC MICE

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Lectins are proteins that bind to glycosylated structures. The seed of Moringa oleifera contains the water-soluble lectin (WSMoL) with antimicrobial and anti-inflammatory activities. Inefficient healing process has been associated with Diabetes mellitus and plants have been investigated as sources of new compounds with healing activity in order to establish new therapeutic approaches. The present work investigated the wound-healing activity of WSMoL in hyperglycemic mice. WSMoL was isolated by chromatography on chitin column. Hyperglycemia was induced by intraperitoneal administration of alloxan (180 mg/kg) in animals fasting for 12 hours and confirmed by determination of glucose level in the blood of treated animals. For determination of wound healing activity, a 1 cm<sup>2</sup> lesion was performed on the dorsal surface of the mice. After, 0.15 M NaCl (negative control, NC), 3 U collagenase (positive control, PC) or WSMoL at concentrations of 10, 25 or 50 mg/kg were topically applied on the injured area for 10 days. The lesions were evaluated daily, for the presence of edema, scabies, hyperemia, granulation and scar tissue. Also, the diameter of the lesions was measured daily. WSMoL at 10, 25, and 50 mg/kg acted as a healing agent, promoting wound contraction by 100%, 77.6%, and 62.8%, respectively. The rate of wound contraction in the NC was 22.33% and WSMoL at the lowest dose tested promoted the same effect of PC (100% of wound contraction). The data revealed that WSMoL has wound-healing activity in hyperglycemic mice.

Keywords: Diabetes mellitus. Cutaneous lesion. Protein.

Acknowledgment: CAPES, CNPq and FACEPE.

#### K - Artrópodes

## K-01. Bioactivity of *Cratylia mollis* seed lectin preparation (cramoll 1,2,3) against the termite *Nasutitermes corniger* and the mite *Tetranychus bastosi*

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The termite Nasutitermes corniger and the mite Tetranychus bastosi are of great economic importance as they are considered urban and agricultural pests. Due to the need to control these pest species and to reduce the use of synthetic compounds which are harmful to the environment and lead to the emergence of resistant populations. Plant extracts, essential oils and plant lectins (carbohydrate-binding proteins) are being evaluated for their termiticidal and/or acaricidal activities. This study investigated the termiticidal and acaricidal activities of a mixture containing three lectins from Cratylia mollis seeds (Cramoll<sub>1,2,3</sub>). The seeds of C. mollis were collected in the municipality of Ibimirim-PE. The seed extract was prepared with 0.15 M NaCl and then centrifuged. The supernatant was saturated in 60% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The precipitated fraction was collected, dialyzed and chromatographed on a Sephadex G-100 column. Cramoll<sub>1,2,3</sub> corresponded to the non-adsorbed fractions of the precipitate. The termiticide test was carried out in Petri dishes containing an artificial diet prepared by mixing Avicel, nest meal and Cramoll<sub>1,2,3</sub> at concentrations of 25, 50, 100 or 200 µg/mL. Then, 16 workers and 4 soldiers were transferred to each Petri dish and mortality was observed for eight days. The effects of Cramoll<sub>1,2,3</sub> on the enzymes endoglucanase, exoglucanase, xylanase, amylase and trypsin were then evaluated. The effect of Cramoll<sub>1,2,3</sub> on the survival of T. bastosi females was carried out on Canavalia ensiformis leaf disks immersed in Cramoll<sub>1,2,3</sub> (0.02, 0.05, 0.1 or 0.2 mg/mL) for 10 s and then 10 females were placed on each disk. Mortality was assessed 48 h after infestation. For the ovicidal evaluation, leaf disks were individually immersed in Cramoll<sub>1,2,3</sub> (0.02, 0.05, 0.1 or 0.2 mg/mL) and then 30 eggs were placed on each disk. The number of hatched larvae was determined after 96 h. The CL<sub>50</sub> of Cramoll<sub>1,2,3</sub> on N. corniger was 0.078 and 0.199 mg/mL for workers and soldiers, respectively. The Cramoll<sub>1,2,3</sub> preparation was able to increase the activity of exoglucanase and endoglucanase by 8% and inhibited the activities of amylase, xylanase and trypsin by 14%, 11% and 56.7%, respectively. Cramoll<sub>1,2,3</sub> was toxic to T. bastosi females with a CL<sub>50</sub> of 0.14 mg/mL and showed ovicidal activity ( $CL_{50}$  of 0.16 mg/mL). In conclusion, Cramoll<sub>1.2.3</sub> proved to be a pesticide against insect pests and mites and was the first lectin preparation with acaricidal potential against T. bastosi. Keywords: Lectin. Insect enzymes. Insecticidal activity. Acknowledgment: CAPES, CNPq and FACEPE.

# K-02. Exposure to sublethal concentrations of the herbicide Imazethapyr results in mitochondrial redox imbalance and oxidative stress during post-embryonic development in Drosophila melanogaster

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Herbicides of the imidazolinone class are widely marketed. They act by inhibiting the enzyme acetolactate synthase, preventing the synthesis of branched-chain amino acids in plants, making it difficult for weeds to grow. Due to its high selectivity, this class of herbicides is considered safe for use. Imazethapyr is a broad-spectrum herbicide belonging to the imidazolinone class. However, few studies evaluate the effect of this compound in non-target organisms. The fruit fly, Drosophila melanogaster, has a fast life cycle, low maintenance cost, and a range of tools. These characteristics make this dipteran an ideal model for evaluating the effect of substances such as Imazethapyr. Thus, the objective of this work was to evaluate the toxicity of the herbicide Imazethapyr in *Drosophila melanogaster*. The specific objectives were: to evaluate the effects of sublethal concentrations on the post-embryonic development of Drosophila, to evaluate the pattern of mitochondrial redox homeostasis and the enzymatic activity of biomarkers of oxidative stress. Sublethal concentrations were defined from the LC50 of adult animals. From this experiment, the concentrations of 0.05, 0.1 and 0.2 mg/ml were defined. In order to evaluate the effects of Imazethapyr on post-embryonic development, first-instar larvae were exposed to flasks containing standard culture medium for Drosophila added with different concentrations of Imazethapyr. After exposure, the subsequent stages of development were followed and the number of pupae formed per day, the total number of pupae formed and the emergence rate were quantified. Using the transgenic lines UAS-mito-roGFP2-Orp1 (H<sub>2</sub>O<sub>2</sub> redox sensor) and UAS-mito-roGFP2-Grx1 (glutathione redox sensor) we accessed through fluorescence microscopy the pattern of mitochondrial redox homeostasis of the fat body of larvae exposed to Imazethapyr. Finally, we evaluated the enzymatic activity pattern of CAT, SOD and GST through spectrophotometric assays. Our results demonstrate that exposure to sublethal concentrations of Imazethapyr results in delayed development, increased larval lethality, and decreased pupation and hatching rates. Exposure to a concentration of 0.2 mg/ml resulted in an increase in mitochondrial H<sub>2</sub>O<sub>2</sub> levels in the fat body, indicating a pattern of redox imbalance, which was not observed for the glutathione sensor. These energetic results indicate impairment in an important tissue. Exposure to the herbicide imazethapyr resulted in significant changes in the enzymatic activity of oxidative stress biomarkers. Indicating exacerbated increase of reactive oxygen species. Thus, we can conclude that sublethal concentrations of Imazethapyr have high toxicity in non-target organisms.

### K-03.PARTIAL PURIFICATION OF A TRYPSIN INHIBITOR EXTRACTED FROM *Tribolium castaneum* LARVA

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INTRODUCTION: Enzyme inhibitors are molecules that can moderate or stop the catalytic activity of enzymes. These substances can be produced by the body itself, and play a crucial role in maintaining homeostasis. Trypsin inhibitors are studied because of their importance in pest control, understanding of inhibitory mechanisms, application in the food industry and use for diagnosis and treatment of diseases. OBJECTIVE: In this research, we aimed to study a trypsin inhibitor found in Tribolium castaneum larvae, and its activity after a partial purification process. MATERIALS AND METHODS: The crude extract was subjected to saline fractionation with ammonium sulfate, followed by the application of the sample to an ultrafiltration membrane. The inhibition of bovine trypsin was detected through the hydrolysis of the substrate L-BapNa, and the performance of the inhibitor at high temperatures was also verified. RESULTS AND DISCUSSIONS: After these steps, there was a decrease in impurities in the larval extract, confirmed by a polyacrolamide SDS-PAGE gel. Notably, the purified extract showed the ability to inhibit around 58% of bovine trypsin activity and retained its effectiveness even under high temperatures. CONCLUSION: These results reinforce the presence of an endogenous trypsin inhibitor in the *T. castaneum* larva, contributing to a better understanding of the insect's physiology and to new research.

Keywords: Trypsin inhibitors; Purification; *Tribolium castaneum*.

Supported by: CNPq, CAPES, FAPEAL

#### L - Educação

#### L-01. Bioplastics as a Teaching Tool in Biomolecule Chemistry: An Approach in Continuing Teacher Education

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Introduction: The study of the chemical constituents of the cell requires a high level of abstraction in the classroom, generating, as a consequence, an outdated and sometimes decontextualized understanding, especially in basic education. By presenting the principles of Creative Learning to teachers, giving themselves the opportunity to insert them into the demand for education that values the protagonist role of the student in their learning process. **Objective:** To produce bioplastic from natural sources of starch as a methodology for teaching biomolecule chemistry. Methodology: The strategy was presented to science teachers from the 6th to the 9th grade, during the continuing education of the municipal education network of Arapiraca-Al. For the production of biodegradable plastic, easily accessible natural sources were used, such as potatoes and tapioca gum, from which the initial extracts were prepared. After 20 minutes, the precipitated starch was removed and mixed in water with the glycerin, acetic acid and dye solutions. This mixture was brought to a boil, stirring until a gelatinous solution was formed, which was then placed in uniform plastic containers. After a week the plastic film was ready. Results and Discussion: Different contents proposed for the Science curriculum were discussed, such as biopolymers, homogeneous and heterogeneous mixtures, chemical transformations, biodegradation, among others. This type of experimental activity allows the development of skills and competences that go beyond understanding the content, making the student reflect, question, interpret and also interact directly with the object of study. Still, according to the BNCC, throughout basic education, the natural sciences play a fundamental role in the development of the student's scientific literacy, and for this purpose, they must use methodologies that favor the understanding and interpretation of the world (natural, social and technological). , as well as the ability to transform it. In this sense, education professionals, at all levels, have been interested and reflected on the possibilities of using aspects of the maker movement in different pedagogical practices, and the continuing education of teachers must bring innovative and creative proposals that also allow the professional the development of new skills and knowledge, since it can strengthen the teaching-learning relationship. **Conclusion:** With this proposal, it was possible to contemplate the perspective of a participative and attractive training for teachers that allows them to approach more complex contents in a playful, technical and constructive way and integrated with the students' reality.

Keywords: Active methodology; teacher training; teaching biochemistry.

We would like to thank the City Hall of Arapiraca, through the Municipal Department of Education and Sports, the Scientific Development Nucleus and the Continuing Education Nucleus of SEMEDE for their support in carrying out this activity.

#### L-02. Flipped classroom: an innovative proposition to enhance the teaching and learning of biochemistry

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Biochemistry is a crucial discipline for the proficient competence of students in the biological sciences program, as it serves as a tool to comprehend biological, environmental, and pathological processes. Nevertheless, numerous students struggle with substantial challenges due to its intricate nature and dense content. As a consequence, the conventional pedagogical framework, grounded in expository lectures and demonstrative practices, has been proven to be unsatisfactory, failing to reach the determined level of comprehension, understanding, and effective application of intricate principles embedded within the discipline. This reveals the need to reevaluate the methodologies and assess innovative approaches that can be cultivated in students to enable the systematic assimilation of the discipline. This challenge was accentuated during remote lessons throughout the pandemic period spanning from 2020 to 2022. This hardship was intensified by the decline in student motivation and engagement in the post-pandemic era. Consequently, the main goal of this study was to evaluate the potential implementation of a hybrid education strategy, particularly focusing on the flipped classroom model, within the teaching and learning process of the Molecular Diversity course within the Biological Sciences program at UFRN. To achieve this goal, the methodology encompassed the development of didactic resources, their practical implementation, and systematic data collection. Firstly, the preparation of video tutorials containing biochemistry topics, then both tangible and virtual educational materials, as well as didactic kits tailored for the study of biomolecules. The instructional process involved the pre-availability of video lectures. During in-person sessions, the content was briefly reviewed, followed by the performance of activities employing the prepared resources. These activities were further deliberated upon and discussed within groups, nurtured by the guidance of the instructor and teaching assistants. Ultimately, qualitative analyses were conducted, aimed at scrutinizing the intricate interrelationship between the efficacy of the active pedagogical methodology and the depth of student engagement with the instructional content. The empirical findings highlighted a substantial surge in student involvement in group discussions, both physical and virtual contexts. This was paralleled by enhanced performance in theoretical evaluations, heightened motivation, augmented dedication, and participation in debates, collectively contributing to a heightened success rate within the discipline. In conclusion, this study substantiates that this proposed instructional model fosters profound learning and cultivates analytical, systematic thinking, and collaborative skills. This refinement in the teaching methodology renders students capable of achieving professional excellence and making impactful contributions to society.

Supported by: CNPq

Keywords: Hybrid teaching, active methodology, biomolecules.

## L-03. Biochemistry beyond university walls: the invisible world through playful practices in high school

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Introduction: Biochemistry is an area of life sciences that studies the biological processes that occur in organisms and the production of biomolecules of interest to several areas. The biochemistry is essential for understanding the relationship between biodiversity and the environment and for producing food, cosmetics, medicines, and biofuels, among others, of industrial and socio-bioeconomic interest. Biochemistry has been taught through pedagogical practices contextualized in everyday life and not restricted to theoretical foundations, lectures, or the use of limited resources without demonstrating the subjects' applicability. Objective: This work aimed to carry out practical biochemistry activities in the 1st, 2nd, and 3rd grades of high school at Colégio Modelo Luís Eduardo Magalhães (Muniz Ferreira-Ba e Camaçari-Bahia), using the theme "Biochemistry beyond the walls of the University: invisible world," aiming to encourage students to build links between theory, practice and everyday life, in addition to promoting the dissemination of scientific knowledge and integration between the University and the Secondary School. Materials and methods: Seed germination practices, fungus observation, toxicity test in Artemia salina, and pH colorimetric determination were carried out, demonstrating the acidity of food, soap, milk of magnesia, and water from the school drinking fountain. Results and discussion: The seed structure, germination, and plant production were presented simply (planting in disposable cups and pet bottles). A red cabbage solution was used to demonstrate a colorimetric pH scale, demonstrating that it is possible to use this food as a universal indicator of acidity. The students showed much interest in these practices, particularly in the cytotoxicity test in A. salina, by using a stereoscope to observe this microcrustacean used in feeding aquarium fish, sensitive to toxic compounds. Therefore, methodologies were used to facilitate understanding and expand knowledge in practice faster and with didactics, which can be easily performed in high school. **Conclusions:** Biochemistry, a university course subject, can be presented in high school practically and playfully to demonstrate biological processes relating them to the students' daily lives. With more practical and playful activities, this methodology is more efficient in ensuring a dynamic teaching/learning process related to everyday activities. This extension activity provides undergraduate and graduate students with important pedagogical experiences for professionalization. It stimulates high school students to search for new knowledge and greater interest in continuing their studies through understanding basic concepts and the importance of scientific investigations in developing new skills and opportunities.

Keywords: Food, Teaching/learning, Playful practices

**Acknowledgments:** UFBA; Capes (CNPq); FAPESB; Colégio Estadual Luís Eduardo Magalhães (Muniz Ferreira-Bahia e Camaçari-Bahia); Prefeitura Municipal de Muniz Ferreira-Ba.

## M-01. Metabolic Effects of Thimerosal Sublethal Doses Exposure on Zebrafish Embryos: A Metabolomics Study

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Increasing concerns surrounding human exposure to heavy metals have arisen due to the alarming escalation in water and vegetation contamination, coupled with instances of occupational exposure. Among these metals, mercury is particularly noteworthy due to its recurrent occurrence in environmental contamination and the bioaccumulation of its organic forms. Thimerosal, an organic compound containing mercury, has a historical application as an antiseptic agent, notably in multi-dose vial vaccines and cosmetic products. However, its use in human-related products remains a subject of controversy due to its potential adverse health effects. This research proposes the utilization of metabolomics in conjunction with zebrafish embryos as an animal model to elucidate the effects of sublethal thimerosal exposure on an organism's metabolism. The selection of zebrafish for this study is justified by the physiological and genetic similarity between zebrafish and humans, allowing for the generalization of findings to human exposure. Zebrafish embryos were obtained from an inhouse bioterium, after the approval of Ethics Committee on animal use at Federal University of Alagoas (CEUA/UFAL), and subjected to thimerosal exposure for 144 hours postfertilization, at concentrations of 0.04µM and 0.08µM, alongside a control group. These sublethal doses were determined through experimental calculations of the lethal dose for zebrafish embryos. Each experimental group comprised 8 replicates, each containing 100 embryos. Sample preparation involved extraction with 80% methanol, followed by vacuum drying and reconstitution in 600µL of D<sub>2</sub>O with a phosphate buffer. Reconstituted extracts underwent centrifugation, and the resulting supernatant was transferred to 5mm NMR tubes. <sup>1</sup>H NMR spectra were acquired using a Bruker Ascend 600 MHz spectrometer, employing the 1D NOESY pulse sequence with water presaturation. Data processing involved an automated script routine through the PepsNMR R package, encompassing phasing, calibration, baseline correction, and segmentation into 0.01 ppm width bins. The ensuing dataset was subjected to multivariate data analysis, employing partial least squares discriminant analysis (PLS-DA) and principal component analysis. The results indicated that thimerosal exposure correlated with elevated levels of methylhistidine, carnosine, ethanolamine, histidine, mevalonate, glutamate, and glutamine. Conversely, reduced levels of histidine, mevalonate, glutamate, glutamine, betaine, histamine, ethanol, adenosine, adenosine monophosphate, inosine, maltose, and sucrose were observed. These findings suggest that thimerosal disrupts intermediates integral to cellular energy production and synthesis pathways, in addition, the disrupted metabolites suggest neurodevelopmental disorders and oxidative stress. In summary, this study demonstrates that thimerosal modulates the metabolism of zebrafish embryos, highlighting the necessity of considering its effects on human health. Keywords: Thimerosal, zebrafish, metabolomics. Supported by: CNPq, CAPES.

### M-02. Investigating Blood Amino Acid Concentrations in Elite Karate Athletes Using the Sportomics Approach

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Introduction: It is known that physical exercise leads to changes in the metabolome, including the increased catabolism of many amino acids (AAs). Karate is a combined exercise with energy turnover that occurs mainly via both hydrolysis of phosphocreatine and glycolysis. However, there is no information available regarding blood AAs concentrations during training or competition in karate athletes. The sportomics approach has been developed to understand the metabolomic changes induced by physical exercise closer to the reality faced during sports performance. Sportomics depends on analytical methods with high processing power to analyze massive volumes of data, such as analyses by mass spectrometry. Objective: To analyze the concentrations of amino acids (AAs) in elite karate athletes, during a training session, using the sportomics approach. Methods: seven karate athletes (3 males and 4 females), in their preparation for the XVII Pan American Games in 2015, held in Toronto, Canada, participated in a training session. Blood samples were collected immediately before and after the training session, imitating a real competition situation (kata), and analyzed using mass spectrometry. Results: The results indicated the presence of 21 AAs which were subdivided into aromatic AAs (AAA), branched-chain AAs (BCAA), ketogenic AAs (KAA), glucoketogenic AAs (GKAA), glucogenic AAs (GAA) and total AAs (AAt). When results were stratified by gender, ornithine concentration increased for female athletes (P = 0.01). On the other hand, male athletes demonstrated an increase in threonine concentration (P = 0.009). In addition, a percentage analysis showed that arginine concentration increased for both genders: female, 51.5%, and male, 39.6%. Also, the values of GAA (-12.5%) for women and GAA (11.8%) for men were different. Conclusion: Differences were found in whole blood AAs concentrations in karate athletes, including gender differences between male and female athletes. We believe such a database will have the potential to improve and personalize practices related to nutrition, training, and recovery. Keywords: Amino acids; Exercise; Metabolism.

### M-03. Investigation of Sodium Nitroprusside Effects as an Anti-Schizophrenia Drug using NMR Spectroscopy

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Schizophrenia (SCZ) is a multifactorial illness, where one of the factors that may be contributing to the pathogenesis is the blocking of N-methyl-D-aspartate (NMDA) receptors. So, the use of the sodium nitroprusside (SNP) has been proposed in the literature as a therapeutic agent for schizophrenia treatment, due to its role as a nitric oxide (NO) donor. Although, there is still great debate about the effectiveness of the SNP to reduce SCZ symptoms. Therefore, our aim was evaluating the SNP effectiveness in animal model through NMR-based metabolomics. The application of dosage of SNP in the rats was performed at the Federal University of Sao Paulo in accordance with the Ethics Committee, and the obtained blood serum samples were stored in freezer at -80 °C. NMR samples were prepared using 250  $\mu$ L of blood serum from rats diluted in 250  $\mu$ L of deuterium oxide (D<sub>2</sub>O). The following groups of samples were analyzed: 1-) Wistar rats as control (4 samples); 2-) Wistar + SNP (2.5 mg kg<sup>-</sup> <sup>1</sup>); 3-) Wistar + SNP (5.0 mg kg<sup>-1</sup>); 4-) spontaneously hypertensive rats (SHR) as control (4 samples); 5-) SHR + SNP(2.5 mg kg<sup>-1</sup>); 6-) SHR + SNP (5.0 mg kg<sup>-1</sup>). Furthermore, a comparison between SNP effects with other antipsychotics of first and second generation such as clozapine and haloperidol are still under investigation. <sup>1</sup>H-NMR spectra were acquired in triplicate with water suppression in a NMR spectrometer (600 MHz, Bruker). Partial Least Squares Discriminant Analysis (PLS-DA) were performed at MetaboAnalyst (version 5.0). All analyses were done using spectral bins with no processing mode. Metabolic profiling indicated the drugs more pronounced effects on aromatic and aliphatic spectral regions, in which NMR peaks were assigned to specific amino acids and fatty acids. In all cases, it was possible to observe in chemometrics analysis an approximation of the SHR/Wistar + SNP group with the control group. However, only when applied the SNP in higher concentration (5.0 mg kg<sup>-1</sup>), occurred a higher approximation of the group of animals treated with SNP with the control group. These results suggest a potential use of SNP for SCZ treatment.

Acknowledgments: FAPESP, Grant numbers: 2018/24069-3, 2019/13112-8, 2019/09207-3, and 2020/01107-7, and JGMP fellowship (2023/03780-9). National Institute of Science and Technology in Bioanalytics (INCT-Bio).

Keywords: sodium nitroprusside; antipsychotics; metabolomics.

## M-04. Lavender essential oil induces oxidative stress and stiffening of membranes and cell walls in *Cryptococcus* spp.

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The Cryptococcus neoformans and Cryptococcus gattii species complexes are the etiological agents of cryptococcosis, a disease responsible for 181,000 deaths annually due to late diagnosis and limited treatment options. Studies focusing on the identification of new substances with antifungal activity, such as essential oils (EO), are urgently needed. The antifungal effects of EO have already been suggested. However, the mechanism of action at the molecular level still needs to be evaluated. In this work, we evaluated the molecular changes caused by the exposure of C. neoformans (H99) and C. deuterogatti (R265) to lavender essential oil (LEO) using a proteomics approach. The identified proteins were categorized by Gene Ontology according to biological processes and molecular functions, and KEGG pathway analysis was also performed. Our results suggest that LEO creates a stressful environment in both strains; however, the response to this stimulus differs in each organism. It was observed that in C. neoformans, there were changes in energy metabolism and pathways related to alternative sources of energy and the response to oxidative stress. In C. deuterogatti, changes were observed in pathways related to cellular architecture, suggesting that the cell underwent morphological changes such as membrane and cell wall stiffening. Acknowledgments: CAPES, CNPq, FAPERGS. Keywords: proteomics; cryptococcosis

#### M-05. α1A-adrenoceptor activation induces S6 ribosomal protein phosphorylation

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**Introduction:** The activation of  $\alpha$ 1A-adrenoceptors ( $\alpha$ 1A -AR) in prostate is linked to prostatic cancer, through mechanisms that are not yet fully understood. On the other hand, phosphorylated S6 ribosomal protein (p-S6RP), a downstream target of the PI3K/Akt/mTOR pathway, plays a crucial role in tumorigenicity. **Objective:** The aim of our study was to assess whether the activation of  $\alpha$ 1A-ARs could lead to the formation of p-S6RP. Methods: p-S6RP induced by α1A -AR activation was evaluated in Chinese Hamster Ovary cells stably expressing human α1A -AR (CHOα1A-AR cells) by Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen). Firstly, we conducted a time course response study for  $\alpha$ 1A-AR agonists: A61603 10  $\mu$ M, methoxamine 10  $\mu$ M, noradrenaline 10  $\mu$ M, oxymetazoline 10  $\mu$ M, or phenylephrine 10 µM. Next, we performed concentration response curves for the mentioned α1A-AR agonists to assess the potency (-LogEC50) and efficacy (Emax) of these ligands in inducing p-S6RP. Results and Discussion: All α1A-AR agonists induced phosphorylation of S6RP in CHO $\alpha$ 1A-AR cells, with a peak response observed within 2 minutes and a return to baseline levels within 10-30 minutes. However, oxymetazoline displayed a sustained response for up to 30 minutes. Concentration response curves revealed the following potency order: oxymetazoline = methoxamine > A61603 > Noradrenaline > phenylephrine. All agonists presented similar Emax values. **Conclusion:** Activation of  $\alpha$ 1A-ARs in CHO cells promoted the phosphorylation of S6RP. Interestingly, oxymetazoline, a clinically utilized drug, demonstrated prolonged and potent production of p-S6RP, implying that the extent of S6RP phosphorylation may depend on the type of ligand. Nevertheless, further investigations are required to assess the impact of a1A-AR-mediated S6RP phosphorylation in prostatic cells, its implications on cell growth and survival, and the potential risks associated with oxymetazoline usage in relation to prostatic cancer. Acknowledgments: We thank Dr. Thomas Chang (Roche Bioscience, Palo Alto, CA) for the gift of plasmid containing the human  $\alpha$ 1A-4-adrenoceptor cDNA. EDdaS Jr was supported by National Council for Scientific and Technological Development (CNPq, Portuguese: Conselho Nacional de Desenvolvimento Científico e Tecnológico) of Brazil, Science without Borders Scholarship program (Portuguese: Programa Ciências sem fronteiras), process 203518/2014-4. This work was supported by an Australian National Health and Medical Research Council (NHMRC) program grant (1055134; RJS). DSH was supported by a NHMRC Career Development Fellowship (545952) and MS by a NHMRC CJ Martin Fellowship (606763).

Keywords: α1A adrenoceptors, p-S6RP, CHO cells

#### N - Processo Redox

#### N-01. Potentially harmful effects of polystyrene exposure in human placentas

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Global microplastic (MP) pollution has become a widespread environmental concern with significant potential implications for human health. The MPs have been found in human lungs, colon, blood, heart, testis, ovaries, placentas and fluids such as urine, semen, and breastmilk. The environmental effects and complications of microplastics in aquatic wildlife have been extensively studied, but their influence in human cells and tissues remains poorly understood, particularly in relation to the placenta and fetal development. As such, this study aims to investigate in vitro whether MPs transpose the placental barrier, and which functions they might change in the placentas. Healthy term placentas were dissected (Ethical Committee approval: 58129422.3.0000.5013) and chorionic villi explants cultured for 72 h with different concentrations of 5 µm polystyrene (with and without red fluorescence). Viability was acessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, cytotoxicity by lactate dehydrogenase (LDH) assay, and proliferation by cell counting kit 8 (CCK-8). Reactive oxygen species (ROS) were accessed by MitoSox RED staining, nitroblue tetrazolium (NBT), and H<sub>2</sub>O<sub>2</sub> production. To prove that MPs were transposing the placental barrier, atomic force microscopy (AFM), and laser confocal scanning microscopy (LCSM) were applied.. Tested concentrations of 0.1 µg/mL, 1 µg/mL, 10 µg/mL, and 100 µg/mL have not changed explants viability, but 100  $\mu$ g/mL exposure increased proliferation in 24, 48 and 72 h (p < 0.01). The LDH assay indicated increased cytotoxicity at 100 µg/mL exposure through time (24 to 72 h, p < 0.01). In all methods for ROS production, the analyses resulted in increased ROS production after 72 h exposure of 100 µg/mL of polystyrene (p < 0.05). AFM and LCSM analyses showed that 44% of the polysterene MPs added in the culture transposed the placental barrier after 72 h, which seems to be occurring by a passive process. These initial findings suggest that polystyrene MPs are able to easily transpose the human placental barrier, increasing cytotoxicity and ROS production through time, which indicate possible repercutions on Redox balance, and in the maternal-fetal metabolism that could potentially harm placental physiology and fetal development.

Funding support: CNPq, CAPES, and FAPEAL.

Keywords: placenta, microplastics, ROS production.

#### N-02. Acute exposure to mercury species impacts on erythrocytes in hypertension animal model.

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**INTRODUCTION:** Hypertension is a systemic disease without a unique origin. Environmental factors such as exposure to mercury species can trigger the manifestation of this cardiovascular disease. Erythrocytes are cells in our blood that have a primary role in the capture and diffusion of oxygen by the protein hemoglobin (Hb). This kind of cell also transports another component to the other cells and could be one carrier of mercury for tissues. Thimerosal (TM) is a mercury-derived compound used as a vaccine preservative that can potentially be a source of mercury exposure. **GOAL:** Our objective is to verify the effects of TM exposure on erythrocytes from hypertensive animals (SHR), evaluating functional and structural parameters and their redox status. METHODOLOGY: The SHR animals were treated with TM (0.5 mg/kg/day) in water intake for 24 h. The animals were divided into TM (TM-SHR) and control (water) groups. Blood was collected via retro-orbital plexus. Oxygen uptake was evaluated using an Oxygen electrode (Oxygraph Hansatech). The assays: Soret band profile (Hb free), Lactate Dehydrogenase activity (Labtest, commercial kit), free thiol groups (DTNB assay), and the antioxidant enzymes activity (Superoxide Dismutase - SOD, and Catalase -CAT), were all spectrophotometric determined (Shimadzu UV-1900i). The procedures were authorized by the ethics committee (23/2021). RESULTS: The acute treatment leads to a decrease of about 45% in oxygen uptake from erythrocytes (SHR-TM) compared to the control, showing a compromise of Hb functionality. The peaks of the Soret band (intensity) from Hb showed that the SHR-TM group has a decrease of 41% compared to the control group; this result also demonstrates that TM leads to structural changes in Hb. Quantifying total free thiol groups found a decrease of 21% in erythrocytes from the TM-SHR group. Besides, no statistical difference ( $\alpha$  = 0,05) was found in SOD and CAT activities comparing both groups. Similarly, the activity of LDH, a cell damage marker, showed no statistical difference ( $\alpha = 0.05$ ) was found between the groups. CONCLUSION: Based on the data above, acute treatment with TM in hypertensive animals demonstrates damage to Hb from erythrocytes, inducing a compromise in its main function that could be related to interaction with thiol groups with mercury species. These data indicate that TM treatment could trigger hypertension by its side effects in red blood cells.

Acknowledgments: CAPES, UFAL, FAPEAL.

Keywords: Hypertension, Mercury, Cardiovascular Disease.

### N-03. Oxidative stress caused by chronic environmental exposure leads to functional and structural changes in fishermen's blood cells

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**INTRODUCTION:** Mercury is a metal that is among the 10 most toxic contaminants in the environment and can cause human health disturbance. The mercury species causes an increase in oxidative stress, consequently leading to cell damage and imbalances in the redox system. Recently, it was determined that, in the water of Complexo Estuarino Lagoa Mundaú-Manguába (CELMM, Maceió-AL, Brazil) and in biological fluids of fishermen (blood and urine) that live in the surroundings of CELMM, there are high levels of total Hg when compared with limit values established by regulatory agencies. GOAL: This study aimed to evaluate, in fishermen that live around the CELMM, the levels of Hg from total blood. Functionality, structure, morphology, and oxidative stress of peripheral blood mononuclear cells (PBMCs) and erythrocytes. **METHODOLOGY:** The experiments were carried out with fishermen (n = 60) and control (n = 65) volunteers (CAAE 57998116800005013). Assays included mercury quantification, oxygen uptake, reactive oxygen species (ROS) generation, antioxidant enzyme activities, markers of secondary oxidative stress, Raman spectral cell profile, fluorescence, and scanning electron microscopy (SEM). RESULTS AND DISCUSSION: Our results showed that the concentration of Hg in the total blood was two times higher in fishermen compared to the control group. Erythrocyte functionality decreased O<sub>2</sub> uptake by 39%. This result corroborates with Raman spectroscopy data, which shows variations in the structure of proteins and lipids. For PBMCs, O<sub>2</sub><sup>•-</sup> and H<sub>2</sub>O<sub>2</sub> production (ROS generation) was observed an increase of 87 and 116% compared to controls. The generation of reactive species was confirmed using fluorescence microscopy, which showed high production of general reactive species and high O<sub>2</sub><sup>•-</sup> in fishermen's PBMCs. Also, in the SEM, it was possible to verify a higher roughness, showing that mercury exposure can lead to changes in the membranes of the PBMCs of the exposed group. Some secondary oxidative stress markers were measured since an elevated ROS production was observed. In this context, reductions of 37 and 41% were obtained in the GSH/GSSG ratio and thiol content. Besides, MDA production increased by 89% over controls. The activity of antioxidant enzymes was also evaluated, which showed an increase in superoxide dismutase and catalase (159 and 22%, respectively), while glutathione peroxidase showed a 33% depletion. CONCLUSION: Our data show that individuals exposed to a polluted environment have increased oxidative stress and changes in the redox system, leading to impairment in the function and structure of blood cells.

**Keywords:** Environmental contamination, Mercury, Blood cells. **Acknowledgments:** FAPEAL, UFAL, CAPES, FAPESP.

# N-04. Chronic exposure to 2,2'-azobis-2-amidinopropane induces oxidative injury and reduces vialibility in larvae *Drosophila melanogaster*

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Introduction: Oxidative stress can be defined as an imbalance between the production of free radicals and antioxidant defenses. When it occurs, it can lead to damage in cellular structures such as membrane lipids and proteins, resulting in various physiological impairments. Embryonic development is a delicate process and is susceptible to various stressors. Therefore, understanding how the presence of reactive oxygen species (ROS) during this period can influence the development of living organisms becomes highly significant. Objective: To assess the effects of exposure to high concentrations of reactive oxygen species (ROS) using 2,2'-azobis-2-amidinopropane as a radical generator during the larval development of Drosophila melanogaster. Materials and Methods: The Canton-S strain of adult flies was utilized to collect eggs. Fly treatment was administered via food immediately after oviposition. 2,2'-azobis-2-amidinopropane (AAPH) concentration of 30 mM. Toxicity assays were conducted on larvae in stage L3. The rate of development from egg to larva and pupa was assessed as an indicator of potential toxic effects of exposition. Larvae were collected three days after oviposition, thoroughly washed, and subsequently fed to ensure accurate quantification for subsequent experiments. The larvae were also homogenized for various biochemical assays. Results and Discussion: Larvae exposed to AAPH and the control group were evaluated for developmental toxicity. Exposure of larvae to 15 mM AAPH led to a reduction in the number of pupae (19%) and adults (16.5%) compared to the control group, which exhibited 80% pupae and 69% adults. Larvae exposed to AAPH showed significantly higher total antioxidant capacity (382 µmol/mL equivalents of trolox) compared to the control group (181 µmol/mL equivalents of trolox). Furthermore, AAPH-exposed larvae increased activity of antioxidant enzymes, such as superoxide dismutase (40 U/ $\mu$ g protein) and catalase (20 U/µg protein), compared to the control group (10 U/µg protein and 11 U/µg protein, respectively). The ROS generated by AAPH in the medium can stimulate the primary antioxidant defense in L3 larvae, resulting in decreased ROS levels. Larvae exposed to AAPH exhibited higher levels of lipid peroxidation (3.1 nmol MDA/µg protein) compared to the control group (0.9 nmol MDA/µg protein). Additionally, advanced oxidation protein products (AOPP), indicating oxidative damage in proteins, were significantly elevated in larvae exposed to AAPH during the L3 stage, as opposed to the control group. Conclusions. Notable alterations were observed in the redox balance, including heightened antioxidant activity and cellular oxidative damage.

Acknowledgment. The authors gratefully acknowledge the Institute of Biotechnology of the Federal University of Uberlândia (UFU). The authors would also like to thank the Dean of Research and Graduate Studies (Pró-Reitoria de Pesquisa e Pós-graduação- PROPP/ UFU) and the Graduate Program in Cell Biology and Structural Biology of the Biomedical Science Institute (UFU).

Keywords. Oxidative Stress, Drosophila Melanogaster, Toxicity.

#### N-05. Functional and structural changes of human erythrocyte exposure to phenylmercury

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**INTRODUCTION:** Phenylmercury (PM) is a mercury species that has been widely used as a herbicide, antiseptic, fungicide, and preservative for latex paints. Human blood has many types of cells, including erythrocytes, commonly known as red blood cells (RBC). The main protein in erythrocytes is hemoglobin (Hb), which binds to oxygen, and its function is to transport oxygen to the entire body. Biological systems such as Hb exposure to mercury and its species can lead to oxidative stress, protein modification, lipid peroxidation, and other changes. GOAL: To analyze the effect of PM exposure on human erythrocytes, evaluating their functionality, redox state, and possible structural changes. METHODS: The erythrocyte's oxygen uptake was carried out in an oxygen electrode (Oxygraph Hansatech). Global reactive oxygen species production,  $H_2O_2$ , and nitric oxide (NO) were monitored spectrofluorimetrically using specific probes - DCF, Amplex-red, and DAF-FM, respectively. The osmotic fragility was assessed using three aqueous solutions of NaCl simulating two hypotonic (0.2 and 0.5% m/v) and one isotonic (0.9% m/v), employing a spectrophotometer (Shimadzu, model UV-19001). Human blood (CAAE 02840318.2.0000.5013) from volunteers (n = 6) was used in the experiments. **RESULTS:** Our results showed that oxygen uptake in erythrocytes decreased at least 34% in the presence of PM, which was dose-dependent (1 - 0.125  $\mu$ M). Global ROS, using DCF, showed an increase of more than 100% (PM from 1 - 0.125 µM) compared to the control. Besides, H<sub>2</sub>O<sub>2</sub> production showed a dose-dependent decrease of 31 to 4% in the 1 - 0.125  $\mu$ M PM range compared to the control. NO production decreased from around 49 to 25% in the presence of PM concentrations (1 - 0.125 μM). In addition, structural alterations of the erythrocytes in the presence of PMA (1 - 0.125  $\mu$ M) were observed in the osmotic fragility assay, wherein the hypotonic buffer, NaCl 0.2 and 0.5% (m/v) showed a decrease of at least 46 and 65%, respectively. In contrast, in the isotonic medium (NaCl 0.9% m/v), there was a significant increase in the percentage of hemolysis in PM presence, which was over 100% compared to the control. These fragility assays showed damage to the RBC membrane caused by PMA. CONCLUSIONS: Our studies showed that exposure to PM impairs the functionality and structure of human hemoglobin and the erythrocyte in a dosedependent way.

Acknowledgments: CAPES, FAPEAL, CNPq.

Keywords: Mercury Species, Red Blood Cells, Toxicity

### N-06. Some methodological problems in the determination of polysaccharide antioxidant activity using DPPH assay.

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The DPPH assay is a widely used method to evaluate the antioxidant activity of compounds. This test is relatively simple to perform and does not require specialized equipment or training. It is sensitive enough to detect low levels of antioxidant activity, therefore it can be adapted for high-throughput screening of large numbers of compounds and several protocols for performing this test have been published. The DPPH reagent is solubilized in organic solvents. However, these solvents are also used to precipitate polymers, such as polysaccharides. Therefore, we determined whether polysaccharide precipitation occurred during the DPPHtest. The antioxidant activity of fucoidans from Undaria pinatiffida and Fucus vesiculosus were evaluated. However, methodological issues affecting the accuracy of results have been identified. The conventional DPPH assay involves gradually adding 0.1 mL of antioxidant solution to 3.9 mL of DPPH solution. Variations in this protocol include altering the ratio or using microplates. Despite these adaptations, the potential for polysaccharide antioxidant determination through this assay presents several challenges. The study evaluated the DPPH scavenging capacity of fucoidan from Undaria pinnatifida. This fucoidan exhibited low DPPH scavenging activity regardless of concentration (0.25-2.0 mg/mL). An adapted microplate protocol and centrifugation revealed that readings after centrifugation were higher, indicating underestimation due to precipitated fucoidan. A "sample blank" approach partially attenuated this error. Different protocols involving tubes and varied solvent volumes were tested on fucoidan and other polysaccharides. Precipitation-related issues were evident, particularly for polysaccharides. Whereas, glucose, sucrose, and raffinose, as well as Malpighia emarginata leaf extract, showed no problems with precipitation, suggesting the DPPH assay is suitable for substances that don't precipitate in testing solvents. Dextrans exhibited mixed results, influenced by molecular weight. Simple sugars, with low risk of precipitation, were also more reliable in the assay. The study cautioned against relying solely on the DPPH assay to assess the antioxidant activity of polysaccharides prone to precipitation. Alternative methods such as ABTS and DMPD assays might be better suited for such assessments. Studies on other polysaccharides and fractions must be conducted to support the conclusions obtained in this study.

Keywords: antioxidant methods; stress oxidative; sugar; carbohydrate; hydrocolloids Acknowledgments: To CAPES and CNPq for the financial support to carry out this research.

### N-07. Effect of non-enzymatic glycation on catalase and remediation via leafs ethanolic extract *Anacardium Humile*

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Introduction. Advanced glycation end products (AGEs) from non-enzymatic reactions involving aminocarbonyl groups. AGEs have the potential to inflict damage on cells and disrupt properties crucial for their homeostasis. The antioxidant defense system serves to inhibit these damages, with catalase responsible for facilitating the breakdown of hydrogen peroxide. This function is particularly important given the highly toxic nature of hydrogen peroxide to cells. Objectives. The aim of this study was to investigate the antiglycation activity of the ethanolic extract of Anacardium humile leaves on the catalase enzyme, which plays a significant role in antioxidant defense, over the course of 1 hour, 1 day, 5 days, and 12 days of incubation with fructose. Materials and Methods. To obtain the ethanolic extract, 400 g of dried Anacardium humile leaves were triturated and subjected to static maceration in two liters of 98% ethanol (1:5 w/v) for seven days at room temperature. The ethanolic extract of A. humile leaves (EE-Ah) was frozen at -20 °C and subsequently lyophilized to completely remove residual water. The following concentrations were tested: 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1562, 0.0781, 0.0390 and 0.0195 mg/mL. The antiglycation analysis using bovine catalase and fructose allowed the evaluation of the protein glycation process. EE-Ah was incubated with 1.25 M fructose and CAT at 4 mg/mL for 1 hour, 24 hours, 120 hours, and 288 hours at 37 °C in the absence of light. Then, 20% trichloroacetic acid was added, and the mixture was centrifuged at 10,000 x g for 10 minutes. The supernatant was discarded, and the pellet was resuspended with phosphate buffer. Results and Discussion. A total of 400 g of dried and triturated A. humile leaves were used to obtain EE-Ah, with a yield of 24.07% (96.29 g) achieved. After 1 hour of incubation, neither enzyme glycation nor any antiglycation effect was observed in the 10 analyzed concentrations. Following 1 day of incubation, concentrations ranging from 20 mg/mL to 0.3125 mg/mL effectively inhibited enzyme glycation, and this inhibition was sustained until day 5 of incubation. After 12 days, concentrations of 0.1562 mg/mL and 0.0781 mg/mL of the extract demonstrated antioxidant activity. Conclusion. The ethanolic extract of Anacardium humile leaves exhibited efficacy in inhibiting the glycation of catalase enzyme, indicating a protective role on an antioxidant enzyme during hyperglycemia situation. Acknowledgment. The authors gratefully acknowledge the Institute of Biotechnology of the Federal University of Uberlândia (UFU) and would also like to thank the Dean of Research and Graduate Studies (Pró-Reitoria de Pesquisa e Pós-graduação- PROPP/ UFU).

Keywords. Catalase, Glycation, Anacardium humile.

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