1st Research Meeting on Biochemistry

9th and 10th December 2021
Maringá, Paraná, Brazil

Post-Graduation Program
in Biochemistry

State University of Maringá

1st Research Meeting on Biochemistry

Organizing Committee

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Vinicius Mateus Salvatori Cheute

Post-Graduation Program in Biochemistry

State University of Maringá

1st Research Meeting on Biochemistry

December 9th – Thursday afternoon

2:00pm – 2:15pm

Welcome

2:15pm – 4:00pm Lecture:

From nature to products: preservatives, dyes and bioactive ingredients.

Dr. Lillian Barros, Mountain Research Center (CIMO) – Polytechnic Institute of Bragança.

4:00pm – 4:20pm

Break and Poster Session

4:00pm – 6:00pm

Oral abstracts presentations

December 10th – Friday Morning

8:30am-12:30am

Seminars Session

December 10th – Friday Afternoon

2:00pm – 4:00pm Lecture:

Metabolic reprogramming in aging and age- related diseases.

Dr. Eduardo N. Chini, Mayo Clinic and Foundation – EUA.

4:00pm – 4:20pm

Break and Poster Session

4:00pm – 6:00pm

Oral abstracts presentations

Oral abstracts presentations

Thursday – December 9th

Elaine Kaspchak 04:20 pm

Effect of saponin on asparagine–glucose Maillard reaction

Maria Gabriela Leichtweis 04:35 pm

Haskap and blackthorn berries anthocyanin profile

Adriana Katherine Molina Vargas 04:50 pm

Study of Prunus spinosa l. fruit epicarp and Lonicera careulea l. fruit: alternative natural colorants with bioactive properties

Alexis Pereira 05:05 pm

Novel antioxidant and fibre– rich food ingredients from quince peel

Beatriz Helena Paschoalinotto 05:20 pm

Effect of fertilization via nutrient solution on the nutritional profile and chemical composition of Chicorium spinosum l.

Nairana Mithieli de Q. E. Melo 05:35 pm

Effects of arsenial compounds on fructose metabolism on the perfused rat liver

Friday – December 10th

Mateus José de Oliveira 04:20 pm

Effects of a high–fat low carbohydrate diet on plasmatic parameters, in vivo glucose metabolism and fatty liver development in rats: a study under different energetic conditions

Ana Cláudia Castro Novais 04:35 pm

Nutritional and chemical analysis and bioactive potential of aromatic and medicinal plants traditionally used as condiments

Ana Paula Ames Sibin 04:50 pm

Characterization and bioactivity of Copaiba essential oil carried in a self–emulsifying system

Mikel Añibarro–Ortega 05:05 pm

Solanaceae crop by– products as renewable sources of bioactive phenolic extracts

Paulo Vinicius M. C. Menezes 05:20 pm

Isocitrate lyase as a molecular target for weed suppression

Gustavo Henrique de Souza 05:35 pm

Effects of a Myrciaria jaboticaba Peel Extract and role of cyanidin–3–O– Glucoside on lipase in mice

SEMINARS

December 10th – 8:30 to 12:20

Heloisa Vialle Pereira Maróstica 8:30 – 8:50
High rate metabolization of triclosan differently modifies metabolic flow in the perfused rat liver

Lucas Costa Cabral 8:50 – 9:10
Morpho-cytopathological analysis of the midgut of Bombyx mori L. Infected with BmNPV and submitted to the antiviral drug BmS

Karina Borba Paulinodos Santos 9:10 – 9:30
Photodynamic effects of Toluidine blue O on mitochondrial ATP production and hepatic gluconeogenesis

Naiara Cristina Lucredi 9:30 – 9:50
Methylglyoxal impairs gluconeogenesis and increases oxidative stress in rat liver

Cynthia Letícia Serra Cabeça 9:50 – 10:10
Whey protein obtained by membrane separation processes fortified with microencapsulated antioxidant fraction from Stevia rebaudiana

Evelyn Silva Moreira 10:10 – 10:30
The short-term effects of berberine in the perfused liver

Break 10:30 – 10:40

Any Carolina Chagas 10:40 – 11:00
Anti-inflammatory activity of the clove oil-isolated beta- caryophyllene carried in a self-emulsifying system

Vinicius Mateus Salvatori Cheute 11:00 – 11:20
Tolerance to Triclosan of white-rot fungi: a preliminary study

Bruna Francini Lupepsa 11:20 – 11:40
Characterization of residues for the production of gibberellic acid by solid state fermentation

Maria Rosa Zorzenon 11:40 – 12:00
Obtaining ice cream sweetened with stevia products.

Jessica Amanda Garcia 12:00 – 12:20
Hepatoprotective action of Luehea divaricata bark extract

Summary

METABOLIC BIOCHEMISTRY

CHARACTERIZATION AND BIOACTIVITY OF COPAIBA ESSENTIAL OIL CARRIED IN A SELF-EMULSIFYING SYSTEM

Ana Paula Ames Sibin[1,2*], Any C. Chagas[1], Tiane C. Finimundy[2], Sabrina Barbosa de S. Ferreira[3], Marcos L. Bruschi[3], Ricardo C. Calhelha[2], Isabel C. F. R. Ferreira[2], Lillian Barros[2], Jurandir F. Comar[1]

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Copaiba essential oil (CEO) is the volatile part of copaiba balsam, which is very rich in sesquiterpenes. The β-caryophyllene is the main sesquiterpene in the CEO and it shares many of the pharmacological properties with the copaiba oil, particularly the anti-inflammatory activity [1]. The health aid properties of copaiba oil have already been widely described [1]. The balsam, and more recently the essential oil, is used topically for a variety of painful and inflammatory conditions, including rashes, dermatitis, and psoriasis in addition to joint pain [2]. Even though, there are some concerns about the oil safety to oral use to treat severe diseases like rheumatoid arthritis [3]. The lipophilic character of the CEO makes it difficult to interact with the gastrointestinal aqueous environment [3]. Self-emulsifying carriers are very effective as systems for oral administration of active compounds with low water solubility [4]. Therefore, they should improve the anti- inflammatory activity of the orally administered CEO and have low toxicity in the effective dose. To test these hypotheses, a lipid-based self-nanoemulsifying drug delivery system (SNEDDS) containing CEO was prepared. The CEO was extracted using the hydrodistillation technic. The formulation (FSNEDDS), consisting of CEO, surfactant, and oily phase, was characterized in relation to morphology and rheology properties. The *in vitro* anti-inflammatory effect on macrophages (RAW 264.7) was evaluated by means of the inhibition of nitric oxide production. In addition, to prove that the effective dose was not harmful, we treated non-tumor (PLP and VERO) and tumor cell lines (NCI-H460, CaCo, and AGS) to find GI_{50} values. The latter corresponds to the sample concentration that inhibits 50% of cell growth assayed by the sulforhodamine B. The FSNEDDS were spherical droplets with an average size of 68 nm and their viscosity was only dependent on temperature, following a Newtonian behavior. On RAW 264.7 mouse macrophage cell line, the IC_{50} of CEO and FSNEDDS was 47.2 ± 0.4 and 42.0 ± 0.4 □g/mL, respectively. Even though, the GI_{50} (μg/mL) of CEO and FSNEDDS was respectively: 56 ± 1 and 77 ± 2 on PLP2 cell, 159 ± 11 and
178 ± 11 on VERO, 36 ± 4 and 225 ± 16 on AGS cells, and 67 ± 1 and 241 ± 12 on NCI-H460. The FSNEDDS was predominantly less toxic than the copaiba essential oil. The formulation showed a superior anti-inflammatory activity (+11%) and the beneficial concentration either for CEO or FSNEDDS was achieved with an amount that was non-toxic neither to hepatic (PLP2) nor to renal cell lines (VERO).

References

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Acknowledgments

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CHOLECALCIFEROL PROMOTES REDUCTION OF CHOLESTEROL AND ITS FRACTIONS IN THE OBESITY MODEL

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One of the issues of obesity is related to reduction of vitamin (Vit) D bioavailability (CHANG 2016; PRAMONO et al., 2019). However, studies have shown Vit D in high content helps the regulation of adipose tissue morphofunction and lipid metabolism (MONTEIRO et al., 2013; YARIBEYGI et al., 2020). Thus, we hypothesized that Vit D_3 supplementation after obesity induction in WD rats might to reduce body weight (BW), adiposity and paraments of biochemistry for this, male Wistar rats were fed on a standard chow [control (CTL n= 6) group] or a hypercaloric diet [induce obesity (WD n=6) group], from 50 day-old. In which the diet followed a menu offering foods considered to be hypercaloric the type of diet known as the cafeteria diet. After these two groups at 90 days of life, the animals were again separated according to the treatment or not of vitamin D_3 [CTL-VD (n=6) and WD-VD (n=6) groups, respectively), the animals, therefore, were supplemented with a dosage of 2800UI/day, from 90 days to 130 days of life, thus totaling 40 days of treatment. The Vitamin D_3 chosen was the commercial SupraD© liquid via gavage. At 131 days of age, WD rats were obese, exhibiting greater waist circumference and white fat stores, and higher plasma levels of total cholesterol, lowered HDL cholesterol ($p<0,005$). Also, high levels of LDL cholesterol were observed in 13% compared to CTL. Supplementation with Vit D_3 decreased body weight gain, abdominal fat deposition and cholesterol fractions were altered, approximately 5% of LDL was reduced and HDL increased by 7%, confirming the role of vit D_3 in the lipid profile plasma in WD-VD rats. In conclusion, Vit D_3 supplementation after obesity induction is a good strategy to attenuate weight gain and abdominal adiposity and to improve the plasma lipid profile in WD rats. These actions further study the indirect actions of vitamin D3 on the obese body.

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Acknowledgments
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COMPARATIVE STUDY OF IN VITRO AND IN VIVO INHIBITION OF AMYLASE BY CATECHIN AND EPICATECHIN AND ACARBOSE

Bárbara Ribeiro Tonsic[1*], Vanesa Gesser Correa[1], Tamires Barlati Vieira da Silva[1], Camila Gabriel Kato[2], Adelar Bracht[1], Rosane Marina Peralta[1]

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The α-amylases are responsible for the hydrolysis of polysaccharides which, depending on their intensity, can cause unwanted rises in postprandial glycemia, especially in diseases such as diabetes and obesity. In consequence, research has been conducted aiming at finding promising molecules that are able to inhibit the activity of those enzymes. Recent studies have shown that some flavonoids like catechin and epicatechin, abundant in many foods and beverages including green tea, inhibit α-amylases in vitro [1]. This work aimed at comparing the in vitro and in vivo inhibitory actions of catechin and epicatechin with those of acarbose, the classic α-amylase inhibitor. The α-amylase inhibition experiments were performed as previously described [2], using potato starch as substrate. After 10 minutes the reducing sugars were determined by the 3,5-dinitrosalicylic acid method [2] and the results were expressed as the concentration required for 50% inhibition (IC_{50}). For the in vivo inhibition tests, healthy male mice weighing 30 ± 2 g were used (Protocol 7049270421-CEUA-UEM). The fasted animals were divided into 5 groups (n=3), treated intragastrically as described in Figure 1. Blood glucose levels were determined with an Accu-Chek® meter. The IC_{50} values for amylase inhibition by catechin and epicatechin were, respectively, 0,023±0,004 and 0,082±0,003 mM not very far from that one reported for acarbose (0,030 mM) [2]. On the other hand, in vivo (Figure 1), the increase in blood glucose caused by the administration of starch was not reduced by catechin and epicatechin upon the administration of doses that are highly effective in the case of acarbose. The preliminary conclusion that can be drawn from these experiments is that, contrary to expectations, catechin and epicatechin are not capable of affecting starch digestion in vivo at least not at the doses that were tested. This set of observations deserves further experiments for clarification.

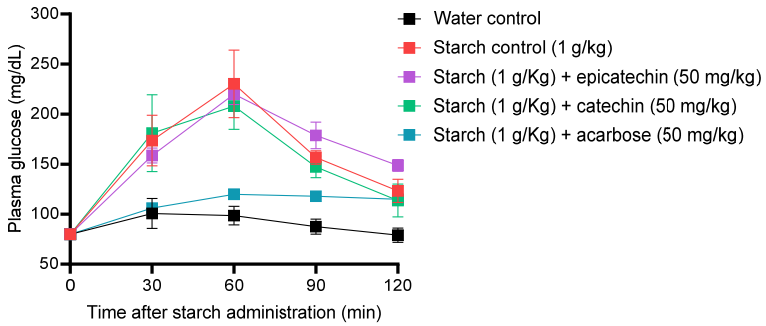

Figure 1. Mice starch tolerance test. Each series of points represents data from the average of 3 experiments (n=3).

References

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DOES HIGH TEMPERATURE THERMAL SHOCK CAUSE OXIDATIVESTRESS IN THE BRAIN OF Astyanax lacustris?

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Stress in vertebrates is considered an adaptive mechanism that allows the animal to deal with the stressor, seeking to maintain its homeostatic state [1]. The assessment of stress caused by temperature change is measured using specific biomarkers that allow understanding the adaptation process, in addition to providing useful information for environmental monitoring and fish farming programs. From the analysis of the enzymatic activity of the antioxidant system and possible cell damage, this study evaluates the effect of oxidative stress under high temperature thermal shock on the brain of *Astyanax lacustris*. The specimens (n=140) were obtained from artificial lakes (União da Vitória - PR) and subjected to thermal shock at high temperature (31°C ±1). The individuals remained in this condition in different time regimes, being: 2, 6, 12, 24, 48, 72 and 96 hours. For each experimental situation there was a control group (23°C ±1). To analyze the possible changes in the enzymatic activity of the antioxidant defense, superoxide dismutase, (SOD) [2], catalase (CAT) [3], glutathione peroxidase (GPx) [4] and glutathione reductase (GR) [5] were measured. In order to check for cell damage, lipid peroxidation (LPO) [6] and protein carbonylation (PCO) techniques were used [7]. After obtaining the data, the ANOVA statistical test was performed, followed by the Tukey post-test. For this work, significant differences were considered between control and treatment within each time regimen tested. There was a decrease in SOD activity at 96h and GPx at 12h and 72h for individuals exposed to high temperature. Also, there was an increase in GPx enzyme activity in 96h for individuals exposed to high temperature. From these results it is concluded that the *A. lacustris* brain submitted to thermal shock at 31ºC presents alterations that decrease or increase the levels of enzymatic activity of SOD (96h) and GPx (12h, 72h and 96h) under different exposure times when observed with their respective controls. These changes in enzymatic activity during different times lead to an imbalance in the brain homeostasis of *A. lacustris* and activate the different pathways of the antioxidant defense system for the acclimation process.

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EFFECT CONCENTRATION-DEPENDENCE OF THE PHLORETIN ON THE PANCREATIC A-AMYLASE: A POTENTIAL TOOL IN GLYCEMIC CONTROL

Thalita F. Demito-Santos[1], Milena Thais F. da Silva[1], Gustavo H. de Souza[1], Lívia Bracht[1], Jurandir Fernando Comar[1], Rosane Marina Peralta[1], Adelar Bracht[1], Anacharis B. de Sá-Nakanishi[1*]

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Phloretin (PL) is a flavonoid of the dihydrochalcone family, mainly found in apples, strawberries, and pears. This natural phenolic has been shown to reduce the glycemic levels and normalized the glucose oral tolerance test in diabetic rats. Some studies already published reveal that phloretin inhibits glucose co- transportation in intestinal epithelial cells (SGLT-1) and stimulates the GLUT-4, Akt, PI3K, IRS-1 genic expression in cells [1]. All these effects contributed to the improvement of the glycemic profile. However, another anti-diabetic mechanism results from inhibition of enzyme pancreatic α-amylase and consequently reduction of the monosaccharides absorption by intestinal cells. In this sense, nothing is known about phloretin action in amylase. Therefore, the present work investigated the effects concentration-dependent of phloretin on the pancreatic α-amylase. The activity of the porcine pancreatic α-amylase (initial reaction rate) was measured as the rate of reducing sugar formation. The reducing sugar was determined by the 3,5- dinitrosalicylate method, using glucose as standard [2]. The final concentration of starch was 1g per 100% and concentrations of phloretin were in the range between 0,25 and 10mM. The phloretin in fact inhibited the pancreatic α-amylase with a well-defined concentration dependence. Inhibition, however, occurred at relatively slow concentration. This is reflected by the IC50 value, which numerical interpolation revealed to be equal to 6,03 ±0,63mM. This result reveals that most likely the enzymatic inhibition of amylase contributes to glycemic control induced to phloretin. Future experiments are necessary to confirm this hypothesis in vivo.

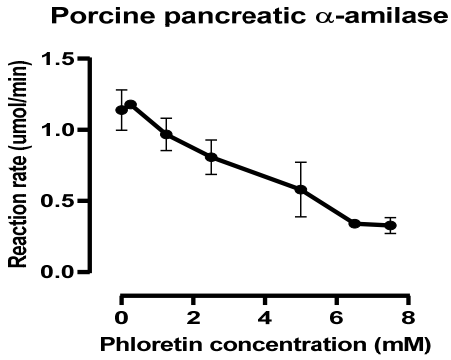

Figure 01. Concentration dependence of the inhibition caused by the phloretin on the porcine α-amylase. Each datum point is the mean ± EPM of four determinations.

References

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Acknowledgments

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EFFECTS OF A HIGH FAT LOW CARBOHYDRATE DIET ON PLASMATIC PARAMETERS, *IN VIVO* GLUCOSE METABOLISM AND FATTY LIVER DEVELOPMENT IN RATS: A STUDY UNDER DIFFERENT ENERGETIC CONDITIONS

Mateus José Oliveira[1], Evelyn Silva Moreira[1], Naiara Cristina Lucredi[1], Anacharis Babeto de Sá-Nakanishi[1], Jurandir Fernando Comar,[1] Lívia Bracht[1*]

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During recent years, increasing amount of evidence suggest that very-low-carbohydrate diets could have a therapeutic role in numerous diseases [1]. However, harmful effects have also already been demonstrated, such as fatty liver. Considering this aspect, it has already been shown that fatty liver development occurs, even in conditions where total caloric intake does not increase [2]. Nonetheless, it is unclear if the same occurs under low caloric intake and weight loss. Therefore, the aim of this work was to investigate the effects of a high fat, low carbohydrate (HFLC) diet on several plasmatic parameters, *in vivo* glucose metabolism and liver lipid accumulation in rats. Male Wistar rats (350-400g) were used. Control group received a standard rodent diet (16.7% fat, 19% protein, and 64.3% carbohydrates in Kcal%; Nuvilab®). HFLC diet consisted of 79% fat, 19% protein, and 2% carbohydrates in Kcal% (6.5 kcal/g) and was administered for 4 weeks under three different energetic conditions: 1) *ad libitum* (hypercaloric); 2) isocaloric (pair-feeding with control group); 3) hypocaloric (energetic reduction of 20%). Energy intake of HFLC *ad libitum* fed rats was 20% higher than control group. Consequently, this group gained 53% more weight compared to the control group. Body weight gain was not significantly different in control and isocaloric groups. As expected, hypocaloric group significantly loosed weight. White adipose tissue deposits were increased both in hypercaloric and isocaloric groups but decreased on hypocaloric group. Fasting blood glucose levels were 35% higher in hypercaloric group, although no differences were observed between the other groups. Plasma triglycerides were reduced by 25% and 38% in hypocaloric and hypercaloric groups, respectively. No alterations were observed for total cholesterol and HDL plasma cholesterol. The area under the curve on glucose tolerance test (GTT) were significantly higher in isocaloric and hypercaloric groups. The amount of total fat in the liver was substantially increased (almost 100%) in all groups, compared to the control condition, indicating fatty liver development. Despite of the low content of carbohydrate in HFLC diet, plasma total ketone bodies were not increased in any condition. However, a significant increase on acetoacetate/β-hydroxybutyrate ratio was observed in all HFLC groups compared to the control condition. Altogether our results demonstrate that fatty liver development occurred independently of diet energetic supply and body weight. Also, this manifestation wasn´t directly correlated with a worse plasma glucose and lipid levels, nor with glucose intolerance. Additionally increased plasma ketone bodies ratio may be a general feature of fatty liver. This study supports the notion that fat intake is more important than total caloric intake for the development of fatty liver.

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Effects of a *Myrciaria jaboticaba* Peel Extract and role of cyanidin-3-O-Glucoside on lipase in mice

Gustavo Henrique de Souza[1*], Milena Thais Francisco da Silva[1], Vitor Gomes Gonçalves [1], Pâmela Alves Castilho,[1] Tamires Barlati Vieira da Silva,[1] Rosane Marina Peralta,[1] Lívia Bracht, [1] Jurandir Fernando Comar,[1] Adelar Bracht, [1] Anacharis Babeto de Sá-Nakanishi[1]

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This study consists of a parallel and comparative investigation of the effects of a *Myrciaria jaboticaba* peel extract and one of its most prominent constituents, cyanidin-3-*O*-glucoside [1], on pancreatic lipase activities. Lipase inhibitors are used in obesity control. All procedures in animals were previously approved by the Ethics Committee for Animal Experimentation. *In vitro* experiments were determined changed both jabuticaba peel extract and the enzymatic substrate. Triglycerides oral tolerance test was quantified in mice after olive oil administration (via gavage 5mL/Kg) in presence of the extract and cyanidin-3-*O*-glucoside doses curve. The peel extract inhibited lipase with IC_{50} of 143,9ug/mL. Cyanidin-3-*O*-glucoside contributed minimally to these inhibitions. Both the extract and cyanidin-3-O-glucoside **(Figure 1)** inhibited triglyceride absorption, but at doses that were considerably smaller than those predicted by their strength in inhibiting the pancreatic lipase *in vitro*. It was concluded that inhibition of triglyceride absorption by extract can result from another action mechanism that is concomitant or even independent of the inhibition of lipase. Due to the low active doses, *Myrciaria jaboticaba* peel extract presents many favorable perspectives as inhibitors of fat absorption, and cyanidin- 3-O-glucoside seems to play a decisive role.

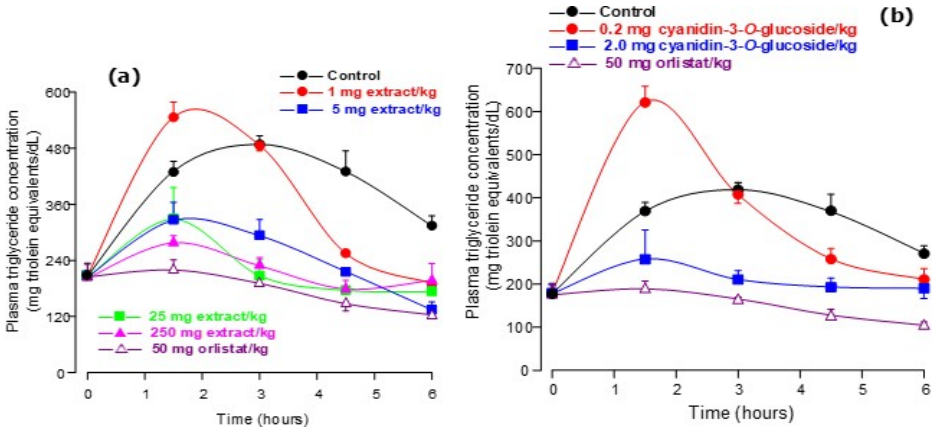

Figure 1: Effect of the different concentrations of *M. jaboticaba* peel extract (a) and the cyanidin-3-O-glucoside (b) on the plasma triglyceride concentration profiles after intragastric olive oil loads in mice.

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Effects of a *Myrciaria jaboticaba* Peel Extract and the contribution of cyanidin-3-*O*-Glucoside on amylase in mice

Vitor Gomes Gonçalves [1*], Gustavo Henrique de Souza[1], Pâmela Alves Castilho,[1] Tamires Barlati Vieira da Silva,[1] Rosane Marina Peralta,[1] Lívia Bracht, [1] Jurandir Fernando Comar,[1] Adelar Bracht, [1] Anacharis Babeto de Sá-Nakanishi[1]

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This study consists of concurrent and comparative research of the effects of a *Myrciaria jaboticaba* hydroalcoholic peel extract and one of its most prominent constituents, cyanidin-3-*O*- glucoside (CYG) on pancreatic α-amylase activities. Amylase inhibitors are used in hyperglycemia control, mainly in diabetic patients. The *M. jaboticaba* skin, usually discarded as a residue by the food industry, is a potential source of bioactive molecules and a functional food [1]. All procedures in animals were previously approved by the Ethics Committee for Animal Experimentation (CEUA-UEM, nº9577260819). *In vitro* experiments were determined by changing both jabuticaba peel extract or CYG and the enzymatic substrate **(Fig.1A)**. The peel extract inhibited amylase with a well-defined concentration dependence (IC_{50} of 1963µg mL^{-1}).The biggest CYG concentration (330uM) caused 13,3% inhibition. Starch oral tolerance test was determined in mice after starch administration (via gavage 1g/Kg) in presence of the extract and CYG doses curve. The extract doses of 500 mg kg^{-1} attenuated the concentration versus time curve in a way that is not very far from the attenuation caused by 50mg/Kg acarbose **(Fig.1B)**. The area under the curves reveals a reduction of 51 and 84%, by 250 and 500mg/Kg **(Fig.1C)**. These extract doses contained 3.2 and 6.1 mg kg−1 cyanidin-3-O-glucoside **(Fig.1C)**. Apparently, cyanidin-3-Oglucoside, at the doses that were given (up to 20mg/Kg), does not inhibit starch absorption, an observation that is consistent with its weak inhibitory activity on pancreatic α-amylase. Therefore, all these observations indicate the participation of other substances present in the extract on the enzyme activity.

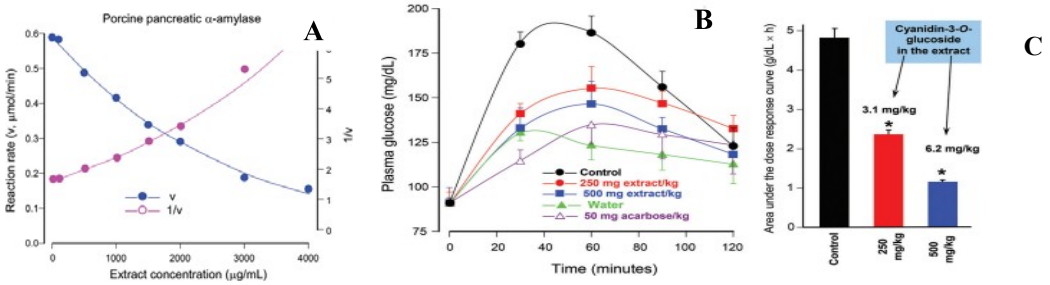

Figure 1: (A) Concentration dependences of the inhibition caused by the Myrciaria jaboticaba peel extract on the porcine α- amylase**.** Reaction rates (v) and reciprocals of the reaction rates (1/v) were represented versus the inhibitor concentrations. **(B)** Blood glucose concentration profiles after intragastric starch loads in mice: the effect of the *M. jaboticaba* residues extract. **(C)** Areas under the curves obtained after the various treatments with of *M. jaboticaba* extract illustrated by panel (B) subtracted from the area under the curve obtained after water administration.

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EFFECTS OF ARSENIAL COMPOUNDS ON FRUCTOSE METABOLISM ON THE PERFUSED RAT LIVER

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Arsenic is a ubiquitous metalloid found in many chemicals, in soil, groundwater, and even food. These compounds are capable of generating different biological effects on cells and tissues and depending on the exposed tissue and exposure time, several responses can be observed [1,2]. The active compounds, organic or inorganic, of arsenic can be divided into two groups: those in which arsenic has a valence of 5, such as sodium arsenate, or those in which arsenic has a valence of 3, such as sodium meta-arsenite (SMA) [3]. The toxicity of arsenic compounds is widely known. Surprisingly, however, detailed measurements of metabolic fluxes in the main pathways supposedly affected by arsenicals (such as gluconeogenesis) do not exist. The aim of this work is to partially fill this gap by measuring the effects of sodium meta-arsenite on fructose metabolism in the perfused rat liver. Male Wistar rats were used for liver isolation. The experimental system was the isolated perfused rat liver. All procedures were previously approved by the Ethics Committee for Animal Experimentation of State University of Maringá (#2535301019). The buffered solution (Krebs/Henseleit-bicarbonate buffer, pH 7.4) contained 0.25 g/L of bovine-serum albumin and was in equilibrium with an oxygen + carbon dioxide atmosphere (95:5). Glucose, lactate, and pyruvate concentrations in the perfusate effluent were determined enzymatically and oxygen consumption was monitored by a platinum electrode. In the liver, fructose undergoes both an anabolic energy-dependent conversion into glucose and a catabolic breakdown into lactate and pyruvate (fructolysis). The introduction of 100 μM SMA produced a small diminution of glucose production (23%) and an even smaller decrease in oxygen uptake (6%). Lactate and pyruvate production, an indicator of the catabolic breakdown of fructose, was also decreased (by 27% and 14%, respectively). Our results indicate that both anabolic and catabolic routes are impaired by SMA. The underlying mechanisms are probably involved with arsenite capability to act as a substitute for inorganic phosphate. More experiments are in course to further elucidate SMA action on other pathways of energy metabolism in the liver.

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EFFECTS OF HIGH TEMPERATURE HEAT SHOCK ON THE LIVER OF *Rhamdia voulezi* HASEMAN, 1911

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The increase in temperature of oceanic and continental waters due to climate change may affect the development and geographical distribution of aquatic species, as well as cause cellular stress in organisms [1]. *Rhamdia voulezi*, an endemic species of the Iguaçu River, presents potential bioindicator/biomonitor of high temperature heat stress conditions due to its endemic characteristic [3]. Thus, the study aims to evaluate the activity of antioxidant defense biomarkers, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glucose-6-phosphate dehydrogenase (G6PDH), reduced glutathione (GSH), lipid lipoperoxidation (LPO) and protein carbonylation (PCO), in *R. voulezi* under the effect of high temperature (31°C) heat shock in the liver for 2, 6, 12, 24 and 96 hours of exposure. With the hypothesis that high temperature heat shock increases antioxidant defense in *R. voulezi* in order to meet the increased oxygen consumption. *R. voulezi* specimens (n = 7 individuals/per experiment) were collected from the Iguaçu River (União da Vitória/PR) and transported to the Ildo Zago Aquaculture Research and Extension Center, where they remained in 1000-liter tanks until the experiments were conducted. At the end of each experiment, the fish were anesthetized with 1% benzocaine, euthanized with medullary section and immediately dissected. The samples were stored in liquid nitrogen and were processed in the Adaptive Biology Laboratory - Department of Cell Biology - UFPR. The effect of the independent variables, temperature (21º and 31º) and exposure time (2, 6, 12, 24 and 96 hours) and their possible interactions were evaluated using two-factor analysis of variance (ANOVA). As main results we observed that GST showed an interaction between the independent variables (F=2.873, P= 0.032). The enzymes that showed variation according to exposure time were SOD, GSH, LPO, and PCO. The enzymes that showed no difference between the independent variables were CAT, GPx and G6PDH. GST status indicates a resistance to oxidative stressors in fish[4]. Despite the increased GST content in *R. voulezi* liver, there was no variation in the activity levels of GPx and GSH, and a similar profile was observed in the liver of the temperate fish *Zoarces viviparus*, and there was no change in the activity of the enzymes SOD and GPx[5]. As a conclusion, we observed that GST activity is related to the defense and elimination of peroxidative products of DNA and lipids.

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EFFECTS OF THE ANTIMICROBIAL TRICLOCARBAN ON RAT LIVER METABOLISM

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Triclocarban (TCC) is a polychlorinated aromatic antimicrobial that is present in personal care products. TCC has been shown in several studies to be harmful to the biological system [1,2,3]. To further elucidate the effects of TCC on liver metabolism, isolated liver perfusion can provide insights into the actual impact of TCC on liver tissue. The catabolic and anabolic pathways, through lactate, were measured, and the results should provide additional information about the interactions of TCC with intact liver cells. As a result, Triclocarban inhibited gluconeogenesis which is a pathway highly dependent on the availability of mitochondrially generated ATP. In addition, Triclocarban stimulated the glycolytic and glycogenolytic pathways, which are compensatory phenomena for inhibiting mitochondrial ATP synthesis. Confirming this association, TCC reduced the mitochondrial ATP content in a situation where the mitochondrial respiratory chain is the primary source of this compound. Therefore, we can suggest that TCC has mainly the electron transport chain as its mechanism of action, having an uncoupling effect with a reduction in energy efficiency. With that, the ATP contents fall, compromising the glucose synthesis in the presence of the substrates. Thus, compensatory mechanisms are stimulated to synthesize ATP, such as glycolysis and glycogenolysis.

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EFFECTS OF *Trichilia catigua* MICROEMULSION ON PLASMA AND LIVER OXIDATIVE STATE IN RATS WITH CEREBRAL ISCHEMIA

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Cerebral vascular accident (CVA) is the second most common cause of dementia and holds the 5th position mortality ranking in the world [1]. The average hospital stay from cerebral ischemia in the Brazilian Unified Health System (SUS) has increased over the years. Furthermore, it is expected that the CVA incident number will increase over nearly decades, due to aging, the rise of obesity and diabetes incidence, as well as to covid-19. Cerebral ischemia is a disease that is not restricted to stopped cerebral blood flow, but it can also modify the hepatic oxidative state and the metabolism [2]. *Trichilia catigua* (Meliaceae), popularly known as catuaba, has shown antioxidant action and memory promoter in ischemic condition3. Studies, however, encourage the search for therapeutic formulations more efficient than the ethyl acetate fraction of catuaba bark crude extraction (FAE). Therefore, this work has aimed to evaluate the effects of the microemulsion of FAE on the plasma and liver oxidative state in ischemic rats. For it was used a model of transient global cerebral ischemia in rats (ICGT) that simulated cardiac arrest in humans. All experimental assays in animals were previously approved by CEUA-UEM (number: n°2102271119). Ischemic animals received the microemulsion (gavage) 4h after cerebral ischemia and reperfusion (I/R) and were euthanized 24 hours after it. Blood was collected with EDTA and the plasma was separated. The liver was removed, and liquid nitrogen clamped. Plasma and liver homogenate, prepared in phosphate buffer (0,1M pH 7,4), were used to determine oxidative markers. Ischemic insult modified the serum and hepatic oxidative state, making these tissues more oxidized. These effects result from a significant reduction in serum thiol groups content (- 54%), and an increase of ROS (+40%), and protein carbonylation levels (+34%) in the liver of ischemic rats. In addition, cerebral ischemia compromised the hepatic antioxidant system, as revealed by the sharp drop of the GSH content (-65%) as well as reducing the activity of catalase (-51%) and SOD (-75%) in the liver. The treatment prevented the systemic oxidative modification induced by brain ischemia and reperfusion. Because, the microemulsion prevented the drop in serum thiols and hepatic GSH content, besides reducing the ROS content in the liver. The results show that cerebral ischemia clearly modifies the systemic oxidative state and the treatment with microemulsion showed a strong antioxidant action, normalizing these changes. Thus, the microemulsion of the catuaba FAE is a promising preparation that needs to be further investigated in order to act as a new therapeutic tool for the treatment of cerebral ischemia.

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HEIGH AND LOW TEMPERATURE THERMAL STRESS AFFECTS Astyanax lacustris BRAIN OF ACETYLCHOLINESTERASE LEVELS?

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Acetylcholinesterase (AChE) is a key enzyme in the nervous system responsible for temporal control of nervous impulse transmission through the rapid hydrolysis of neurotransmitter acetylcholine (ACh) [1]. Considered a biomarker of neurotoxicity in aquatic organisms, the study proposes the assess levels of acetylcholinesterase in the brain of *Astyanax lacustris* high and llow temperature thermal stress. Specimens of *A. lacustris* were collected in artificial lakes (União da Vitória - PR) with the help of nets and fishing lines. After acclimation, specimens of *A. lacustris* (n=280) were subjected to thermal shock at high (31°C±1) and low temperature (15°C ±1), both with a control group (23°C ±1), for 2, 6, 12, 24, 48, 72 and 96 hours. Then, brain AChE activity was quantified according to Ellman et al. [2], with modifications to microplate by Silva de Assis [3] and submitted to the statistical test (ANOVA). The results showed that there were no significant differences in AChE levels in the brain of *A. lacustris* exposed to high and low temperature during 2, 6, 12, 24, 48 and 72 when compared to their respective controls. However, fish exposed to high temperature for 96 hours had lower AChE values when compared to exposures of 6, 12, 24 and 48 hours. It was also observed that fish exposed for 2 hours maintained higher AChE levels compared to those exposed 12, 24, 48, 72 and 96 hours. While AChE levels in the low temperature heat shock showed lower at 2 hours when compared to fish exposed for 12 hours (Figure 1A and B). It is concluded that the AChE levels of the *A. lacustris* brain did not change with the high and low temperature thermal shock when observed with their respective controls, but there were changes in the behavior of AChE levels over the exposure time.

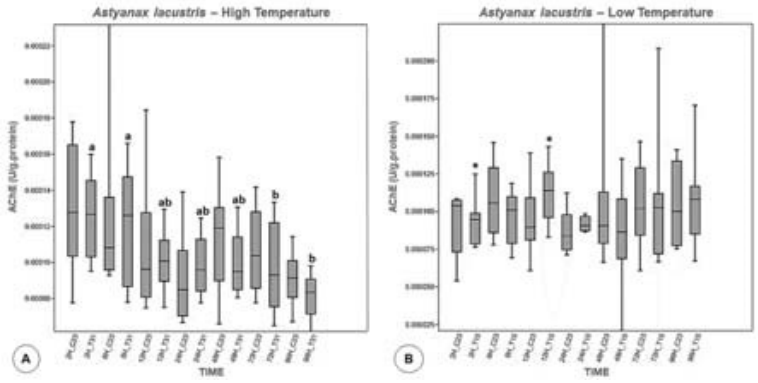

Figure 1: Acetylcholinisterase (AChE) levels of the Astyanax lacustris brain subjected to high (31°C ±1) and low temperature (15°C ±1) heat shock for 2, 6, 12, 24, 48, 72 and 96 hours. Asterisks indicate significant differences between exposure times (ANOVA, $p<0.05$). Activities expressed in nmol.min-1 .mg of protein – 1.

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HEPATOPROTECTIVE ACTION OF *Luehea divaricata* BARK EXTRACT

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Luehea divaricata Martius is a large tree belonging to the Malvaceae family, popularly known in Brazil as "açoita-cavalo". The plant is found mainly in the South and Southeast regions of Brazil [1]. Its leaves and barks are used in folk medicine for the treatment of dysentery, leucorrhea, rheumatism, blennorrhoea, and tumors [2]. The widespread use of *L. divaricata* barks and leaves infusions in folk medicine has demanded a series of scientific investigations about their biological activities and chemical compositions. The objective of this work was to evaluate the hepatoprotective action of *L. divaricata* barks with the view of expanding the current knowledge on the potentialities of this material. The barks of *L. divaricata* were collected and dried under ventilation in an air-flow chamber and pulverized. The powder was submitted to extraction using 70% ethanol. Hepatoprotective activity of the hydroalcoholic extract in rats was evaluated as described previously with some modifications [3]. One group of healthy rats (n = 6) received daily 200 mg/kg of the extract intragastrically during 14 days; another group of healthy rats (n = 6), under identical conditions, received saline (0.9% NaCl). After 14 days half of the animals in each of these two groups were injured by oral administration of 2 g/kg paracetamol [3]. Blood and liver samples were collected for measuring the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (units per liter, U/L). The catalase (CAT) and superoxide dismutase (SOD) activites were measured in liver homogenates using the traditional methodologies (change in absorbance at 240 nm due to H_2O_2 transformation and inhibition of pyrogallol auto-oxidation in alkaline medium, respectively). The plasma levels of AST and ALT in control rats (not injured) were low (43 and 66 U/L, respectively), as expected for healthy animals. The administration of the hydro-alcoholic extract caused no modifications. Paracetamol injury, on the other hand, caused a very pronounced increase in the plasma levels of both AST and ALT (402 and 348 U/L, respectively). These increases were partially prevented by the previous treatment with the extract with 50% and 57% reductions, respectively, for AST and ALT. The paracetamol injury caused a 43% diminution in the hepatic SOD activity. The treatment with the hydro-alcoholic extract fully prevented this decrease and caused no modifications in rats that were not injured with paracetamol. The hepatic CAT activity was also substantially decreased by the paracetamol injury (66% diminution). In treated rats, however, this decrease was much less pronounced as it remained only 26% below the normal level. In conclusion, the hydro-alcoholic *L. divarcata* bark extract presents favorable perspectives at least as an adjuvant for preventing and treating liver damage.

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HIGH RATE METABOLIZATION OF TRICLOSAN DIFFERENTLY MODIFIES METABOLIC FLOWS IN THE PERFUSED RAT LIVER

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Triclosan (5-chloro-2'-[2,4-dichlorophenoxy]-phenol) is a polychlorinated biphenolic antimicrobial with a broad spectrum of action. It is widely used as an antiseptic and preservative in hygiene products and medical equipment [1]. The compound causes mitochondrial dysfunction (uncoupling, inhibition of electron flow), mainly in isolated rat liver mitochondria [1-3]. The purpose of the present work was to investigate how these effects in isolated mitochondria can be translated to the whole and intact hepatocyte. For this purpose, isolated perfused rat liver was used, a system that preserves microcirculation and cell-cell interactions. Gluconeogenesis flow was determined in 18 hours fasted rat livers, alanine was infused as a substrate. Livers from fed rats were used to investigate the action of triclosan on catabolic flows (e.g., glycolysis and glycogen metabolism). Additionally, it was determined the degree of TCS hepatic metabolization from TCS quantification in output perfusion. Gluconeogenesis from alanine was practically not inhibited by concentrations of up to 50 μM. However, the introduction of 100 μM triclosan strongly progressively decreased glucose production, reaching levels near to basal state. The production of lactate, ammonia, and oxygen consumption was increased, without affecting the production of pyruvate and urea. Glycolysis was poorly stimulated at a triclosan concentration that was already strongly inhibitory to gluconeogenesis, i.e., 100 μM. Thus, the triclosan concentration was increased to 200 μM, which induced an increase in oxygen consumption and lactate production and consequently increased the lactate/pyruvate ratio, an indicator of the cytosolic NADH/NAD+ ratio. This contrasts sharply with the action of other strong uncouplers. The peak area of triclosan was strongly reduced when compared to the area of the input perfusate. The most important cause of this behavior is probably the very high single-pass transformation of triclosan, which was greater than 95% at the 100 μM portal concentration. Therefore, we conclude that the triclosan concentration gradient along the sinusoidal bed is very pronounced and the liver response mainly reflects that of the periportal cells.

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HYDROXYCHLOROQUINE INHIBITS THE GLUCONEOGENESIS IN THE LIVER OF RATS

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Hydroxychloroquine (HCQ) is an anti-malarial medication also used against some auto-immune diseases [1]. With coronavirus pandemic (SARS-CoV-2), many countries initially used HCQ for treatment of persons hospitalized with COVID-19 since some studies showed that it is able to inhibit the replication *in vitro* of the virus [2]. However, the drug was not formally approved through clinical trials, on the contrary, it was even associated with decreased in-hospital survival and an increased frequency of ventricular arrhythmias when used for treatment of COVID-19 [3]. HCQ absorbed by the enterocytes first pass through the liver before reaching the systemic circulation. This drug is metabolized in the liver and would produce hepatotoxicity, and the inducible liver injury can impair the metabolic function of the organ. In this regard, the liver is involved in a number of physiological functions, including a pivotal role in the body metabolic homeostasis, so that the organ is the site of several pathways linked to intermediary metabolism, such as gluconeogenesis [4]. An impaired energetic metabolism of the liver could be even more harmful to the already failing health of patients with COVID-19. Therefore, this study aimed to investigated the effects of HCQ on gluconeogenesis in the livers of rats. Gluconeogenesis was evaluated in the perfused liver of 15 h fasted rats using 2 mM lactate and fructose as precursor. After cannulation of the portal and cava veins, the organ was perfused with Krebs/Henseleit (KH) buffer. Glucose, pyruvate and lactate were quantified in the effluent fluid. Oxygen uptake was monitored by polarography. The perfusion protocol was: 10 min with KH, followed by 26 min with KH + lactate or fructose and another 24 min with KH + lactate or fructose + hydroxychloroquine at concentrations in the range up to 200 µM. Liver mitochondria were isolated by differential centrifugation and respiration measured by polarography. When lactate was used as a gluconeogenic substrate, HCQ inhibited the oxygen uptake only at a concentration of 200 µM, however, the production of glucose was inhibited by 54% at the dose of 200µM and approximately 28% at doses of 10 - 100 µM. When fructose was used as substrate, HCQ did not inhibit the oxygen uptake or glucose production, but completely inhibited the pyruvate production and increased in 121% the lactate production at the dose of 200 µM. The respiratory activity of isolated hepatic mitochondria was not significantly inhibited by HCQ. Considering the latter and that the glucose production was not inhibited when fructose was substrate, the HCQ must be inhibiting the gluconeogenesis by another additional mechanism that still require further investigation, such as a direct inhibition of the final enzymes of the gluconeogenic pathway (below the fructose entry point). The inhibition of gluconeogenesis was observed at relatively high concentrations of HCQ, however, the effect should not be also neglected whether the overdosage is considered and could be associated with the liver injury and metabolic alterations in patients.

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METABOLIC IMPLICATIONS OF PROTEIN RESTRICTION ON ADOLESCENCE IN WISTAR RATS

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The DOHaD concept points out that insults such as malnutrition, when occurring in periods of great organ plasticity, such as adolescence, can program for cardiometabolicdisorders in adulthood [1]. This study aims to evaluate the effect of exposure to a low-protein diet during adolescence on glucose metabolism and biometric and biochemicalparameters. The research ethics committee approved the study under CEUA (nº2910011021). 30-day-old Wistar rats were fed a low-protein diet (LP, 4% protein) for 30 days. Control animals (NP) were fed a 20.5% protein diet throughout the protocol. Murinometric parameters were evaluated during adolescence and at 60 days the basal plasmatic values of glucose, cholesterol, triglycerides, and HDL-cholesterol were quantified from serum samples collected after euthanasia. And at 60 days, glucose concentrations were quantified in animals submitted to both the oral glucose tolerance test and the intraperitoneal insulin tolerance test. The LP group had lower body weight (P=0.0027) and nasal-anal length (P=0.0001), with a reduction in food consumption (P=0.0512). Regarding biochemical dosages, the LP grouphad higher levels of glucose (P=0.0079), however, levels of triglycerides (P=0.0024), totalcholesterol (P=0.0163), and HDL (P< 0.0001) were lower in LP animals. In the assessment of glucose metabolism, the LP group had a lower glucose tolerance (P=0.0183) and a higher insulin tolerance (P=0.0073). Protein restriction induces metabolic, biochemical, and biometric dysfunctions during adolescence. Thus, these results demonstrate that malnourished animals are at greater risk for the development of metabolic syndrome in adulthood.

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METHYLGLYOXAL EFFECTS ON MITOCHONDRIAL RESPIRATORY ACTIVITY OF RATS LIVE

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Diabetes mellitus (DM) is associated with several liver abnormalities [1]. The hyperglycemia that characterizes DM is responsible for increasing the flux of several glycotoxic pathways, such as the production of methylglyoxal (MG), a highly reactive α-oxoaldehyde metabolite, produced mainly as a toxic product of glycolysis [2]. MG can cause several damages [3], however, little is known about its effects on respiration in isolated liver mitochondria. There are several pieces of evidence that mitochondrial dysfunction plays a key role in the pathophysiology of diabetes and also in liver diseases, numerous mitochondrial alterations are observed in these pathologies, suggesting that mitochondrial dysfunction can be a link between DM and liver diseases [4]. This work aimed to investigate the effects of MG in aspects of mitochondrial function from rats' livers. Adult male Wistar rats (60 days old) were treated with intraperitoneal injections (i.p) of saline or methylglyoxal 100 or 200 mg/kg for 7 days. Subsequently, the animals were anesthetized (9 $mg.kg^{-1}$ xylazine + 90 $mg.kg^{-1}$ ketamine) i.p, where the liver was collected and homogenized in an isolation solution containing sucrose, mannitol, EGTA, PMSF, and BSA FFA and centrifuged in four cycles. In intact mitochondria were evaluated states II, III, and IV as well as respiratory control, ADP/O ratio, and mitochondrial potential. In ruptured mitochondria were measured complexes I, II, and IV activities. All experiments were conducted in strict adherence to the guidelines of the Ethics Committee for Animal Experimentation of the State University of Maringá (Certificate Nº. 9185221019). The administration of MG for 7 days increased the state IV of respiration with both substrates: succinate and α-ketoglutarate. The respiratory control with both substrates decreased in treated groups when compared to control, indicating that respiration cannot return to its basal state. Mitochondrial potential decreased in treated groups; furthermore, the ADP/O ratio was only modified in respiration with α-ketoglutarate. In respiration with ruptured mitochondria, we obtained alteration in complex IV activity only in MG 200 $mg.kg^{-1}$ group. These results show us that mitochondria are uncoupled after treatment with MG. Therefore, we can conclude that treatment with this compound generates a mitochondrial dysfunction and it can contribute to the hepatic disorders frequently observed in DM-affected patients.

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METHYLGLYOXAL IMPAIRS GLUCONEOGENESIS AND INCREASES OXIDATIVE STRESS IN THE LIVER

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Methylglyoxal (MG) is a highly reactive alpha-oxoaldehyde produced at substantial levels when the cellular glucose increases. In certain metabolic disorders, such as diabetes, obesity and steatosis, the cellular levels of MG are greatly increased and associated with oxidative injuries that affect many tissues and organs, including the liver [1]. This organ plays a central role in body metabolic homeostasis as it is the almost exclusive site of many pathways linked to intermediary metabolism, such as gluconeogenesis. Similarly, these metabolic disorders per se are associated with modifications in liver metabolism, which have been in part attributed to the increased oxidative stress in the organ [2]. The knowledge of MG actions in liver metabolism is limited and little has been done to clarify its role in the evolution of a healthy organ towards liver diseases. Therefore, this study investigated the actions of MG on gluconeogenesis and oxidative state of rat livers. Male Wistar rats weighting 200-250 g (60 days old) received intraperitoneally saline (controls) or MG (100 and 200 mg/kg) daily for 7 days. An additional group received MG at the dose of 25 mg/Kg for 30 days to evaluate the gluconeogenesis on long-term exposure to MG. After this period, the peritoneal cavity of 18 h fasted rats, previously anesthetized with xylazine (9 mg/kg) plus ketamine (90 mg/kg; ip), was exposed, the liver removed, clamped in liquid nitrogen and used to measure the levels of protein carbonyl groups, reduced (GSH) and oxidized (GSSG) glutathione, reactive oxygen species (ROS) and advanced glycation end-products (AGEs), and the activity of myeloperoxidase (MPO), superoxide dismutase (SOD) and catalase. Gluconeogenesis was assessed in separated groups of rats using livers perfused with Krebs-Henseleit buffer and lactate as substrate. The liver oxygen uptake was monitored by polarography and glucose and pyruvate production was measured using enzymatic assays. The animal protocol was approved by the Ethics Committee for Animal Experimentation of UEM (Certificate Nº. 9185221019). MG at both doses for 7 seven days decreased the lactate-induced glucose production by 35% (compared to the controls). The oxygen uptake was not significantly modified, but the pyruvate production was 67% higher in rats treated with 200 mg/Kg MG. At the dose of 25 mg/Kg for 30 days MG decreased the increment of glucose production by 26,25% and the oxygen uptake by 34,50%, but not modified the pyruvate production. MG (100 and 200 mg/Kg) increased the hepatic levels of protein carbonyl groups by (37 and 135%, respectively), ROS content by around 40% and MPO activity by (84 and 104%, respectively
- compared to the controls). MG at the dose of 200 mg/Kg increased the hepatic levels of AGEs by 14% and decreased the GSH/GSSG ratio by 35,70% and SOD activity by 26,74%. MG at both doses decreased the catalase activity by 19 and 39% for MG 100 and 200 mg/kg, respectively. The results show that MG intraperitoneally administered impairs the gluconeogenesis in the liver of rats and the consequence seems to be the organ injury caused by an increase of inflammation, AGEs content and oxidative stress, therefore MG could be associated with the liver injury and metabolic alterations found in diabetic patients.

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PHENOLICS AND NOT POLYSACCHARIDES IN THE *CAMELLIA SINENSIS* AQUEOUS EXTRACTS ARE THE MAIN INHIBITORS OF THE PANCREATIC ALPHA AMYLASE

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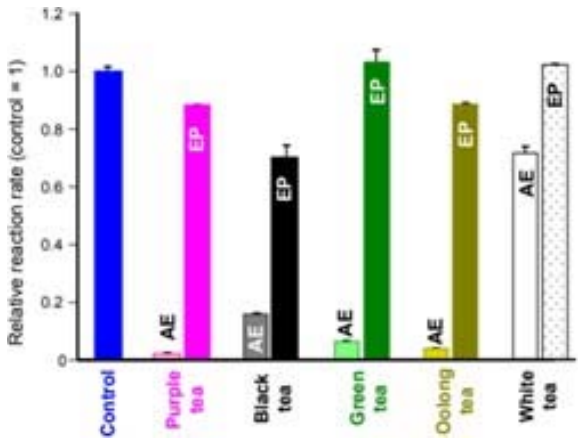
It has been shown that polysaccharides of Pu-erh tea are able to inhibit the pancreatic α-amylase [1]. Traditionally this inhibitory activity has been attributed mainly to polyphenolics. The purpose of this work was to quantify the participation of polysaccharides in the inhibition of α-amylase by several tea varieties. For this purpose, the actions of total aqueous extracts were compared with the actions of resolubilized ethanol precipitates which contain mainly polysaccharides. Fig. 1 allows to compare the activities of the pancreatic α-amylase in the presence of the total aqueous extracts (AE) of the various varieties of *C. sinensis* with the activities in the presence of the corresponding re-solubilized ethanol precipitates (EP). For each pair of columns (AE and EP), the extract concentrations were those that produced the maximal inhibition observed in the experiments. For purple tea, green tea and oolong tea, the contribution of the re-solubilized ethanol precipitate to the total inhibitory activity was either small or non-existent. This also holds for white tea, but in this case even the aqueous extract was not a very strong inhibitor. Black tea, on the other hand, was the only one for which a significant inhibition of the α-amylase by the re-solubilized precipitate was found (29%). It can be concluded that polysaccharides in the black tea preparations may contribute significantly to the inhibition of the □-amylase. For all the other varieties, however, it is likely that polyphenolics are the main inhibitors. This is a conclusion that is substantiated by the content in polyphenolics of these varieties and by computer simulations that suggest inhibitory activity for these compounds. Details can be seen in [2].

Figure 1: Comparison of the activities of the pancreatic α-amylase in the presence of the total aqueous extracts (AE) of C. *sinensis* varieties with the activities in the presence of the corresponding re-solubilized ethanol precipitates (EP).



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PHOTODYNAMIC EFFECTS OF TOLUIDINE BLUE O ON MITOCHONDRIAL ATP PRODUCTION AND HEPATIC GLUCONEOGENESIS

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Toluidine blue O (TBO) has been studied due to its applicability as a photosensitizer (P) in photodynamic therapy (PDT), an ascension modality for the treatment of some types of cancer [1]. The interaction between the photoactivated P and oxygen results in the formation of reactive species of oxygen, which are responsible for promoting cytotoxic effects on the target cells [2]. Previous studies performed by our research group indicated that TBO inhibits mitochondrial oxidative phosphorylation, which can compromise diverse biosynthetic pathways. Thus, the present study aimed to evaluate the direct and photodynamic effects of TBO (40 μM) on the ATP biosynthesis rate in isolated mitochondria. In addition, the intrinsic effects of TBO on hepatic gluconeogenesis, a metabolic pathway strictly dependent on ATP were also evaluated. Male Wistar rats (180─280 g) were used for the experiments. Hepatic mitochondria were isolated by differential centrifugation. Afterward, mitochondria were pre-incubated for 10 min in the absence or presence of TBO and irradiated (or not) with a red light-emitting diode system (636 nm, 2.6 mW □ cm^2), thus providing a dose of energy (fluence) of 1.6 J □ cm^{-2}. Subsequently, mitochondrial ATP biosynthesis rate was measured in intact coupled mitochondria (3.33 mg protein □ mL^{-1}), in the presence of succinate (10 mM) and rotenone (5 μM) or malate (10 mM) and glutamate (10 mM), and ADP (1 mM). The ATP produced was quantified by high-performance liquid chromatography at 254 nm. Liver perfusion was executed using 12 h fasted rat livers. Lactate (2 mM) and pyruvate (0.2 mM) were used as the gluconeogenic substrates. Glucose production was evaluated enzymatically and oxygen consumption was measured polarographically. The results show that the biosynthetic rate of ATP on isolated mitochondria was inhibited both under irradiation (-95%) and in the absence of irradiation (-85%) when malate and glutamate were utilized as the substrates. When succinate was employed as the oxidable substrate there were also inhibitions on the biosynthetic rate of ATP in both conditions (-80% without irradiation and -97% with irradiation). In the hepatic perfusion experiments, TBO progressively inhibited gluconeogenesis, reaching a maximum inhibition of 68%. After the interruption of TBO infusion, there was a partial recovery in glucose production. No significant difference in oxygen consumption was found, although a slight stimulus was noticed. The gluconeogenesis inhibition is probably a consequence of the inhibition of mitochondrial oxidative phosphorylation, which happened under or without irradiation. According to the results, TBO seems to exert an uncoupling effect. Considering the similarity of mitochondrial metabolism in different tissues, other metabolic ATP-dependent processes might be affected. A possible adverse effect caused by the TBO action observed in this study could be hypoglycemia due to the inhibition of glucose production. These metabolic alterations could limit the use of TBO on PDT and other clinical modalities.

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THE SHORT-TERMS EFFECTS OF BERBERINE IN THE PERFUSED LIVER

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Berberine is an alkaloid present in plants belonging to the Berberidaceae, Papaveraceae, Ranunculaceae, Rutaceae, Annonaceae and Menispermaceae families. It has numerous bioactive activities, such as antioxidant, anti-inflammatory, anti-tumor, anti-mutagenic, anti-diabetic, lipid-lowering activities and is freely marketed in capsules for therapeutic use, without the need for medical advice [1]. Studies show that the liver is a central organ in the biodistribution and biotransformation of berberine, being the organ where berberine accumulates the most. Additionally, the liver is an important site of the pharmacological action of berberine, as it has been shown to inhibit hepatic gluconeogenesis. Toxicological data, on the other hand, suggest that berberine may be harmful to liver tissue [2]. Furthermore, studies show that berberine interferes with cellular respiration, decreasing the generation of ATP. Considering that substances that impair mitochondrial function also interfere with the liver's energy metabolism, the aim of this work was to perform a systematic study on the effects of berberine on hepatic metabolism. The experimental system was the isolated perfused rat liver. The results demonstrated that at low portal concentrations (up to 10 μM) berberine can significantly modify several pathways in the liver. It inhibited gluconeogenesis from different substrates (lactate, alanine and fructose) and ammonia detoxification, which are dependent of ATP generated within the mitochondria. The strongest effect of berberine found in this work was that on lactate gluconeogenesis, with 50% inhibition at the portal concentration of 17 μM. Conversely, it stimulated glycolysis and fructolysis, which are compensatory phenomena for an inhibited mitochondrial ATP generation. Experiments with intact rat liver mitochondria showed that berberine inhibited state III respiration (presence of exogenous ADP), especially when complex I substrates (pyruvate and α-ketoglutarate) were used. Inhibition of state III respiration was nearly 3-fold stronger in the presence of pyruvate than in the presence of α-ketoglutarate. However, there was no inhibition of respiration in freeze-thawed mitochondria, in the presence of different substrates. As a result of the inhibition of mitochondrial respiration, berberine decreased ATP levels, as well as the ATP/ADP and ATP/AMP ratios in the liver, a set of modifications that clearly justify the inhibition of gluconeogenesis, as well as the synthesis of urea from of alanine, processes that are strictly ATP-dependent. These results are strong evidence that the direct acute effects of berberine in the liver represent a great contribution to its clinical efficacy as an antihyperglycemic drug. Safety concerns also arise, and toxic symptoms can include lack of circulating glucose due to gluconeogenesis inhibition, metabolic acidosis due to excessive lactate production, impairment of ammonia detoxification and cell damage due to a deficient maintenance of its homeostasis.

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β-MYRCENE INHIBIT THE LEUKOCYTE RECRUITMENT AND PHAGOCYTIC ACTIVITY OF NETROPHILS

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β-myrcene, a monoterpene, is derived from essential oils of many plants such as lemongrass, verbena, *Rosmarinus officinalis*, and others, which are commonly applied in cosmetics and food industries. Many studies demonstrated pharmacological activities of β-myrcene, as antioxidant, analgesic, antiulcer, anti-inflammatory, and antimicrobial activities. This study aimed to investigate the effects of β-myrcene in leukocyte behavior. Chemotaxis *in vitro* was carried out in Boyden chambers using leukocytes from the peritoneal cavity of mice with zymosan-induced peritonitis. The cells were incubated with β-myrcene at concentrations of 3, 10, 30, and 90 µg/mL for 30 min and fMLP was the chemoattractant. Cell viability, by MTT methods, and phagocytosis assay was performed with β-myrcene at the same concentrations. The animal protocol was approved by the Ethics Committee for Animal Experimentation of UEM (CEUA/UEM nº 3919220419). Our results showed that β-myrcene at 10 µg/mL (57,35%) promoted a significant decrease in leukocyte chemotaxis when stimulated by fMLP (Figure1). The treatment with this compound did not modified the cell viability, measured by the cytotoxicity assay. We also observed that β-myrcene at concentrations of 3, 10, 30, and 90 µg/mL significantly reduced the phagocytic activity of neutrophils in 30.13; 51.02; 61,56 and 56.19% respectively. This study showed that β-myrcene has potential anti-inflammatory effects by reducing migration and phagocytic ability of leukocytes.

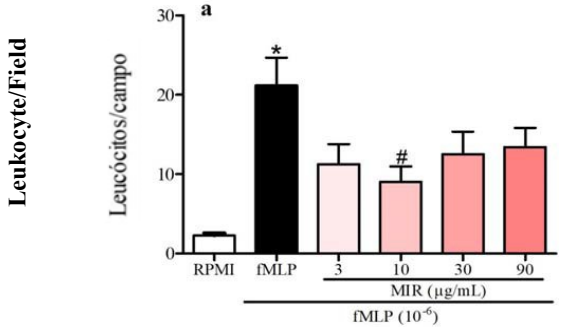

Figure 1: Effect of β-myrcene (MIR, a) on leukocyte chemotaxis. Leukocytes obtained from the peritoneal cavity of mice were treated with β- myrcene concentration of 3, 10, 30, 90 µg / ml.

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BIOTECHNOLOGY AND MOLECULAR BIOLOGY

ALPHA-BISABOLOL TREATMENT IN COLYTICS RATS REDUCES ACTIVITY OF MYELOPEROXIDASE PLASMATIC, COLONIC AND HEPATIC

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Ulcerative colitis and Crohn's disease are characterized as Inflammatory Bowel Diseases (IBD). They affect the colonic mucosa, as well as have hepatic and systemic involvement [1]. The pathophysiology of both involves intense hyperplasia of the intestinal mucosa triggered by pro-inflammatory cytokines [2]. The uncontrolled production of these cytokines stimulates activated neutrophils and macrophages to secrete reactive species and inflammatory enzymes, which cause tissue damage and oxidation of tissue components, including membrane proteins and lipids [3-4]. Alpha-bisabolol is a natural monocyclic sesquiterpenic alcohol, the main constituent of the essential oil of chamomile-vulgar and has anti-inflammatory activity [5- 6]. The aim of this work was to evaluate the effects of alpha-bisabolol on inflammation in healthy rats and those with experimental colitis. Male Wistar rats were induced to colitis by TNBS (2,4,6- trinitrobenzenesulfonic acid) via enema and treated with alpha-bisabolol 50 mg/kg for 7 days, via gavage. After this period, they were anesthetized and euthanized, and blood was collected from the vena cava. For the analysis of myeloperoxidase (MPO) enzyme activity, plasma, liver and colon samples were frozen in liquid nitrogen and analyzed by spectrophotometry with o-dianisidine. The MPO enzyme is present in neutrophils and participates in processes where there is inflammation. Thus, an increase in the activity of this enzyme was observed in the plasma, liver and colon of colitic animals, and treatment with alpha- bisabolol was able to significantly reduce this parameter. In conclusion, it can be inferred that alpha- bisabolol treatment was able to reduce neutrophil-mediated inflammation.

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ANTI-INFLAMMATORY ACTIVITY OF THE CLOVE OIL-ISOLATED B-CARYOPHYLLENE CARRIED IN A SELF-EMULSIFYING SYSTEM

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(-)-β-Caryophyllene is a sesquiterpene reported to present anti-inflammatory activity that can be obtained from asymmetric synthesis or, alternatively, isolated from many essential oils, including clove oil. However, the low water-solubility limits the application of β-caryophyllene in the pharmaceutical field [1]. The aim of this study is to investigate and compare the oral bioavailability and the effects of the clove oil-isolated β- caryophyllene free and carried in a lipid-based self-emulsifying drug delivery system (SNEDDS) on the carrageenan-induced paw edema in rats. β-Caryophyllene (91% purity) was isolated from clove oil using preparative thin-layer chromatography and copaene (0.7%) and α-humulene (8.2%) were also identified in the preparation by GC/MS. The SNEDDS system, consisting of a self-emulsion formed by βcaryophyllene, cremophor (surfactant), and ethyl linoleate, was characterized in relation to size and rheology (continuous flow and oscillatory). The SNEDDS thus obtained was a pseudoplastic and viscoelastic nanostructured self- emulsion with homogeneous sizes in the water, PBS and simulated intestinal fluid [2]. βcaryophyllene orally administered was effective in improving the carrageenan induced paw edema in rats, but when compared to the free form, the higher effectivity of the compound carried in SNEDDS was found only at a low dose of 50 mg/Kg. The latter shows that β-caryophyllene could be reducing the paw edema at low concentrations, which are achieved in the paw tissue when administered even in the free form at the doses of 100 or more mg/Kg. In fact, the leukocyte's chemotaxis in vitro was considerably and equally inhibited by free β- caryophyllene at concentrations of 3-90 μg/mL, a phenomenon that did not modified the cell viability. Comparison on pharmacokinetic parameters of free and formulated β-caryophyllene showed that when it is carried in SNEDDS its oral bioavailability is improved. Therefore, SNEDDS proposed in the present study seems to be an appropriate carrier to increase the solubility and bioavailability of β-caryophyllene for clinical applications.

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ASSESSMENT OF TOLERANCE TO THE HERBICIDE DIQUAT AND LACCASE PRODUCTION BY BASIDIOMYCETE ISOLATES

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To degrade lignocellulosic materials, filamentous fungi produce enzymes such as laccase, a lignin-oxidizing enzyme that can degrade phenolic and non-phenolic compounds and several environmental pollutants including synthetic dyes, toxic substances in industrial effluents, herbicides, and pesticides in soil [1]. A potential source of this enzyme is fungi of the Basidiomycetes class, known as white-rot fungi, which play a pivotal role in the ecosystem owing to their rich enzymatic apparatus. Due to their ability to degrade and detoxify various environmental pollutants, laccases have been widely applied in several biotechnological processes. In order to facilitate new and more efficient bio-catalytic applications, the biochemical properties of fungal laccase must be evaluated. Diquat dibromide is a fast-acting, non-selective contact herbicide, photosystem I inhibitor, which regulates plant growth, causing injury only to the parts of the plant where it is applied. In the field, the product is used as a desiccant for crops and control of aquatic macrophytes. In this study, some isolates of basidiomycetes were investigated regarding their tolerance to Diquat and the production of laccases in a medium containing the herbicide. Tolerance to the herbicide was evaluated in solid medium (MEA) containing Diquat at concentrations ranging from 5 to 1000mg/l. Laccase production in the presence of Diquat (10mg/mL) was studied by inoculating three discs of fungal mycelium in minimal Vogel medium containing glucose (1%). The cultures were stopped by filtration after 10 days. All material was autoclaved before use and the herbicide was diluted in sterile water and membrane filtered (0.22µm). The plaque tolerance test revealed that isolates SE, P2AG and C3 tolerated up to 250mg/l of herbicide, and M5, M3, CM and M2 showed growth at a dilution of up to 100mg/l. Isolate A6 demonstrated slower growth, but it grew in medium with up to 500 mg/l of Diquat. *Pleurotus pulmonarius*-CCB19 and *Phanerochaete chrysosporium* tolerated the herbicide up to 50 mg/l. In the assessment of tolerance in a liquid medium, there was no growth in the medium containing herbicide, that is, even at a low concentration, the herbicide was able to completely inhibit fungal growth, in opposition to what occurred in solid medium. The fungi that produced the greatest amount of laccase in the control crops were C3 (271.95 U/L), M2 (156.06 U/L), and M3 (129.42 U/L), however, M5 and M2 produced the greatest amounts of biomass, 0.180g, and 0.177g, respectively. It is concluded that herbicide tolerance, verified only in a solid medium, was affected by herbicide availability in the medium. Diquat is very soluble in water and, therefore, the liquid medium must have provided greater contact of the herbicide with the mycelium, unlike what occurs in solid medium. New experiments are being conducted to assess tolerance in liquid medium at lower concentrations and the consequent action of the herbicide in the production of laccases of these fungi. Thus, we intend to seek information about the possible form of degradation of this herbicide to contribute with new technologies in the processes of bioremediation of surface water and soil.

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CHARACTERIZATION OF A STRAIN OF *Herbaspirillum seropedicae* MUTANT IN GENE glnE

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The bacterium *Herbaspirillum seropedicae* is and endophytic nitrogen fixing bacteria. Amongst the plants that *H. seropedicae* associates with, grasses with economic interest are included, such as sugarcane, wheat and corn. The nitrogen fixed by *H. seropedicae* is incorporated into the plant host biomass. In addition, *H. seropedicae* can also induce plant growth through the production of phytohormones.

The main source of nitrogen for most bacteria is ammonium, which can be assimilated by two distinct metabolic pathways. The GS pathway, target of the present work, is strongly regulated in response to environmental nitrogen levels. The metabolic flux through the GS pathway is regulated by a sensor enzyme, GlnE. GlnE is a bifunctional enzyme, capable to catalyze the reversible adenylylation at a conserved tyrosine residue of the GS, decreasing its biosynthetic activity at high concentrations of ammonium. At low concentrations of ammonium, GlnE promotes deadenylylation of GS, activating it [1].

Understanding the metabolism of nitrogen assimilation requires further understanding of the regulatory mechanism of GlnE. For this, a strain of *H. seropedicae* with deletion in phase of the *glnE* gene was constructed, named as ELΔglnE and we started its characterization.

We verified the GS activity of wild and ELΔglnE mutant strains in high ammonium (20mM) and low glutamate (5mM) through the biosynthetic reaction and the γ-glutamyl transferase (γ-GT) reaction, as shown in Figure 1.

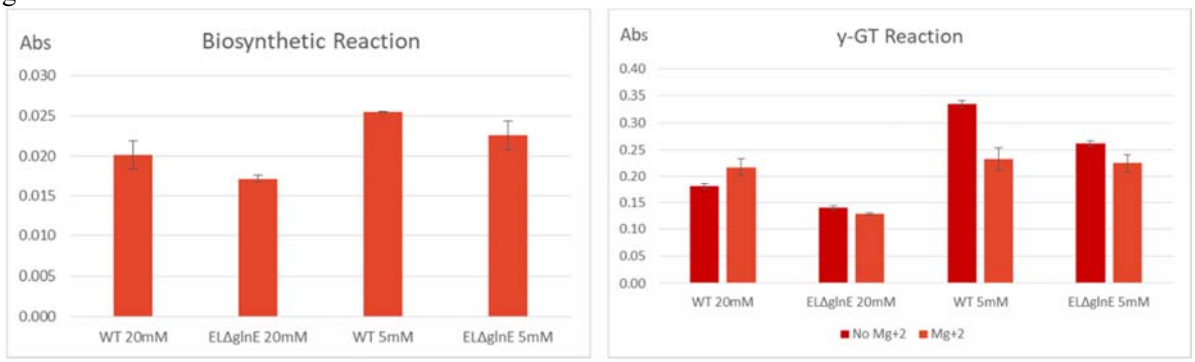

Figure 1: Biosynthetic reaction and γ-GT reaction to verify GS activity of wild (WT) and mutant ELΔglnE strains. Being high ammonium the culture grown with ammonium chloride at 20mM and low ammonium the condition with glutamate 5mM.

The biosynthetic reaction showed that GS activity, both in the wild strain and in the mutant strain, was regulated according to nitrogen availability, with higher activity in 5mM glutamate. The γ-GT activity is inhibited by Mg^{+2} ions, suggesting that GS is not adenylylated. The characterization of the ELΔglnE will continue to be carried out in the future.

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CHARACTERIZATION OF THE ACTIVITY OF THE MUTANT VARIANT OF THE GLUTAMINE SYNTHETASE PROTEIN FROM *Herbaspirillum seropedicae*

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Herbaspirillum seropedicae is a gram-negative, vibrioid bacterium, member of class β of the phylum proteobacteria, capable of colonizing internal plant tissues without causing any apparent damage to the host and promoting plant growth [1]. These factors make *H. seropedicae* a potential biofertilizer, being a more efficient and less polluting alternative to nitrogen fertilizers. The preferred source of nitrogen in bacteria is ammonium, whose main assimilation pathway includes the Glutamine Synthetase (GS) enzyme [2]. In this pathway, glutamate is aminated to glutamine, which has its amide group transferred to 2-OG to form two glutamate molecules [3]. The regulation of the rate of metabolic flux through this pathway depends on the regulation of GS activity, providing a way to keep intracellular glutamine levels constant to meet the metabolic needs of the cell. The mechanism of regulation of the GS enzyme of *H. seropedicae* has not yet been fully described. The present work aimed to express and characterize an enzyme variant containing a site-directed point mutation in the GS enzyme of *H. seropedicae*, G54C. By using a PCR method with complementary mutagenic primers, the gene variant with the point mutation was constructed. In order to overexpress the mutant GS variant, the mutant gene was cloned in the expression vector pETM11. The resulting plasmid expresses the His-tagged mutant protein suitable for purification by affinity chromatography by using a Nickel column. GS activity was evaluated by colorimetric assays through either two reactions catalyzed by this enzyme, named biosynthetic and γ- glutamyltransferase. In addition, we determined protein-ligand interactions using Differential Scanning Fluorimetry (DSF). *In vitro* assays showed that the mutation G54C conferred, in comparison to the wild- type enzyme, a higher biosynthetic activity when the reaction cofactor was Mn^{2+}, and reduced activity when the cofactor was Mg^{2+}. The DSF assays indicated that the G54C variant was apparently unable to bind both ATP and ADP in buffer containing Mg^{2+} ions, which possibly explains the lack of enzyme activity of this variant in the presence of this cofactor. Unlike the wild type, the G54C mutant variant was able to bind ATP and ADP exclusively when Mn^{2+} ions were present, consistent with the conditions that this enzyme variant showed activity. Thus, the data indicate that the G54C mutation caused a perturbation in the nucleotide-binding site such that it prevents MgATP binding, but with the allowing the MnATP coordination. The purified protein will continue to be further characterized *in vitro* in continuing work.

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Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES; Complexo de Apoio a Pesquisa – COMCAP

COMPARATIVE DETOXIFICATION OF REMAZOL RRILLIANT BLUE R BY FREE AND IMMOBILIZED LACCASE OF *OUDEMANSIELLA CANARII*

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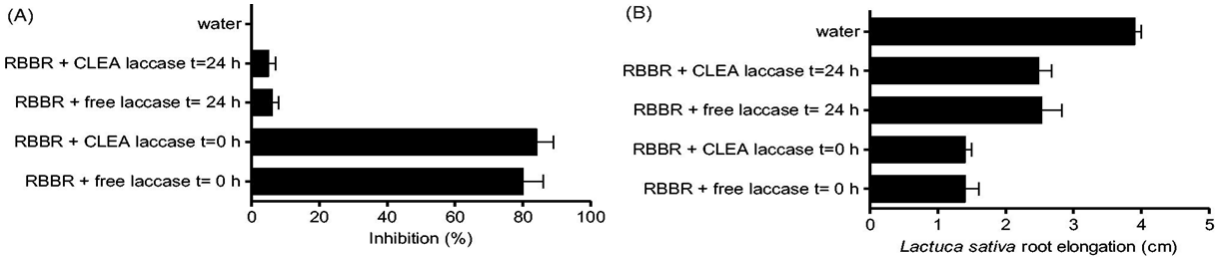
Laccases (EC 1.10.3.2) are multicopper oxidases capable of oxidizing a broad range of substrates with the concomitant reduction of O_2 to water. A laccase from *Oudemansiella canarii* was immobilized using the crosslinked enzyme aggregate (CLEA) methodology and applied in the degradation of the anthraquinonic dye Remazol Brilliant Blue R (RBBR). The immobilized laccase was superior to the free laccase in both thermal and storage stabilities. Both immobilized and free laccase decolourized 100 mg/L RBBR within 24 h at 30 °C and pH 5.0, but the former was still efficient in degrading the dye after at least 6 cycles. The relationship between the decolorization rate and the RBBR concentration obeyed Michaelis–Menten kinetics, with K_M of 0.126 ± 0.044 mmol/L and V_{max} of 1.412 ± 0.295 µmol/min for free laccase and K_M of 0.159 ± 0.050 mmol/L and V_{max} of 1.214 ± 0.242 µmol/min for immobilized laccase. Fourier transform infrared spectroscopy (FTIR) and mass spectrometry allowed to conclude that the *O. canarii* laccase acts not only on the chromophore group of the dye, but that it also cleaves other covalent bonds, causing an effective fragmentation of the molecule. The Microtox assay detected a significant diminution in toxicity, a finding corroborated by the phytotoxicity test performed with lettuce seeds (Fig. 1). Our results indicate that the immobilized laccase from *O. canarii* could be useful in biological strategies aiming at degrading unwanted dyes in the environment [1]. The full version of this work can be accessed at https://doi.org/10.1080/10242422.2020.1835873.

Figure 1. Evaluation of toxicity of RBBR before and after treatment with laccase from *O. canarii*. In A: Changes in percent luminescence inhibition of *Vibrio fischeri* caused by RBBR treated for 0, and 24 h with free and immobilized laccase from *O. canarii*. In B: Evaluation of phytotoxicity of RBBR before and after treatment with free and immobilized laccase using lettuce seeds (*Lactuca sativa*) seeds.



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Farnesol affects laccase production in white-rote fungi

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Laccases (EC 1.10.3.2) are multicopper oxidases capable of oxidizing a broad range of substrates with the concomitant reduction of O_2 to water. So far, its great potential for biotechnological applications has been hampered by its insufficient production [1]. Among the strategies for improving production, the addition of farnesol – a quorum-sense molecule – has proved to be a powerful choice for inducing laccase production in submerged cultures [2]. Its use in solid-state cultures, however, was not evaluated before. Thus, this work aimed at evaluating the ability of farnesol in inducing laccase production by several white-rote fungi (*Trametes versicolor*, *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Oudemansiella canarii,* and *Pycnoporus sp*) on solid-state cultivation (SSC). Media were composed of pineapple crown (5.0 g), glucose (1.0 %), and yeast extract (0.1%), in the presence (5 mM) and absence of farnesol. The initial moisture content was adjusted at 95% with a mineral solution. The SSC was run for 7 days (10 days for *O. canarii*) at 28±2 ºC. The laccase activity was measured with ABTS (2,2′-azinodi-[3-ethyl-benzo-thiazolin-sulfonate]) as substrate. One activity unit of laccase (U) is the amount of enzyme that catalyzes the transformation of 1 µmol of substrate per minute. Fairly distinct results were found (**Figure 1**). A significant increase in laccase production was observed in both *T. versicolor* and *Pycnoporus sp* farnesol-induced cultures, which produced 1.88 and 1.52-fold more laccase, respectively, compared to the control without farnesol. Differently, while no significant changes were observed for *P. ostreatus* laccase yield, the presence of 5 mM of farnesol in the medium caused a suppression effect on laccase production in the *O. canarii* and *P. pulmonarius* cultures. These data suggest that farnesol influences laccase secretion in a strain-dependent way: while some isolates are not affected by this molecule, increased or decreased laccase yields are observed in others. Thus, the use of farnesol represents a key aspect in the greater laccase biosynthetic *T. versicolor* and *Pycnoporus sp* cultures. Furthermore, it brings important additional data in the search for novel and efficient approaches aiming at improving laccase production.

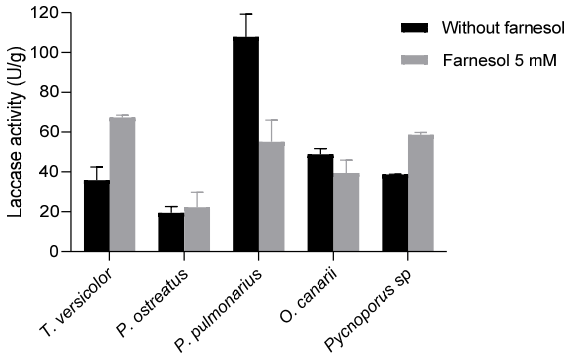

Figure 1: Effects of farnesol addition (5 mM) on laccase production by various white-rot fungi.

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IDENTIFICATION OF PROTEINS FROM *Hebaspirillum seropedicae* THAT INTERACT WITH GlnB PROTEIN

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The nitrogen metabolism in bacteria is controlled by a set of proteins that form a system called Ntr. This system is capable of sensing the quality and quantity of environmental nitrogen and modulating bacterial metabolism to adjust to these conditions [1]. Proteins of the PII family play a central role in the Ntr system: they are signal transducers, capable of regulating many target proteins through physical protein-protein interaction [2]. PII proteins are sensitive to ATP/ADP and 2OG/glutamine ratios, being able to integrate molecular information about nitrogen, carbon, and energy to regulate cell metabolism [3,4]. Although they were initially described as regulators of nitrogen metabolism, recent evidence indicates that they play a much broader role than what has been predicted in the literature, and can act as a central metabolic regulator, coordinating the interface between the pathways of nitrogen metabolism nitrogen, and carbon [5,6,7]. Thus, we want to better understand the regulatory function by determining the network interactions of the PII GlnB protein from the bacterium *Herbaspirillum seropedicae*. In the present work, the objective was to determine the interactome of the *H. seropedicae* GlnB protein bound to the allosteric effectors ATP and 2-OG. For this purpose, an extract of soluble proteins from *H. seropedicae* was submitted to an affinity chromatography in a column containing immobilized GlnB. The mobile phase of the chromatography included, at the injection, the effectors ATP and 2-OG, followed by elution with ADP. The eluted proteins were analyzed by SDS-PAGE to confirm the elution. After confirmation, the fractions containing proteins of interest were precipitated with acetone and subjected to protein digestion by trypsin. The generated peptides were injected into the nano ACQUITY mass spectrometer (Waters) and the result analyzed in the Protein Lynx Global Server v.25.2 program (PLGS). In this analysis, about 200 proteins from the organism
H. seropedicae were identified, including important enzymes in the metabolism of nitrogen, carbon and in the synthesis of amino acids. The regulatory role of PII proteins in these metabolic pathways will be described in the continuation of the work.

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IN TRANS REGULATION OF THE GLND PROTEIN FUTILE CICLE OF *Herbaspirillum seropedicae* BY THE ACT DOMAIN

Lorena Gomes Polizelli*[1], Ana Paula Alves dos Santos Silva[1], Amanda Ruoso Lazzari Almeida[1], Bárbara Barizão Nogueira[1], Eduardo Sabatine Lopes[1], Josielle Abrahão de Souza[1], Larissa Fonseca Tomazini[1], Marco Aurélio Schuler de Oliveira[1]

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Herbaspirillum seropedicae is a nitrogen-fixing bacterium nitrogen that associate with economically important plants stimulating their growth [1]. Nitrogen metabolism in many gram-negative bacteria is controlled by the general nitrogen regulatory (NTR) system, in which PII proteins play a key role in controlling the activity of target proteins through protein-protein interaction [2]. The bifunctional enzyme GlnD is responsible for controlling the post-translational modification state of PII family proteins, catalyzing both uridylation (UTase) under nitrogen-limiting conditions, and deuridylation (UR) under nitrogen-excess conditions, of PII. Among the four domains of the GlnD protein are two C-terminal ACT domains, presumably involved in sensing environmental signals [3]. The purpose of this study was to characterize *in vitro* the regulation of uridylation activity of *H. seropedicae* GlnD protein by truncated versions of the regulatory GlnD domains. Through alignment analysis of the GlnD amino acid residues of *H. seropedicae*, three versions of truncated ACT domains for expression of the ACT domains of this protein were defined, denominated ACT1, ACT2 ACT3 and constructed through PCR reactions. The GlnDΔACT protein, lacking the ACT regulatory domains, catalyzes the futile cycle of PII uridylylation and deuridylation [4]. To attempt restoring the proper regulation of GlnDΔACT, we expressed, purified and tested the regulatory capacity of truncated versions of the ACT domains by non-denaturing polyacrylamide gel electrophoresis, which allows separation of the four possible forms of the PII proteins (non uridylylated, PII (UMP), PII $(UMP)_2$ and PII $(UMP)_3$) (5). Here, the ability of the truncated versions of the ACT domains to prevent *in trans* the futile cycle catalyzed by the GlnDΔACT enzyme in the presence of the 2-OG, as it happens in wild GlnD, was tested. The truncated GlnD ACT1 domain was able to control the futile cycle, indicating that we successfully developed a tool to study the regulatory mechanism of enzyme activities of GlnD. In addition, ACT2 and ACT3 also appear to prevent in lesser degree the futile cycle catalyzed by GlnDΔACT.

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IN VITRO CHARACTERIZATION OF THE AspA, NadK, AND ThrA PROTEINS OF *Herbaspirillum seropedicae*

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The PII family of proteins is ubiquitously spread between Bacteria, Archaea, and plants [1]. These proteins have a pivotal role in the so-called Ntr system, responsible for the regulation of nitrogen metabolism in bacteria. In this system, the PII proteins transduce molecular clues through protein-protein interactions, modulating the activity of many enzymatic targets [2]. In order to describe the role of the *Herbaspirillum seropedicae* GlnB protein, a member of the PII family, we previously characterized its interactome, indicating several candidate interaction target-proteins, which are potentially regulated by GlnB. Amongst the identified proteins at the interactome we picked AspA, NadK and ThrA, the focus of the present work. AspA has an aspartate ammonia-lyase activity, forming fumarate and ammonium from aspartate [3]. NadK phosphorylates NAD^+, consuming ATP, to form $NADP^+$ [4]. ThrA, also known as HSD, is an NADP- dependent dehydrogenase that catalyzes the oxidation of L-homoserine to form L-aspartate 4-semialdehyde [5]. *H. seropedicae* is a nitrogen-fixing bacteria that live in association with Poaceae plants, having the potential to transfer nitrogen to them, and can then be used as a biofertilizer to promote plant growth [6]. These facts make *H. seropedicae* a good model to study the regulation of nitrogen metabolism. The present work aims to in vitro characterize the activity of the AspA, NadK, and ThrA proteins from *H. seropedicae*, and the ability of GlnB to modulate their activities. For the amplification of each gene, direct primers containing a restriction site for the NdeI enzyme and reverse primers for the BamHI enzyme were designed. The PCR reactions contained the 1X enzyme buffer, dNTP, the primers, DNA polymerase, and the genomic DNA of the bacterium. For the NadK primer set a 918 bp fragment was obtained, for the ThrA set a 1311 bp fragment was obtained, and for the AspA set no amplified fragment was obtained yet. The genes are currently being cloned into the pETM11 expression vector by using T4 DNA ligase. Once the cloning is confirmed, the proteins will be overexpressed and their activity and interaction with PII proteins characterized.

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MORPHO-CYTOPATHOLOGICAL ANALYSIS OF THE MIDGUT OF *Bombyx mori* L. INFECTED WITH BmNPV AND SUBMITTED TO THE ANTIVIRAL DRUG Bm5

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The creation of silkworms for the production of cocoons for the industry is called sericulture. The biggest problems that threaten sericulture in Brazil and the world are diseases caused by various pathogens. Viral diseases are the most serious and represent a serious problem for the world's sericulture sector [1]. *Bombyx mori* nuclear polyhedrosis virus (BmNPV) is a highly infectious pathogen that causes serious disease in the species. Caterpillars become infected after ingesting viral occlusion bodies (OBs) present in contaminated mulberry leaves. After the death of the infected caterpillar, its integument easily disintegrates, releasing large amounts of OBs into the environment, which serves as an inoculum to infect other caterpillars. This process is facilitated by the synergistic interaction between the viral gene products *V-cath* (a viral cathepsin) and *chiA* (a viral chitinase) [2]. As the virus is polyorganotrophic, there are several target tissues, with the midgut being the primordial one, as it is related to the digestion and absorption of nutrients. In this work, the effects of the drug Bm5 (built through modeling and molecular docking to inhibit the viral cathepsin of BmNPV [3]) on the cytopathological morphology of the midgut of *B. mori* caterpillars infected by BmNPV were analyzed. Morphological analysis was performed following the techniques of histology and electron microscopy. The results revealed that more earnest alterations occurred in the tissue of caterpillars infected with BmNPV and not treated with the drug Bm5, indicating that it acted by minimizing the effects of the viral action in the midgut, thus reducing the viral proliferation of the contaminated host.

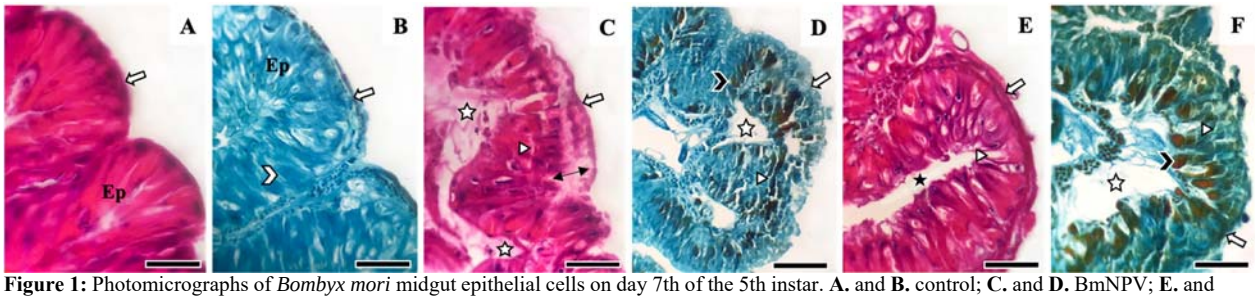

Figure 1: Photomicrographs of *Bombyx mori* midgut epithelial cells on day 7th of the 5th instar. **A.** and **B.** control; **C.** and **D.** BmNPV; **E.** and **F.** BmNPV treated with Bm5. Epithelium (Ep), musculature (white arrow), epithelial cells without infection (white chevron), epithelial cells with infection (black chevron), epithelial disruption (white star), epithelial spacing (black star), basal lamina detachment (arrow double), and cell disruption (isosceles triangle). Color: Hematoxylin and eosin (A, C, and E); Modified Azan for occlusion bodies (B, D, and F). Scale = 50 µm.

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PERSISTENCE OF SARS-COV-2 IN UPPER RESPIRATORY TRACT SAMPLES OF PATIENTS ATTENDED AT THE REGIONAL UNIVERSITY HOSPITAL OF MARINGÁ

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SARS-CoV-2 is a respiratory virus that has an incubation period of 5 to 6 days, with an interval of 1 to 14 days. Therefore, the Center for Disease Control and Prevention (CDC) of the United States of America recommends 14 days of isolation for individuals with severe acute respiratory syndrome (SARS), while in Brazil 10 days of isolation is recommended for individuals with only flu syndromes and 20 days for SARS. However, Li, Wang, and Lv [1] demonstrated the possibility of the virus being present for more than 14 days, and this fact raises concerns and doubts regarding the time when the individual may be eliminating the virus and consequently interfering with the clinical management criteria. Thus, this study aimed to evaluate the persistence of SARS-CoV-2 in the upper respiratory tract of patients with COVID-19 admitted to the Reginal University Hospital of Maringá (HUM). Clinical and epidemiological information were obtained from de medical records of patients contained in the Sistema de Gestão Hospitalar e Ambulatorial do SUS (GSUS). Nasopharyngeal swab samples were collected using a Rayon swab packaged in Viral Transport Medium. The extraction, analysis, and amplification of viral RNA were carried out with: MagMax™ Pathogen RNA/DNA kit, Mastermix SuperScript™ III Platinum One-Step qRT-PCR kit, and Applied Biosystems™ 7500 Real-Time PCR System equipment, respectively. The study was approved by the Ethics Committee for Research involving Human (COPEP) of the State University of Maringá (Protocol nº 4.634.043). In this study, data were obtained from 50 patients (30 in the ward, 20 in the ICU) with the mean age of 59.0 years (SD, ±12.4), and the majority being male (52%). The median time from onset of patient symptoms to the date of sample collection was 13.0 days (IQR, 5.00). The shortest time to detect viral RNA in the samples was 6 days, while the longest time was 29 days. In addition, in this study, it was possible to observe that 70.2% of the samples with more than 7 days of onset of symptoms had a detectable result by qRT-PCR. It was also possible to observe that the viral RNA detection rate in samples with more than 7 days of symptoms was higher in samples obtained from patients admitted to the ICU (78.95%) when compared to patients admitted to the ward (64.3%). Although this result does not deny the possibility of detecting the remnants of viral genetic material, since the technique only detects the presence of nucleic acid, it is important to consider that all samples with a high viral load were obtained from patients with, in the minimum, 11 days from onset of symptoms. Based on these results, we verified that it was possible to detect the viral RNA of SARS-CoV-2 for a time longer than 7 days (recommended time to perform the molecular test) and 14 days (recommended isolation time for symptomatic individuals) of symptoms. Although we cannot confirm the presence of viable infective viruses, these results may serve as a warning for reviewing clinical management criteria and recommending the length of isolation.

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TOLERANCE TO TRICLOSAN OF WHITE-ROT FUNGI: A PRELIMINARY STUDY

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Triclosan (TCS), a widely used antimicrobial and preservative agent, is an emerging contaminant in aqueous and soil environments [1,2]. TCS has been detected in water, agricultural soil, and biological samples such as human breast milk, blood, and fish. Some physico-chemical methods, such as adsorption and oxidation have been applied to remove TCS. Microbial degradation of TCS has not been reported frequently because of its inhibition of microbial growth. However, in recent years, biodegradation of TCS, especially by white-rot fungi has gained significant attention. The objective of this work was to evaluate the tolerance to TCS of 5 species of white-rot fungi: *Pleurotus ostreatus*, Pleurotus *pulmonarius, Picnoporus* sp, *Oudemansiella canarii* and *Trametes versicolor*. The cultures were developed under stationary liquid conditions using wheat bran as substrate in the presence or absence of 40 µg/mL TSC dissolved in ethanol. Ethanol cultures were developed in parallel to check solvent effects. After 7 days, the cultures were filtered and the fungal biomasses were dried at 45º C. The dry fungal biomasses that were obtained are shown in Figure 1. *P. ostreatus,* and *Picnoporus* sp were less tolerant to TSC than *O. canarii, P. pulmonarius*, and *T. versicolor*. These preliminary results suggest that the last 3 fungi can be more effective in the degradation of TSC.

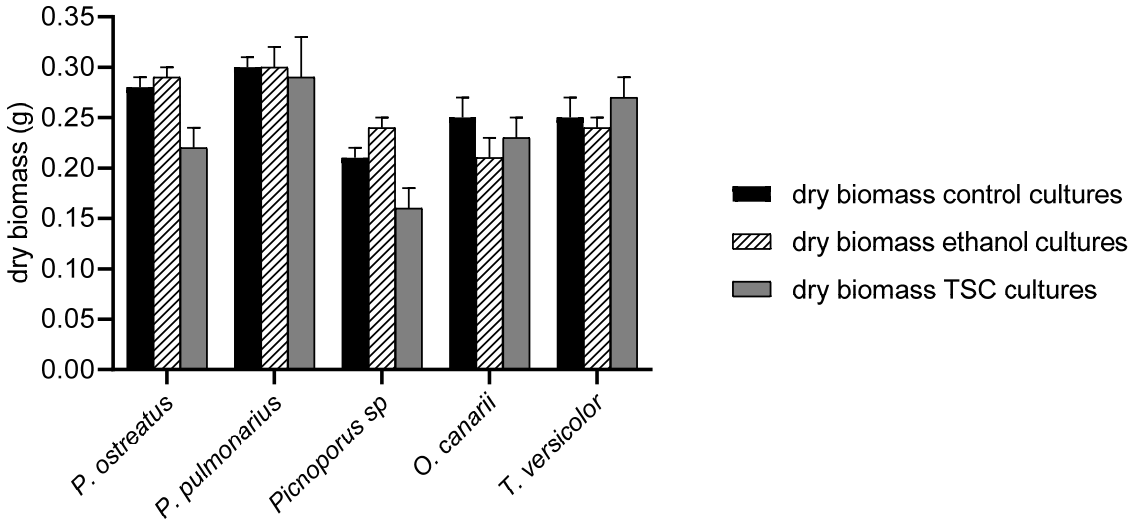

Figure 1. Dry biomass of white-rot fungi obtained in stationary liquid cultures in the absence and presence of TSC

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FOOD BIOCHEMISTRY

EFFECT OF SAPONIN ON ASPARAGINE-GLUCOSE MAILLARD REACTION

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Saponins are molecules commonly found in foods of plant origin (*e.g.* yerba mate and quinoa) or can be added to foods as foaming and emulsifying agents. These molecules are composed by a triterpenoid or non-polar steroid aglycone attached to hydrophilic oligosaccharides [1,2] and are capable of interact with proteins [3]. Therefore, its effect on reactions involving proteins and aminoacids is demanded. In this context, the aim of this work was to study the effect of saponin on asparagine-glucose Maillard reaction at pH 7 and 150 °C. The effect of different saponin concentration and time of reaction on the formation of melanoidins and acrylamide was studied by UV-VIS [4] and HPLC [5], respectively. Results obtained by this work (Figure 1) showed that the melanoidins and acrylamide content of asparagine-glucose model system increase linearly in function of saponin concentration. The effect of saponin is more evident after 20 min of reaction. Thus, the addition of saponin and plant extracts rich is these substances may be evaluated when products submitted to heating are developed mainly due to the acrylamide formation that can be carcinogenic.

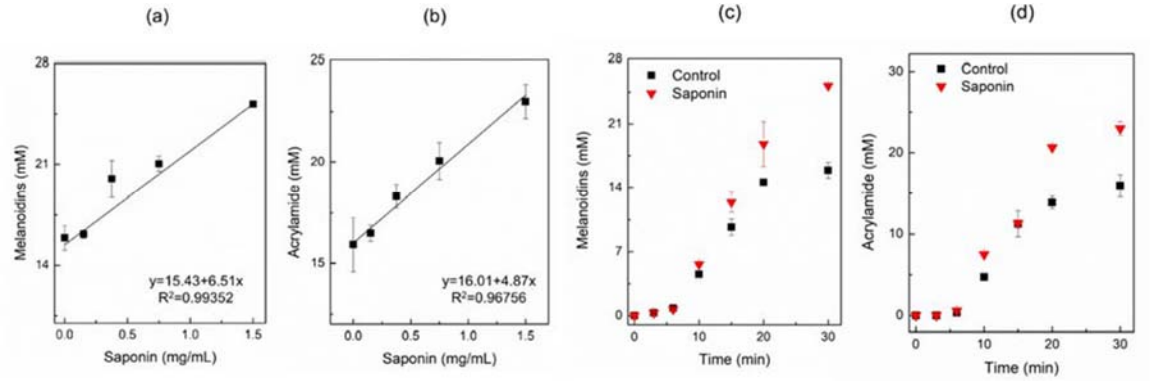

Figure 1: Effect of saponin on the formation of melanoidins (a, c) and acrylamide (b, d) by Maillard reaction (150 °C; pH 7) in function of saponin concentration (a, b) and time of reaction (c,d). The effect of reaction time (c, d) was performed with a saponin concentration of 1.5 mg/mL. The concentration of asparagine and glucose was 0.1 mol/L for all experiments.

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HASKAP AND BLACKTHORN BERRIES ANTHOCYANIN PROFILE.

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Anthocyanin compounds are pigments that have a red, blue, and/or violet coloration widely found in nature, mainly in fruits [1]. Given the current need to replace synthetic colorants with healthier natural alternatives, this study investigated the potential of haskap (*Prunus spinosa* L.) and blackthorn (*Lonicera careulea* L.) fruits as sources of anthocyanins. For this purpose, the characterization of these fruits (the hydroethanolic extract of blachthorn epicarp and the haskap juice) was carried out by high performance liquid chromatography coupled to a diode array detector and a mass spectrometer (HPLC-DAD/ESI-MS). The identification was carried out using standards, when available, comparing their retention times, UV-Vis spectra, and mass spectra. In the absence of standards, the identification was carried out by the fragmentation profile and by comparison with the information available in the literature. The quantification was performed from the peak areas recorded at a wavelength of 520 nm, compared to the standard calibration curves.
The blackthorn epicarp showed two anthocyanin compounds, being the molecules present in the highest concentrations cyanidin-3-*O*-rutinoside ($[H]^+$ at *m/z* 595) and peonidin-3-*O*-rutinoside ($[H]^+$ at *m/z* 609). In haskap berries, six anthocyanins were identified, namely cyanidin-*O*-hexoside-*O*-hexoside ($[H]^+$ at *m/z* 611), cyanidin-*O*-rhamnoside-*O*-hexoside ($[H]^+$ at *m/z* 595), pelargonidin-3-*O*-glucoside ($[H]^+$ at *m/z* 433), peonidin-3-*O*-glucoside ($[H]^+$ at *m/z* 463), peonidin-*O*-rhamnoside-*O*-hexoside ($[H]^+$ at *m/z* 609), and cyanidin-3-*O*-glucoside ($[H]^+$ at *m/z* 449), with the latest as the most abundant one. These results demonstrate that the blackthorn fruit epicarp and haskap fruits can be considered great sources of pigments in the red-purple colour range, having applicability both in the food and pharmaceutical industries.

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NANOENCAPSULATION OF SUMAC EXTRACT: THE EFFECT ON ANTIOXIDANT CAPACITY AND ALFA-AMYLASE INHIBITION

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Sumac (*Rhus coriaria* L.) is a spice widely consumed by the Arab population [1] and its extract present different biological properties (antioxidant, antifungal, cytotoxic and hypoglycemic) [2]. One of the strategies used to maintain the stability and bioactivity of natural extracts is the encapsulation [3]. Thus, the objective of this work was to compare the effect of free (ES) and nanoencapsulated (ESE) *Rhus coriaria* L. extract in relation to alpha-amylase activity (Porcine pancreatic alpha-amylase, Type VI-B, 74 $U.mL^{-1}$, Sigma-Aldrich [4]) and antioxidant capacity (DPPH [5]). Before the extraction step, sumac was deffated (maceration with n-hexane for 24h, 10%wt/v) and after solvent evaporation it was submitted to extraction (10%wt/v) with an ethanolic solution (80:20, v:v) in an Ultra-Turrax (IKA T25, 15 min at 15,000 rpm). The solids were separated by vacuum filtering and the solvent was evaporated in a convection oven. The encapsulation was performed *in situ* (during extraction) by solid dispersion (PVP 0.57%wt/v as encapsulating material and Tween 80 0.057%wt/v as surfactant) [6]. Results showed that (**Table 1**) there was a significant difference ($p<0.05$) between the IC_{50} values obtained for ES and ESE for both, antioxidant capacity and alpha-amylase inhibition. The ES sample presented IC_{50} for DPPH 2.2-fold lower when compared to ESE for both analyses. Such difference may be a result of the proportion of extract in the ESE sample, since PVP, the encapsulating agent represents 1/3 of its weight, and does not present bioactivity. These results suggest that the application of sumac encapsulated extract may be viable as an antioxidant agent and natural inhibitor of this enzyme, as well as in the future, it can be used in the development of new products with increased stability due to encapsulation.

Table 1: Results in IC_{50} of antioxidant activity and alpha-amylase inhibition.

Sample	ES	ESE
DPPH IC_{50}[1]	$0.396^{a} \pm 0.014$	$0.898^{b} \pm 0.007$
Porcine pancreatic α- amylase IC_{50}[2]	$0.367^{a} \pm 0.015$	$0.805^{b} \pm 0.028$

Sample averages (n = 3) followed by the same letter in the same row do not differ statistically ($p>0.05$) by the t-Student's test.
[1]IC_{50} expressed in $mg.mL^{-1}$, initial concentration. [2]IC_{50} expressed in $mg.mL^{-1}$, final concentration.

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NOVEL ANTIOXIDANT AND FIBRE-RICH FOOD INGREDIENTS FROM QUINCE PEEL

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Quince (*Cydonia oblonga* Mill.) is a sour and astringent fruit usually processed into various food products, such as jam, jelly, and quince pudding, or marmalade. Although the peel is often discarded as a by-product during the processing steps, it has been reported as a valuable source of bioactive phytoconstituents [1-3]. Therefore, this work was carried out to characterize the quince peel composition in phenolic compounds and dietary fiber, and to evaluate its antioxidant activity. The dry peel powder was subjected to extractions by hydroethanolic maceration (HM) and hot water (HW). The obtained extracts were characterized for their phenolic composition by HPLC-DAD-ESI/MS^n [4] and their antioxidant activity was evaluated *in vitro* by their ability to inhibit the oxidative hemolysis and the formation of thiobarbituric acid reactive substances (TBARS) using sheep erythrocytes and porcine brain cells, respectively [4]. The fiber content in the solid residues from the extractions was determined by an enzymatic-gravimetric method [5]. The analysis allowed to identify of 16 phenolic compounds, including caffeoylquinic acids, flavan-3-ols, and flavonol glycosides. Flavan-3-ols accounted for about 57% and 48% of the total phenolic fraction of the HM and HW extracts, respectively. The HM extract showed greater antioxidant activity than the HW extract in both *in vitro* assays, a result that strongly correlated with the higher content of flavan-3-ols. In turn, both extraction residues revealed fiber contents that reached nearly 37 g/100 g. Overall, this study demonstrated that it is possible to obtain antioxidant phenolic extracts and novel fiber-rich ingredients from quince peel, which could be used in food and beverage formulation. Future work is planned to optimize the extraction processes and assess their effectiveness as natural food preservatives and fortifiers.

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NUTRITIONAL AND CHEMICAL ANALYSIS AND BIOACTIVE POTENTIAL OF AROMATIC AND MEDICINAL PLANTS TRADITIONALLY USED AS CONDIMENTS

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Aromatic and medicinal plants are highly appreciated and used worldwide as condiments, tinctures, and preservatives. Due to their nutritional value and chemical composition, related to properties beneficial to health, their inclusion in the human diet has gained increasing expression [1]. Certain mixtures of plants have greater potential when compared to isolated plants, due to synergistic effects, and these properties make them of great interest in the food, pharmaceutical, and cosmetic industries. They have been consumed directly in prepared dishes, but also by incorporation into foods, making them bioactive and functional [2]. In the present study, four mixtures of aromatic plants used to season poultry, meat, fish, and salads were characterized in terms of nutritional value, according to the AOAC procedures, and chemical composition, namely in free sugars (HPLC-RI), organic acids (UFLC-PDA), tocopherols (HPLC-fluorescence), fatty acids (GC-FID), and phenolic compounds (HPLC-DAD-ESI/MS). The antioxidant, antimicrobial, anti-inflammatory, and anti- tumour capacities were also evaluated, validating their bioactive properties. Carbohydrates were the main macronutrients found in mixtures of condiments, followed by proteins and ash. Fructose, glucose, and sucrose were detected in all samples, as well as three organic acids, namely oxalic, citric, and malic acids, being malic acid the most abundant. The four tocopherol isoforms (α, β, γ, and δ) and a total of 23 different fatty acids were detected, with a predominance of polyunsaturated fatty acids (PUFA), with high percentages of linoleic acid and linolenic acid. In terms of phenolic composition, twenty-five compounds were identified, with apigenin-*O*-malonyl-pentoside- hexoside as the most abundant compound in all extracts. Regarding bioactive properties, to antioxidant activity, the extracts of mixtures for meat and salads presented the best results in the TBARS test, while those of mixtures for meat and poultry stood out in the OxHLIA assay. The mixtures for poultry and fish showed the highest anti-inflammatory activity and the mixtures for salads showed the best anti-tumour properties. On the other hand, mixtures for meat and salads revealed the greatest antimicrobial activity. In conclusion, these seasoning mixtures demonstrated valuable bioactive properties, conferred by their chemical composition and cumulative and synergistic effects observed in the mixtures, which corroborates the importance of their inclusion in the human diet.

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OBTAINING ICE CREAM SWEETENED WITH STEVIA PRODUCTS

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The formulation of innovative edible ice cream depends on the quality and differentiation of the ingredients used in order to confer its sensory and functional properties. The Brazilian ice cream market has grown almost 80% in ten years. Brazil is now the fourth largest ice cream market in the world, behind the USA, China and Japan. In this sense, this paper aimed to employ differentiated ingredients, among which rebaudioside A extracted from Stevia leaves pre-treated with ethanol, as a sweetening and functional agent, and fruits like strawberry and guava, rich in anthocyanins and widely used in the formulation of functional food products, due to the health benefits of their compounds. Five formulations were made, using as sweetening agent: sucralose (formulation 1 - F1), sucrose (formulation 2 - F2), rebaudioside A (formulation 3 - F3). In order to standardize the formulation, two new ice creams were made by changing only the amount of concentrated guava juice in the sucralose and sucrose formulations (formulations 4 and 5). The ice creams were analyzed chemically and the results showed that the reb A extracted from pre-treated leaves has great potential to sweeten ice cream formulations when compared to sucrose and sucralose, since the results suggest that there was no loss of sweet taste quality. Thus, rebaudioside A can be an alternative for replacing sugar, which is caloric, and the sweetener sucralose, which, although it is not caloric, is synthetic, which can bring risks to the consumer's health.

OPTIMIZED EXTRACTION OF CHLOROPHYLLS FROM Solanum lycopersicum L. VAR. cerasiforme BY-PRODUCTS

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The increasing worldwide consumption of cherry tomatoes (*Solanum lycopersicum* L. var. *cerasiforme*) is explained by the great acceptability by the consumer, along with the ease in its trade and distribution. Despite being a fruit that provides essential nutrients such as lycopene, vitamin C, and phenolic acids, its consumption is closely related to its sensory characteristics such as taste, color, and appearance. Nevertheless, along the production chain, some by-products are not used and are, therefore, discarded, generating large amounts of bio-residues [1–3]. The use of such bio-residues, namely the aerial parts, as a source of valuable compounds that can find other applications in food industry as, for example, food colorants, is a growing tendency. In this context, the following work aimed to explore the hydroethanolic extracts obtained from the aerial parts of cherry tomato, in terms of chlorophylls. For this purpose, two extraction methodologies were used, namely ultrasound assisted extraction for 15 minutes at 400 W and maceration assisted extraction for 120 minutes, both using 90% ethanol (*v/v*) as solvent. The chlorophyll pigments were identified and quantified by HPLC-DAD/ESI-MS. Chlorophyll *a*, *b*, and their isomers (*a'* e *b'*) were identified in both extracts, as well as direct derivatives of chlorophyll and phaeophytin *a* and *a'*, compounds commonly found in fruits of cherry cultivars. Regarding ultrasound assisted extraction, the most expressive compounds found were chlorophyll *b* and its *b'* isomer. As for maceration assisted extraction, chlorophylls *a* and *b* were the most abundant compounds in the extract. These results demonstrate the great potential of using cherry tomato by-products as sources of natural pigments, presenting a basis for deeper investigations regarding the optimal extraction conditions of chlorophylls and their possible uses within several industrial sectors.

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ORAL GAVAGE OF FREE AND MICROENCAPSULED CAPSICUM OLEORESIN IN MICE FED WITH THE HIGH-FAT DIET

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Capsicum oleoresin (CO) is high in compounds responsible for weight loss through the thermogenicity and has shown promising results for obesity treatment. Nonetheless, its application is limited due to its strong pungency and low bioaccessibility. With the purpose to overcome these drawbacks, Capsicum oleoresin microparticles were obtained by spray drying, and their effect on mice fed with a high-fat diet was investigated. At first, two emulsions containing 95% wall material (gum arabic and modified malt (50:50)) and 5% oil phase were atomized: one using only Capsicum oleoresin as oil phase (CO) and another containing CO plus corn oil (1:1, w/w), named Formulation 1 (F1) and Formulation 2 (F2), respectively. Thirty Swiss mice were divided into five groups (n= 6) where the lean control group received a commercial diet, and the other four groups received a high-fat (HF) diet for four weeks. The four HF groups were divided into HF control, HF + free Capsicum oleoresin (218 µL of CO/kg/day by gavage), HF + F1, and HF + F2 (both receiving 1250 mg microparticles/kg/day by gavage). The effects of the compound administration were assessed through diet consumption, weight gain, glucose tolerance test (GTT), insulin tolerance test (ITT), and oxidative stress parameters. Results showed a slightly higher consumption (4.53 g diet/per day) by the HF control group when compared to the group treated with F1 (3.75 g diet/day, p=0.05). After four weeks, treated groups (F1 and F2) gained the same weight as the lean group, pointing to a better effect of Capsicum oleoresin encapsulated than free oleoresin on weight gain. However, no effects on GTT and ITT were observed in animals treated with oleoresin, F1, and F2 microparticles, indicating no reversion of insulin resistance. In addition, although endogenous antioxidant enzymes were reduced in HF groups compared to the lean group, there were no significant differences among the groups. The protective effects of Capsicum oleoresin on obesity were not observed in the present study, maybe because of the doses or time used in the experimental protocol.

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SOLANACEAE CROP BY-PRODUCTS AS RENEWABLE SOURCES OF BIOACTIVE PHENOLIC EXTRACTS

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The upcycling of agri-food by-products into high added-value products has been promoted in recent years. Solanaceae is one of the main plant families supplying important vegetable and staple food crops worldwide. Bell pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) are two good examples, and their agricultural production generates million tons of valueless crop remains (especially plant aerial parts) [1], whose insertion in the value chain needs to be promoted and investigated to ensure the efficient use and circularity of these natural resources. Moreover, while the fruits of these species are well characterized for their nutritional value [2], the residual biomass of these crops remain unexplored, and little is known about their composition in bioactive constituents. Therefore, this work aimed at characterizing the phenolic compounds of bell pepper and eggplant crop by-products and evaluating the bioactive activities in order to find possible industrial applications. The phenolic profiles of both plant materials were characterized in the hydroethanolic extracts by the HPLC-DAD/ESI-MS^n technique [3]. The extracts were also used to evaluate *in vitro* antioxidant activity, with regard to their ability to inhibit lipid peroxidation and oxidative haemolysis [4], and antimicrobial effects against foodborne microorganisms, by serial microdilution methods [3]. The bell pepper by-product extract showed a qualitative predominance of flavonoids and a better performance in the lipid peroxidation and oxidative haemolysis inhibition assays, as well as greater antifungal activity. In turn, phenolic acids stood out as main compounds in the eggplant by-product extract, with presented higher activity against the tested bacterial strains. Overall, the obtained extracts seemed to be a promising material for application in the food and nutraceutical industries, among other sectors, given their high potential to be used as natural preservative ingredients.

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STABILITY OF PHENOLIC COMPOUNDS OF YERBA MATE AFTER COLONIC FERMENTATION WITH HUMAN INOCULUM

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Phenolic compounds are an extensive group of structures, with one or more hydroxyl groups, frequently associated to health benefits by the current literature. Although they exert pronounced functional activities, it is known that they undergo many structural transformations during the digestion process. Nature and extent of these transformations have not yet been fully characterized. The objective of this work was to verify how the microbiota of humans modifies the yerba mate compounds using a simulation of colonic fermentation. Yerba mate was obtained at the local commerce of Maringá, State of Paraná, Brazil and the extraction was carried out by simulating the preparation of the traditional beverage known as *chimarrão*. The latter consists basically in a hot water extraction. For mimicking the digestion and fermentation we used the methodology described by Correa *et al.* [1], but with human inoculum, obtained from female donors aged 25-30 years, who had not used antibiotics during the past 3 months and who maintained a diet low in phenolics for 3 days prior to the analysis. Phenolic compounds were analyzed in a HPLC system coupled to a diode-array detector and a linear ion trap mass spectrometer equipped with an electrospray ionization source. The results are given in Table 1 as mg per g of extract. Stomach digestion generally diminished the phenolic contents, whereas the opposite generally occurred for the small intestine digestion. Use of the inoculum from humans, on the other hand, resulted in much lower amounts of some compounds to the point that the total phenolics after FHFI comprised 51% of the crude extract. Diminution was more pronounced for trans-3-O-caffeoylquinic acid (peak 3). However, for 5-O-caffeoylquinic acid (peak 4) and 4,5-O- dicaffeoylquinic acid (peak 9), the contents after fermentation with the human inoculum were increased. Although both stomach and small intestine also modify the phenolic compounds, colonic fermentation represents a much more drastic event in terms of metabolic transformation.

Table 1. Quantification of phenolic compounds (mg/g) of yerba mate after stomach and small intestine digestion and after colonic fermentation with human inoculum (mean ± SD).

Peak	Crude extract	Stomach digestion	Small intestine digestion		Colonic fermentation
1	*cis* 3-*O*-Caffeoylquinic acid	27.74±0.25^{a}	17.08±0.32^{b}	48.33±1.39^{c}	10.56±0.25^{e}
2	4-*O*-Caffeoylquinic acid	17.40±0.03^{a}	16.94±0.56^{a}	19.75±0.63^{b}	4.85±0.15^{d}
3	*trans* 3-*O*-Caffeoylquinic acid	35.30±0.70^{a}	30.12±1.11^{b}	24.78±0.41^{c}	4.80±0.27^{d}
4	5-*O*-Caffeoylquinic acid	22.93±0.30^{a}	29.19±0.78^{b}	25.39±0.12^{c}	31.04±0.55^{d}
5	Quercetin-3-*O*-rutinoside	2.81±0.06^{a}	2.26±0.06^{b}	3.05±0.04^{c}	5.80±0.09^{d}
6	3,4-*O*-diCaffeoylquinic acid	9.74±0.03^{a}	3.67±0.19^{b}	12.84±0.17^{c}	Nd
7	*cis* 3,5-*O*-diCaffeoylquinic acid	3.58±0.02^{a}	0.69±0.02^{b}	1.83±0.01^{c}	Nd
8	*trans* 3,5-*O*-diCaffeoylquinic acid	7.49±0.02^{a}	0.43±0.04^{b}	0.70±0.01^{c}	Nd
9	4,5-*O*-diCaffeoylquinic acid	6.70±0.06^{a}	2.47±0.21^{b}	10.48±0.02^{c}	10.63±0.57 ^{c}

Data are means ± SD. Different letters denote $p<0.05$ among stages.

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STUDY OF *PRUNUS SPINOSA* L. FRUIT EPICARP AND *LONICERA CAREULEA* L. FRUIT: ALTERNATIVE NATURAL COLORANTS WITH BIOACTIVE PROPERTIES

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Natural matrices that are rich in anthocyanin compounds are increasingly explored by the food industry due to their colouring properties. As examples, *Prunus spinosa* L. and *Lonicera careulea* L. fruits are excellent sources of anthocyanins and are, therefore, increasingly explored for their colouring properties to be applied as food colorants, in addition to providing beneficial properties to the consumer [1,2]. Therefore, the aim of this study was to evaluate the anthocyanin profile (HPLC-DAD/ESI-MS) and the antioxidant (TBARS and OxHLIA) and antimicrobial properties of the hydroethanolic extract of *P. spinosa* fruit epicarp and *L. careulea* fruit juice.

A high content of cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside was found in the juice of *L. caerulea* berries and in the hydroethanolic extract of the epicarp of *P. spinosa*, respectively. As for the antioxidant activity, in the TBARS assay, the *L. caerulea* berries showed a higher capacity (IC_{50} of 29.9±0.3 μg/mL) than the positive control, trolox (IC_{50} of 139±5 μg/mL). Similarly, the epicarp of *P. spinosa* showed the ability to inhibit lipid peroxidation, revealing an EC_{50} value of 204±2 μg/mL. Regarding the ability to retard oxidative haemolysis, both extracts showed activity not only at 60 min, but also at 120 min, allowing EC_{50} values of 145±5 μg/mL and 938±49 μg/mL, respectively, for *L. caerulea*, and 296±4 and 509±3 μg/mL, respectively, for *P. spinosa*. On the other hand, both colouring extracts revealed great antimicrobial properties. Through this work, it was possible to conclude that *L. caerulea* and *P. spinosa* berries have a high colouring capacity and bioactive potential, being suitable for the development of new products for food industry.

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WHEY PROTEIN OBTAINED BY MEMBRANE SEPARATION PROCESSES FORTIFIED WITH MICROENCAPSULATED ANTIOXIDANT FRACTION FROM Stevia rebaudiana

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The aim of this study was to develop a whey protein concentrate obtained by membrane separation processes and fortify it with a microencapsulated fraction of *Stevia rebaudiana*, rich in antioxidant compounds, and thus evaluate its antioxidant and antidiabetic potential in vitro. A fraction of the elite variety Stevia UEM-13 was obtained by fractionating the methanolic extract of its leaves, using ethyl acetate as solvent. This fraction already has proven antioxidant and anti-diabetic properties, and it has the potential to enhance the health benefits of whey protein, mainly immunological and in the treatment and prevention of metabolic syndromes such as diabetes mellitus. The microencapsulation of the ethyl acetate fraction was performed in order to preserve the antioxidant compounds for a longer time and also to increase their solubility. The vehicle used was maltodextrin and the microencapsulation ratio was 1:4 (w/w). The Stevia UEM-13 leaves, the fraction in ethyl acetate, the microencapsulated fraction, the membrane concentrate, and a commercial concentrate were submitted to physicochemical analyses for their characterization (proteins, lipids, ash, moisture, fiber, carbohydrates, glycosides, lactose, phenolic compounds, flavonoids, and antioxidant activity; also, solubility, microencapsulation efficiency, and stability). In addition, the fractions, concentrates, and also two formulations of microencapsulated fraction added to the membrane concentrate were tested for their potential to inhibit the enzyme α-amylase (in vitro antidiabetic activity). The microencapsulated fraction and the formulation containing 20g/100g of membrane concentrate showed inhibitory potential for the enzyme, not significantly differing from the official inhibitor (acarbose). Thus, this work developed a *Stevia rebaudiana* fraction, microencapsulated it, obtained a protein concentrate by the membrane separation process, characterized them, and also showed that the microencapsulated fraction increases the solubility and keeps the compounds of interest more stable for twice the time. Still, it showed that this fraction presents great potential to fortify the antioxidant and antidiabetic activity of products such as Whey protein.

PLANT BIOCHEMISTRY

CHARACTERIZATION OF RESIDUES FOR THE PRODUCTION OF GIBBERELLIC ACID BY SOLID-STATE FERMENTATION

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Gibberellic acid (GA3) is a phytohormone and is involved in several aspects of plant development such as induction of germination and sprouting of yolks, development of stems and flowers, interruption of seed latency status. GA3 production can be performed by fermentation in a solid medium, with aeration through the growth of *Gibberella fujikuroi* ascomycete fungi. After the growth of the inoculum, in BDA medium (Potato–Dextrose–Agar), one of the forms of GA3 production is solid-state fermentation (FES) with different nutritive media obtained from agroindustrial residues. These residues present promising results not only as of the extraction of an economically viable product but also becomes a way to maintain the conservation of the environment since it decreases the deposition of these materials in the soil [1]. The residues characterized in this first stage of the work were brewery residue (BDM), corn residue (RM), sugarcane (BCA), and cassava (RBM). This first phase includes the characterization of all residues and the subsequent selection of only two to continue the next stage of the research (phase 2), which will be the inoculation, production, extraction, purification, and quantification of GA3. All residues were quantified according to the percentage of proteins, organic carbon, nitrogen, pH, moisture, and total, volatile, and fixed solids. The residues that presented the best conditions for GA3 production were BDM, corresponding to moisture content (77.55%), pH 6.33, proteins (13.87%), organic carbon (39.06%), and nitrogen (2.22%), which was the most discrepant result, since the C: N ratio would normally be 6:1 to obtain the secondary metabolite [1, 2]. For cassava bagasse residue (RBM), nitrogen content was (0.09%), organic carbon (30.87%), pH 5.31, and proteins (0.59%), both demonstrated favorable conditions for GA3 production, being selected for the follow-up of phase 2 of the research.

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CLONING, EXPRESSION AND PURIFICATION OF PIRUVATE-ORTOPHOSPHATE DIKINASE (PPDK) FROM ZEA MAYS

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Pyruvate-orthophosphate dikinase (PPDK) is an enzyme which plays an important role in plants with C4 photosynthetic metabolism, being an interesting target to the development of new herbicides [1]. In this work, molecular biology techniques were used to express and purify PPDK protein (chloroplastic) from *Zea mays* to assess the inhibitory potential of some compounds *in vitro*. First of all, the RNA from maize leaf (10 days after germination (DAG)) was extracted and then used the reverse transcription reaction to synthesize the cDNA. Then, this cDNA was used to perform the polymerase chain reactions (PCRs) to amplify the gene of interest. Since the gene which encodes PPDK protein is too large (2650 pb), we amplified separated the 5' region and the 3' region of the gene. After that, we overlap these two sequences, to get the final gene amplified (Figure 1A). Thereby, the recombinant plasmid (pETM11) containing the gene that corresponds to the *Z. mays* chloroplastic protein PPDK was successfully synthesized. Protein expression was performed using the pET system, with 1 mM IPTG, 25 degrees, during 20 hours. The protein was purified by nickel affinity chromatography with a high purity. It had an apparent molecular weight of 97 kDa, corresponding to the theoretical molecular weight (Figure 1B). We intend to carry out the activity and inhibition experiments to complement this work.

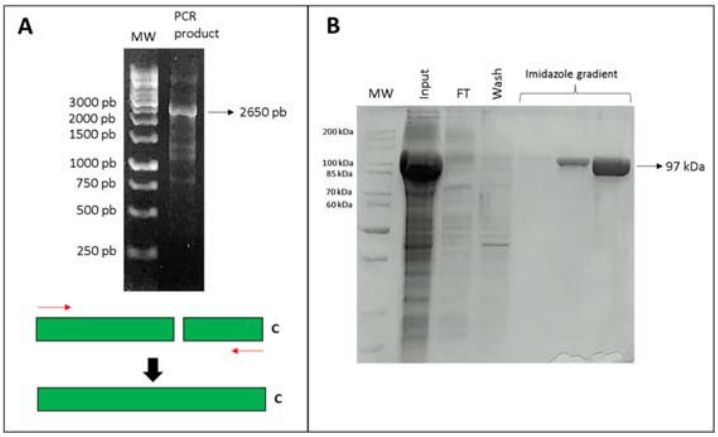

Figure 1: **Cloning and expression of PPDK**. **A)** Gene product of overlap PCR. MW: molecular weight; PCR Product: arrow indicationg 2650 pb; at the bottom: scheme of overlap PCR. **B)** SDS-PAGE of affinity chromatography of PPDK. MW: molecular weight; Input: supernatant from lysis; FT: flow through; Wash: buffer wash; Imidazole gradient: buffer wash with imidazole 40-250 mM; arrow: PPDK with 97 kDa.

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EFFECT OF FERTILIZATION VIA NUTRIENT SOLUTION ON THE NUTRITIONAL PROFILE AND CHEMICAL COMPOSITION OF *CHICORIUM SPINOSUM* L.

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The availability of healthy and functional food is a worldwide concern to meet the increasing demands of consumers. Currently, there is a new trend in relation to the consumption of wild edible plants, with high nutritional and functional properties, which until now were little explored. *Chicorium spinosum* L. is a wild edible plant that occurs in different Mediterranean climates, which has already been described and correlated with the prevention of chronic diseases and disorders [1–3]. Thus, the aim of the present study was to evaluate the effect of fertilization with nutrient solutions that contained different ratios of nitrogen, phosphorus, and potassium on the nutritional profile (AOAC methods) and chemical composition (organic acids obtained by UFLC-PDA, free sugars identified by HPLC-RI, and fatty acids determined by GC-FID) of *C. spinosum* leaves. The samples fertilized with 300:100:100 ppm of N:P:K (C311) stood out for its high crude protein and total dietary fiber content, followed by the sample fertilized with 200:200:200 ppm of N:P:K that also showed promising fat values and carbohydrates. The control sample (without fertilization) showed the lowest values in all the studied parameters, except for the protein content in which there were no significant differences compared to the C311 sample. In terms of organic acids, quinic acid was present in the highest amounts, while malic and oxalic acids presented low content. The most abundant individual sugar was glucose, followed by sucrose and fructose. Regarding fatty acids, the presence of unsaturated fatty acids was highlighted, mainly linoleic acid. Overall, the sample treated with 300:200:200 ppm of N:P:K (C322) was the one presenting the highest amounts of sugars and fatty acids, while C311 presented the highest amounts of organic acids. The results demonstrated that it is possible by selecting the proper nutrient solution to improves the nutritional profile and functional compounds, thus enabling the cultivation of *C. spinosum* in controlled cropping systems, increasing yield and product availability, and making it available to a larger number of consumers.

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ISOCITRATE LYASE AS A MOLECULAR TARGET FOR WEED SUPPRESSION

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Weeds cause serious damage to crops yields, and the repeated use of herbicides over the past few decades has favored the emergence of several resistant weed biotypes. There is currently a growing demand for the discovery of new natural compounds that can act through alternative herbicidal mechanism of actions, without causing environmental impact [1]. The enzyme Isocitrate lyase (ICL) plays a central role during the germination of oil seeds, enabling the conversion of lipid reserves into glucose through the glyoxylate cycle [2]. In addition to its importance during the establishment of seedlings, this enzyme has some desirable characteristics to act as an herbicide molecular target, such as not being expressed in mammals. In the present study, computational biochemistry techniques were used to model the structure of the *Arabidopsis thaliana* ICL enzyme (Figure 1A) in order to perform a virtual screening of ligands. Using the molecular docking technique, the itaconic and tartaric acids were found as potential ICL ligands (Figure 1C, D). Their effects on the initial growth and on the ICL activity were examined in *Sesamum indicum* and *Euphorbia heterophylla*, two species dependent on lipid reserves. Seeds were sown in gerbox plates (50 seeds per plate) using 0.8% agar as the culture medium, and the tested compounds were dissolved at the concentrations of 50, 100, 500 and 1000 µM, using the Tukey's test to determine statistical significance. ICL activity was evaluated in seedlings cotyledons extracts according to [3]. The results revealed that the structural model of ICL presented the required quality, with a rmsd average of 0.571 Å (Figure 1B). *S. indicum* and *E. heterophylla* grown in the presence of itaconic acid presented a significant inhibition in the length of their roots, reaching a reduction of 68.7% and 27.5%, respectively, at the concentration of 1000 µM (Figure 1E). When the ICL activity of the cotyledon's extracts was measured in the presence of itaconic and tartaric acid, a dose-dependent inhibition by itaconate was observed in both tested species, without influence of tartaric acid (Figure 1F). Previous studies had already described itaconic acid as an ICL inhibitor [3], and the remarkable ability of this compound to inhibit the early development of oilseed species may be a strong indication of the importance exerted by ICL throughout the glyoxylate cycle, suggesting a potential herbicide target.

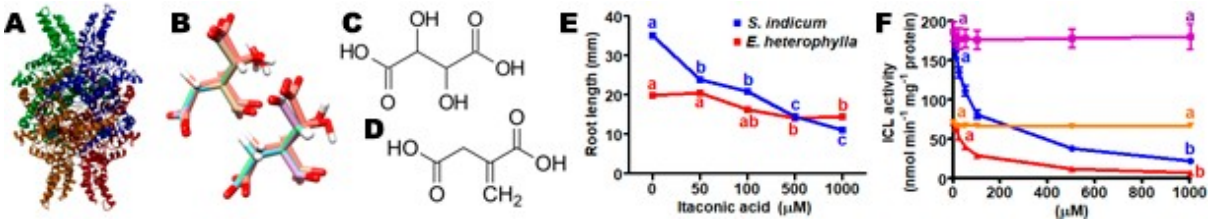

Figure 1: ICL model (A), superposition of the substrate found on redocking (Vina and Molegro protocols) (B), tartaric acid (C), itaconic acid (D), effects of itaconic acid on the root lengths (E) and effects of tartaric and itaconic acid on ICL activity of *S. indicum* and *E. heterophylla* (F).

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LEAF APPLICATION OF LIGNIFICATION INDUCER INCREASES LIGNIN CONTENT IN SOYBEAN GRAIN TEGUMENT

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Soy is a crop of significant importance to the world economic scenario [1]. Damage to grains, in the stages of production, harvesting, drying, processing, storage, transport and marketing, can compromise final productivity. Mechanical damage is one of the agents that most influence grain processing, as soybean is very sensitive to impacts due to its thin seed coat and low lignin content. Lignin is a polyphenolic polymer that guarantees plant rigidity and resistance against pathogen attacks, in addition to promoting greater mechanical resistance to the grain [3]. The absorption of phenylpropanoid allelochemicals is still being studied, however, it is known that the exogenous application of lignification inducers increases the lignin content in soybean [4], as they are absorbed and channeled to the phenylpropanoid pathway [5]. Therefore, the objective of this work was to evaluate the lignin content in the seed coat after foliar application of lignification inducer in field- grown soybean plants. The experiment was carried out, in the 2018/19 season, at the Cocamar Technological Diffusion Unit, located in Floresta / PR, and the experimental design used was in five replications in completely randomized blocks. The sowing was carried out with the cultivar BMX Garra I PRO, with spacing between rows of 0.45 m. The lignification inducer (Intellectual Property) was applied by foliar spray with spray volume equivalent to 200 L/ha. The experiment presented control plants, without the application, and plants treated with the lignification inducer, in a single dose, at a concentration of 1 mM. After harvesting (step R8), the grains of the plants in each plot were separated and, later, immersed in water for 12 hours to remove the integuments. After drying (60 °C), the biomass was crushed and standardized with $0.149 \geq 0.053$ mm. The lignin content was determined according to the adapted method Acetyl Bromide [4] and the values were expressed in mg.g-1 of PCIP (Protein Free Cell Wall) according to the standard curve. The results were submitted to the Student t test, with a 95% confidence level, using the Graph Pad Prism ® program. The seed coat of plants treated with the lignification inducer showed a significant increase of 13.54% of lignin, when compared with the seed coat of the control plants. Thus, the lignification inducer has great potential for the development of new agrochemicals in order to increase grain resistance and, consequently, contribute to increased productivity. Furthermore, the method presented is an alternative to the use of genetically modified organisms.

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NUTRITIONAL AND BIOACTIVE CHARACTERIZATION OF *Apium graveolens* L.

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Plants are an important source of active natural products that are divided into several classes based on their chemical characteristics. Among the existing vegetables, celery (*Apium graveolens*) is a medicinal native to Europe with a wide range of use and cultivation. In this context, this work aimed to perform a nutritional and bioactive characterization of the different properties of this matrix, envisioning the development of a possible preservative for the food industry. The samples obtained from a local commercial establishment were freeze-dried and then extracted using a maceration process with ethanol: water (80:20) on a shaker plate for two hours, filtered, and then the evaporated ethanol was taken for freeze-drying to obtain an extract for bioactivities. Afterward, a nutritional characterization of celery was carried out: crude fat (Soxhlet extractor), protein (method Macro-Kjeldahl), organic acids (High-performance liquid chromatography coupled with a photodiode detector (HPLC-DAD), ash (AOAC methodology 923.03), moisture (PMB 163 Moisture Analyzer, Adam Equipment, Oxford (AOAC methodology 925.09)), sugars (HPLC coupled with a refractive index detector (HPLC-RI), fatty acids (Gas chromatography coupled to a flame ionization detector (CG-FID). Furthermore, the antimicrobial potential was also accessed through the microdilution method and the antioxidant capacity by the Thiobarbituric acid reactive substances (TBARS) assay and the cellular antioxidant method (CAA). According to the results of the nutritional analysis, celery consisted mainly of 95% water, followed by ash content (16.40 ± 0.04 g/100 g dw), protein (10.6 ± 0.4 g/100 g dw), and low-fat contents (1.50 ± 0.01 g/100 g dw), leading to an energy value of 341.8 ± 0.2 kcal. Of the sugars quantified glucose obtained the highest content (42 ± 1g/100 g dw). Among the 19 fatty acids identified, linoleic obtained the highest results, followed by total saturated fatty acids (SFA) of 34 ± 0.10 %, polyunsaturated fatty acids (PUFA) 61 ± 0.007%, and monounsaturated fatty acids (MUFA) 3.55 ± 0.01% present in celery. Regarding organic acids, malic (6.8 ± 0.3 g/100 g dw) and oxalic (5.15 ± 0.09 g/100 g dw) acids were the main compounds. For antifungal activity, both *Aspergillus brasiliensis* and *Aspergillus fumigatus* were inhibited with minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MBC) values of 10/>10 mg/mL, respectively. Regarding bacterial strains, the extract that caused the highest inhibition for gram-negative bacteria was Salmonella Typhimurium (2.5 mg/mL), while for gram-positive bacteria *Staphylococcus aureus* (5 mg/mL) was the most sensitive strain. As for the antioxidant potential, an EC50 value of 2315 ± 143 µg/mL was determined for the TBARs assay and no activity was seen in the CAA at the maximum tested concentration of 2000 µM. It can be concluded that celery is a good source of nutrients; thus, exploring this food in terms of nutritional benefits in addition to its good bioactive properties is a valuable research topic, especially in the development of an extract with a strong antibacterial activity that may have application in different industries, especially in food, where the demand for natural preservatives is huge.

Acknowledgments

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PHENOLIC COMPOSITION OF *Ruscus aculeatus* L.

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Ruscus aculeatus L. is a subshrub used in traditional medicine in different parts of the world, namely in Europe and the Iberian Peninsula [1]. According to reported folk knowledge, the aerial parts are mainly used as diuretics and the underground parts are used for the treatment of disorders of the urinary system and as laxatives [2]. The present work reports a comprehensive study on *R. aculeatus* phenolic compounds, intending to contribute to the knowledge of the chemical composition of this unexplored species. The plant material of *R. aculeatus* was harvested in April 2019 inside woodlands and hedgerows, in Valpaços, Portugal. Two distinct parts were gathered, the aerial part (cladodes or laminar stems and lateral branches) and the underground organs (rhizomes with roots). After lyophilization, the distinct parts of the plant were further analyzed. Hydroethanolic extracts and aqueous (infusions and decoctions) preparations from the two mentioned parts of the plant were prepared. Phenolic compounds were determined by high-performance liquid chromatography (HPLC) coupled to a diode array detector (DAD) and mass spectrometry (MS) using the electrospray ionization interface (ESI). Nine phenolic compounds were detected in all extracts, one caffeic acid derivative and eight flavonoids (six C-glycosylated derivatives of apigenin and two O-glycosylated derivatives of quercetin and kaempferol). Apigenin-C-hexoside-C-pentoside isomer II was the major compound in aqueous extracts and, in the hydroethanolic extract, the most abundant one was quercetin-O-deoxyhexoside-hexoside, followed by apigenin-C-hexoside-C-pentoside isomer II. The hydroalcoholic extracts of the aerial part (Figure 1) presented the highest levels of phenolic compounds (107±3 mg/g extract), followed by aqueous extracts, the decoction (18±1 mg/g extract), and the infusion (14.6±0.3 mg/g extract). These innovative results are not enough to relate the empirical uses with the chemical properties demonstrated by the extracts obtained from both the aerial and the underground parts. It will be essential to continue exploring the compounds and the mentioned activities so that it is possible to corroborate and substantiate the use of this species in traditional medicine.

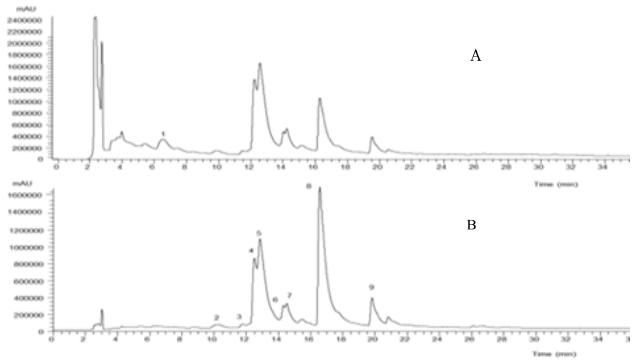

Figure 1: Phenolic profile of hydroethanolic extract of the aerial part, recorded at 280 nm (A) and 370 nm (B). 1- Caffeic acid hexoside, 2- Apigenin-C-hexoside-C- pentoside isomer I, 3- Apigenin-C-pentoside-C-hexoside isomer I, 4- Apigenin-C-pentoside-C-hexoside isomer II, 5- Apigenin-C-hexoside-C-pentoside isomer II, 6- Apigenin-C-pentoside-C-hexoside isomer III, 7- Apigenin-C-hexoside-C-pentoside isomer III, 8- Quercetin-O-deoxyhexoside-hexoside, 9- Kaempherol-O- deoxyhexoside-hexoside.

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