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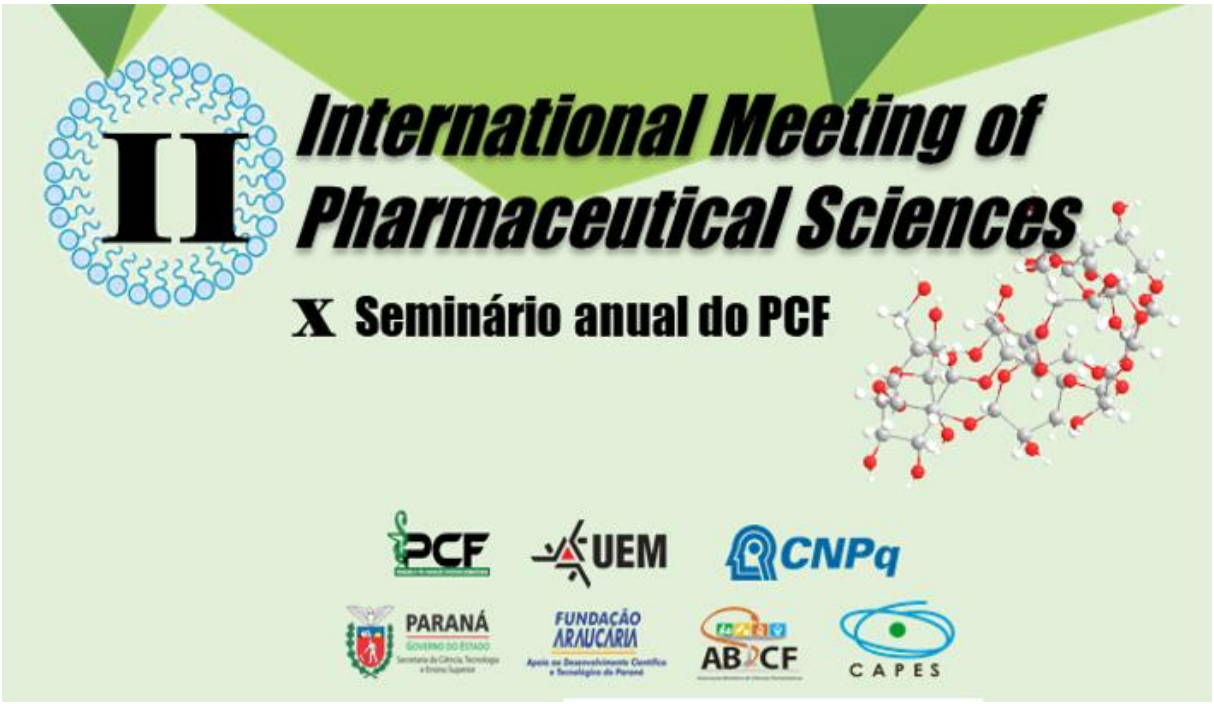

19th – 22nd November 2019

State University of Maringá
Health Sciences Center
Departament of Pharmacy

Annals of the II International Meeting of Pharmaceutical Sciences and X Annual Seminar of the Pharmaceutical Sciences Graduate Program

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Maringá – Paraná – Brazil
2019

PRESENTATION

Annals of the II International Meeting of Pharmaceutical Sciences and X Annual Seminar of the Pharmaceutical Sciences Graduate Program (PCF), took place between 19th – 22nd November 2019, in Block K68, State University of Maringá. The event promoted integration among undergraduate, master and doctoral students linked to PCF and from other postgraduate programs at the State University of Maringá and Universities in Brazil and abroad.

During the event there were lectures and courses given by professionals from different areas of activity within the Pharmaceutical Sciences and related areas, which aimed to provide participants who are at the beginning of their research, as well as those who are in thee more advanced stages of the knowledge the opportunity to glimpse new directions for their projects through new interpretations, propose solutions to problems, update and improve on specific subjects or opportunities for collaborations with renowned invited researcher.

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II International Meeting of Pharmaceutical Sciences

X Seminário anual do PCF

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SPEAKERS

Dra. Graciette Matioli
State University of Maringá
Short Course – Ciclodextrinas: moléculas versáteis de aplicações abrangentes

Dr. Flávio A. V. Seixas
State University of Maringá
Short Course – Introdução ao Docking molecular uma abordagem "in silico" no estudo da interação proteína ligante.

Dr. Adriano Valim Reis
State University of Maringá
Short Course – Tecnologias sub e supercrítica podem ser utilizadas nas áreas, farmacêutica, ambiental e de alimento?

Dra. Ana M. Castellani
Pitágoras Londrina College
Short Course – Tecnologias e Práticas Educativas: O Desafio da Profissão Docente

Dr. Frederico S. Martins
Galapagos Pharmaceutical
Short Course – Model Informed Drug Development

Dra. Audrey A. S. G. Lonni
State University of Londrina
Short Course – Ciência genética, inovação e tecnologia no desenvolvimento de dermocosméticos específicos

Dra. Stefanie Steiger
Ludwig Maximilians University Munich
Short Course – Bone marrow isolation

Dra. Stefanie Steiger
Ludwig Maximilians University Munich
Short Course –Flow cytometry (FACS)

Dr. Waldiceu Verri Junior
State University of Londrina
Lecture – Mecanismos envolvidos no controle da inflamação e dor por lipídeos pró-resolução

Dra. Kelly Ishida
University of São Paulo
Lecture – Alternativas para o tratamento das micoses

Dr. Celso Vataru Nakamura
State University of Maringá
Lecture – Os Desafios do Laboratório B- 08 na obtenção de fármacos no combate a Leishmaniose e a Doença de Chagas

Dra. Joyce M. da Silva
Azidus Brasil
Lecture – Pesquisa clínica, aspectos éticos e regulatórios

Dr. Paulo C. Vieira
Federal University of São Carlos
Lecture – Velhas plantas para novas utilidades

Dr. Paulo V. Farago
State University of Ponta Grossa
Lecture – Nanocápsulas poliméricas: conceitos e aplicações na tecnologia farmacêutica

Dra. Débora M. G. Sant'Ana
State University of Maringá
Lecture – Neurociência explica as emoções

Dr. Diego A. R. da Silva
Paraná Adventist Institute
Lecture – Saúde Mental na Pós-graduação: desafios no cenário atual

Dr. Frederico S. Martins
Galapagos Pharmaceutical
Lecture – D Good Practices in Model - Informed Drug Discovery and Development: Practice, Application, and Documentation

Dra. Stefanie Steiger
Ludwig Maximilians University Munich
Lecture – Hyperuricemia and the immune system

Dr. Flavio S. Emery
University of São Paulo
Lecture – Entre ABCF e USP – uma ponte de conhecimento

Dra. Lillian Barros
Polyclinic Institute of Bragaça
Lecture – Da Natureza aos Produtos: ingredientes conservantes, corantes e bioativos

PROGRAM

19/11/19			
08:00 - 11:40	**Short Course:** Ciclodextrinas: moléculas versáteis de aplicações abrangentes. Profa. Dra. **Graciette Matioli** - UEM/PCF	**Short Course:** flow cytometry (FACS) Dra. **Christine Stefanie Steiger** - University of Munique	**Short Course:** Introdução ao Docking molecular uma abordagem "in silico" no estudo da interação proteína ligante. Prof. Dr. **Flávio Augusto Vicente Seixas** - UEM
13:30 - 17:30	**Short Course:** Tecnologias sub e supercrítica podem ser utilizadas nas áreas, farmacêutica, ambiental e de alimento? – Prof. Dr. **Adriano Valim Reis** - UEM	**Short Course:** flow cytometry (FACS) Dra. **Christine Stefanie Steiger** - University of Munique	**Short Course:** Introdução ao Docking molecular uma abordagem "in silico" no estudo da interação proteína ligante. Prof. Dr. **Flávio Augusto Vicente Seixas** - UEM

20/11/19			
08:00 - 11:40	**Short Course:** Clínica aplicada à descoberta e ao desenvolvimento de medicamentos. Dr. **Frederico Severino Martins** – Galapagos Pharmaceutical	**Lecture:** Neurociência explica as emoções. Profa. Dra. **Débora de Mello Gonçales Sant'Ana** – UEM/PCF **Lecture:** Saúde Mental na Pós-graduação: desafios no cenário atual. Prof. Dr. **Diego Alexandre Rozendo da Silva –** Instituto Adventista Paranaense	
13:30 - 17:30	**Mini-Curso:** Tecnologias e Práticas Educativas: O Desafio da Profissão Docente. Profa. Dra **Ana Mauriceia Castellani** - Faculdade Pitágoras de Londrina		

21/11/19 Manhã	
07:40-8:40	Entrega de Materiais e abertura
08:40-9:00	Homenagem ao prof. Dr. Diógenes Aparício Garcia Cortez pelos professores Prof. Dr. **Paulo Cézar Vieira** - FCF/RP-USP e Prof. Dr. **Roberto Bazotte** – UEM/PCF
09:00-09:40	**Lecture:** Velhas plantas para novas utilidades - Prof. Dr. **Paulo Cesar Vieira** -FCF/RP-USP
09:40-10:00	Coffee break
10:00-10:40	**Lecture:** Descoberta e desenvolvimento de medicamentos utilizando modelos matemáticos - **Prof. Dr. Frederico Severino Martins** – Galapagos Pharmaceutical – Paris - França
10:40 - 11:40	Apresentação oral
21/11/19 Tarde	
13:30-14:10	**Lecture:** Os Desafios do Laboratório B- 08 na obtenção de fármacos no combate a Leishmaniose e a Doença de Chagas. Prof. Dr. **Celso Vataru Nakamura**– UEM/PCF

14:10-15:00	**Lecture:** Hyperuricemia and the immune system - Dra. **Christine Stefanie Steiger –** Ludwig Maximilians University- Munique - Alemanha
15:00-15:20	Coffee break
15:20-16:10	**Lecture:** Da Natureza aos Produtos: ingredientes conservantes, corantes e bioativos - Profa. Dra. **Lilian Barros** – Profa. do Instituto Politécnico de Bragança - IPB (Portugal)
16:10-17:30	Apresentação oral

<table>
<tr><td colspan="3">22/11/19 Manhã</td></tr>
<tr><td>8:00-8:40</td><td colspan="2">Lecture: Nanocápsulas poliméricas: conceitos e aplicações na tecnologia farmacêutica– Prof. Dr. Paulo Vitor Farago - UEPG</td></tr>
<tr><td>8:40-09:20</td><td colspan="2">Lecture: Mecanismos envolvidos no controle da inflamação e dor por lipídeos pró-resolução – Prof. Dr. Waldiceu Verri Junior - UEL</td></tr>
<tr><td>9:20-10:00</td><td colspan="2">Lecture: Pesquisa clínica, aspectos éticos e regulatórios - Dra. Joyce Macedo da Silva - Azidus Brasil</td></tr>
<tr><td>10:00-10:30</td><td colspan="2">Coffee break</td></tr>
<tr><td>10:30-11:30</td><td colspan="2">Apresentação oral</td></tr>
<tr><td colspan="3">22/11/19 Tarde</td></tr>
<tr><td>13:30-14:20</td><td>Lecture: Alternativas para o tratamento das micoses – Profa. Dra. Kelly Ishida – ICB-USP/SP</td><td rowspan="2">Short Course: Ciência genética, inovação e tecnologia no desenvolvimento de dermocosméticos específicos – Profa. Dra. Audrey Alesandra Stingen Garcia Lonni - UEL</td></tr>
<tr><td>14:20-15:30</td><td>Apresentação de Poster</td></tr>
<tr><td>15:30-16:00</td><td colspan="2">Coffee break</td></tr>
<tr><td>16:00-16:40</td><td colspan="2">Lecture: Entre a ABCF e USP - uma ponte de conhecimento - Prof. Dr. Flávio da Silva Emery - FCF/RP-USP</td></tr>
<tr><td>16:40-17:30</td><td colspan="2">Encerramento</td></tr>
</table>

SUMMARY

QUALITY CONTROL OF VEGETABLE SPECIE OF TRADITIONAL CHINESE MEDICINE: *LIGUSTICUM STRIATUM*

Amanda Benites Mansano[1]; Thalita Prates da Silva[1]; Emilly Isabelly dos Santos Teodoro[1]; Tânia Maria Antonelli Ushirobira[2]; Naiara Cássia Gancedo[1]; Vinicius Fukumi Takegava[3]; Viviane Bergamo Morgeu Takegava[3]; Daniela Cristina de Medeiros Araujo[2]; João Carlos Palazzo de Mello[1].

[1]Laboratory of Pharmaceutical Biology (PALAFITO), State University of Maringá, Maringá, Paraná. [2]Centro Universitário Ingá (UNINGA), Maringá, Paraná. [3]Fitofórmula, Santos, São Paulo. ***e-mail:*** *amandab.mansano@gmail.com; mello@uem.br*

Key-words: Pharmacopoeia; Thin layer chromatography; Purity assays.

Introduction: Due to the increase Western interest in alternative therapies and the growing interest of scientific knowledge about the use of millenary Traditional Chinese Medicine (TCM) the control to ensure quality of the used plants is necessary. **Aim:** The aim of this work was to perform the quality control of *Ligusticum striatum* DC., one of the plants normally used in the TCM. **Methods:** The dry samples were provided by Fitoformula Laboratory in May of 2019. The morphoanatomical analysis (macroscopic and microscopic identification), identification by thin layer chromatography (TLC), colorimetric and purity assays (water, total ash and extractives) were carried out. The methods were based by Chinese and Brazilian Pharmacopoeia. **Results:** In the morphoanatomical analysis *L. striatum* presented the characteristics according to the specifications of the Chinese Pharmacopoeia. In the identification by TLC the retention factor (Rf) was 0.13; 0.3; 0.4; 0.58; 0.7 and 0.83 compatible with Rf of the *L. striatum* standard. In the colorimetric assay, the color obtained was the specified by the Chinese Pharmacopoeia; a visually purplish-red color was produced. For the purity assays, the tested sample presented 10.17% ± 0.08 (coefficient of variation (CV) 0.83%); 5.34% ± 0.10 (CV 1.91%); 41.85% ± 0.59 (CV 1.41%) for water, total ash and extractives, respectively. The limits for the analyzes specified by the Chinese Pharmacopoeia of, no more than 12.0% for water, no more than 6.0% for total ash and no less than 12.0% for extractives. **Conclusion:** The results obtained are within the specifications by the Chinese Pharmacopoeia Commission (2010) and Brazilian Pharmacopoeia (2019), this suggests that this sample of *L. striatum* is proper for therapeutic use.

References:

(1) China Medical Science Press. Pharmacopoeia of the people`s republic of China. China, 2010.
(2) National Health Surveillance Agency. Brazilian Pharmacopoeia. Brasilia, DF. Brazil, 2019.

Acknowledgments: Fitoformula Laboratory[1] and CNPq[2].

EVALUATION OF THE SEMIPURIFIED FRACTION OF *Poincianella pluviosa* BY UHPLC

Ana Carolina Guidi; João Carlos Palazzo de Mello

Laboratory of Pharmaceutical Biology - Palafito, State University of Maringá, Maringá, Paraná. ***e-mail*** *acguidi2@gmail.com; mello@uem.br*

Key-words: chromatography, tannins, sibipiruna.

Introduction: The species *Poincianella pluviosa* (DC.) L.P. Queiroz, popularly known as sibipiruna, it is a very abundant species in cities of Brazil, and its extracts and isolated substances have already demonstrated pharmacological activities (1). **Aim:** Development of the chromatographic profile of *P. pluviosa* by ultra-high-performance liquid chromatography (UHPLC) for identification of the substances present. **Methods:** For development, a semipurified fraction of *P. pluviosa* was used. It was dissolved (1 mg/mL) in 20% methanol and eluted through a solid-phase extraction cartridge (SPE, C18, Chromabond®) and was filtered in membrane filters (0.45 µm, Millipore®). The analysis was performed using a UHPL*C UltiMate 3000 (*Thermo Scientific) equipped with a diode array detector, controller software (*Chromeleon*®), autosampler, with a 20 µL loop (total injection), integral pumps and degasser. A Phenomenex® Onix Monolithic C18 column (100 mm × 4.6 mm, 130 Å, 2 µm) and Phenomenex guard, were used. The mobile phases were: phase A (acetonitrile with 0.05% formic acid) and phase B (water with 0.05% formic acid). The gradient elution program was set as follow: 0–10 min, 10-15% A; 10–30 min, 15-20% A, after re-equilibration of the system. The analysis was carried out at a flow rate of 0.5 mL/min and detection at 280 nm. The column temperature was maintained at 35 °C. Standard solutions of gallic acid and ellagic acid (Sigma-Aldrich), and geraniin (Phytopurify) were prepared by dissolving in 20% methanol (100 mg/mL) and analyzed under the same conditions. **Results:** The chromatogram showed a total time of 40 min, confirming the compounds: gallic acid (rt= 3,723), geraniin (rt= 10,633), and ellagic acid (rt= 17,660). **Conclusion:** The described method was able to separate substances effectively, presenting peaks with good resolution. Mass spectroscopy is being performed to identify other substances present in the extract.

References:

(1) BUENO, F. G.; MOREIRA, E. A.; MORAIS, G. R.; PACHECO, I. A.; BAESSO, M. L.; LEITE-MELLO, E. V. S.; MELLO, J. C. P. Enhanced cutaneous wound healing in vivo by standardized crude extract of *Poincianella pluviosa*. **Plos One,** v.11, p.1-13, 2016.

Acknowledgments: Thanks to CAPES for financial support.

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MITOCHONDRIAL MEMBRANE POTENTIAL ALTERATIONS IN *Leishmania (Leishmania) amazonensis* TREATED WITH LAPACHOL AND β-LAPACHONE

Áquila Carolina Fernandes Herculano Ramos-Milaré[1]; Bruna Gomes Sydor[1]; Aline Ávila Brustolin[1]; Daniele Stéfanie Sara Lopes Lera-Nonose[1]; Expedito Leite Silva[2]; Wilker Caetano[2]; Izabel Galhardo Demarchi[1]; Maria Valdrinez Campana Lonardoni[1]

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Key-words: leishmaniasis; cutaneous leishmaniasis; naphthoquinones;

Introduction: leishmaniasis is a noncontagious infectious disease and presents a broad spectrum of clinical forms. Cutaneous leishmaniasis (CL) affects approximately 1 million people worldwide and threatens 350 million people who live in high-risk areas, mainly in developing countries (1). The available treatments have severe limitations. The development of new therapeutic strategies with less toxicity and more accessibility is essential. Lapachol and β-lapachone present a series of pharmacological compounds (2,3), and the knowledge about the action of these compounds may direct better applicability for therapeutic use. **Aim:** this study aimed to investigate the activity involved in promastigotes forms of *L. amazonensis* treated with lapachol and β-lapachone. **Methods:** lapachol and β-lapachone were obtained in the chemistry laboratory at State University of Maringá, Paraná, Brazil. Lapachol was extracted from the bark of Purple "Ipê" (*Tabebuia avellanedae*), and β-lapachone was obtained through an acid treatment. The viability of *Leishmania* promastigotes was evaluated using the Rh123 probe thought the determination of mitochondrial potential ($\Delta\Psi m$) using on BD FacsCalibur Flow Cytometer. Hydrogen peroxide was used as a positive control, and results were expressed as the percentage of fluorescence. **Results:** the compounds caused the loss of mitochondrial membrane potential, and for β-lapachone the effect occurred concurrently with the longer exposure to treatment. **Conclusion:** the naphthoquinones lapachol and β-lapachone have activity on promastigotes of *L. amazonensis*, being strong candidates for the research on new drugs derived from natural products with anti-*Leishmania* activity.

References:

(1) Epidemiological situation W. WHO | Epidemiological situation [Internet]. WHO. World Health Organization, 2017. Available from: http://www.who.int/leishmaniasis/burden/en/

(2) Hussain H, Green IR. Lapachol and lapachone analogs: a journey of two decades *of patent research* (1997-2016). Expert Opin Ther Pat [Internet]. 2017;0(0):1–11.

(3) Tseng CH, Cheng CM, Tzeng CC, Peng SI, Yang CL, Chen YL. Synthesis and anti-inflammatory evaluations of β-lapachone derivatives. Bioorganic Med Chem [Internet]. 2013;21(2):523–31.

Acknowledgments: this study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES).

SYNTHESIS OF NOVEL 5-ARYL-3-TETRAZOLYL-4-HYDROXIMETHYL-*N*-ARYLPYRAZOLES DESIGNED AS POTENTIAL ANTICANCER AGENTS

Arthur Antunes Ferrarezi[a]; Michael Jackson Vieira da Silva[b], Arildo José Braz de Oliveira[a], Fernanda Andreia Rosa[b], Regina Aparecida Correia Gonçalves[a].

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[b] *Heterocycles Synthesis Group (Sinthet), State University of Maringá, Maringá, Paraná.*

Key-words: bioisosterism, aromatase, tetrazole.

Introduction: Cancer is a set of multifactorial diseases which is characterized by an abnormal growth of cells and their spread into other organs or tissues (1). Ovarian cancer is the third most common gynecological malignancy (2), whose number of cases in Brazil was estimated to be around 6.150 in 2018 (3). Estrogens are closely related to this pathology and one therapeutic approach is the use of Aromatase inhibitors, which is the key enzyme in the estrogens biosynthesis and a target for this disease (4). The diaryl pyrazole scaffold represents an important pharmacophore in the design and synthesis of anticancer agents, specially for those molecules with anti-estrogen activity (5). **Aim:** The aim of this work was to optimize the synthetic route for the obtainment of novel diaryl pyrazoles designed as potential anticancer agents. **Methods:** Molecules were designed by applying the bioisosterism approach (COOH↔Tetrazole); each reaction step was optimized according to the methods in the literature; products were characterized by means of ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR). **Results:** The first step was the obtainment of a primary amide (**I**) by reacting the corresponding ethyl ester with ammonia in ethanol at 25 °C for 24 hours (88% yield). ^{1}H NMR showed the two characteristics large singlets at 7.79 and 8.16 ppm. Dehydration of **I** with diphosphoryl chloride in DMF, for 2h30 min at 25 ºC, led to the carbonitrile (**II**) with ^{13}C NMR signal at 111.7 ppm. Reduction of the aldehyde with $NaBH_4$ in acetonitrile at 25 ºC for 30 min led to the 4-hydroxymethyl-3-carbonitrile intermediate (**III**, 90% yield), whose ^{1}H NMR signals appeared as one doublet at 4.43 ppm and a singlet at 5.58 ppm. The target compound was obtained *via* a 1,3-dipolar cycloaddition with **III** and triethylammonium azide, under toluene reflux for 16 h; the product (80% yield) showed characteristic signal of tetrazole carbon at 149.7 ppm in ^{13}C NMR. **Conclusion:** The target compound, as well as its intermediates, were obtained with good to excellent yields and were properly characterized by NMR. The counterpart series is being synthesized by above described methods and will be used to determine the structure-activity relationships towards ovarian cancer cells.

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(1) World Health Organization. Available at: http://www.who.int/cancer/en/ access in 30/10/2019.
(2) Chen S. et al. The positivity of estrogen receptor and progesterone receptor may not be associated with metastasis and recurrence in epithelial ovarian cancer. Sci. Reports 2017; 7:1-7.
(3) Instituto Nacional do Câncer. Available at: https://www.inca.gov.br/tipos-de-cancer/cancer-de-ovario access in 30/10/2019.
(4) Miller W. R. Aromatase inhibitors: mechanism of action and role in the treatment of breast cancer. Semin. Oncol. 2009; 22:191-196.
(5) Farag. A. M. et al. Design, synthesis and structure-activity relationship study of novel pyrazole-based heterocycles as potential antitumor agents. Eur. J. of Med. Chem. 2010; 45: 5997-5898.

Acknowledgments: UEM; COMCAP; CAPES; CNPq.

II International Meeting of Pharmaceutical Sciences
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ACTIVITY OF *LIMONIUM BRASILIENSE AGAINST H. PYLORI IN VITRO* AND *IN VIVO* AND INVOLVED PATHOGENIC MECHANISMS

Augusto Santos Borges[a], Mariana Nascimento de Paula[a], Tânia Mara Antonelli-Ushirobira[a], Rodrigo Rezende Kitagawa[b], Rita Ribeiro Gonçalves[b], Marco Aurélio Valadão Fagundes[a], Daniela Cristina de Medeiros[a], João Carlos Palazzo de Mello[a].

[a] *State University of Maringa, Postgraduate Program in Pharmaceutical Sciences, Pharmaceutical Biology Laboratory, Palafito, Maringa, PR, Brazil.*
[b] *Federal University of Espirito Santo, Postgraduate Program in Pharmaceutical Sciences, Biological Screening of Natural Products Laboratory, Vitoria, ES, Brazil.*

Key-words: *Helicobacter pylori*, Gastritis, *Baicuru*.

Introduction: Gastric diseases are considered of great importance worldwide because of the high number of cases, and *Helicobacter pylori* (HP) infection is the most relevant. This microorganism can colonize the harsh stomach environment and can leads to an intense activation of the immune system and high production of reactive oxygen species. The ability to survive the acidic pH of the stomach is given by the urease enzyme (1). The WHO includes HP in a high priority list of bacteria for which new antibiotics are urgently needed. In this way, it is well justified the search for new substances capable to control HP. *Limonium brasiliense*, commonly named as "Baicuru" is a medicinal plant species used by folk medicine mainly to treat and prevent menstrual disorders. Some studies demonstrated good antioxidant and anti-inflammatory activity of the *L. brasiliense* rhizomes (2). **Aim:** The objective of this study was to evaluate the anti-HP activity *in vitro* and *in vivo* as well the anti-urease and antioxidant activities of an ethyl-acetate fraction (EAF) and its microencapsulated formulation (mEAF), of *L. brasiliense* rhizomes extract. **Methods:** The in vitro test was evaluated by broth microdilution method and anti-urease assay. The *in vivo* assay was evaluated by a gavage infection method in Balb/c mice, previously approved by the ethics committee of UEM (CEUA no. 4554021215), with histology analyzes. The antioxidant was evaluated against the HOCl, $O2^{\bullet-}$ and NO. **Results:** The EAF demonstrated a good capacity to inhibit the enzyme urease with an IC_{50} of 601.02 µg/mL. Also, the EAF showed outstanding antioxidant activity against the HOCl, NO and $O2^{\bullet-}$ showing IC_{50} values of 6.02, 69.94, and 20.93 µg/mL, respectively. EAF showed an *in vitro* MIC value of 1024 µg/mL for ATCC 43504 strain. For the *in vivo* assay, histology analyses showed no significant inflammation or high presence of the HP. This data leads us to try a clinical strain of HP, obtained from patients diagnosed with gastritis or gastric ulcer caused by HP infection. This project was already been submitted to the human ethic committee of UEM (COPEP), and is under consideration. All mEAF data are still being obtained. **Conclusion:** The *L. brasiliense* EAF can be a good source of substances capable to control some of the HP pathogenic mechanisms, but we still need to improve the *in vivo* model for better understanding of *L. brasiliense* actions.

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Acknowledgments: The authors thank CAPES for the scholarship granted to ASB.

PRELIMINARY STUDY OF THE ANTIOXIDANT CAPACITY OF *Eugenia neoverrucosa* SOBRAL (MYRTACEAE)

Bianka Carolina Welker[a]; Camila Cristina Iwanaga[a]; Yvine de Souza Moraes [a]; Maria da Conceição Torrado Truiti[a];

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Key-words: Myrtaceae; Antioxidant; Oxidative stress;

Introduction: Different intrinsic and extrinsic factors can lead to excessive production of reactive oxygen species and consequent oxidative stress, which is associated with various damages to the organism. Plants are a vast source of biologically active substances of different classes, from its secondary metabolism[1]. Many of them are profuse in phenolic substances with antioxidant capacity, such as *Eugenia* species, thus promising source of plant material for prevention or treatment of various alterations or diseases caused by oxidative stress. **Aim:** The aim of this study was evaluate total phenolic content (TP) and antioxidant capacity (AC) of *Eugenia neoverrucosa* Sobral plant materials. **Methods:** Ethanol extract (EE) was obtained by percolation of dried and ground leaves, it was concentrated under vacuum and lyophilized. Part of EE was submitted to liquid-liquid partition, providing the hexanic (HF), ethyl acetate (EAF) and hydromethanolic (MF) fractions. The TP was measured using Folin-Ciocalteau reagent[2] (expressed in mg of gallic acid equivalents per gram of dry sample) and AC was determinate by DPPH (2-2-difenil-1-pricril-hidrazil) method[3] (expressed in IC_{50}). **Results:** The EFA showed the highest level of TP and great antioxidant potential by DPPH method (502.8 mg GAE/g; IC_{50} 10.4 μg/mL). EE (430.0 mg GAE/g; IC_{50} 14.6 μg/mL) and MF (417.3 mg GAE/g; IC_{50} 12.8 μg/mL) also showed antioxidant potential, higher than the antioxidant butylated hydroxytoluene (IC_{50} 12,44 μg/mL). **Conclusion:** These results show that *E. neoverrucosa* leaves is a important source of natural antioxidants. Further studies are needed to confirm the biological activity and identify substances responsible for its potential.

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FORMULATION AND *IN VIVO* STUDY OF SOLID EFFERVESCENT SYSTEM AS A NEW STRATEGY FOR ORAL GLUTAMINE DELIVERY

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Keywords: Microparticulate system; bioavailability; bioactive food components.

Introduction: Glutamine (GLN) is used as dietary supplement for endurance athletes, surgery, trauma, transplants, cancer, wound healing, critically ill neonates, and HIV patients. However, GLN is practically insoluble in water, which reduces its bioavailability after oral administration. **Aim:** The purpose of this work was to develop a formulation of solid effervescent system as a new strategy for oral glutamine delivery. **Methods**: Effervescent GLN (EBGLN) was prepared by micronization with an effervescent base (EB) that enhances its water solubility. EB composed by citric acid, tartaric acid and sodium bicarbonate were used in the ratio 1:2:3. This formulation was characterized in the liquid state (solubility studies, pH, effervescence time, glutamine content) and solid state (sieving, residual moisture, flow properties studies, differential scanning calorimetry, thermogravimetric analysis, powder x-ray diffraction and Fourier transformed infrared Raman spectroscopy). *In vivo* studies were performed with adult overnight fasted (15 h) male Wistar rats. The animals were euthanized by decapitation 30, 60 and 120 min after the administration of water (vehicle of GLN), EB (vehicle EBGLN), GLN or EBGLN. Blood was collected and the aminogram of plasma was obtained by HPLC analysis. **Results:** Physicochemical characterizations indicated that EBGLN was present as a physical mixture with lower crystallinity and higher solubility, when compared with pure GLN. *In vivo* experiments demonstrated improved oral GLN biovailability from EBGLN (500 mg/kg - 30 min and 60 min). **Conclusion:** The solid effervescent system represents a new strategy for oral glutamine delivery and suggests it is worthy of clinical evaluation.

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IN VITRO EVALUATION OF THE 2-ACETYLPHENOTHIAZINE ANTIOXIDANT EFFECT AGAINST OXIDATIVE DAMAGES IN FIBROBLAST L929 UVB-IRRADIATED

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Key words: 2-acetylphenothiazine, UVB radiation, antioxidants, photoprotection.

Introduction: Chronic UVB exposure might promotes oxidative stress, due to the accumulation of reactive oxygen species (ROS) (1), which leads to oxidative destruction of the cell components through oxidative damage of membrane lipids, nucleic acid, and protein (2,3). **Aim:** To evaluate *in vitro* the antioxidant and photoprotectant activity of 2-acetylphenothiazine (ML171) in UVB-irradiated L929 fibroblasts. **Methods:** The antioxidant capacity of ML171 was studied in a cell-free system using the 2,2- diphenyl-1-picrylhydrazyl (DPPH%) method and the xanthine/luminol/xanthine oxidase system. The neutral red method was used to determine cell viability. The markers 2',7'-dichlorodecafluorescein diacetate (H_2DCF-DA) and tetramethylrhodamine ethyl ester (TMRE) were used to evaluate ROS production and mitochondrial membrane potential, respectively. A fluorescence microscopy was performed using propidium iodete (PI) and Hoechst for cell membrane integrity. **Results:** ML171 showed antioxidant activity in both assays (DPPH and XOD) and the results were similar to quercetin (QT). ML171 showed no cytotoxicity to L929 fibroblasts at concentrations of 1-200 μM. In the cytoprotection assay, concentrations 1, 5 and 10 μM prevented 73, 72, 65% of unviability caused by UVB radiation, respectively. ML171 pretreatment (1 and 5 μM) also significantly decreased UVB-induced ROS production (approximately≅85% lower compared with UVB group), and prevented the loss of $\Delta\psi m$ (higher than 45% compared with UVB group). In PI and Hoechst assay, the pretreatment with ML171, increased cell membrane integrity like quantified and visualized by fluorescence microscopy. **Conclusion:** These results indicate that ML171 decreased oxidative stress induced by UVB radiation.

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PHENOLIC GLYCOSIDE FROM *Eugenia hiemalis* PROTECTS L929 FIBROBLASTS AGAINST UVB-INDUCED PHOTODAMAGE

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Key-words: Ultraviolet radiation, Plant materials, Skin disorders

Introduction: Chronic exposure to UVB radiation (280-320 nm) causes several harmful effects on the skin, including photoaging and skin cancer. Plant materials (extracts, fractions and isolated compounds) have the ability to exert inhibitory effects on several cellular and molecular events and are gaining considerable attention for the prevention of UVB-induced skin damage[1]. *Eugenia hiemalis* Cambess. (Myrtaceae) represents a promising source of natural agents with photochemoprotective potential, since in preliminary assays the ethyl acetate fraction (EAF) showed a high antioxidant capacity and phenolic compounds content. **Aim:** To evaluate the cytotoxicity and photochemoprotective potential of Eh-1, a phenolic compound isolated from EAF of *E. hiemalis*on UVB-irradiated L929 fibroblasts. **Methods:** The EAF, obtained by liquid-liquid partition from the ethanolic extract of dried and ground leaves from *E. hiemalis*, was subjected to silica gel column chromatography affording 17 fractions. Size exclusion chromatography of the subfraction 12 on Sephadex LH-20 provided the isolation of Eh-1, which was identified by spectroscopic analyses. The cytotoxicity was determined after 24 h incubation of L929 cells with Eh-1 (3.2μg/mL; DPPH IC_{50}previouslydetermined) by the Neutral Red assay[2]. TheEh-1photochemoprotective potential[2] was determined by the percentage of cell viability on pre-treated (3.2 μg/mL) and UVB-irradiated (500 mJ/cm^2) L929 cells. **Results:** Eh-1 was isolated as a white amorphous powder. Their structure was determined on the basis of spectroscopic evidence (^{1}H and ^{13}C NMR, HSQC and HMBC) in comparison with literature values[3] and was identified as 2,6-di-*O*-galloylarbutin. The compound 2,6-di-*O*-galloylarbutin presented no cytotoxic effects on L929 fibroblasts. In comparison to UVB control (62.56 ± 0.374% cell viability), the pre-treatment significantly (p>0.05) prevented cell death preserving the proliferative ability of cells (83.57 ±0.943% cell viability). **Conclusion:** The data indicated that the phenolic compound galloylarbutin isolated from *E. hiemalis* showed photochemoprotective potential against UVB, justifying the continuation of the studies to determine the mechanisms involved in the photoprotection.

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DEVELOPMENT OF HIGH AVAILABLE ZINC DERMOCOSMETIC TO PROLONG THE EFFECT OF BOTULINUM TOXIN (BTA)

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Key-words:dermocosmetic; botulinum toxin; effect prolongation;

Introduction: Botulinum toxin is synthesized by the anaerobic bacteria *Clostridium botulinum*, being the type A (BTA) with the greatest effect in preventing presynaptic secretion of acetylcholine in neuromuscular junction(1). BTA is a zinc-dependent endoprotease(2). Thus, the presence of available zinc at the site of action favors the cleavage process of polypeptides that command exocytosis, prolonging the effect of toxin A. In addition to the aesthetic effect, notably the most widely used in facial harmonization, BTA has been widely used in dental area for the control of bruxism, as well as in the medical area, for the treatment of tension-based migraines(3).Although the use of TBA has become popular, the cost of treatment is still high, especially due to its short action. **Aim:** To develop a high available zinc-dermocosmetic for prolonging the effect of BTA. **Methods:** A nonionic O/W emulsion was prepared and the dermatological actives (ferulic acid, sodium pyrrolidonecarboxylate, Argireline® and PCA zinc) were incorporated. Quality control was carried out in accordance with Collegiate Board Resolution nº 67/2007.**Results:** Serum presented an emulsion appearance with white color and pH of 5.4, a hydrogen ionic potential value suitable for application to the facial skin epidermis. **Conclusion:** The developed product presented sensory qualities suitable for topical therapy, aiming at prolonging the effect of botulinum toxin A and complementing the orofacial harmonization treatment.

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EFFECTS OF ANETHOLE + METHOTREXATE COMBINATION ON INFLAMMATORY PARAMETERS OF ARTHRITIC RATS

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Introduction: Rheumatoid arthritis (RA) is a chronic multisystemic disease, whose main feature is persistent inflammatory synovitis (1). Methotrexate (MTX) is considered the drug of choice for RA treatment, but its main limitation is the appearance of adverse effects (2). Anethole (AN), a constituent of anise essential oil, attenuated the inflammatory response caused by the disease. **Aim:** To investigate the effect of the combination AN+MTX on RA. **Methods:** Male Holtzman rats (180 to 220 g) were used (CEUA no. 9937300419). Arthritis (AIA) was induced by intradermal injection of 0.1 ml of Freund's complete adjuvant suspension (0.5% in mineral oil) on the plantar surface of the left paw. Animals were treated daily with AN (oral gavage) at doses of 62.5 and 250 mg/kg; MTX once a week (i.p., 6, 12 and 24 mg/kg) or the AN + MTX combination (following the same protocols as above at the dosage of 62.5 + 6 mg/kg, respectively). Treatments began on the day of induction of AIA and continued for 21 days. **Results:** AN treatment (250 mg/Kg) significantly reduced paw edema in the injected paw by 29%, on the 20th day after inoculation. Treatment with AN at the dose of 62.5 mg/kg caused only a small, non-significant, reduction (7%) on paw edema. MTX efficiently reduced paw edema by 32%, 36% and 52% at the doses of 6, 12 and 24 mg/Kg, respectively. The combination AN+MTX significantly reduced paw edema by 40%. This combination was also efficient to reduce the counting of total blood leucocytes of arthritic rats and to reduce the appearance of secondary lesions. It is important to note that the effects observed for AN+MTX combination were comparable to the ones observed for MTX 12 mg/Kg, and superior to the ones observed for AN 250 mg/Kg. None of the treatments were able to prevent the diminished weight gain observed in the arthritic condition. MTX (12 mg/Kg) and AN+MTX were able to attenuate the increase in plasmatic ALT activity seen in the arthritic condition, although none of the treatments reduced the increased plasmatic AST activity of AIA animals. **Conclusion**: The present study demonstrates that AN administration potentiates the anti-arthritic effects of MTX. Therefore, the combination AN+MTX may represent a novel and highly effective strategy for RA treatment.

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INFRARED PHOTOACOUSTIC SPECTROSCOPY FOR EX VIVO EVALUATION OF SKIN DIFFUSION OF EMULSIONS CONTAINING CELLULOSE NANOCRYSTALS AND SAPONINS

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Key-words: Permeation, saponins, infrared photoacoustic spectroscopy.

Introduction: Emulsions are interesting for the formulation of skin cosmetics and health products, acting as drug delivery systems. Saponins extracted from *Sapindus saponaria* L. act as substitutes for synthetic surfactants (1, 2). Cellulose nanocrystals settle around the inner phase of the emulsion, resulting in greater stability. **Aim:** To evaluate the skin permeation of emulsions containing saponins and cellulose nanocrystals by the FTIR-PAS technique. **Methods:** Emulsions were prepared with ultra-purified water, oil extracted from *S. saponaria* L., cellulose nanocrystals (CNC) and *Sapindus* saponins (S). There were 4 emulsions named as: A, B, C and D, with concentrations of: 0.5% CNC and 5% S; 0% CNC and 5% S; 5% CNC and 8% S; 10% CNC and 6.5% S, respectively. Systems were subjected to high intensity sonication (Bioblock Scientific, VibraCell 75115, Autotune series, 750 watts model) at room temperature. After 10 µL each emulsion was applied to the epidermis of swine ears from the Experimental Farm of UEM. After one hour, the excess emulsion was removed and measurements were taken from the epidermis illumination and then the dermis using a Fourier Transform Infrared Spectrometer (BRUKER, Vertex 70v). The spectra were acquired in rapid scan mode in the spectral range from 3,725 to 1,000 cm^{-1}. **Results:** There were no changes in skin bands due to the emulsion application, i.e. there was no appearance, disappearance, dislocation or change, it can be said that there were no chemical interactions of the formulations with the skin, therefore no adverse effects occur as a result of the application. Emulsion bands were identified in the dermis spectra that received the emulsion in the epidermis, suggesting that there was permeation. Both emulsions reached skin depths equivalent to 718 µm, but emulsion D presented a lower permeation rate, which may be due to the gelation of the medium. **Conclusion:** The four formulations were able to penetrate through the skin and there was no physicochemical alteration in the skin after emulsion application.

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ANALYSIS OF GENE EXPRESSION OF *Candida albicans* ISOLATED FROM EXPERIMENTAL SERIAL CANDIDEMIA

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Key-words: *Candida albicans*, virulence factors, real-time PCR.

Introduction: *Candida albicans* commensally inhabits the mucosal surfaces of the gastrointestinal and vaginal tracts without causing damage. The candidemia development will depend on host susceptibility and fungal virulence. **Aim:** To evaluate virulence genes expression profile from *C. albicans* after serial experimental candidemia. **Methods:** After literature review, virulence genes: *CYS3*, *SOD1*, *SOD3*, *YHB1* and *YWP1* were selected and the expression profile was analyzed by real-time PCR. Three serial experimental candidemia were performed and yeast recovered from kidney. These yeasts were used to RNA extraction and conversion to cDNA. Standardization and validation assays were performed to obtain the optimal concentration for primers and samples. Furthermore, *CEF3* was chosen as a housekeeping gene due to its low variation in expression among the samples. Relative quantification by real-time PCR was performed and the gene expression of *C. albicans* isolates recovered from serial candidemia was compared to the expression of the reference strain *C. albicans* SC5314. **Results:** For the *CYS3* gene, there was a higher expression in yeasts recovered from both the first (P1) and third (P3) passages. For *SOD1*, P1 presented lower expression and P3 higher expression. Regarding *SOD3*, there was also lower expression in P1 and higher expression in P3. For *YHB1*, in both P1 and P3, there was an increased expression. For *YWP1*, in P1 the expression was lower and in P3 higher. **Conclusion:** The differential expression from genes related to virulence factors such as biofilm formation, filamentation and stress response, could have an important role during the infection and may have contributed to the higher virulence observed from *C. albicans* isolated from serial infection.

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ETHYL-ACETATE FRACTION OF *TRICHILIA CATIGUA* (CATUABA) ATTENUATES THE MEMORY IMPAIRMENT AND OXIDATIVE STRESS CAUSED BY GLOBAL CEREBRAL ISCHEMIA IN RATS: AN ANALYSIS OF THE TIME WINDOW OF EFFICACY

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Key-words: Cerebral ischemia, memory, oxidative stress.

Introduction: Transient, global cerebral ischemia (TGCI) leads to severe neuropsychological deficits that are associated with oxidative stress, inflammation and neurodegeneration. We reported that an ethyl acetate fraction (EAF) of *Trichilia catigua* (catuaba) reduced the memory deficit, oxidative stress and inflammation caused by TGCI when treatment initiated before ischemia (1). **Aim:** To investigate whether the EAF remains effective when treatment is initiated post-ischemia. **Methods:** In a first experiment, the EAF (400 mg/Kg, p.o.) was administered 1, 4 or 6 hours after TGCI, and the oxidant/antioxidant status of the brain was measured after 24 h of reperfusion. In a second experiment, the EAF treatment begun at 4 or 6 hours after ischemia and continued for 7 days. Retrograde memory was assessed in the aversive radial maze task at 10, 17 and 24 days after TGCI and expressed by three parameters: (i) latency to complete the task, (ii) number of reference memory errors and (iii) number of working memory errors. This protocol had the approval of internal Ethical Committee (CEUA nº 7481261017). **Results:** In the analysis of oxidative stress, TGCI strongly reduced the levels of antioxidant enzymes and increased the concentration of carbonylated proteins. EAF was able to reestablish the levels of antioxidant enzymes and markers of oxidative damage. In the cognitive analysis, TGCI caused the rats to spent more time (latency) to complete the task, and to commit more reference and working memory errors, indicating they forgot the task that was learned prior to ischemia. The treatment with EAF significantly decreased the latency and number of errors, indicating a memory-protective effect. **Conclusion:** The data indicate that the neuroprotective effect of EAF can be observed with a time window of efficacy of up to 6 h.

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MAIN CHARACTERISTICS OF CELL CULTURE MODELS FOR ATTENTION DEFICIT HYPERACTIVITY DISORDER

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Key-words: cell model, methodological parameter; systematic review

Introduction: Cell cultures techniques a key role in health area, including of drug efficacy, safety, and toxicity, as well as for the production of vaccines and biopharmaceuticals[1]. **Aim:** The objective was to summary the main characteristics of studies that used cell culture models used to investigate therapeutic options for Attention Deficit Hyperactivity Disorder (ADHD). **Methods:** Systematic search was performed in the electronic databases PubMed, Scopus, and DOAJ. *In vitro* experimental studies using any type of cell line were included. This research was designed according to the recommendations from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[2] and others guidelines. **Results**: A total of 328 studies were screened, after duplicate removal, of which 83 were included for full-text appraisal. Finally, 16 studies were eligible for qualitative synthesis. Studies were published between 2007 and 2017 and conducted in different countries. The reported types of cell cultures were: neuronal cell lines and non-neuronal cell lines such a HEK293rtTA, HEK-293, TsA201, and JAR, cells isolated from patients such as human lymphocytes, lymphoblastoid cell lines, peripheral blood mononuclear cells, and cells isolated from animals (L929sA, CHO, MDCK). The studies reported data on the cell culture conditions, evaluation cell viability using the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazoline bromide (MTT) assay, lactate dehydrogenase (LDH) activity, or the adenosine triphosphate (ATP) content and evaluation gene expression for reverse transcription polymerase chain reaction (RT-PCR). **Conclusion:** This research to summary information on the use of these models for the investigation of potential therapeutic substances for ADHD and contribute for development of laboratory protocols and further *in vitro* investigation.

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DEVELOPMENT AND VALIDATION OF INDICATIVE STABILITY METHOD BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) FOR QUANTIFICATION OF DAPACONAZOLE TOSILATE AND TOSYLATE DAPACONAZELE CREAM 2%.

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Key-words: Dapaconazole Tosylate, Degradation Products, Development

Introduction: In Brazil, for a new active pharmaceutical ingredient (API) and / or drug to be registered and commercialized, it is necessary that the National Health Surveillance Agency (ANVISA) evaluates the administrative and technical-scientific documentation related to quality, safety and efficacy of this drug. One of the regulatory requirements is the demonstration of product quality and safety in relation to degradation products, that may be generated during the production and / or storage process.[1,2,3.] **Aim:** To develop and validate an indicative stability method for the quantification of degradation products from Dapaconazole Tosylate API as well as from the 2% Dapaconazole Tosylate Dermatological Cream®. **Methods:** For the present study, ultra-performance liquid chromatography (UPLC) methods were established and defined using a project-based quality by design approach (QbD) for the quantification of the analytes of interest. For the generation of degradation compounds, the active pharmaceutical ingredient and the product were exposed to stress conditions: acid, basic, oxidative, thermal, metal ions, moisture and photolytic. “In silico” prediction softwares were also being used to evaluate mutagenicity and genotoxicity of compounds with already elucidated structures. **Results:** The results show that the method is indicative of stability, since under stress conditions where there was degradation, the method presented a satisfactory mass balance. Furthermore, with the aid of the diode array detector it was possible to verify the absence of coelution between the compounds. For impurities with elucidated structures, the prediction test “in silico” is negative, corroborating the classification of these impurities as non-mutagenic and / or carcinogenic. **Conclusion:** The present study demonstrates that the developed method is indicative of stability for the quantification of degradation products derived from the dapaconazole tosylate API, as well as for the medicine Dapaconazole Cream 2%®. As expected, dapaconazole tosylate is more stable compared to the other antifungals of its class (azolics), probably due to the presence of the CF3 group linked to a benzene ring. Therefore, this method can be submitted to the National Health Surveillance Agency (ANVISA) for stability evaluation of both the dapaconazole tosylate API and the medicine Dapaconazole Tosylate Cream 2%®.

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II International Meeting of Pharmaceutical Sciences
X Seminário anual do PCF

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR QUANTITATION OF LAMOTRIGINE

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Key-words: HPLC, simple system, reverse phase.

Introduction: Lamotrigine (LTG) is an antiepileptic drug used to treat epilepsy in adults and children. LTG can be used in children as young as 2 years old when it is given as part of a combination of seizure medications. However, epilepsy is a disease that affects 0.5 to 1% of the pediatric population, with most syndromes beginning during the first years of life. So, the main question is: What is the pharmacokinetic profile of LTG for the pediatric population? But to start answering this question, a method capable of identifying and quantifying LTG is required. Thus, the **aim** of this work was develop and validate an analytical method for LTG quantitation. **Methods:** The HPLC analytical method was elaborated and adapted from American Pharmacopoeia Lamotrigine Tablet Monograph. After, the method was validated according to ANVISA (RDC166/2017) and ICH (2005). The parameters evaluated were: selectivity, robustness, linearity, quantification limit, detection limit, precision and accuracy. **Results:** The chromatographic parameters were evaluated using a RP18 150 x 4,6 mm, 5 µm column. After the evaluation LTG UV spectrum in the range of 200–400 nm, the wavelength of 210 nm was selected for detection. The mobile phase selected was 0.1% (v / v) trifluoroacetic acid in ultrapurified water pH 4.5 and methanol (40:60 v / v). The flow rate was 1.0 mL/min in 30° C column temperature. The injection volume of sample was 20 µL (loop volume). Once the conditions were defined, the validation was performed. The method showed to be specific, robust, linear, precise and accurate. The chromatogram obtained with mobile phase and diluent showed no interfering peaks in the same retention time of LTG. The method showed to be robust for changes in proportion of mobile phase and temperature with stability of 193 h. According to the determined signal-to-noise ratio, LTG presented limits of detection of 0,05 µg/mL and limits of quantitation of 0,3 µg/mL. A linear correlation was found between the peak areas and the concentrations of LTG (R^2>0,99). The method showed to be precise and accurate within the acceptance range adopted by the Horwitz equation. **Conclusion:** A method capable of identifying and quantifying LTG has been developed and validated. Therefore, the method will be used in future analyzes.

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II International Meeting of Pharmaceutical Sciences
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ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF THE LEAVES OF *Cinnamomum verum* J. PRESL (LAURACEAE)

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Key-words: Extraction, antimicrobial activity, chemical composition.

Introduction: Essential oils are volatile chemical compounds produced by the secondary metabolites of plants, are mainly composed of terpenic substances which confer their lipophilic and organoleptic characteristics.[1] The intensification of biological studies of essential oil constituents has resulted in the discovery of natural products that can be used to control pests and diseases.[2] Many of the species belonging to the Lauraceae family are considered medicinal and of varied use, performing different functions against various diseases, chemically they are a family rich in secondary metabolites.[3] **Aim:** The objectives of this work are extraction of the essential oil from *Cinnamomum verum* leaves, its chemical characterization and to perform antimicrobial activity tests of this essential oil. **Methods:** Plant material was collected in the urban area of Naviraí, in the state of Mato Grosso do Sul. Extraction was performed by the method of hydrodistillation in Clevenger apparatus. The essential oil has been subjected to chromatographic analysis (GC-MS), and the chemical composition can be determined by comparing the calculated Kovats index (KI) with Adams literature and equipment library (NIST). Antimicrobial activity tests were performed, being the disk agar diffusion test and minimum inhibitory concentration (MIC) test, the tests were performed with gram-positive *Staphylococcus aureus* and *Bacillus cereus* bacteria, both gram-negative *Escherichia coli*. **Results:** After extracting the essential oil from the leaves the yield was calculated at 0.12%. It was possible to identify 82.43% of the essential oil compounds, 77.70% of monoterpenes and 4.73% of sesquiterpenes. All bacteria tested in the agar diffusion disc method were oil-sensitive in their crude form. The MIC test showed the concentration required to inhibit the growth of the tested bacteria, being 2250 μg/mL for *Staphylococcus aureus*, 9000 μg/mL *Bacillus cereus* and 2250 μg/mL for *Escherichia coli*. **Conclusion:** Fifteen essential oil compounds were identified, the main ones being eucalyptol, followed by ρ-cimene, α-pinene, β-felandrene and α-terpineol. The essential oil obtained a weak antimicrobial activity, requiring a higher concentration of essential oil than recommended in the literature, and the MIC above 1600 μg/mL is considered weak activity.[4]

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II International Meeting of Pharmaceutical Sciences
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ANTIINFLAMMATORY ACTIVITY OF MICROENCAPSULATED ANETHOLE IN AN EXPERIMENTAL ACUTE INFLAMMATION MODEL

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Keywords: Edema, Anethole, Microparticles.

Introduction: Anethole (AN) is an aromatic compound traditionally used in pharmaceutical formulations because of its antioxidant, gastroprotective, and hepatoprotective effects A recent study demonstrated that AN has antinociceptive and antiinflammatory activities in different models of inflammation (1). However, these effects were obtained with high doses of AN, which may be one limitation of its therapeutic use. Drug encapsulation systems (microparticles) are promising due to their ability to protect compounds against physical and chemical degradation and to improve their bioactivity. **Aim:** To obtain AN-loaded microparticles and to evaluate their antiedematogenic activity using a carrageenan-induced paw edema model. **Methods:** Microparticles were obtained using the hot homogenization method (2) with Compritol® as encapsulant and Tween 80 as stabilizer. Differential Scanning Calorimetry (20°C/min; 30 to 300°C) was used to determine the thermal properties of the encapsulated oil. Wistar rats (200-220g) were treated orally with single doses of AN at dose 250mg/kg and AN- microparticles (MLSAN) at doses 12.5; 25 e 50 12.5mg/kg. After 1h the rats (n = 5-7) received an intraplantar injection of 100 µl of carrageenan (Cg) solution in the left hind paw (200µg). The right hind paw was injected with the same volume of 0.9% saline solution. The volume of paws was determined 1, 2, and 4h after the Cg injection using a plethysmograph. The edema was calculated by subtracting the volume of the paw that received saline solution from the volume of the paw that received Cg. The results were expressed as mean±SEM (ANOVA). All of the procedures were approved by the CEEA/UEM (3001210819). **Results:** FTIR results showed the efficient encapsulation of AN in the Compritol matrix. Melting temperature of the particles were detected at 71°C. The edema presented an maximum intensity 4h after the Cg injection, resulting from the release of numerous proinflammatory mediators (3). AN at a dose of 250mg.kg^{-1} and MLSAN at doses 12.5, 25 and 50mg.kg^{-1} significantly reduced (by 29% e 20%, 29% and 35%, respectively) the formation of edema 4h after the Cg injection. **Conclusion**: The antiedematogenic effect may be at least partially explained by inhibitory effects on the synthesis or release of inflammatory mediators. The data show that the micro-encapsulation process of anethole is important for protection against biodegradation after oral administration improving this biodisponibility.

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EFFECT OF MAGNETIC NANOPARTICLES FUNCTIONALIZED WITH DOXORUBICIN APPLIED TO BREAST ADENOCARCINOMA CELLS (MCF-7)

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Key-words: breast cancer, nanotechnology, doxorubicin.

Introduction: Nanotechnology is an area of interdisciplinary and multidisciplinary research that has been used in various biomedical areas. Currently one of the cancer treatments choice is chemotherapy, but problems such as drug resistance, lack of selectivity and accelerated metabolism result in toxicity to healthy cells thus reducing the effectiveness of this treatment (1). Therefore, it is important to study the use of nanocarriers that can be used on specific targets and consequently can reduce side effects caused by anti-tumor drugs in healthy organisms. In this respect magnetic nanoparticles are the most promising candidates due to their physicochemical and magnetic characteristics (2, 3). **Aim:** Therefore, this work aimed to evaluate the cytotoxic potential of magnetite (NPMag), silica-coated magnetite (NPMag+Si) nanoparticles and doxorubicin-functionalized nanoparticles (NPMag+Dox, NPMag+Si+Dox) on human breast adenocarcinoma cells (MCF-7) and immortalized human keratinocytes (HaCat), using the MTT assay. **Methods:** For the MTT assay, MCF-7 and HaCat cells were treated with different concentrations (5, 10, 25µg/mL) of NPMag, NPMag+Si, Doxorubicin (Dox), NPMag+Dox and NPMag+Si+Dox for 24 and 48 h. **Results:** The MTT assay showed that MCF-7 cells treated with NPMag+Dox and NPMag+Si+Dox present decrease in viability after 24 and 48 h when compared with untreated cells. HaCat cells also presented a decrease in viability but it was less cytotoxic compared with the cancer cell line. NPMag an NPMag+Si did not show any cytotoxicity alone against MCF-7 cells or HaCat cells. Nonetheless more studies should be conducted to demonstrate the effects of these magnetic nanoparticles on tumor cells.

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DEVELOPMENT OF ANTIOLEOSITY NATURAL FACIAL CLAY MASK

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Keywords: Acne; salicylic acid; plant extracts

Introduction: The formation of acne has as its main causes: sebaceous overproduction, follicular hyperkeratinization, high colonization by *Propionibacterium acnes*, periglandular dermal inflammation, or from hormonal causes[1]. Treatment should be early and appropriate and may be topical or systemic. The main actives used in topical treatment are: retinoids, topical antibiotics, benzoyl peroxide, salicylic acid, azelaic acid, nicotinamide, and alpha hydroxy acids (AHA)[2]. In addition, there are plant actives that have anti-inflammatory and astringent activity, such as chamomile and calendula extracts. Green clay can also be used as an adjunct to anti-acne therapies, indicated to reduce the appearance of greasiness, invigorate the skin, as it provides deep cleansing, toning action, astringent, moisturizing and healing. **Aim**: Development of antiacne dermocosmetic with suitable rheological profile and pleasant sensory characteristics. **Methods**: For the preparation, the clay was incorporated into the propylene glycol, followed by the extracts and water. The evaluation of organoleptic characteristics, determination of tactile sensation and pH was assessed according to RDC 67/2007. Rheological profile was determined using MARS II rheometer (Haake®), with C35/2 spindle. Experiments were carried out at room temperature (25 ± 0,5 °C). **Results:** The formulation presented moss green color, characteristic odor, pasty appearance, having an exfoliating sensation at the time of application and quick drying. The pH was neutral (7.50), which does not cause skin irritation. Flow behavior analysis determined a shear thinning fluid with thixotropic properties. **Conclusion:** The face mask is an indispensable product for the treatment of acne, helping to reduce oiliness and acting as an anti-inflammatory manner. Rheologic behavior described a non-Newtonian fluid. Considering the observed aspects, it can be concluded that the product has good applicability and spreadability, which can increase adhesion and consequently improve treatment.

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COMPARISON OF QUALITY OF THE TRADICIONAL CHINESE MEDICINE *PAEONIA LACTIFLORA* PALL OBTAINED FROM DIFFERENT SUPPLIER.

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Key-words: Quality Control; Zhong Yi Xue; Paeoniae Radix.

Introduction: *Paeonia lactiflora* Pall or also known as Bai Shao, has in its composition monoterpene glycosides, tannins, and flavonoids, but its main active ingredient is paeniflorine. Bai Shao is widely used in Traditional Chinese Medicine (TCM), and due to the growth of TCM in the west it is important that the quality control of the plants used is performed. **Aim:** Perform quality control by comparing suppliers A and B of the vegetable drug *Paeonia lactiflora* Pall. **Methods:** Morpho-anatomical analyzes (macroscopic and microscopic identification), identification by thin layer chromatography (TLC), purity tests (water, extractives content, and total ash) were performed for both suppliers. **Results:** In the morpho-anatomical analysis (macroscopic and microscopic identification) it was possible to observe characteristic structures of *Paeonia lactiflora* Pall for both suppliers; For TLC a retention factor of 0.51 was observed for sample A and B compatible with standard SRC paeoniflorin substance. For purity tests supplier A presented 10.33% ± 0.06 (CV 0.57%) and supplier B 11.28% ± 0.01 (CV 0.13%) for water being ≤ 14 %; for extractives content being ≥ 22%, sample A showed 39.74% ± 0.017 (CV 4.22) and sample B 30.94% ± 0.007 (CV 2.42%); in the total ashes being ≤ 4% A indicated a result of 3.81% ± 0.10 (CV 2.65%) and B of 3.65% ± 0.05 (CV 1.29). Being the limits set according to the Chinese Pharmacopoeia Commission (2010). **Conclusion:** The results show that both suppliers are within the limits recommended by the Chinese Pharmacopoeia Commission (2010).

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QUALITY OF THE TRADICIONAL CHINESE MEDICINE *WOLFIPORIA EXTENSA* FUNGUS

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Key-words: Quality Control; Fu Ling; Zhong Yi Xue.

Introduction: *Wolfiporia extensa* (Peck) Ginns (fungus) or also known as Fu ling, presents in its composition mainly triterpenes, polysaccharides, and steroids. Fu ling is widely used in Traditional Chinese Medicine (TCM), and due to the growth of TCM in the west it is important that the quality control of the plants used is performed. **Aim:** Perform quality control comparing suppliers A and B of the *Wolfiporia extensa* fungus. **Methods:** Morpho-anatomical analyzes (macroscopic identification), identification by thin layer chromatography (TLC), purity tests (water, extractives content, and total ash) were performed for both suppliers. **Results:** In the morpho-anatomical analysis (macroscopic identification) it was possible to observe characteristic structures of the *Wolfiporia extensa* for both suppliers; In TLC a retention factor of 0.53 was observed for the reference drug, and 0.53 and 0.55 for the samples A and B respectively, thus showing that the suppliers have the same compounds as the reference drug of Fu ling. For purity tests supplier A presented 12.76% ± 0.27 (CV 2.10%) and supplier B 15.65% ± 0.21 (CV 1.31%) for water being ≤ 18 %; for extractives content being ≥ 2.5%, sample A showed 2.74% ± 0.001 (CV 3.56) and sample B 2.39% ± 0.001 (CV 2.98%); total ashes ≤ 2% A showed a result of 0.44% ± 0.01 (CV 2.60%) and B of 0.18% ± 0.02 (CV 9.62) sample B showed a high CV due to material loss that occurs during the temperature change to 400 ºC making it impossible to perform the method with a low CV. The limits being set according to the Chinese Pharmacopoeia Commission (2010). **Conclusion:** The results show that both suppliers are within the limits recommended by the Chinese Pharmacopoeia Commission (2010) except for the determination of extractives and total ash content for supplier B.

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CBD PREVENTS MEMORY IMPAIRMENTS AND STIMULATES NEUROPLASTICITY IN ISCHEMIC RATS

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Key-words: Cannabidiol, brain ischemia, neuroprotection.

Introduction: Transient and Global Cerebral Ischemia (TGCI), an immediate and severe outcome of reversible cardiac arrest, has been characterized by a global reduction of cerebral blood flow, hippocampal neurodegeneration and, deficits in cognitive functions[1]. Neuroplastic phenomena may occur in neurons that survive an ischemic insult, as adaptive changes possibly supporting neuronal and functional recovery. Cannabidiol (CBD), a non-psychoactive constituent of *Cannabis sativa*, has been proposed as a potential therapeutic strategy for the treatment of several neuropsychiatric conditions[2]. **Aim:** To investigate whether CBD is effective to attenuate the impact of TGCI on cognitive performance, neuronal viability, dendritic remodeling and, neuroplastic proteins. **Methods:** Rats were trained to learn the eight-arm aversive radial maze (AvRM) task and then submitted to the sham or TGCI surgery through permanent occlusion of the vertebral arteries with subsequent transient occlusion of the carotid arteries for 15'. Vehicle or CBD 10mg/Kg were administered (i.p.) 30 min before, 3 h after reperfusion and daily during 14 days. On the 7th and 14th days the retention memory trials (RMT) were carried out. Another group of sham or ischemic animals receiving the same pharmacological treatment were exposed to the object location test (OLT) to evaluate spatial memory. The brain of the animals was processed for immunohistochemistry, Western blot analyses and, Golgi-cox staining. **Results:** CBD treatment decreased the memory deficits caused by ischemia evaluated in the OLT and AvRM tasks. CBD attenuated CA1 neurodegeneration, while increased the BDNF and restored the PSD-95 levels in the hippocampus of TGCI rats. Additionally, CBD protected neurons from the deleterious effects of TGCI on the dendritic spine number and length of dendritic arborization. **Conclusion:** CBD neuroprotective effects are, at least in part, dependent on mechanisms underlying synaptic plasticity. These findings indicate that CBD may be useful for memory recovering and functional improvement in cerebral ischemic conditions.

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ALTERNATIVE IN PRODUCTION SUSTAINABLE OF THE PHENOLIC COMPOUNDS USING CULTURE OF THE CALLUS FROM *Cereus peruvianus* Mill.

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Key-words: Callus; Mandacaru; Mass spectrometry.

Introduction: *Cereus peruvianus* Mill. (*Cactaceae*) popularly is known as "Mandacaru", is native in arid and semiarid regions of the world, where Brazil is included as the third-largest center in *Cactaceae* biodiversity[1]. In search of a large-scale production of plant tissue and its biocompounds, callus culture of *C. peruvianus* was started[2]. **Aim:** The objective of the work was to establish and bioproduce phenolics compounds, using an alternative culture of callus of *C. peruvianus*, in which the conventional agar medium is replaced with cotton, along with the preliminar chemical characterization, using UHPLC-QTOF-ESI. **Methods:** Callus cultivated in conventional method were grown in Murashige e Skoog (MS) medium[2] and incubated for 45 days, and the alternative callus was cultured in Petri dishes, containing a thin layer of cotton covered with filter paper soaked with MS medium for 90 days. These cultures were maintained at 32 °C during a photo-period of 16 h (15 µmol m^{-2} s^{-1}, light intensity). Cladodes of *C. peruvianus* were collected in State University of Maringá. The crude extracts obtained by Soxhlet with ethanol 70% were extracts from plant cladodes (SEPC); conventional agar-grown callus (SECAC) and alternative cotton-grown callus (SEACC). Futhermore, crudes extracts were obtained by maceration with methanol, followed by a partition with dichloromethane (DCM) and hydromethanol fractions (HF), fractions from plant cladode (DCMPC and HFPC); conventional agar-grown callus (DCMCAC and HFCAC) and alternative cotton-grown callus (DCMACC and HFACC). Extracts and fractions were analyzed by UHPLC-QTOF-ESI, after the total phenolic content (TPC) and total flavonoid content (TFC) were determined by spectrophotometry. **Results:** Detected the presence of phenolic compounds in UHPLC-QTOF-ESI (derived ferulic acid and narcissin). The highest concentrations the TPC (µg equivalent gallic acid/mg) and TFC (µg equivalent quercetin/mg) in the callus were obtained with the dichloromethane fractions 384.4 ± 2.9 and 38.8 ± 1.9 (DCMACC); 279.9 ± 2.9 and 72.1 ± 1.7 ((DCMCAC), respectively. For cladodes samples 541.5 ± 3.1 ((DCMPC) in and 443.1 ± 2.9 (HFPC) the opposite occurred (P<0.05). In general, the maceration technique presented a greater extraction of phenolic compounds when compared to Soxhlet. **Conclusion:** Alternative callus culture has shown promise in the sustainable production of phenolic compounds i*n vitro*, therefore having potential antioxidant activity.

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DEVELOPMENT AND CHARACTERIZATION OF GASTRORETENTIVE FLOATING DRUG DELIVERY SYSTEMS OF SILDENAFIL CITRATE

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Key-words: quality-by-design, dissolution, incremental innovative.

Introduction: Gastroretentive drug delivery systems are modified release dosage forms designed with the purpose of prolonging the residence time in the upper gastrointestinal tract. These systems with prolonged residence time in the stomach can be useful for drugs with a narrow absorption window in the upper small intestine. Sildenafil citrate has this absorption behavior. It is a weak base with high solubility at acid pH and very low in pH above 6.0 and consequently, low bioavailability[1,2,3]. **Aim:** Development and characterization of a gastroretentive floating drug delivery system of sildenafil citrate using combination of rate controlling hydrophilic polymers by implementing quality by design approach (QbD). **Methods:** The design space and optimum formulation were established through a mixture design. Quality target product profile and critical quality attributes were defined according to the preliminary studies. The drug release kinetics were evaluated through the dissolution profiles using basket apparatus (100 rpm) and 0.1 M hydrochloric acid for 24 hours. In addition, buoyancy studies, friability, hardness, assay and content uniformity were evaluated. **Results:** The formulations with different amounts and viscosity of hydroxypropyl methylcellulose (HPMC) generated different dissolution profiles, actually proving to be the modulator of drug release kinetic. However, the all the formulations presented first-order kinetics. The flotation lag time was less than 20 seconds for four of five formulations as well the total floating time that was about 24 h for the formulations with more sodium bicarbonate. All the formulations showed good results of assay, friability and content uniformity. **Conclusion:** The present study demonstrated the potential of QbD for development of tablet formulations. Most of the formulations in the design space are regarded as they show sustained release for 24 h with first-order release. The floating property of tablets should be secured prior to use and the relationship between polymer and sodium bicarbonate showed be important for this quality attribute. Consequently, application of experimental design was shown to be a useful tool for QbD.

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ANETHOLE AND IBUPROFEN COMBINATION AT LOW DOSES PRESERVES GASTRIC MUSIC OF ARTHRITIC RATS

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Key-words: Antiinflammatory drug, adjuvant-induced arthritis, gastric lesion.

Introduction: Gastric lesions are one of the most common adverse effects of nonsteroidal antiinflammatory drug (NSAID) treatment when used at high doses or for prolonged periods of time, ranging from stomach discomfort to severe ulcerations that are characterized by perforation and bleeding.[1] Thus, the discovery of therapeutic alternatives with good antiinflammatory effects and lower gastric adverse effects is important. Previous results showed that the combination of anethole (AN), a compound of natural origin, and ibuprofen (IB), a traditional NSAID, both at low doses, was effective for reducing the inflammatory parameters in the adjuvant-induced arthritis (AIA) model.[2] Therefore, we find it important to investigate the effect of the AN + IB combination on the gastric mucosa. **Aim:** The present study evaluated the effects of the AN + IB combination compared with AN and IB monotherapies on the gastric mucosa in arthritic rats. **Methods:** After induction of arthritis by a subcutaneous injection of Freund's complete adjuvant into the plantar surface of the left paw, Holtzman rats (n=5/group) were orally administered once daily with AN + IB (62.5 mg/kg + 8.75 mg/kg), IB (35 mg/kg), and AN (250 mg/kg) for 21 days. The stomachs in the different groups were removed and processed for histological analysis using hematoxylin and eosin (HE) and Periodic Acid Schiff (PAS) staining. Immunohistochemical analysis with proliferation cell nuclear antigen (anti-PCNA) was also performed. The experimental protocol was approved by the Animal Ethics Committee of the State University of Maringá (protocol no. CEUA 7896220716). The data were analyzed using ANOVA - Tukey's test ($P < 0.05$). **Results:** The histological analysis showed structural and morphological alterations in the stomach in arthritic animals, including disorganization of the mucosal architecture, a dilated glandular structure, parietal cell hypertrophy, and an increase in mucin content. Treatment with the AN + IB combination did not influence these alterations, whereas IB treatment alone caused additional alterations (e.g., ulcerations, decrease in mucosal thickness, and increase in mucin content) and AN treatment alone caused mucosal thickening and intense cell proliferation in the gastric mucosa. **Conclusion:** The results showed evidence that the arthritis-induced gastric histological changes in rats. Additionally, the data indicate that AN + IB combination at lower doses (4-fold lower) than AN and IB treatment alone did not cause gastric lesions.

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ESSENTIAL OIL OF *LAVANDULA OFFICINALES* PROTECT AGAINST ACUTE ACETAMINOPHEN HEPATOTOXICITY IN MICE

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Key-words: Acute hepatic injury; Lavender essential oil; Hepatotoxicity

Introduction: Acetaminophen (N-acetyl-p-aminophenol, APAP), overdose is one of the most common causes of drug-induced acute liver failure. *Lavandula officinalis* is a natural product with antioxidant and anti-inflammatory activities. **Aim:** Investigate the protective effects of lavender essential oil (LEO) on liver damage induced by paracetamol in mice. **Methods:** In the present study, the hepatoprotective effects and the underlying mechanisms of lavender essential oil (LEO) in APAP-induced hepatotoxicity were investigated. Balb/c mice were pretreated with LEO (200 and 400 mg/kg), Silymarin or vehicle once daily for seven days. On the seventh day, mice were injected with a single dose of APAP (250 mg/kg) to induce acute liver injury. After 12 hours of APAP administration, the animals were anesthetized with thiopental and the peritoneal cavity was exposed. Blood was collected for biochemical analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (γGT) [1]. Results are reported as the mean ± standard error of the mean. Significance was assessed by one-way analysis of variance (ANOVA), followed by Tukey's test. **Results:** ALT, AST, ALP and γGT values were significantly increased with the administration of APAP, indicating hepatic injury. However, the LEO 200 mg/kg and LEO 400 mg/kg groups significantly prevented changes in AST activity (85 % and 90%, respectively), ALT (86% and 87%, respectively), ALP (43% and 48%, respectively) and γGT (53% and 68%, respectively), compared to the APAP group. Treatment with 200 mg/kg silymarin inhibited APAP toxicity and the values of the above mentioned serum parameters were significantly reversed. **Conclusion:** Our results demonstrated that treatment with LEO improves hepatic functions in paracetamol-induced hepatotoxicity as evidenced by reduction in serum liver enzymes.

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ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *Plinia rivulares* (CAMBESS) ROTMAN (MYRTACEAE)

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Key-words: chromatography, kaurene, diterpeno

Introduction: *Plinia rivularis* is popularly known as guamirim, jaboticabarana, guapuriti and baporeti. This species belongs to the Myrtaceae family and is one of the most important families in Brazil. **Aim:** This work aims to identify and characterize the oil obtained from species *Plinia rivularis* (Myrtaceae). **Methods:** Extraction of the essential oil was performed by hydrodistillation using a Clevenger apparatus, at the end of extraction the yield will be calculated in relation to fresh material. To identify the chemical constituents, the oil was subjected to gas chromatography coupled to mass spectrometry (GC-MS), and it was possible to determine the chemical composition by comparing the calculated Kovats index (IK) and comparing it to the Adams literature, 2017. Antimicrobial activity was performed by the Minimum Inhibitory Concentration (MIC) method using two gram positive bacteria *Staphylococcus aureus* and *Enterococcus Faecalis* and one gram negative *Escherichia coli*. **Results:** The average yield of *P. rivularis* essential oil was 0.29%. The oil presented in it composition 2.06% of oxygenated monoterpenes, 17.15% of hydrocarbon sesquiterpenes, 49.98% of oxygenated sesquiterpenes and 11.82% of diterpene. The main compounds identified were sesquiterpenes rosifoliol (12.53%), β-eudesmol (7.63%) and diterpene Kaurene (11.82%). *P. rivularis* oil presented MIC of 0.56 mg/mL for *S. aureus* and *E. faecalis* strains and for *E. coli* MIC of 2.25 mg/mL. **Conclusion:** A highlight of this oil is the presence of a diterpene (Kaurene) in significant concentrations, as diterpenes are found with low frequency in volatile oil compositions. For antimicrobial activity the oil showed strong activity for *S. aureus* and *E. faecalis* bacteria and weak activity for *E. coli*.

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EVALUTION OF THE EFFECT OF NUX VÔMICA ON METABOLIC ALTERATIONS IN MICE SUBMITTTED TO ANTIRETROVIRAL THERAPY

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Key-words: HIV/AIDS, HAART, Nux vômica

Introduction: Highly Active Antiretroviral Therapy (HAART) is associated with a significant reduction in mortality in patients with HIV / AIDS.[1] Every year, the interest and demand of the Brazilian population and also in other countries for "Unconventional Health Practices" (PNCS) increases, emphasizing phytotherapy, homeopathy and acupuncture. The OMS encourages the use of Homeopathy.[2] Nux vômica is a homeopathic medicine produced from desiccated seeds of the tree called Nux vômica (*Strychnos nux-vomica*). Pharmacologically it has been tested as anti-inflammatory, anti-microbial, antioxidant and anti-cancer.[3] **Aim:** This project proposes to evaluate the effect of Nux vômica in homeopathic formulation in mice submitted to HAART therapy. **Methods:** Experimental groups of 10 Swiss mice with 38-40 days were constituted, treated for 15 days: (I) HAART-treated animals diluted in 1.2 mL of water and administered by gavage / day, (II) HAART-treated animals diluted in 1.2 mL of water per gavage / day + diluted Nux vômia 1x1012 added to drinking water (1:10 ml) and made available *ad libitum*, (III) HAART treated animals diluted in 1.2 ml water per gavage / day + diluted Nux vômica 1x1060 added to drinking water (1:10 mL), (IV) untreated control group receiving 1.2 mL of water per gavage / day. Weight gain, triglycerides, creatinine, AST, ALT, cholesterol, liver and spleen weight were evaluated. Results were analyzed using *Graph Pad Prism*. **Results:** Compared to the results of the groups administered with HAART and HAART + Nux vômica, we obtained: increased body weight, showing that the rats were able to feed more; increased creatinine, which indicates kidney failure; increased AST, indicating liver failure; and enlargement of the liver and spleen, indicating overload of these organs. And in return we obtained: decrease of ALT; lowering cholesterol, decreasing chances of dyslipidemia; and decreased triglyceride, improving for the non-appearance of heart disease.[4] **Conclusion:** With the results obtained in this study, we conclude that the drug Nux Vômica in the combination administration with antiretroviral cocktail is beneficial in relation to cholesterol, triglycerides, and weight gain, but it should be further studied further its toxic effects on liver and kidney levels.

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PHARMACOTHERAPETIC MONITORING OF PATIENTS WITH METABOLIC SYNDROME

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Key-words: Metabolic Syndrome, Pharmaceutical Care, Pharmaceutical Monitoring

Introduction: Metabolic syndrome (MS) is a patology characterized by several metabolic disorders that lipid metabolism disorders, elevation of blood pressure and obesity[1]. Obesity should therefore be the main target of treatment of Metabolic Syndrome. Weight loss alone improves lipid profile, lowers blood pressure and blood glucose. **Aim:** Perform pharmacotherapeutic follow-up to improve the quality of treatment of patients with Metabolic Syndrome[1,2]. **Methods:** Interviews were conducted from August 2017 to July 2019 with employees of the State University of Maringá, registered in the Occupational Safety and Medicine Service (SESMT) fit the patterns of MS. In the first interview answered about medication, physical activity, diet and other pathologies. After the case study, intervention plan was elaborated and in a second interview, each patient was oriented. In a thrid interview the results were evaluated. **Results:** The study was conducted with 52 patients. The average age was between 40 to 49 years and there was a prevalence of womans (75%). 70% of patients described PRM (treatment-related problems). Of these, 90% had PRM 7 which is related to treatment adherence, 7% PRM 1, where therapy was unnecessary and 3% PRM 2, where the medical condition requires additional treatment. The most frequent of all pathologies of SM were Hypertension. The most commonly used drugs for hypertension were Hydrochlorothiazide and Losartan. In addition, all patients reported weight problems, including obesity (91%) and overweight (9%). At the end of the interviews and the pharmaceutical care provided (involving time shifts, substitutions and withdrawals of medications, plus encouragement of dietary and eating reeducation practices), were observed the resolution of all PRMs and a significantly improves weight in 15% of patients. **Conclusion**: It can be concluded that a healthy life practice, including exercise and a balanced diet along with the correct pharmacological therapy, where attention and pharmaceutical care are present, are essential for the treatment of MS.

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EVALUATION OF THE PERMEATION OF EMULSIONS CONTAINING SAPONINS AND CELLULOSE NANOCRYSTALS IN THE SKIN BY UV-VIS PHOTOACOUSTIC SPECTROSCOPY

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Key-words: Emulsions; Permeation; UV-Vis photoacoustic spectroscopy.

Introduction: Emulsions are pharmaceutical forms of topical administration that need to be evaluated for their skin permeation ability to ensure drug delivery and consequently the efficiency of therapeutic properties (1, 2). **Aim:** Evaluate the permeation of emulsions in pig ear skin *ex vivo* by ultraviolet and visible (UV-Vis) photoacoustic spectroscopy. **Methods:** Emulsions were prepared from different concentrations of cellulose nanocrystals, saponins, oil extracted from the seed of *Sapindus saponaria* L. and water. They were sonicated in a sonicator (Bioblock Scientific, VibraCell 75115, Autotune series, model 750 watts), with agitation duration of 5 minutes, pulse on 5" and pulse off 3" at room temperature. The experimental procedure was performed using pig ears skin samples, with thicknesses of 756 μm and diameters of approximately 5 mm. Measurements were made in triplicates and from the spectra of each emulsion group an average was made. It was applied with a pipette 10 μL of the emulsion on the skin surface and kept for one hour before performing the analysis. The experiments were performed with photoacoustic spectroscopy (Oriel, modelo 68820; 77250, 1/8 m; 77296; Stanford Research Systems, modelo SR 540; Brüel & Kjaer, modelo BK 2669; EG&G PAR Instruments) in the ultraviolet-visible spectral regions, between 270 and 600 nm. **Results:** The results showed the oil bands in the epidermis and dermis, considering that the oil droplets are the internal phase of the emulsion, this predicts that the emulsion permeated. Therefore, all formulations presented a good permeation performance, except emulsion 04 (10% nanocrystals + 6.5% saponins) which, through area calculation, presented a lower permeation rate than the others, probably due to the higher concentration of cellulose nanocrystals that increase the viscosity of the emulsion, making it difficult diffusion through the layers of the skin. **Conclusion:** The analysis revealed the efficiency of all emulsions to permeate through skin tissues suggesting that they can be used as carriers for topically applied drugs.

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PRECLINICAL PHARMACOKINETIC ANALYSIS OF CANNABIDIOL AFTER ORAL ADMINISTRATION

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Key-words: noncompartmental analysis, absorption, cannabinoids

Introduction: The clinical use of cannabidiol (CBD) as antiepileptic is increasing and improving information about pharmacokinetics and pharmacodynamics profiles is important to make this drug safer and more effective. **Aim:** The present work aimed to determine preclinical pharmacokinetic parameters of CBD administered orally in Wistar rats, as well as to optimize the analytical conditions for CBD quantification in rat plasma by UPLC-MS/MS. **Methods:** The animal experiments were approved by Animal Ethical Committee (protocol number CEUA/UEM: 5520250918, ID: 002242). CBD was dissolved in sunflower oil and administered by gavage in a single dose (120 mg/kg). Chromatographic separation was achieved on ACE® 3 C18 column (50x4,6 mm, 3 µm). The mobile phase was composed by water (0,1% v/v ammonium formate 5 $mM.L^{-}$) (A) and methanol (B) under the following gradient profile: 0-2 min (85% B), 2-3 min (100% B), 3-7 min (100% B), 7-7.1 min (85% B), 7.1-11 min (85% B). The analytes were prepared with 10 µL of diazepam (IS, 2 µg/mL), 150 µL of acetonitrile and 740 µL of hexane added to 100 µL plasma. The mixture was centrifuged, the hexane phase was dried under vacuum condition and the dried extract was resuspended with methanol. Mass spectrometry was performed in MRM mode using electrospray ionization in positive mode. All results were used to calculate pharmacokinetic metrics using PKanalix®2019R2 (Lixoft, Antony, France). **Results and discussion:** The linear range of quantification was 0.025-1.0 µg/mL (r^2 = 0.996). The mean pharmacokinetic parameters obtained (*n* = 8) were: AUC_{0-24} (µg*h/mL) = 4.52 ± 1.79, C_{max} (µg/mL) = 0.71 ± 0.22, t_{max} (h) = 3.34 ± 1.34, λ_z (h^{-}) = 0.09 ± 0.04, $t1/2\_{\lambda z}$ (h) = 8.30 ± 4.19, Cl/F_{Clast} (L/h) = 8.89 ± 4.03, V/F (L/kg) = 103.69 ± 60.08, MRT_{last} (h) = 6.70 ± 1.44. The wide variability observed in pharmacokinetic parameters suggest that, for humans, this variability could happen as well. Another important point is related to test a final formulation, since absorption is also related to the formulation. Therefore, biopharmaceutical data is as important as PK-PD information. **Conclusion:** All pharmacokinetic parameters showed that CBD is slowly absorbed and relatively slowly eliminated. The results also indicate a wide distribution in the body.

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THE PHOSPHODIESTERASE TYPE 4 INHIBITOR ROFLUMILAST CONFERS NEUROPROTECTION AGAINST CEREBRAL ISCHEMIA BY TARGETING NEUROINFLAMMATION

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Key-words: transient global cerebral ischemia, roflumilast, memory.

Introduction: Phosphodiestarese 4 (PDE4) inhibitors have been shown to present beneficial effects in ischemic cerebral injury because of their ability to improve cognition and target different phases and mechanisms of cerebral ischemia, including apoptosis, neurogenesis, angiogenesis, and inflammation. **Aim:** The present study investigated whether repeated treatment with the PDE4 inhibitor roflumilast prevented memory loss, impact neuroplasticity and attenuated neuroinflammation in rats following transient global cerebral ischemia (TGCI) (1). **Methods:** Wistar rats (CEUA 5529100517) underwent 4-vessel occlusion model of TGCI. Roflumilast or vehicle was administered for 21 days after ischemia. On day 7, 14 and 21 the rats were tested in the aversive radial maze (AvRM), to evaluated retrograde memory. After behavioral testing, the hippocampus were examined for neuroplasticity markers including doublecortin (DCX) and phospho-cyclic AMP-response element binding protein (CREB) and inflammation markers such as the ionized calcium binding adaptor molecule 1(Iba-1), *glial fibrillary acidic protein* (GFAP), Arginase-1 (Arg-1), the cytokines IL-10 and IL-4. **Results:** TGCI caused memory impairments, neuronal loss, compensatory neurogenesis, and increase in the protein levels of inflammatory markers in the hippocampus in TGCI rats. Repeated treatment with roflumilast prevented cognitive deficits without promoting hippocampal histological neuroprotection in ischemic animals. Roflumilast also attenuated hippocampal pCREB expression that was induced by TGCI in the CA1 and dentate gyrus (DG) of the hippocampus. Roflumilast did not affect the hippocampal levels of neuroinflammatory markers, but it increased the levels of arginase-1, IL-4, and IL-10 21 days after TGCI, indicating an antiinflammatory effect. **Conclusion** Altogether, these data indicate a protective effect of roflumilast against sequelae of cerebral ischemia which might be related to its antiinflammatory properties.

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SYNERGISTIC ACTIVITY *in vitro* OF KETOCONAZOLE AND DOXORUBICIN INDUCES OXIDATIVE STRESS IN PROSTATE CANCER

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Key-words: Synergism, oxidative stress, prostate cancer.

Introduction: Prostate cancer is the most common cancer in the male population in Brazil and the second in the world[1]. This neoplasm can be characterized as castration-resistant (CPRC) and, at this stage, the chemotherapy options are restricted. Ketoconazole is an antifungal used as an off-label in the second line of therapy in patients with CPRC[2], but its action mechanisms still unknown. The doxorubicin is a broad-spectrum antineoplastic agent used to treat some tumors. In this context, an alternative for the treatment of this pathology is the association between drugs. **Aim:** It was evaluated the synergism and the *in vitro* action mechanism of ketoconazole and doxorubicin against castration-resistant prostate cancer (PC-3). **Methods:** Cytotoxicity and synergism were evaluated by MTT method, cell morphology, reactive oxygen species (ROS) by H_2DCFDA marker, hydrogen peroxide by Amplex Red®, thiol levels by Ellman's reagent and reduced glutathione by OPT reagent. Statistical analysis was performed by GraphPad Prism 5.0 software (San Diego, CA, USA) and the synergic effect by CompuSyn v.1.0. **Results:** For the MTT assay, the IC_{50} for ketoconazole was 34.93 ± 2.72 µM and for the doxorubicin was 2.88 ± 0.34. When combined, the IC_{50} was 14.57 ± 2.08 and 1.39 ± 0.16 µM for ketoconazole and doxorubicin, respectively, with a combination index was 0.6, indicating synergism. Morphological changes such as size increase and cells containing two nuclei were observed. There was a higher ROS production after the treatments, especially with the combination, and the co-treatment with antioxidant N-acetylcysteine (NAC) was not significantly different. Doxorubicin and the combination significantly increased hydrogen peroxide levels and, in the presence of NAC, this activity was reduced. In the quantification of the antioxidant system, we observed that there was a suppression of total thiol levels, but only the combination reduced glutathione levels. Increased ROS and suppression of the antioxidant system characterize oxidative stress[3]. **Conclusion:** The combination of ketoconazole and doxorubicin may be a good candidate for prostate cancer treatment, but further studies are needed.

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APPLICATION OF RHEOLOGY AND BIOPHYSICS METHODS IN *FACE CARE*® MOISTURIZERS AND ITS VEGAN VERSION

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Key-words: Photoprotection; Skin biometrics; Rheology

Introduction: Skin is an important organ of the body human, its functions are thermoregulation, vitamin D production, besides being a protective barrier against external agents[1]. There are several causes for skin-related conditions, including ultraviolet radiation, so it is extremely important to use photoprotective[2] and multifunctional formulations. Multifunction products are cosmetic preparations which UV protection is an additional benefit of the product, not the primary intuit[3]. In addition, studies have demonstrated efficacy, good compatibility and a variety of applications of vegan formulations, which have not animal origin[4]. **Aim:** Thus, the aim of this paper was to compare two multifunctional formulations, one of which is vegan, using biometric and rheological methods. **Methods:** Skin biometry tests were performed using the Courage Khazaka Multi Probe Adapter equipment. The study involved 28 volunteers who were administered the formulations topically, and it was approved by the ethics committee (2,990,495). The equipment used for rheological analysis was a MARS II (Haake) with parallel cone-plate geometry of 35 nm in diameter, at temperatures of 4, 25, 34 and 40°C ± 0,1 °C. **Results:** Biometric analysis showed that cutaneous pH remained in normal condition and no significant differences between time and the products tested. Skin sebum content increased significantly in both formulations, and that was repeated in the hydration parameter only in males. Rheological analyzes showed pseudoplastic and thixotropic profiles. Besides that, viscosity decreased as temperature increased. **Conclusion:** Both formulations described similar rheological behavior, in addition to improving some biometric parameters of the skin. It is important to study vegan products to reach different audiences.

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METABOLITES OF *Burkholderia pyrrocinia* WITH ACTIVITY IN SENSITIVE ANDRESISTANT STRAINS OF *Acinetobacter baumannii* AND *Klebsiella pneumoniae*

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Keywords: *Burkholderia pyrrocinia;* bacteria secondary metabolites, clinical isolated bacteria.

Introduction: The selection of resistant microorganisms requests the discovery of new antibiotics. One of the most utilized sources in researches are bacterial secondary metabolites. *Burkholderia pyrrocinia*RV1R2 strain was isolated in Rio Verde-Itararé/SP (Brazil) and identified previously by gene sequencing (data not shown) and has demonstrated to produce compounds with antimicrobial activity. **Aim:** The aim of this research was to test grown inhibition of clinical isolated Gram-negative bacteria of *Klebsiella pneumoniae* (*Kpn.*) ATCC 10031(sensitive)and *Acinetobacter baumannii* (*Ac.*) S 171/15 (sensitive) and OXA 23 (resistant) strains. **Methods:** The strain was cultivated in 1L of nutrient broth, with an inoculum of 1% v/v ($1,5x10^8$ $CFU.mL^{-1}$) and incubated in shaker for 7 days at 28°C and 170 rpm. The supernatant was obtained by centrifugation and dried until almost 10% of initial volume. Antimicrobial activity of a raw supernatant was tested by diffusion well test in Petri dishes with Mueller Hinton Agar (MHA) with 1mL of a bacteria inoculum ($1,5x10^8$ $CFU.mL^{-1}$). After the confirmed activity, produced metabolites were separated by partition liquid-liquid, 3 times, with ethyl acetate (2:1 of supernatant) and was obtained the EAP (ethyl acetate phase). The activity of EAP was evaluated by disk diffusion test with a $500\mu g.disk^{-1}$ of EAP in cultures inoculated with 10^8 CFU of each bacteria's strains. **Results**: In the raw supernatant test, the inhibition zone was 21mm to *Kpn* ATCC 10031, 21mm for *Ac.* 171/15 and 25mm for *Ac.* OXA-23. In disk diffusion test was obtained zones of 14mm for *Kpn* ATCC 10031, 16mm for *Ac.* 171/15 and 18mm for *Ac.* Oxa-23. **Conclusion:** The raw supernatant and EAP fraction of *Burkholderia pyrrocinia* secondary metabolism has shown great results. Therefore, we will continue the research to improve the production and extraction, besides the identification of the antimicrobial activity compounds.

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SEPARATION OF CATECHIN STEREOISOMERS BY SUPERCRITICAL FLUID CHROMATOGRAPHY

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Key-words: supercritical fluid, chromatography, catechins.

Introduction: A supercritical fluid is a state of mater where liquids and gases are indistinguishable from each other. Upon critical point the fluid has compressibility and diffusivities as a gas, and density and viscosity as a liquid. These unique characteristics confer to supercritical fluid a better option as a mobile phase in chromatography[1]. **Aim:** In this study was explored the capacity of carbon dioxide supercritical to separate (+) and (-) catechin stereoisomers. **Methods:** The Supercritical Fluid Chromatography used a Jasco system, with detectors Circular Dicroism (CD-4095) and Photodiode Array (MD-4015), Autosampler (AS-4350), Oven (CO-4065), Back Pressure (BP-4340), Module PU-4386 and Interface LC-NetII/ADC, coupled OD-H Chiralpack column (250 mm and 3 µm particles). **Results:** Carbon dioxide supercritical without add of modifiers was tested, the solvation powder was not sufficient to eluate all catechin injected. So, ethanol, methanol, and water were used to eluate catechin from system. **Conclusion:** The use of organic modifiers in carbon dioxide supercritical chromatography is necessary for catechins elution from OD-H Chiralpack column. Other system conditions will be applicate employing different amounts of organic modifiers and anothers columns in further studies.

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EVALUATION OF MONOSACCHARIDE COMPOSITION OF EXOPOLYSACCHARIDE PRODUCED BY *KLEBSIELLA OXYTOCA*.

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Key-words: Polysaccharides; Determination; Chromatography.

Introduction: The bacteria *Klebsiella oxytoca*, isolated from the soil, produces an exopolysaccharide (EPS) that has shown biological activity[1]. **Aim:** Characterization of the monosaccharides that compose the EPS produced by *K. oxytoca* through High Performance Liquid Chromatography - Refractive index (HPLC-RI) and Gas Chromatography–Mass Spectrometry (GC-MS). **Methods:** A EPS sample (2 mg) was converted to alditols-acetates and analyzed in GC-MS according Sassaki et al. (2008)[2], with modifications, inositol was used as internal standard. HPLC-RI evaluation was performed according Sigma-Aldrich (2019)[3], with modifications, was used a EPS sample (103.5 mg). The mobile phase was prepared by the mixture of 75:25 acetonitrile: water. In both analyzes, the results were analyzed by comparing the chromatograms of sample with monosaccharide standards (Sigma®). **Results:** The chromatographic profile obtained by GC-MS showed two peaks, the first more intense with RT: 12.36 min and the second with a lower intensity of RT: 20.57 min. Compared to the standards analysis rhamnose (RT: 12.43 minutes) and galactose of (20.73 minutes), indicating the presence of this monosaccharides, in a concentration of 413.19 µg (77.33%), 121.12 µg (22.66%) respectively. In the analysis of HPLC - IR, EPS showed two peaks, the first one at 5.43 minutes and the second one at 9.48 minutes, indicating the presence of rhamnose (TR: 5.43 min) (63,35%) and galactose (RT: 9.50 min) (36,64%). **Conclusion:** In this work it was possible to identify the monosaccharides rhamnose and galactose in the chemical composition of bacterial EPS produced by *K. oxytoca* and determine the concentration of these compounds through chromatography techniques HPLC-RI and CG-MS.

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CHEMICAL CONSTITUENTS AND *IN VIVO* EFFECT OF ETHYL ACETATE FRACTION FROM *Campomanesia guaviroba* ON UVB-INDUCED CATALASE DEPLETION AND SUPEROXIDE ANION PRODUCTION

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Key-words: photochemoprotection; oxidative stress; Myrtaceae.

Introduction: Supplementation of the skin barrier with antioxidant substances, especially substances of plant origin, has gained attention in dermocosmetology, because sunscreens alone do not act on biochemical mechanisms of the skin that are affected by excessive exposure to ultraviolet radiation, including reactive oxygen species production and endogenous antioxidants depletion. *Campomanesia guaviroba* is a species that remains poorly studied chemically and biologically and may be a promising source of compounds that have activity against the deleterious effects of UVB radiation on the skin. **Aims:** To evaluate the effect of ethyl acetate fraction from *C. guaviroba* (EAF) on catalase activity and superoxide anion ($O_2^{\cdot-}$) production and identify the main chemical constituents of the EAF. **Methods:** The ethanolic extract (EE) from leaves of *C. guaviroba* D.C. Kiaersk (Myrtaceae) was concentrated under vacuum and lyophilized. Part of the EE was dissolved in methanol: H_2O (1:1) and partitioned with hexane and ethyl acetate. The EAF underwent column chromatography on silica gel, affording Cg-1 by recrystallization. To measure catalase activity and $O_2^{\cdot-}$ production the mice were randomly distributed into four groups (n=5/group): G1 (non-irradiated and untreated), G2 (irradiated and untreated), G3 (irradiated and treated with formulation base), and G4 (irradiated and treated with formulation that contained 1% EAF). The mice received the respective treatments on the dorsal surface of the skin 12 h, 6 h, and 5 min before and 5 min after UVB irradiation (4.14 J/cm²)[1]. The animals were euthanized 2 h after UVB exposure. Catalase activity and $O_2^{\cdot-}$ production assays was conducted[1]. The study was approved by the Animal Ethics Committee of the State University of Londrina (CEUA/UEL process no. 15805.2015.99). **Results:** Significant differences were found between G3 and G4 in both assays. Treatment with the EAF (G4) restored catalase levels (0.084 U catalase/mg of skin/min) and inhibited $O_2^{\cdot-}$ production (0.32 optical density at 620 nm [OD_{620}]/10 mg of skin) compared with G2 (0.047 U catalase/mg of skin/min and 0.75 OD_{620}/10 mg of skin). Cg-1 was isolated as a white amorphous powder and spectroscopically analized (^{1}H NMR, ^{13}C NMR, HSQC and HMBC) and in comparison with literature[2] identified as ellagic acid. **Conclusion:** The results demonstrated that the EAF of *C. guaviroba* may be a promising source of antioxidant substances, such as ellagic acid, that can attenuate the deleterious oxidative effects of UVB on the skin.

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DEVELOPMENT AND PHYSICOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF MICROPARTICLES CONTAINING THE COMBINATION OF BERBERINE AND FLUCONAZOLE ANTIFUNGAL AGENTS

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Key words: Berberine; Fluconazole; *Candida* spp.

Candida spp. it is a commensal microorganism that can remain in the body without major damage. However, if there is any kind of imbalance in the host, they may manifest orally, intestinally or vaginally. The berberine, an isoquinoline alkaloid, has shown wide varieties of benefits in antifungal filters. Fluconazole is a well-tolerated antifungal triazole and a safe agent that exhibits good clinical activity against most *Candida* spp, but already shows fungal resistance due to its indiscriminate use. The objective of this work is to produce microparticles containing two substances that present synergism, to obtain the chemical and microbiological physical analyzes for subsequent biological tests in patients who present oral candidiasis by total prosthesis. The microparticles were produced by spray drying technique to encapsulate Berberine and Fluconazole substances, morphology, scanning electron microscopy, Raman spectroscopy and FT-IR, the minimal inhibition assay (MIC), anti-biofilm effect in the formation phase. The results obtained were, a circular shape morphology, with some holes in its characteristic microparticle surface. Scanning electron microscopy made it possible to verify the treatment with a microparticle compared to the selected drugs. The microorganism and damage to its cellular structure. Raman and FT-IR spectroscopy show the presence of Berberine and Fluconazole in the microparticles through their characteristic peaks: 1065 cm^{-1}, 1681 cm^{-1} and 1276 cm^{-1}, 1421 cm^{-1} respectively.

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STUDY OF THE INTERACTION OF PATHOLOGICAL BACTERIA WITH FUNGI ISOLATED FROM HUMAN SKIN

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Key-words: Co-culture; fungus; microorganisms.

Introduction: Fungi and bacteria are important sources of bioactive compounds[1]. The co-culture technic is an efficient way for prospecting metabolites, since, it simulates ecological interactions for competition of two or more microorganisms on an enclosed space[2]. For elucidation of these metabolites, techniques and identification tools based on mass spectrometry and data analysis can be used. In this study, was applied the metabolomic approach using the co-culture methodology of commensal fungi isolated from human skin, against potentially pathogenic yeasts or bacteria, in order to induce production of secondary metabolites, followed by analysis by ultra-high performance liquid chromatography (UHPLC), with MS/MS, in addition with the use of the Molecular Networking (MN) tool to identify metabolites. **Aim:** Study a co-culture environment between the fungus *Phoma sp*. (F40) with *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) bacteria using UHPLC-MS/MS and MN. **Methods:** Commensal fungi F40 co-culture relationship with bacteria PA and SA were monitored for 7 days by photographs, to evaluate morphological alterations and interactions between species. With 7 days of incubation, the region with inhibition were occupied by the fungi, this interaction can trigger metabolites with potential antibacterial activity[2]. The interaction was removed, extracted with solvent and analyzed with MS tool and the data were treated with MN. **Results:** MN provided information about the ion distribution. About 704 compounds were detected, 7% (56 chemical entities) were matched with the database available in the GNPS platform[3], 9% (63 ions) came exclusively from the co-culture. Through *in silico* analysis were possible identify compounds from lactam family and one Benzodioxole compound. These compounds showcase biological activities and came exclusively from the co-culture. **Conclusion:** MS and MN tool helps the compound characterization, indicating that the co-culture provides production of different substances from pure cultures, demonstrating the potential of this methodology for discovering new molecules.

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EXPERIMENTAL MODEL OF AN ESTABLISHMENT PARA ASSESSMENT OF POTENTIAL CARCINOGEN 7,2-DIMETILBEZENTRACENO USING THE TECHNIQUE OF REAL-TIME PCR (RT-QPCR)

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Keywords: DMBA; RT-qPCR; breast cancer.

Introduction: The 7,12- Dimethylbezantracene is widely used in experiments related to breast carcinogenesis. **Objective:** To establish an experimental model based on the RT-qPCR technique to evaluate gene expression of carcinogenesis-related genes. **Material and methods:** Twenty one 50-day-old *Wistar* rats were divided into two groups and treated with a single dose of 65 mg / kg DMBA diluted in 1 mL corn oil (test group) or 1mL corn oil (control group). The rats were euthanized after 140 days and breast tissue RNA was extracted through lysis with Trizol . Subsequently, cDNA was synthesized and the RT-qPCR technique was used to determine the gene expression of six genes involved in breast carcinogenesis. The software StepOne TM Real Time PCR System equipment gives u the value of Relative Quantification (QR) equal to 1.0 for all genes evaluated in the control group. **Results:** The QR values neither of the test group samples can be I nor to or greater than 1.0; it represents increase or decrease of gene expression of genes evaluated. There were no significant differences in weight evaluation of the two groups evaluated, nor were palpable tumor masses observed. However, the *AR* genes; *MAPK1; MAPK3 and PGK1* presented QR values between 8.2 and 16.7 while *CTNNB1 and CDH1* genes presented values of 62.6 and 67.3 respectively. **Conclusion:** Despite the lack of formation of palpable tumor masses, the results show that DMBA was able to induce mammary carcinogenesis.

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MODIFIED HYALURONIC ACID HYDROGEL CONTAINING DIHYDROCAFFEIC ACID PROTECT FIBROBLASTS AGAINST UVB IRRADIATION

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Key-words: phenolic acid, modified hyaluronic acid hydrogel, anti-photoaging.

Introduction: UVB exposure can induces a set of skin alterations, including photoaging (1). Recently, we demonstrated that the phenolic acid dihydrocaffeic acid (DHCA) showed antioxidant and anti-photoaging properties on L929 fibroblasts irradiated with UVB (2). The polymer hyaluronic acid (HA) can be chemical modified with boronic acids, such as phenylboronic acid (PBA), and with saccharides molecules, to produce hydrogels crosslinked by boronate ester bonds formed between saccharide catechol group and PBA (3). Thus, we previously incorporated DHCA in a PBA-modified HA hydrogel (HG). **Aim:** Evaluate the cytotoxicity and the cytoprotective effect of the HG developed on L929 fibroblasts irradiated with UVB. **Methods:** L929 fibroblasts monolayer culture was treated with DHCA (7 - 35 µM), HG alone and HG containing DHCA (amount of HG containing 7 - 35 µM of DHCA) for 24 h. Thereafter, the cellular viability was evaluated by neutral red assay. To verify the cytoprotective capacity, the L929 fibroblasts monolayer culture was treated with the same samples concentrations previously described for 1 h before UVB irradiation (600 mJ/cm^2), incubated for 24 h and submitted to neutral red assay. All analyses were performed in triplicate. **Results:** The samples were not toxic to L929 cells in all range of concentrations evaluated, with a cell viability of 98%, 97% and 94% for the highest dose of DHCA (35 µM), plain HG (23 mg of HG) and HG containing DHCA (35 µM of DHCA in 23 mg of HG), respectively. In the cytoprotective assay, UVB irradiation significantly decreased cell viability at 600 mJ/cm^2 (cell viability of 52%) compared to nontreated and nonirradiated cells. On the other hand, DHCA alone showed a cytoprotective effect in a dose dependent manner, with a significant increase in the cell viability at higher dose evaluated compared to cells only irradiated (cell viability of 65% at 35 µM of DHCA). Interestingly, plain HG also protected L929 cells against death induced by UVB (cell viability of 68% for 23 mg of HG), and the HG containing DHCA provided a significant protective effect in all doses used (cell viability of 76% at 35 µM of DHCA in 23 mg of HG). **Conclusion:** The present work demonstrated that the innovative HG containing DHCA developed prevented UVB-induced fibroblasts death. Therefore, it is a promising anti-photoaging product.

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CELL VIABILITY AND ANTIADHESIVE ACTIVITY OF *MAYTENUS ILICIFOLIA* EXTRACT IN AGS CELLS AND *HELICOBACTER PYLORI*

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Key-words: *Maytenus ilicifolia*, *Helicobacter pylori*, AGS cells.

Introduction: *Maytenus ilicifolia* Mart. Ex Reissek (Celastraceae) leaves are popularly known in South America as *espinheira-santa*. In Brazil the herbal material is commercialized as a phytotherapeutic remedy mainly for treatment of gastric disorders (1,3). **Aim:** The aim of this work was evaluating the activity of crude extract obtained from the leaves of *M. ilicifolia*, anti-*H. pylori*, evaluating its toxicity in AGS cells and anti-adhesion activity. **Methods:** The crude extract was obtained from the leaves by extraction with acetone: water (7:3, v/v) and lyophilisation (herb: extract ratio = 100:24). For evaluation of functionality the influence of the extract on the cell viability of human stomach AGS cells (ATCC CRL 1739) was tested by MTT Assay; potential cytotoxicity against *Helicobacter pylori* (strain J99, ATCC 700824) was tested by agar diffusion assay and influence of the extract on bacterial adhesion was investigated by FACS adhesion assay (2). **Results:** AGS cell viability was not influenced significantly by extract (1000 to 1 µg/mL). Against *H. pylori* no direct cytotoxicity was found in the concentration range from 2000 to 1 µg/mL. Inhibition of bacterial adhesion (32.08%, 62.24%, and 71.81%) was found at 1000, 500, and 250 µg/mL, respectively. **Conclusion:** The extract does not exert cytotoxic effects to the cells and bacteria, and presents a promising antiadhesive activity against *H. pylori*.

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ANTIFUNGAL ACTIVITY OF ISOLATED COMPOUNDS FROM *Trichilia catigua* AGAINST *Candida glabrata*

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Key-words: Medicinal plants; Catuaba; *Candida glabrata*.

Introduction: *Trichilia catigua* A. Juss., known as Catuaba, is a tree widely found in Brazilian territory. Presents antimicrobial, antiamnesic, antioxidant, neuroprotective, and antinociceptive activities[(1,2)]. *Candida glabrata* is a dimorphic fungus that has emerged as an infectious agent, especially in hospital environment. It has high mortality rates in immunocompromised patients and rapid development of antifungal resistance[(3)]. **Aim:** Evaluate the antifungal activity of isolated compounds from *T. catigua* against *C. glabrata*. **Methods:** Cinchonain Ia, Cinchonain Ib, Cinchonain IIb, Epicatechin, and Procyanidin B2 (PB2) were isolated from ethyl acetate fraction of catuaba by column chromatography and high speed counter current. The evaluation of the anti-Candida activity was performed by determining the minimum inhibitory concentration (MIC) according to the CLSI[(4)] and evaluation of the synergistic activity of compounds in combination and with standard antifungals caspofungin and amphotericin B, by the checkerboard technique. The strain used was *C. glabrata* ATCC 2001. The cytotoxicity was evaluated in HeLa, Vero, and macrophages J774 cell lines by MTS technique. **Results:** The MIC values (µmol/L) were: 8.64 for Cinchonain Ia and Ib; 5.27 for IIb; 29.93 for Epicatechin and 13.51 for PB2. All compounds were synergistic with each other, and the best results were observed with Epicatechin and Cinchonain Ib, which showed strongly synergistic activity. The combination with epicatechin and PB2 present strongly synergistic activity with amphotericin B, and the best results of synergistic activity with caspofungin were observed for Cinchonain Ib in combination with Epicatechin and PB2. **Conclusion:** Isolated compounds of catuaba present potential for the development of new treatment strategies against *C. glabrata*.

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A HIGH-CARBOHYDRATE DIET INDUCES GREATER INFLAMMATION THAN A HIGH-FAT DIET IN MOUSE SKELETAL MUSCLE

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Key-words: Fatty acids; SFA/n-3 PUFA ratio; inflammation.

Introduction: Skeletal muscle has essential function on glucose uptake and fatty acids (FA) oxidation. Muscular deposition of FA as triacylglycerols and inflammation in the tissue can be influenced by the diet (1). Diet-induced obesity promotes insulin resistance, lipid accumulation and inflammation (2,3) in skeletal muscle. Considering that obesity *per se* causes lipid accumulation and inflammation in skeletal muscle (2), it is necessary to differentiate the direct effects of macronutrients and the effects of obesity itself. We previously reported in mice that the replacement of commercial diets with a high-carbohydrate diet (HCD) for 2 months leads to similar body weight gain in comparison with a high-fat diet (HFD) (4), which allows the evaluation of diet-induced changes without the influence of obesity. **Aim:** Investigate whether a dietary intervention with a high carbohydrate diet (HCD) or high fat diet (HFD) influence FA composition and inflammation in skeletal muscle. **Methods:** Male Swiss mice were fed with HCD or HFD for 0 (before starting given the diets), 1 or 2 months. After this period the mice were euthanized and gastrocnemius muscle was collected. FA composition was evaluated by gas chromatography and inflammation was evaluated by myeloperoxidase (MPO) activity and gene expression of F4/80, tumor necrosis factor-α (TNF-α), interleukin (IL)-4, IL-6, IL-10. In addition, de novo lipogenesis (DNL), the activities of stearoyl-CoA desaturase-1 (SCD-1), Δ-6 desaturase (D6D) and elongase were estimated. All experiments were approved by Scientific Advisory Committee on Animal Care of the State University of Maringá (protocol 3105210717). Data was analyzed by one-way ANOVA, followed by the post-test of Tukey or Student's t-test considering significant $p<0.05$. **Results:** The HCD led to lower ($p<0.05$) deposition of SFA, MUFA, n-3 PUFA, and n-6 PUFA compared to HFD. However, the HCD increased ($p<0.05$) arachidonic acid levels, SFA/n-3 PUFA ratio, activities of DNL, SCD-1, D6D MPO, and expression of IL-6. **Conclusion:** The HCD was more potent to induce skeletal muscle inflammation than the HFD, regardless of the lower lipid accumulation.

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ANTIPROLIFERATIVE CAPACITY OF CRUDE EXTRACTS OF *Croton floribundus* BARK

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Key-words: Prostate Cancer; Euphorbiaceae; Pharmacognosy.

Introduction: *Croton floribundus* Spreng., Euphorbiaceae, popularly known as "capixingui" is a native tree of the Atlantic Rainforest. It is reported in folk medicine the tea activity of *C. floribundus* bark for the treatment of leukemia, tumors, and syphilis. Plants of the genus *Croton* are rich in secondary metabolites of the class of diterpenes, alkaloids, tannins, and flavonoids. **Aim:** Evaluating the in vitro antiproliferative capacity, determining of half-maximal inhibitory concentration (IC_{50}) and selectivity index (SI) of the crude extracts (CE). **Methods:** The CE was prepared from the dried bark of *C. floribundus* (20.0 g) using a mix of solvents (v/v): water; ethanol:water (1:1; 7:3 and 9.5: 0.5); methanol; methanol:water (1:1); ethyl acetate; dichloromethane and acetone:water (7:3) and, an Ultra-Turrax (IKA T25; 12000 rpm, 4 times x 5 min, temperature < 40 ºC) at the proportion 1:10 (w/v). The CE was filtered, the organic phase was removed in a rotavapor (Büchi R-200), frozen in liquid nitrogen and the residue was lyophilized. The antiproliferative capacity of CE was determined using the human adenocarcinoma prostate cell line (PC-3), human carcinoma prostate cell line (LNCaP), and human normal prostate cell line (RWPE-1). Cells were cultured in cell culture bottles (CELLSTAR®) with 5 mL RPMI-1640 medium (Roswell Park Memorial Institute, Gibco®) for PC-3 and LNCaP cell lines, and Keratinocyte medium (Gibco®) for RWPE-1 cell line, supplemented with L-glutamine and phenol red with 2.0 g/L sodium bicarbonate ($NaHCO_3$, Synth®) and 10% (v/v) heat-inactivated fetal bovine serum (Gibco®), 1% (v/v) antibiotic-antimycotic solution (Gibco®), under incubation conditions of 37.0 °C in a humid atmosphere with 5.0% CO_2. The cells were subcultured with 0.25% trypsin EDTA after ≥90% confluent. The Neutral red[1] (NR) assay was performed using the concentrations of 200, 100, 50, 10, and 1 µg/mL of CE, culture medium without antibiotic-antimycotic solution, 100 µL $2.5x10^5$ cells/mL (PC-3); $4x10^5$ cells/mL (RWPE-1) and $5x10^5$ cells/mL (LNCaP) per well were plated in a 96-well plate and, maintained for 24 h at 37.0 °C in 5.0% CO_2. The incubation was continued for a total duration of 48 h after the addition of CE solutions. Wells containing culture medium with 0.5% DMSO as the negative control (NC) and wells with culture medium was taken as a control group (CG). After the incubation period, the supernatant was removed, the wells were washed with PBS and 200 µL of the NR solution (40 µg/mL) was added. After 3 h of incubation, the supernatant was removed and the wells washed rapidly with 200 µL of the fixative solution (1% $CaCl_2$ and 2% formaldehyde, v/v). NR solution into viable cell lysosomes was solubilized with 200 µL of the acid-alcohol solution (1% glacial acetic acid in 50% ethanol, v/v). The absorbance was measured in a spectrophotometer (EPOCH 2) at 540 nm. The experiments were performed on three separate days in quadruplicate techniques of each condition. IS was determined by the IC_{50} ratio of each treatment between normal (RWPE-1) and neoplastic (PC-3 and LNCaP) cell lines. **Results:** The 70% acetone crude extract was the best antiproliferative capacity (IC_{50} = 132.43±3.43 µg/mL (RWPE-1), IC_{50} = 42.11±6.32 µg/mL and SI 3.20 (PC-3), IC_{50} = 21.5±2.99 µg/mL and SI 6.27 (LNCaP)). **Conclusion:** The results of the analyzes contributed to biological studies of *C. floribundus* bark and can help to discover new compounds with anticancer activity.

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POTENTIAL USE OF AMANO® TRADE ENZYME FOR CYCLODEXTRIN PRODUCTION

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Key-words: cyclodextrins, magnetic nanoparticles, commercial Amano® CGTase

Introduction: Cyclodextrins (CDs) are cyclic oligosaccharides formed by the action of the enzyme cyclomaltodextrin glucanotransferase (CGTase) through the intramolecular transglycosylation reaction. The most produced CDs are α-CD, β-CD and γ-CD, have a truncated cone-shaped structure with hydrophilic exterior and hydrophobic cavity, which favors the formation of inclusion complexes that modify physicochemical properties, such as increased stability and/or solubility. This property makes CDs interesting for applications in the pharmaceutical and cosmetics industries. (1) However, CD production is costly. Alternatives for production involve the use of commercial enzymes such as Amano®, CGTase from a selected strain of *Paenibacillus macerans*, which converts starch to α-CD and β-CD, as well as enzymatic immobilization, which promotes economically viable production. of CDs by reusing the enzyme in various production cycles.(2) Magnetic nanoparticles can act as a support for immobilization because with the use of a magnet, the isolation and separation of the biocatalyst from the reaction medium is facilitated.(3) **Aim:** The objective of this work was to evaluate the conditions of the use of the Amano® enzyme with subsequent immobilization on magnetic nanoparticles and CD production. **Methods:** The protein content of the Amano® enzyme was tested by the Bradford method. The best enzyme concentration was evaluated at 0.2, 0.1 and 0.05% (V/V) dilutions from reaction with 1% maltodextrin substrate in a reactor for 30 min. The best substrate was evaluated considering the best enzyme dilution between maltodextrin, cassava starch, potato starch and corn starch at concentrations of 1 and 5% (w/V). A 24 h reaction with the best enzyme concentration and substrate was performed. The magnetic nanoparticles were obtained by alkaline coprecipitation of iron sulfate II and iron sulfate III in solution. **Results:** The protein content of the Amano® enzyme was 2.71 mg / mL. Enzyme activity using enzyme dilution at 0.2% (V/V) showed the best correlation between the concentration of β-CD produced as a function of reaction time. Among the substrates evaluated corn starch, 5% (w/V) presented the best result, with enzymatic activity 2,375 μmol CD/(min x mL). After a 24 h reaction, the production of 4.59 mg / mL β-CD was observed. The synthesized nanoparticles showed superparamagnetic action. **Conclusion:** The enzyme Amano® produces β-CD in adequate quantities and has the potential to be immobilized on magnetic nanoparticles.

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DEVELOPMENT OF ORAL MUCOADHESIVE FILM FOR MODIFIED RELEASE OF TRAMADOL HYDROCHLORIDE

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Key-words: tramadol, prolonged release system, pharmaceutical films.

Tramadol hydrochloride (TrHC) is a synthetic analogue of codeine and has action in the central nervous system as an opioid agonist and inhibitor of the recapitation of serotonin and norepinephrine. TrHChas been used for the control of moderate to severe pain. The pharmaceutical films for buccal administration are currently one of the strategies of the oral administration of drugs. Oral films may show greater patient compliance, when compared to the more commonly used oral dosage forms, such as tablets, capsules and syrups, mainly due to flexibility and comfort during their administration. Moreover, ethylcellulose (EC) polymer has been used for providing sustained drug release, polyvinylpirrolydone (PVP) has adhesive characteristics and polyvinyl alcohol (PVA) shows excellent film-forming properties (1). Therefore, the aim of this study was to develop a formulation in the form of oral administration film for the modified release of TrHC. Ten films were produced using a factorial design 2^3 plus repetition of central point, with different proportions between polymers, by solvent casting technique. The main analyzes performed were tension and elongation and mucoadhesion analysis, both using the TA-XTplus. Texture Analyzer (2). In addition to these, swelling index, density and macroscopic analyzes were also performed to evaluate the films for flexibility, integrity, homogeneity, presence of bubbles and touch adhesion. For tension and elongation, the values observed were, Young's modulus, where displayed values from 7.01 to 68.11P; maximum tensile stress, with values between 2.9 to 56.5P; fracture and tension, displayed values from 12.7 to 75.7P and elongation, displayed values from 7.5 and 104.1P. For mucoadhesion analysis, the values found ranged from 5.2 to 28.3N. Therefore, it was possible to obtain films with different characteristics and dependent on the polymeric composition, where the factorial design helped to select the best formulations for TrHC buccal release platform.

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DEVELOPMENT OF PHYSIOLOGICALLY-BASED PHARMACOKINETICS (PBPK) MODEL FOR KETAMINE.

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Key-words: Analgesia; Pharmacometrics; Simulation.

Introduction: Ketamine (KET) is a dissociative anesthetic used in clinical practice for decades. Low dose of this drug has been used for analgesia because of the antagonistic effect on N-methyl-D-aspartate (NMDA) receptors. In humans, analgesia studies with KET used intravenous route (IV). Pharmacometry has been used to assess dose adequacy for drugs. Thus, the development of a Physiologically-based pharmacokinetics (PBPK) model for KET IV will help to predict the doses required to use KET as an analgesic in different populations. **Aim:** To develop KET IV PBPK model. **Methods:** KET plasma data and its molecular chemical descriptors were obtained from literature (1,2) and used to establish the PBPK model (1,2). The Simcyp® Simulator Version 17 (SV) was used to generate the model. The population selected in SV was healthy adult patients (50% of women) aged 20-50 years. The performance of simulations was assessed by the mean fold error (MFE, MFE = Pharmacokinetic (PK) parameter predicted mean/PK parameter observed mean) for PK parameters area under the curve (AUC), Cmax and tmax extracted from SV. The model was only accepted if all predicted PK parameters has MFE between 0.5–2.0. **Results:** The parameters used to obtain the PBPK model IV that had the best fit to the observed data were: molecular weight (237.7 g/mol); monoprotic base; log P (7.16); pKa (3.35); blood/plasma ratio (0.733); hematocrit (45%); fraction unbound (0.53), scalar kp value (0.1); clearance IV (60 L/h with a coefficient of variation of 30%); The full PBPK method was assumed. Three different methods were tested to predict the steady state distribution volume value and the method 2(3) was chosen for the simulation tests, KET 0.125 mg/kg, 0.25 mg/kg and 10 mg IV bolus doses were used. The PBPK model could successfully predict the KET disposition in healthy adult patients, with MFE values of 0.86–1.08, 0.78–1.41 and 1.26-1.47 for AUC, Cmax and tmax, respectively. **Conclusion:** The KET PBPK model developed for healthy volunteers was predictive and could be extrapolated to estimate the plasma exposure of KET in different populations in order to achieve dose adequacy in analgesic protocols.

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COMPATIBILITY STUDY FOR OMEPRAZOLE PELLETS AND EVALUATION OF THE QUANTITATIVE IMPACT OF BUFFER AND UNDERCOAT LAYER ON THE STABILITY OF THE PRODUCT

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Key-words: compatibility study, omeprazole, stability.

Introduction: The compatibility test is an important step towards implementing the requirements and predicting the stability behavior of the formulation[1,2]. **Aim:** Carry out a compatibility study for omeprazole pellets in order to analyzing the impact of increased undercoating layer and buffer amount develop a new generic stable formulation. **Methods:** The active pharmaceutical ingredient (API) was mixed with the excipients in the proportions of the formulation. Mixtures, as well as API and isolated excipients, were analyzed initially without stress and compared after stress condition: 40ºC and 75% of humidity for 28 days in a glass bottle open and closed**.** The visual results were evaluated through changes of the coloration. The infrared spectroscopy (FT-IR) analyzes were performed with 10 scans using Spectrum ES. The criteria was to contrast the spectral band changes. The samples for thermal analyzes were weighed in aluminum crucibles, subjected a heating rate of 10°C/min between 30 and 250°C, flow of 50 mL/min of N_2 and calculated using STARe. Above variation of ±2.0°C in the onset temperature was considered incompatible. Next, the samples that presented incompatibility were subjected to chromatographic (HPLC) for the quantitative evaluation, based on the USP, 2016[3]. Lastly, in order to verify the stability of the final formulation, we varied the concentrations of the excipients dibasic sodium phosphate (buffer) and hypromellose (undercoat layer). **Results:** Visual analysis showed a change of the omeprazole in all conditions. The FT-IR analyses showed that none of the mixtures changes the spectra of API. During the thermal analysis, there were only incompatibility between omeprazole and mannitol mixture. Therefore, it was sent to HPLC but it was in the specification. The stability tests showed that all of them kept within the limits for omeprazole content and impurities. However, we observed that the increased hypromellose provided lower and smaller amounts of impurities in the formulation and combined with dibasic phosphate dihydrate improved the results. **Conclusion:** Our study demonstrated that the increase of these two excipients provides better stability results for the omeprazole pellets.

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ACTIVATION OF 5-HT1A POSTSYNAPTIC RECEPTORS BY NLX-101 RESULTS IN FUNCTIONAL RECOVERY AND AN INCREASE IN NEUROPLASTICITY IN MICE WITH BRAIN ISCHEMIA

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Key-words: 5-HT1A receptor; brain ischemia; neuroprotection.

Introduction: Pharmacological interventions that selectively activate serotonin 5-hydroxytryptramine-1A (5-HT1A) receptors may prevent or attenuate the consequences of brain ischemic episodes[1]. **Aim:** The present study investigated whether the preferential 5-HT1A postsynaptic receptor agonist NLX-101 (a.k.a. F15599) mitigates cognitive and emotional impairments and affects neuroplasticity in mice that are subjected to the bilateral common carotid artery occlusion (BCCAO) model of brain ischemia. **Methods:** Sham and BCCAO mice received daily doses of NLX-101 (0.32 mg/kg, i.p) or Esc (20 mg/kg, i.p) for 28 days. During this period, they were evaluated for locomotor activity, anxiety- and despair-related behaviors and hippocampus-dependent cognitive function, using the open field, elevated zero maze, forced swim test and object location test, respectivelly. The mice's brains were processed for biochemical and histological analyses (CEUA nº. 7200220818). **Results:** BCCAO mice exhibited high anxiety and despair-like behaviors and performed worse than controls in the cognitive assessment. BCCAO induced neuronal and dendritic spine loss and decreases in the protein levels of neuronal plasticity markers, including brain-derived neurotrophic factor (BDNF), synaptophysin (SYN), and postsynaptic density protein-95 (PSD-95), in prefrontal cortex (PFC) and hippocampus. NLX-101 and Esc attenuated cognitive impairments and despair-like behaviors in BCCAO mice. Only Esc decreased anxiety-like behaviors due to brain ischemia. Both NLX-101 and Esc blocked the increase in plasma corticosterone levels and, restored BDNF, SYN and PSD-95 protein levels in the hippocampus. Moreover, both compounds impacted positively dentritic remodeling in the hippocampus and PFC of ischemic mice. In the PFC, NLX-101 increased the BDNF protein levels, while Esc in turn, attenuated the decrease in the PSD-95 protein levels induced by BCCAO. **Conclusion:** The present results suggest that activation of post-synaptic 5-HT1A receptors by biased agonists such as NLX-101 might constitute promising therapeutics for treatment of functional and neurodegenerative outcomes of brain ischemia.

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DEVELOPMENT OF MINI-TABLETS FOR MODIFIED RELEASE OF MORPHINE SULFATE

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Key-words: morphine sulfate, multiparticulate pharmaceutical forms, drug modified release.

Introduction: Opioids are the class of analgesics most commonly used for chronic pain therapy, and morphine remains the standard for comparisons used for decades. This drug acts as an agonist for opioid receptors, but despite its great potential against pain, it has a short half-life with immediate-release formulations, providing only about four hours of pain relief. Immediate release of morphine is accompanied by a rapid increase, and followed by a large decline, in serum levels that can lead to loss of pain control and subsequent increase. In this way, the patient needs on average six doses per day to maintain the level within the therapeutic window, becoming the main inconvenience for its use[2]. Pharmaceutical and technological approaches that provide prolonged release of the active ingredient over time and, if possible, in a controlled manner, constitute a strategy to solve these problems presented by morphine. Recently, an increasing number of studies have emphasized the development of multiparticulate pharmaceutical dosage forms for prolonged drug release, in preference to monolithic unitary systems, due to their potential benefits, such as better distribution, easier disintegration, greater bioavailability of the active agent, lower risk of toxicity systemic and local irritation[1]. The aim of this work was to develop mini-tablet as a platform for modified morphine sulfate release. For characterization of the raw material, DSC, TGA, IR, SEM and DRX analyzes were performed. For the production of mini-tablets, a formulation screening was performed using the Hyperstart® software. From this, three formulations were made and compressed into punches with a diameter of 3 mm. For in-process control, weight, hardness, friability, thickness and disintegration analyzes were performed. Regarding the characterization of the raw material, all analyzes corroborate to confirm that it is the asset in question. In-process control analyzes guarantee the quality of the final product that will still be analyzed in dissolution profile so that the release profile can be identified. From the formulations created, we expect to obtain a formulation that gradually releases the active and decreasing the number of daily doses.

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EVALUATION OF SEMIPURIFIED FRACTIONS OF *Limonium brasiliense* IN THE INHIBITION OF BIOFILM FORMATION BY *Porphyromonas gingivalis*

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Key-words: *Limonium brasiliense*, *Porphyromonas gingivalis*, antibiofilm assay.

Introduction: *Limonium brasiliense* (Boiss). Kuntze (Plumbaginaceae), popularly known as *baicuru* [1], is used in Brazil for the treatment of menstrual disorders [2]. The crude extract has specific inhibitory activity against *Porphyromonas gingivalis* (*Pg*) [3]. *Pg* is a pathogen strongly involved in chronic and aggressive forms of periodontitis, that is a complex microorganism-induced inflammation of periodontal tissue, and if left untreated leads to the destruction of the tooth supporting system with eventually tooth loss, and this microorganism is also related with Alzheimer disease [4] and cardiovascular disease [5]. **Aim:** Evaluate the activity of semipurified fractions of *L. brasiliense* against *Pg* biofilm formation. **Methods:** The crude extract (CE) of the rhizomes was obtained by turbo-extraction with acetone:water 70:30 and was partitioned with water (aqueous-fraction - AQF) and ethyl acetate (ethyl-acetate-fraction - EAF). EAF was fractioned by Sephadex LH20 column, obtaining the subfraction FLB7 that contains the three majorities compounds of this fraction: Samarangenin A, Samarangenin B and Epigallocatechin-3-*O*-gallate. The antibiofilm activity was analyzed by the violet crystal method, in which fractions were incubated at different concentrations (1000, 500, 250, 125, 62,5 µg/mL) with *Pg* (ATCC 33277) at 37 ºC and anaerobic conditions for 24 h. After incubation, the plates were washed and incubated for 15 min with violet crystal solution and washed again with subsequent addition of 96% ethanol and reading on a 550 nm microplate reader. **Results:** The biofilm formation inhibition tests showed fractions activity at all concentrations tested, with inhibition of 97 ± 1% at the highest concentration and 64 ± 5% at the lowest concentration compared to the untreated control (UC) for AQF, 96 ± 1% and 54 ± 11% for EAF and 95 ± 1% and 55 ± 7% for FLB7. **Conclusion:** The *L. brasiliense* fractions showed promising results in inhibiting the formation of *Pg* biofilm and may be used to prevent infections caused by this microorganism and to reduce periodontitis and the related diseases in the world population.

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EVALUATION OF THE ANTIADHESIVE ACTIVITY AND ANTIBIOFILM FORMATION OF MICROPARTICLES CONTAINING *Limonium brasiliense* AGAINST *Porphyromonas gingivalis*

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Key-words: *Limonium brasiliense*, *Porphyromonas gingivalis*, microparticles.

Introduction *Limonium brasiliense* (Boiss). Kuntze (*Lb*), popularly known as baicuru, is a specie belonging to the Plumbaginaceae family. The rhizome of this plant has proven activity against *Bacillus mycoides*, *Escherichia coli*, *Staphylococcus aureus*, and *Porphyromonas gingivalis* (*Pg*) [1, 2]. *Pg* is a bacterium belonging to the red complex, most relevant group in periodontal disease. The disease is the leading cause of tooth loss in adults and can also lead to systemic problems such as rheumatism, diabetes, and cardiovascular [3]. Polymeric microparticles were produced with *Lb* for increase the therapeutic efficiency by providing a prolonged release and allow lower doses administration [4]. **Aim:** Evaluate the protective activity of microparticles containing ethyl-acetate fraction of *L. brasiliense* (LM) against *Pg* adhesion in KB cells and the antibiofilm activity. **Methods:** LM were produced with the Surelease® and polycarbophyl polymers in different concentrations by the spray drying method. Microparticle morphology analysis was evaluated by scanning electron microscopy (SEM). The influence of LM on cell viability of KB cells (ATCC CCL-17) was monitored by MTT assay and the influence of LM with 8 hours of pre incubation to prevent the adhesion of *Pg* (ATCC 33277) to KB cells was monitored by plate fluorimetric assay. Besides that, the antibiofilm formation assay was performed by violet crystal method. **Results:** The production yield of the prepared batches was found to be in 49,8%. LM micrograph by SEM showed the presence of polydisperse systems. There was no toxicity against KB cells from the lowest concentration tested to the concentration of 50 µg/mL of LM. The antiadhesive effects with the LM showed results around 73% and 88% reduction of bacterial adhesion to KB cells for all concentrations tested and around 12% and 44% with the free extract. The biofilm formation inhibition tests showed LM activity at all concentrations tested, with inhibition of 95% at the highest concentration and 75% at the lowest concentration compared to the untreated control (UC). **Conclusion:** The microparticles tested showed promising protective effect against *Pg* adhesion in KB cells without affecting the viability of the cells and inhibits the formation of *Pg* biofilm. The LM can be used to prevent periodontal disease caused by *Pg* and improve the systemic health of population.

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DEVELOPMENT, CHARACTERIZATION AND SAFETY ASSESSMENT FOR TOPICAL DELIVERY OF SOLID LIPID NANOPARTICLES AND NANOSTRUCTURED LIPID CARRIERS CONTAINING PROTOCATECHUIC ACID OR ETHYL PROTOCATECHUATE

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Keywords: Protocatechuic acid, nanostructured lipid carriers, topical drug delivery.

Introduction: Excessive exposure to solar radiation induces deleterious effects on human skin, provoking photoaging and photocarcinogenesis on a long-term basis[1]. Our previous study proved that protocatechuic acid (P0) and its alkyl ester derivative ethyl protocatechuate (P2) act against photodamage caused by UVB radiation in L929 fibroblasts. **Aim:** In this present study, we developed solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) for topical delivery of P0 and P2. **Methods:** The lipid nanoparticles were physiochemically characterized. The cytotoxicity evaluation was performed using keratinocytes (HaCat) and fibroblasts (HFF-1). NLCs-P0 or -P2 were also evaluated for their *in vitro* drug release and UVB photodegradation profiles. Additionally, the *ex vivo* permeability and histological evaluation were assessed in Franz cells assembly, using human excised skin, with approval of the Bioethics Committee of the São João Hospital (Portugal). **Results:** Lipid nanoparticles exhibited mean particle size, polydispersability index, zeta potential and association efficiency in the range of 200 to 400 nm, 0.160 to 0.460, -2.20 mV to -5.20 mV, and 60% to 80%, respectively. TEM images exhibited spherical morphology for bare nanoparticles. P0- SLN/NLC showed minor cytotoxicity effects compared to P2-SLN/NLC, in skin cell lines. NLC systems exhibited controlled release for P0 and P2, and were able to protect the compounds against UVB degradation. The *ex vivo* experiments showed that P0 and P2 associated with NLCs modulate their retention profiles on skin layers, but did not change their permeation profiles, avoiding systemic absorption. Moreover, no changes in skin structure were observed after 24h of treatment, except for NLCs-P2. **Conclusion:** Based on the results the NLCs are potential dermatological nanocarriers for P0 delivery.

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PREPARATION, CHARACTERIZATION AND ANTIBIOFILM EFFECT OF FREE AND NANOENCAPSULATED *Tetradenia riparia* (Hochst). Codd ESSENTIAL OIL AGAINST *Staphylococcus aureus*

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Key-words: Biofilm, essential oil, nanoparticle.

Introduction: *Staphylococcus aureus* is an important microorganism that have the ability to form biofilm. This microbial community live in an extracellular matrix composed of proteins, extracellular DNA and polysaccharides. Factors contributing to the reduction of the effectiveness of the treatment are the development of resistance to antimicrobial drugs, as well as the appearance of undesirable effects of certain antimicrobial agents. Thus, arises the need to search for new agents with low toxicity and side effects[1]. Antimicrobial agents of natural origin are effective and economical alternatives, as essential oils (EO). Although, these compounds show rapid oxidation, nanoencapsulation is an alternative that improves stability, reduces toxicity and controls the release of oil[2]**. Aim:** Preparation, characterization and evaluation of antibiofilm activity of free and nanoencapsulated essential oil of *Tetradenia riparia* (Hochst). Codd against *S. aureus*. **Methods:** Nanoprecipitation with Poly-lactide (PLA) was used to obtain nanoparticles containing EO. The nanoparticles (NP) was characterized by Dynamic light scattering (DLS), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) for morphology and size distribution. Thermal analysis was realized by Dynamic Scanning Calorimetry (DSC). The antimicrobial and antibiofilm effect of EO and NP was observed by broth microdilution method according to CLSI. The effect of EO and NP in biofilm formation was observed with SEM. A cytotoxic assay was performed using a VERO cell line. **Results:** Nanoparticles were found to be nanometric, round with regular structures, as observed in SEM, TEM and DLS. ΔHm values decreased with the incorporation of *T. riparia* EO, that suggest the encapsulation of EO in the PLA matrix. The minimum inhibitory concentration of EO and NP was 125 and 250 µg/mL, respectively. The Minimum bactericidal concentration (MBC) was 250 µg/mL to EO and NP. The biofilm minimum concentration of 50% cells (BIC50) was 310 and 330 µg/mL of OE and NP, respectively. SEM confirmed the reduction of biofilm formation and altered morphology. NP was less cytotoxic than EO. **Conclusion**: EO and NP could be effective as an antibiofilm alternative treatment.

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IN VITRO VERIFICATION OF SUPEROXIDE ANION RADICAL SCAVENGING ACTIVITY AND IRON CHELATOR OF *SOLIDAGO CHILENSIS* EXTRACT

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Key-words: *Solidago chilensis***,** oxidative stress, antioxidant.

Introduction: Oxidative stress is defined by the imbalance between the formation of pro-oxidant and antioxidant substances in our body (1). One of the main effects observed is the reduction of endogenous antioxidant stores. Plant extracts rich in antioxidant substances, such as *Solidago chilensis*, are good candidates for the prevention or treatment of oxidative stress. **Aim:** The objective of this work was to perform *in vitro* assays to evaluate the antioxidant effect of *S. chilensis* extract. **Methods:** The 70% hydroethanolic extracts of *S. chilensis* leaf (SCF) and root (SCR) were prepared by adding the mixture of drug and solvent (1:10 (drug/solvent), m/v) in the ultrasonic bath for 2 hours, followed by maceration for 7 days under light protection. SCF and SCR extracts were added at concentrations of 0.24-19.40 and 0.22-18.13 µg/mL, respectively, to the tube containing the reaction mixture composed of buffer pH 9.4 (glycine 0.1 M + EDTA 1 mM), xanthine (6 mM), luminol (6 mM) and xanthine oxidase (20 UI/mL) (2). The reaction evaluate the ability of the samples to scavenging the superoxide anion formed by the xanthine/XO quantified by chemiluminescence. In addition, SCF and SCR extracts were added at 2.6-65 and 3-75.90 µg/mL in due order, to the tube containing the reaction mixture composed of buffer pH 7.4 (KCl 130mM + tris-HCl 10 mM), $(NH4)_2Fe(SO4)_2$ (50 µM) and bathophenanthroline (0.2mM) (3). The reaction evaluate the chelating capacity of ferrous ions by the compounds present in the extract. The absorbance was obtained at 530 nm and 700 nm. **Results:** SCF and SCR extracts showed antioxidant activity with IC_{50} values of 2.08 µg/mL for SCF and 1.69 µg/mL for SCR in relation to xanthine/XO chemiluminescence assay. Moreover, *S. chilensis* extract exhibited iron chelating activity with IC_{50} values of 21.53 µg/mL for SCF and 55.93 µg/mL for SCR. **Conclusion:** *S. chilensis* extract showed antioxidant capacity on the reduction of superoxide anion, as well as chelating effect of ferrous ion. Thus, *S. chilensis* extract can help treat diseases caused by oxidative stress.

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UHPLC CHROMATOGRAPHIC PROFILE DEVELOPMENT OF THE ETHYL-ACETATE FRACTION OF *Guazuma ulmifolia* BARKS

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Key-words: *Guazuma ulmifolia*, chromatographic profile, UHPLC

Introduction: *Guazuma ulmifolia* Lam. (Malvaceae), popularly known as Mutamba in Brazil, originates from Tropical America, and extends from Mexico to South America. In folk medicine its use has been reported in several countries for the treatment of gastrointestinal diseases, hypertension, fever, diabetes, and in childbirth (uterine contractions). *G. ulmifolia* barks are rich in tannins and proanthocyanidins, compounds that have been reported for their various biological activities, including: protective effects against neurodegenerative diseases, antiparasitic activity, antimicrobial properties, antihypertensive effects, antidiabetic activity, antidiarrheal activity, antiviral activity, and hair growth promoter (1,2). **Aim:** UHPLC chromatographic profile development of the ethyl-acetate fraction of *G. ulmifolia* barks. **Methods:** The crude extract of the barks was obtained by turbo-extraction with acetone:water (70:30, v/v) and was partitioned with water and ethyl acetate (ethyl-acetate-fraction - EAF). A methodology development for EAF was carried out based on the work of Lopes and collaborators (3). UHPLC analysis was performed using an UHPLC^{+} focused (Thermo Scientific) Dionex UltiMate 3000, mobile phase: solvent A: water + 0.05% formic acid, solvent B: Acetonitrile + 0.05% formic acid, (gradient: minute/%A:%B: 0/87:13, 10/83:17, 16/81:19, 20/78:22, 23/71:29, 25/65:35, 28/87:13, 32/87:13, 40/87:13), flow rate: 0.3 mL/min, sample manager (inj.-vol.: 20 µL), detection at 210 nm, column heater (40 °C), stationary phase: Phenomenex Onyx Monolithic C18 column, (100 × 4,6 mm, 2 µm). **Results:** According to tests performed, the decrease in temperature is a factor that contributed positively, improving the chromatographic profile. The temperatures analyzed were 40.0, 35.0, 30.0, 27.0, 25.0, 23.0, 20.0, and 17.0 ° C. The best condition observed was at 17.0 ° C, where there was a more efficient peak separation. **Conclusion:** Further analysis is required, but it was possible to develop a UHPLC chromatographic profile for the ethyl-acetate fraction of *G. ulmifolia* barks.

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SEQUENTIAL EXTRACTION AS PROMISING STRATEGY TO OBTAIN BIOACTIVE COMPOUNDS FROM WASTE *Stevia rebaudiana*

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Key-words: *Stevia rebaudiana*; inulin; phenolic compounds.

Introduction: *Stevia rebaudiana* (Bertoni) is widely studied due to its steviol glycosides in its leaves.[1] The stems are waste generated by industries due to the low amount of steviosides. The use of industrial waste from the plant is an alternative to obtain biologically active compounds. **Aim:** The aim of this study was to identify the primary and secondary metabolites from industrial waste of *S. rebaudiana* by sequential extraction using ultrasound-assisted (UAE) and reflux method. **Methods:** The *S. rebaudiana* stems utilized in this study were provided by Stevia Soul sweetener industry. In UAE extraction process, 5.0 g of powdered *S. rebaudiana* was added with 250 mL of hydroethanolic solvent (75% v/v). The UAE was continued for 50 min and hydroethanolic extract (HE) was separated and filtered. Then, the same *S. rebaudiana* stem was extract with water under reflux for 4 h (3x). The ratio of solid to solvent was analogous to UAE extraction process. The crude aqueous extract was precipitated with EtOH, resulting in two fractions: supernatant (AES) and precipitate (AEP). The total carbohydrate content in the extracts were determined by the phenol-sulfuric acid method[1] with D-fructose as the standard and of total phenolic compounds were realized by the Folin-Ciocalteu's method[2] with gallic acid as the standard. All experiments were accomplished in triplicate. The AES and AEP were analyzed by ^{1}H NMR. **Results:** The yield of HE, AES and AEP extracts were 6.46, 10.38 and 3.47%, respectively. Qualitative assays indicated total polyphenol content of 16.78% in HE, and the presence of sugar in AES (31.97%) and AEP (23.83%). The ^{1}H NMR spectra of AES and AEP showed characteristic signs at 5.46 ppm of the terminal glucose hydrogen of the fructan. Signal referents of the residue (→2-β-Fru) were observed at δ 4.25 (H3-Fru) and δ 4.12 (H4-Fru)[1]. The polymerization degree of AES and AEP were 9.31 (fructo-oligosaccharides) and 12.94 (inulin), respectively. **Conclusion:** The use of sequential extraction shows be a promising strategy to obtain bioactive compounds as polyphenols, fructo-oligosaccharides and inulin from waste industrial of stem *S. rebaudiana*.

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DMAE ANTI-AGING SERUM DEVELOPMENT

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Key Words: Dermocosmetic; anti-aging; rheology.

Introduction: The skin aging is a complex process and depends on both intrinsic (such as genetics, metabolic process, hormones) and extrinsic factors (such as light exposure, pollution, radiation, chemicals, toxin), that over time goes through slowing of the epidermal turnover rate and cell cycle, leading a slower healing and less effective desquamation, putting in evidence thickened epidermis, wrinkles, laxity, dullness and roughness.Dermocosmetics are products with active substances for dermatological treatment. Serum is a highly concentrated product based in oil or water, with faster action and more effective therapeutic. The actives present in this serum were carefully chosen in order to keep water in the epidermis, besides maintains the skin's hydrophobic barrier and assists in cell renewal and healing. This personal care product, formulated for skin rejuvenation and biocompatibility, contains Hidrovital, silk proteins, D-panthenol and extract of *Aloe vera*. Dimethylaminoethanol (DMAE), an acetylcholine precursor molecule, was also employed in order to preclude onset of wrinkles and sagging skin. **Aim:** Development of anti-aging dermocosmetic with suitable rheological profile and pleasant sensory characteristics for greater therapeutic compliance. **Methods:** The base serum was prepared and active ingredients were incorporated. The determination of pH was performed using pHmeter. Rheological analysis rotational was assessed with the MARS II rheometer (Haake®), with titanium parallel cone-plate geometry with 35 mm diameter and 2° angle. The experiments were carried out at temperature of 25 ± 0.5 °C. **Results:** The pH of the formulation was characterized as 5.92, being suitable for facial skin care. Flow behavior analysis determined a Herschel-Bulkley behavior, with consistency index (K) of 2.269, flow index (n) of 0.4158 and yield point of 2.68 mPa. The serum showed thixotropic properties, being able to restructure itself back to its initial state. Oscillatory analysis revealed that viscous modulus (0.808 Pa) was higher than elastic modulus (0.305 Pa). **Conclusion:** Rheologic behavior properly described a non-Newtonian fluid with thixotropic, shear thinning and elastoviscous conduct, that affording good spreadability and topical applicability.

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QUALITY CONTROL OF VEGETABLE SPECIES *ANGELICA SINENSIS* (OLIV.) DIELS BELONGING TO TRADITIONAL CHINESE MEDICINE (TCM).

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Key-words: Danggui, Apiaceae, Zhong Yi Xue

Introduction: Chinese Angelica, know in China a Danggui, are the dried roots of *Angelica sinensis* (Oliv.) Diels belonging the family Apiaceae and used for millennia in Traditional Chinese Medicine (TCM) (1,2). **Aim:** The purpose that study was carry out the quality control of *A. sinensis* and your dry extract. **Methods:** It were used samples of roots of *A. sinensis* (Mestre Kim supplier) and dry extract (Infinity Pharma supplier), provided by Fitofórmula Laboratory, being performed morphoanatomical analysis, identification by thin layer chromatography (TLC), and purity assays (water, total ash and acid-insoluble ash, extractives, and assay volatile oil) present in the Chinese Pharmacopoeia Commission. **Results:** The form and length could not be described because the plant drug (Mestre Kim) was fragmented into diagonal sections. In the TLC was observed a retention factor (Rf) of 0.17 for both suppliers and 0.15 for ferulic acid standard. For water values were founds of 9.57% ± 0.15 and 6.58%±0.25 for Mestre Kim and Infinity Pharma, respectively. For total ash and acid-insoluble ash the values obtained are of 6.00% ± 0.06 and of 0.48% ± 0.02, respectively for Mestre Kim supplier; and for extractives and assay volatile oil the results obtained were 53.12% ± 1.56 and 0.45% (mL/g), respectively for Mestre Kim supplier. **Conclusion:** Although the macroscopic analysis of plant drug (Mestre Kim) be incomplete, the analyses carried out aided in to prove the vegetable specie. Already for the dry extract was not possible to perform macroscopic and microscopic analyses, total ash and acid-insoluble ash. But the quality control analyses carried out on both samples are within the limits specified by the Chinese Pharmacopoeia Commission (2010).

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QUALITY CONTROL OF VEGETABLE SPECIES *GLYCYRRHIZAE RADIX ET RHIZOME* BELONGING TO TRADITIONAL CHINESE MEDICINE (TCM).

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Key-words: Alcaçuz, Leguminosae, Zhong Yi Xue.

Introduction: *Glycyrrhizae radix et rhizome*, know too as alcaçuz, are the dried roots and rhizomes of *Glycyrrhiza uralensis* Fisch, *Glycyrrhiza inflata* Batalin, and *Glycyrrhiza glabra* L. belonging the family Leguminosae (1) and used for millennia in Tradicional Chinese Medicine (TCM) (2). **Aim:** The purpose that study was carry out the quality control of *Glycyrrhizae radix et rhizome*. **Methods:** It were used samples of roots and rhizomes of *Glycyrrhizae radix et rhizoma* (Mestre Kim and Sim Sim suppliers), provided of Fitofórmula Laboratory, being performed morphoanatomical analysis, identification by thin layer chromatography (TLC), and purity assays (water, total ash, acid-insoluble ash, and extractives) present in the Chinese Farmacopoeia Commission. **Results:** The form and length of both samples could not be described because the plant drug was fragmented into diagonal sections. In the TLC was observed a retention factor (Rf) of 0.15 for both samples compatible with the Rf of the ammonium glycyrrhizinate standard. For water values were founds of 9.79% ± 0.10 and.67% ±0.23 for Mestre Kim and Sim Sim, respectively. The results for total ash were 6.46% ±0.22 and 3.34% ±0.13, respectively for Mestre Kim and Sim Sim; acid-insoluble ash the values were of 0.46% ±0.02 and of 0.42% ±0,02 for Mestre Kim and Sim Sim, respectively; and for extractives the values obtained were 34.12% ± 1.25 and 34.22% ±1.14 for Mestre Kim and Sim Sim, respectively. **Conclusion:** Although the macroscopic analyses of plant drug be incomplete, the analyses carried out aided in to prove to be the vegetable specie. Thus, the results obtained are within the limits specified by the Chinese Pharmacopoeia Commission (2010).

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ALPHA-BISABOLOL: THE LIVER AEGIS

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Key-words: alpha – bisabolol, hepatotoxicity, APAP, hepatoprotection

Introduction: α-Bisabolol (BISA) is an unsaturated monocyclic sesquiterpenes compound, that is mainly found in the chamomile (*Matricaria chamomilla*), has generated considerable economic interest for its organoleptic and biological characteristics. This compound has several biological activities and it is reported in the literature, as a terpenoid that has great anti-inflammatory, healing, anti-mutagenic and antioxidant activity (1,2,4). Paracetamol self-medication (APAP), a non-steroidal anti-inflammatory drug is the leading cause of acute liver failure. The APAP-induced hepatotoxicity model is the most widely model used to investigate the potential hepatoprotective of plant drugs. **Aim**: The aim of the study is to evaluate the hepatoprotective ability of BISA in an animal model of APAP overdose-induced liver injury, in different concentrations. **Methods**: Swiss mouse were used and the animals received single dose of BISA, by gavage, at 50, 100 and 200 mg. kg-1, for 7 days and were euthanized 12 hours after APAP overdose. The samples obtained were submitted to cell viability analysis, antioxidant activity, liver markers (ALT and AST), chemotaxis, myeloperoxidase activity, nitric oxide production, antioxidant defenses and histopathological analysis (3). Data were expressed as the mean ± SEM for each experimental group. **Results:** will be statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. The software used will be GraphPad Prism version 6.0, GraphPad Software, Inc. Significance index of $p < 0.05$. All of the procedures were approved by the CEEA/UEM (4286240419). Results: Treatment with APAP caused liver damage demonstrated by elevation of liver enzymes ALT (alanine aminotransferase), AST (aspartate aminotransferase), Gamma GT (gamaglutamiltransferase) and FSA (alkaline phosphatase). Pretreatment with BISA at concentrations of 50, 100 and 200 mg.kg -1 was effective in preventing the elevation of liver enzymes that indicate hepatotoxicity, when compared with the control. The reduction averages were: 43.9 %, 57.9 % and 73.8 % for AST; 17.0 %, 47.6 % and 95.2 % for ALT and 40.3 %, 42.7 % and 48.2 % for alkaline phosphatase, respectively. Gamma GT values did not change. The hepatoprotective effect of BISA behaved in a dose dependent manner. **Conclusion**: We conclude that BISA is a promising compound for the treatment of APAP-induced drug hepatitis. Its effect may be justified, at least in part, by its antioxidant and anti-inflammatory potential.

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N-ACYLIDRAZONE DERIVED AS A POTENTIAL AGENT AGAINST *Paracoccidioides* spp.

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Key-words: New antifungal; *in vitro*; *Paracoccidioides* spp.

Introduction: Paracoccidioidomycosis (PCM) is a systemic mycosis caused by the genus *Paracoccidioides* spp. The prolonged antifungal treatment and nephrotoxicity are limitations that require searching for new antifungals. **Aim:** To evaluate *in vitro* antifungal activity of N '-(2-hydroxy-5-nitrobenzylidene)-4-methoxy-1-naphthohydrazide (AON3). **Methods:** The minimum inhibitory concentration (MIC) was determined by the broth microdilution method as recommended by CLSI (M27-A3), with modifications. Yeast (Pb18, Pb01, Pb0113, Pb0116 and Pb0117, ~$2.8x10^6$ cell) were incubated with different AON3 concentration (0.25 to 128 µg/mL) at 35°C/7 days. Resazurin (0.02%) was added at 6th day of incubation, and visual reading was performed after 24 h. For minimum fungicidal concentration (MFC) determination, aliquots from all wells were plated on BHI agar (35°C/7 days). For kill-time curve, Pb18 was cultivated in McVeigh Morton medium (35°C/7 days) with shaking. Subsequently, yeast ($2.5x10^3$ CFU) were treated with three AON3 concentration (2, 1 e 0.5 µg/mL) and incubed until 14 days at 35°C. At time of 1, 3, 5, 7, 10 and 14 days, cells were diluted in PBS and plated on supplemented BHI agar. **Results:** MIC and MFC values ranged from 1 to 8 µg/mL, corroborating previous research (1). The kill-time curve showed that ANO3 presented a fungistatic effect. The best activity was observed on 10° day (2 µg/mL) with reduction of 91.20%, but this effect was not preserved on 14º day reduction was 67.08%, as compared to control. Treatment with 1 µg/mL concentration reduced the number of viable fungal cells by approximately 67.91% at 3 days, but did not show the same effectiveness from day 5º, thus reducing to 31.59% at 14º days. In 0.5 µg/mL concentration, ANO3 not showed activity. **Conclusion:** Promising *in vitro* results of compound AON3 make it a candidate for the new antifungals development.

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ANTIFUNGAL ACTIVITY OF A NEW COMPOUND AGAINST *Paracoccidioides brasiliensis*

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Key-words: New antifungal; histopathological; *Paracoccidioides brasiliensis*

Introduction: Paracoccidioidomycosis (PCM) is a granulomatous systemic mycosis caused by the genus *Paracoccidioides* spp. The prolonged therapy, toxicity, pulmonary sequelae and drug interactions still require the urgent search for new antifungal compounds. **Aim:** To evaluate *in vivo* antifungal activity of AOB1 compound. **Methods:** Balb/c mice were inoculated in the lateral vein of the tail with *Paracoccidioides brasiliensis* ($1x10^5$ CFU) and treated with AOB1 for 14 days (CEUA approved - number 9810191015). After euthanasia, the lungs were removed for histopathological analysis. For this, organs were fixed in 4% paraformaldehyde, embedded with paraffin wax and stained with Hematoxylin & Eosin and Gomori-Grocott. For the fungal burden determination, the lung area in the histological section was determined and expressed as the ratio of colony-forming units by organ extension (CFU/mm^2). Three parameters were evaluated in lung tissue: necrosis, inflammatory infiltrates and the number of fungal cells. In semiquantitative analysis, an intensity scale from 1 to 3 of the tissue damage in each parameter was determined by microscopy (X10, X40, X60), and subjected to statistical analysis ($p<0.05$). **Results:** The present study showed promising *in vivo* antifungal activity of AOB1, by semiquantitative, quantitative and qualitative histopathological analysis. In semiquantitative analysis, both groups treated with AOB1 and itraconazole (ITZ) showed a significant reduction ($p<0.05$) of parameters: Necrosis and inflammatory infiltrates as compared to the untreated control group. In the quantitative analysis (CFU/mm^2), AOB1 tended to reduce the fungal burden in the lung, being more efficient in relation to the antifungal currently used in the PCM treatment (ITZ), corroborating with previous studies (1). **Conclusion:** Promising *in vivo* results of AOB1 compound make it a candidate for the development of new antifungals.

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BOTANICAL CHARACTERIZATION AND QUALITY CONTROL ANALYSIS OF *CRATAEGUS PINNATIFIDA* FRUIT

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Key-words: traditional Chinese medicine, Chinese Hawthorn, flavonoid.

Introduction: Chinese Hawthorn, widely used in traditional Chinese medicine (TCM), are fruits of species *Crataegus pinnatifida*, family Rosaceae. It has several beneficial effects, such as hypocholesterolemic, protector of cardiovascular system, antinflammatory and antioxidant properties[1]. **Aim:** In this sense, the objective of this work was to develop a quality control protocol to evaluate the quality of *C. pinnatifida* commercialized in Brazil. **Methods:** The fruits used were purchased from accredited pharmacies in the city of Hong Kong in China. The visual examination and microscopic inspection were performed[2,3]. The physicochemical tests were made according to Brazilian Pharmacopoeia[2] for determination of foreign matter, moisture content and total ashes. All results were compared to Chinese Pharmacopoeia[3]. The chemical marker was flavonoid content and the extract was prepared as ratio 1:10 plant:solvent using ethanol 80% and turbolysis as extractive technique. The hydroalcoholic extract was lyophilized. This dried extract was used to analyze flanonoid contents by ultraviolet spectroscopy (Shimadzu UV-1800 spectrophotometer) using quercetin as standard. All spectrometer analysis (quercetin and extract) were performed according Calendula Monograph in Brazilian Pharmacopoeia. **Results**: *C. pinnatifida* fruit can be classified as pomos or pomidium fruits. The fruit powder analysis presented remarkable elements that correspond to those described in the Chinese Pharmacopoeia[3]. According botanical macro and microscopic analysis, the results showed that the samples used in this work were comparable to description on Chinese Pharmacopoeia, then was assumed to be *C. pinnatifida*. The physicochemical analysis indicated that foreign material, moisture content and total ash were adequate according to the Chinese Pharmacopoeia, also. Assuming that the samples were according to the quality stablished by Chinese Pharmacopoeia, the chemical content was determined assuming literature methodology[2], because Chinese Pharmacopoeia does not bring information about chemical contents. The assay method for total flavonoids was linear, selective and precise. The total flavonoid of dried extract was 0.0167%, which represents 0.1% of total flavonoids in one gram of vegetable drug. **Conclusion:** From this assay, it was possible to stablish a methodology for quality control *Crataegus pinnatifida*.

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ANTIOXIDANT CAPACITY OF INPUTS OBTAINED FROM *Eugenia gracillima* (LEAVES) BY ABTS AND FRAP METHODS

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Key-words: Myrtaceae, Natural antioxidants, Oxidative stress

Introduction: Oxidative stress is associated with the development of several diseases that affects people all around the world, such as cancer, cardiovascular, degenerative and neurological diseases, among others. Moreover, it is directly related to premature aging. Disorders in the body generate oxidative stress, such as the imbalance between reactive oxygen species (ROS) and endogenous antioxidants in the human body. Among the most studied antioxidant compounds, plants stand out for having secondary metabolites capable of intervening in oxidative damage, neutralizing free radicals that affect DNA, lipids and proteins[1]. **Aim:** The aim of this study was to investigate the *in vitro* antioxidant activity of inputs obtained of *Eugenia gracillima* Kiaersk. **Methods:** The dried and ground leaves of *E. gracillima* were extracted by percolation using absolute ethanol as solvent. The obtained ethanolic extract (EE) was submitted to liquid-liquid partition with solvents of different polarities, resulting in hexane (HF), ethyl acetate (AF) and hydromethanolic (MF) fractions. Antioxidant activity of the EE and fractions was tested by the 2,29-azinobis-3-ethylbenzotiazoline-6-sulfonic acid ($ABTS^{\bullet+}$)[2] and ferric reducing antioxidant power (FRAP)[3] assays. Quercetin (QT) was used as a positive control. **Results:** The results were expressed as µmol of trolox per gram of sample. AF showed higher antioxidant activity in both methods, with the value of 4631.34 ± 153.86 µmol TE/g on ABTS assay (QT: 14214.21 ± 149.12 µmol TE/g) and 4925.75 ± 97.04 µmol TE/g on FRAP method (QT: 12629.22 ± 492.01 µmol TE/g). **Conclusion:** The results show that *E. gracillima* is a promising source of antioxidants and should continue research related to biological systems, as well as the isolation and identification of bioactive substances, which in the future may contribute as new alternatives for the treatment and/or prevention of diseases associated with oxidative stress.

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