ANAIS

VIII SEMINÁRIO ANUAL DO PCF 2017

Programa de Pós-Graduação em Ciências Farmacêuticas

22 a 24 de novembro de 2017

Universidade Estadual de Maringá
Centro de Ciências da Saúde
Departamento de Farmácia

Anais do VIII Seminário Anual do Programa de Pós-graduação em Ciências Farmacêuticas

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Apresentação

O **VIII Seminário Anual do Programa de Pós-graduação em Ciências Farmacêuticas** (PCF), aconteceu entre os dias 22 e 24 de novembro de 2017, no Bloco B33 - PDE, Câmpus sede da Universidade Estadual de Maringá. O Seminário Anual do PCF promoveu a integração entre os alunos de iniciação científica, mestrado e doutorado vinculados ao PCF ou oriundos da graduação e de outros programas de pós-graduação da Universidade Estadual de Maringá e de Universidades da região. Foram ministradas palestras por profissionais de diferentes áreas de atuação dentro das Ciências Farmacêuticas e áreas afins, vindos de diferentes partes do mundo.

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Palestrantes

Dr. Andreas Hensel
Universidade de Münster - Alemanha
Tema: "*Antiadhesive Natural Products against Parthogens*".

Dr. Bruno Filipe Carmelino Cardoso Sarmento
Universidade do Porto – Portugal
Tema: "*Bioengineered nanomedicines for the delivery of anticancer drugs in the gastrointestinal tract*"

Dra. Claudia Maria Padovan
Universidade de São Paulo - Brasil
Tema: "*Princípios e Normas Éticas na utilização de Animais no Ensino e Pesquisa*"

Dr. Jos Prickaerts
Universidade de Maastrich - Holanda
Tema: "*Combining Academia and Industry: can you do that?*"

Dr. Juliano Bordignon
Instituto Cralos Chagas
Tema: "*The in vitro infection of human cells by Zyka virus is impaired by the citrus flavonoid naringenin*".

Dr. Roberto Barbosa Bazotte/ Dr. Rui Curi
Universidade Estadual de Maringá/ Universidade de São Paulo - Brasil
Tema: "*Mecanismos de regulação da glicemia vs. mecanismos de desregulação da glicemia e seus tratamentos*".

Dr. Rodrigo Cristofoletti
Agência Nacional de Vigilância Sanitária – ANVISA. Departamento de Bioequivalência
Tema: "*Uso de modelagem e simulação (M&S) na avaliação de segurança e eficácia de medicamentos novos e genéricos: realidade e perspectivas futuras*".

Dr. Soumen Das.
Universidade Central da Florida– EUA
Tema: "*Nanoparticles for regenerative medicine*".

Programação

PAINEIS - Apresentação de trabalhos

Data	Horário	Avaliadores	Apresentadores
Quinta-feira (23/11)	09:00 - 09:20	1. Daniela Cristina de Medeiros 2. Danielle Lazarin Bidóia 3. Bianca A. Ratti	André Oliveira Fernandes da Silva Camila Felix Vecchi Clara Beatriz de Lima Daniela Cristina de Medeiros Fernanda Pilatti da Silva Gustavo Angeoletto Scramim
Quinta-feira (23/11)	15:00 - 15:20	1. Lucas de Alcantara Sica de Toledo 2. Ligia dos Santos Lenis Mendes Soares 3. Larissa Carla Lauer Schneider	Karen de Melo Silva Mariana Nascimento de Paula Naiara Cássia Gancedo Rafaela Said dos Santos Raquel Garcia Isolani Thaila Fernanda Oliveira da Silva

Curso: Desenvolvimento de métodos de dissolução in vitro biorrelevantes. Rodrigo Cristofoletti

Data	Horário
20 de novembro de 2017	08:00 - 11:00 e das 14:00 às 17:00
21 de novembro de 2017	08:00 - 11:00 e das 14:00 às 17:00
22 de novembro de 2017	08:00 - 11:00 e das 14:00 às 17:00
25 de novembro de 2017	08:00 - 11:00

VIII Seminário Anual do PCF

Quarta-feira, 22 de novembro de 2017

Horário	
18h00 - 19h00	**Credenciamento**
19h00 - 19h30	**Cerimônia de abertura**
19h30 - 20h30	**Palestra 1 - 40 min. palestra/ 10 min. de perguntas** Palestrante: Rodrigo Cristofoletti **Tema: Uso de modelagem e simulação (M&S) na avaliação de segurança e eficacia de medicamentos novos e genéricos: realidade e perspectivas futuras.**
20h30 - 21h00	**Coffee break**
21h00 - 22h00	**Palestra 2 - 40 min. palestra/ 10 min. de perguntas** Palestrante: Andreas Hensel **Tema: Antiadhesive Natural Products against Parthogens**

Quinta-feira, 23 de novembro de 2017

08h00 - 09h00	**Palestra 3 - 40 min. palestra/ 10 min. de perguntas** Palestrante: Juliano Bordignon **Tema: The in vitro infection of human cells by Zyka virus is impaired by the citrus flavonoid naringenin**
09h00 - 09h30	**Coffee break**
09h30 - 10h30	**Apresentação dos discentes do PCF - GRUPO 1** 1. Amanda Nunes S. Hubner 2. Maria Fernanda Alves Aguiar 3. Bruna Luiza Pelegrine 4. Camila Caviquiole Sehaber 5. Carla Maria Mariano Fernandez
09h30 - 10h30	**Apresentação dos discentes do PCF - GRUPO 2** 6. Camila Biesdorf de Almeida 7. Erica Benassi Zanqueta 8. Fabiana Brusco Lorenzetti 9. Gabriele Gregodin Gimenez 10. Hélen Cássia Rosseto
10h30 - 11h30	**Apresentação dos discentes do PCF - GRUPO 3** 11. Bruna Juliano W. Ferrari 12. Jhonatan Christian Maraschin 13. Camila Cristina Iwanaga 14. Débora Botura Beaniot 15. Daniela Velasques Oliveira
10h30 - 11h30	**Apresentação dos discentes do PCF - GRUPO 4** 16. Erika Meyer 17. Fernanda Pilatti da Silva 18. Flávia Cristina Vieira Froz 19. Franciele Queiroz Ames 20. Heitor Augusto Otaviano Cavalcanti

14h00 - 15h00	**Palestra 4 - 40 min. palestra/ 10 min. de perguntas** Palestrante: Somen Das **Tema: Nanoparticles for regenerative medicine.**
15h00 - 15h30	**Coffee break**
15h30 - 16h30	**Apresentação dos discentes do PCF - GRUPO 5** 21. Jaqueline Godinho 22. Juliana Kovalczuk de Oliveira 23. Karinan Inácio de Oliveira 24. Lilian dos Anjos Oliveira Ferreira 25. Camila Caviquioli Sehaber
15h30 - 16h30	**Apresentação dos discentes do PCF - GRUPO 6** 26. Caio Cesar Sestile 27. Karen Elaine Poloi 28. Larissa Lachi da Silva 29. Leticia Pin Coltri 30. Rogério Aparecido Minini Santos
16h30 - 17h30	**Apresentação dos discentes do PCF - GRUPO 7** 31. Lorena Gimenes da Silva Santi 32. Ludmila Pini Simões 33. Gabriele Gregolin Gimenez 34. Mariana Maciel de Oliveira 35. Larissa Machado Valone
16h30 - 17h30	**Apresentação dos discentes do PCF - GRUPO 8** 36. Mariana Nascimento de Paula 37. Mariana Volpato Junqueira 38. Raquel Garcia Isolani 39. Rafael Pazinatto Aguiar

Sexta-feira, 24 de novembro de 2017	
08h00 - 09h00	**Palestra 5 - 40 min. palestra/ 10 min. de perguntas** Palestrante: Jos Prickaerts **Tema: Combining Academia and Industry: can you do that?**
09h00 - 09h30	**Coffee break**
09h30 - 10h30	**Apresentação dos discentes do PCF - GRUPO 9** 40. Priscila Miyuki Outuki 41. Regina Gomes Daré 42. Vanderson Carvalho Fenolon 43. Sirlene Adriana Kleinbing 44. Vanessa Kaplum
09h30 - 10h30	**Apresentação dos discentes do PCF - GRUPO 10** 45. Regina Yasuko Makimori 46. Tamara Borges Mariano 47. Sabrina Barbosa de Souza Ferreira 48. Thaysa Kaiaskiewcz Karam 49. Taisa Dalla Valle Raig Ribeiro
10h30 - 11h30	**Palestra 6 - 40 min. palestra/ 10 min. de perguntas** Palestrante: Roberto Barbosa Bazotte/ Rui Curi **Tema: Mecanismos de regulação da glicemia vs. mecanismos de desregulação da glicemia e seus tratamentos**
14h00 - 15h00	**Palestra 7 - 40 min. palestra/ 10 min. de perguntas** Palestrante: Claudia Maria Padovan **Tema: Princípios e Normas Éticas na utilização de Animais no Ensino e Pesquisa**
15h30 - 16h30	**Palestra 8 - 40 min. palestra/ 10 min. de perguntas** Palestrante: Bruno Filipe Carmelino Cardoso Sarmento **Tema: Bioengineered nanomedicines for the delivery of anticancer drugs in the gastrointestinal tract**
16h30 - 17h30	**Encerramento-Premiação**

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Programa de Pós-Graduação em Ciências Farmacêuticas
Curso:
de 20 a 25/11/2017
Demais atividades:
de 22 a 24/11/2017
Universidade Estadual de Maringá

RESUMOS

USE OF COMPLEXING AGENTS FOR THE DIRECTED PRODUCTION OF CYCLODEXTRINS BY COMMERCIAL ENZYME.

[1] Maria Fernanda Alves Aguiar*; [1]Vanderson Carvalho Fenelon; [1] Nathalia Maria Valerio; [1] Aline Satome Noce; [1] Graciette Matioli
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Key words: cyclodextrins, CGTase, complexing agents.

Introduction: Cyclodextrins (CD) are cyclic oligosaccharides, formed by the intramolecular transglycosylation reaction provided by the enzyme cyclodextrin glycosyltransferase (CGTase). The most commonly produced CDs are α-CD, β-CD and γ-CD, composed of 6, 7 and 8 glucose molecules, respectively. They can form non-covalent inclusion complexes with various molecules, increasing their stability and / or solubility**. Aim:** Due to the importance of CDs and the various industrial applications, this work aimed to use the commercial enzyme Toruzyme® to obtain CDs and orient their production to β-CD using 3-phenylpropionic acid and cyclohexanecarboxylic acid, as complexing agents in the reaction medium. **Methods:** The production was carried out in a glass reactor coated at 65 °C, and the production medium contained 15% cassava starch. The addition of ethanol to favor the production of CDs was also evaluated. The samples were boiled to the inactivation of the enzyme, then diluted and centrifuged at 9500 xg and 40 ºC, the supernatant was separated and the β-CD was quantified by spectrophotometry**. Results:** When β-CD production was evaluated for a period of 24 h, the best result was obtained with the presence of 10% ethanol in the reaction medium and 3-phenylpropionic acid as a complexing agent. In this case, the production was 14.4224 mmol L^{-1} of β-CD. However, when the production was for a longer period, i.e., 168 h, the complexing agent which proved most effective was cyclohexanecarboxylic acid, with a β-CD production of 41.4913 mmol L^{-1}. **Conclusion:** This study showed that the complexing agents used in this research were able to direct the production of CDs along with the addition of ethanol in the medium. The results obtained, therefore, are favorable, allowing a reduction of costs in the production of β-CD, due to the increase in selectivity and yield.

Acknowledgments: CAPES for financial support.

References:

[1]Challa, R., Ahuja, A., Ali, J., Khar, R. K., 2005. Cyclodextrins in Drug Delivery: An Updated Review. AAPS PharmSciTech. 6, 329-357.
[2]Del Valle, E. M. M., 2004. Cyclodextrins and their uses: a review. Process Biochemistry. 39, 1033-1046.

THE CANNABINOID TYPE-1 RECEPTOR IS INVOLVED IN THE ANTI-STRESS EFFECTS OF ESCITALOPRAM OBSERVED IN THE TAIL SUSPENSION TEST

[1]Rafael Pazinatto Aguiar*; [2]Franciele Franco Scarante; [2]Eduardo Junji Fusse; [1]Rubia Weffort de Oliveira; [2]Alline Cristina de Campos.

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Key words: Stress, antidepressants, CB1 receptor.

Introduction: Stress is a disruptive condition that occurs in response to adverse experiences. Chronic stress leads to changes in cognitive, emotional and, physiological processes and plays a pivotal role in the pathogenesis of psychiatric disorders[1]. Both serotonergic and cannabinoid neurotransmission have been implicated in neurobiology and behavioral consequences of stress[2]. Despite their therapeutic efficacy, antidepressant (AD) treatment is associated with a delayed response, considerable side effects and low rates of response due treatment-resistance[3]. Classically, AD facilitates monoamine neurotransmission, however, some studies also suggested that this class of drugs may also interfere with endocannabinoid system function[2]. **Aim:** To determinate if the type 1 cannabinoid receptor (CB1) participates in behavioral effects of escitalopram, a selective serotonin reuptake inhibitor, in mice submitted to the chronic unpredictable stress (CUS) paradigm. **Methods:** C57Bl/6 male mice were exposed to the CUS paradigm for 21 days and daily treated with AM251 or vehicle (VEH) (CB1 receptor inverse agonist, i.p., 0.3mg/kg) and 1h later with escitalopram (i.p., 10mg/kg) or VEH. On 20th and 22nd day of the CUS protocol, the animals were submitted to tail suspension test (TST) and novelty suppressed feeding test (NSF), respectively. **Results:** Our results suggested that CUS induced decreased coping strategies represented by the immobility time in TST ($t_{(24)}$= 2.207, $p<0.05$) and increased defensive behaviors evaluated in NSF ($t_{(18)}$= 2.82, $p<0.05$). ANOVA analysis has shown that escitalopram chronic treatment prevented stress-induced behavioral in TST ($F_{(3,39)}$=4.48 $p<0.05$) and NSF ($F_{(3,51)}$=3.301, $p<0.05$). In CUS-VEH mice, AM251 administration did not promote significant behavioral effects in TST or NSF. However, in mice that received escitalopram, pretreatment with AM251 prevented the behavioral effects of escitalopram observed in TST but not in NSF. **Conclusion:** Our preliminary results suggest that the positive effects of escitalopram on stress-induced decreased coping behavior observed in the TST, but the anxiolytic-like detected in NSF, rely on the activation of the CB1 receptor.
Acknowledgments: CNPQ; FAPESP, L'oreal-UNESCO-ABC.

References:
[1]Selye, H., 1976. The stress concept. Can Med Assoc J, 115(8), 718.
[2]Haj-Dahmane, S., Shen, R.Y., 2011. Modulation of the serotonin system by endocannabinoid signaling. Neuropharmacology. 61(3), 414-420.
[3]Blier, P., de Montigny, C., 1994. Current advances and trends in the treatment of depression. Trends Pharmacol Sci. 15(7), 220-222

COMPARISON OF HIGH FAT DIET AND HIGH CARBOHYDRATE DIET ON SERUM LIPID COMPOSITION IN SWISS MICE

[1]Camila Biesdorf de Almeida*; [1]Lorena Gimenez da Silva-Santi; [1]Marina Massetto Antunes; [1]Guilherme Godoy; [2]Fabiana Carbonera; [2]Jesuí Vergílio Visentainer; [1]Roberto Barbosa Bazotte
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Key words: Blood fatty acid, high carbohydrate diet, high fat diet.

Introduction: Blood fatty acid composition reflects the diet composition[1], and has been shown to predict the risk obesity[2], metabolic syndrome, insulin resistance, diabetes, and cardiovascular disease[3]. However, studies with humans present some limitations in terms of the extensive variability in diet composition. In addition, it has been shown that high carbohydrate diet promote more intense lipid accumulation in liver[4]. **Aim:** Compare the changes in serum FA composition in male Swiss mice fed with high carbohydrate diet (HCD) or high fat diet (HFD). **Methods:** Male Swiss mice (*Mus musculus*) weighing about 35 g (six-week-old), were used. The animals were randomly divided into two groups and were allocated one per cage. One group was fed with a high carbohydrate diet (HCD) and another one with a high fat diet (HFD). Amounts (g/100 g) of protein, carbohydrate and total fat in the HCD were 14.2, 73.8 and 4, respectively. Quantities (g/100 g) of protein, carbohydrate and total fat in the HFD were 20.3, 36.5 and 35.2, respectively. The mice were fed with the HFD or HCD for 0 (before starting the diets), 1, 7, 14, 28 or 56 days. After receiving HCD or HFD, mice were fasted from 17:00 to 08:00 h, killed by decapitation and the blood was collected, centrifuged to obtain the serum and stored at -80°C until analysis by gas chromatography**.** Activities of stearoyl-CoA desaturase-1 (SCD-1), ∆-6 desaturase (D6D), elongases and *de novo* lipogenesis (DNL) were estimated as the product/precursor ratio of individual fatty acid as follows: SCD-1 activity as the ratios of 16:1n-7/16:0 and 18:1n-9/18:0, D6D as the ratio of 18:3n-6/18:2n-6, elongase as the ratio of 18:0/16:0, and DNL as the ratio of 16:0/18:2n-6. Results are reported as means ± standard deviation of the means and were analyzed by ANOVA (one-way). P-values < 0.05 indicate statistical significance. **Results:** We observed predominance of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9), linoleic acid (18:2n-6), arachidonic acid (20:4n-6), and docosahexaenoic acid (DHA; 22:6n-3) in comparison with other fatty acid, either in HFD or HCD group. Serum from the HFD group had higher polyunsaturated fatty acid (PUFA) and elongase activity associated with lower monounsaturated fatty acid (MUFA), DNL, SCD-1 and D6D activities. **Conclusion:** The dietary carbohydrate and lipids modulate differently serum fatty acid composition as well as the estimated activities of desaturases, elongases and DNL. Serum from the HFD group had higher PUFA and elongase activity associated with lower MUFA, DNL, SCD-1 and D6D activities.

Acknowledgments: Capes, CNPq and Fundação Araucária.

1. Kotronen, A. et al. Comparison of Lipid and Fatty Acid Composition of the Liver, Subcutaneous and Intra-abdominal Adipose Tissue, and Serum. **Obesity**, vol. 18, n. 5, p. 937-944, 2010.
2. Pietiläinen, K. H. et al. Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects—a monozygotic twin study. **PLoS ONE**, vol. 2, p. 218, 2007.
3. Warensjö, E. et al. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. **Diabetologia**, vol. 48, p. 1999–2005, 2005.
4. da Silva-Santi, L. et al. Liver Fatty Acid Composition and Inflammation in Mice Fed with High-Carbohydrate Diet or High-Fat Diet. **Nutrients***,* vol. *8*, p. 682, 2016.

EFFECTS OF ANETHOLE, IBUPROFEN OR COMBINED ANETHOLE + IBUPROFEN ON INFLAMMATION AND LIVER METABOLISM OF L-ALANINE IN ARTHRITIC RATS

[1] Franciele Queiroz Ames*; [2] Lívia Bracht; [1] Letícia Aparecida de Oliveira; [1] Emanuele Parreira de Lima; [1] Ciomar Aparecida Bersani-Amado
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Key words: Anethole, anti-inflammatory drugs, chronic inflammation, liver metabolism.

Introduction: Previous studies showed that the combination of anethole (AN), a natural compound, and ibuprofen (IB), a non-steroidal anti-inflammatory drug, both at low doses, was effective for reducing the acute inflammatory response [1]. However, it is well known that some treatments which exhibit therapeutic efficacy in acute inflammatory diseases do not show the same efficacy in chronic diseases such as arthritis. **Aim:** The effect of AN, IB or combined AN + IB on inflammation and liver metabolism in rats with adjuvant-induced arthritis (AIA) were compared. **Methods:** Holtzman rats were divided into groups (n=7/group): (*i*) normal; (*ii*) AIA; (*iii, iv*) AIA treated with AN (62.5 and 250 mg/kg); (*v, vi*) AIA treated with IB (8.75 and 35 mg/kg) and (*vii*) AIA treated with AN + IB (62.5 + 8.75 mg/kg). Treatment with AN, IB or AN + IB was done by gavage, once daily from day 0 until day 21. Hind paw volume, appearance of secondary lesions and the number of recruited leukocytes into femorotibial joint cavity were evaluated. Moreover, rats that received these treatments were used to evaluate the liver metabolism of L-alanine. For this purpose the liver was isolated and perfused with L-alanine. Perfusion fluid samples were collected to determine the concentrations of glucose, pyruvate, L-lactate, urea, ammonia production, and oxygen consumption. Experimental protocol was approved by Ethics Committee (CEUA/UEM-7896220716). Data were analyzed using ANOVA-Tukey's test ($P<0.05$). **Results:** Treatments with 250 mg/kg AN, 35 and 8.75 mg/kg IB, and 62.5 + 8.75 mg/kg AN + IB reduced both injected and non-injected paws volume on the 13th, 17th and 21st days after adjuvant injection. Treatments with 35 mg/kg IB and AN + IB were the most effective. 62.5 mg/kg AN did not reduce paws volume. Treatments with AN and IB in the highest doses and AN + IB delayed the appearence of secondary lesions and reduced the number of leukocytes into the joint cavity. No significant difference was found between these treatments. On the other hand, treatments with AN and IB at low doses did not change these parameters. Treatments with 250 mg/kg AN, 35 mg/kg IB and AN + IB increased L-lactate and pyruvate production, but did not alter the low rates of oxygen uptake, glucose and urea production, and the high rate of ammonia production induced by AIA. **Conclusion:** AN + IB has an important anti-inflammatory effect and partially normalized the liver metabolism in arthritics rats.

Acknowledgments: CNPq, CAPES and Fundação Araucária.

Reference:
[1] WISNIEWSKI-REBECCA, E.S. et al. Synergistic effects of anethole and ibuprofen in acute inflammatory response. Chemico-Biological Interactions, 242:247-53, 2015.

EFFECT OF (-)-α-BISABOLOL ON THE LEUKOCYTES RECRUITMENT IN EXPERIMENTAL SEPSIS MODEL

[1]Heitor Augusto Otaviano Cavalcante*; [1]Luiz Alexandre Marques Wiirzler; [2]Saulo Euclides Silva-Filho; [1]Ciomar Aparecida Bersani-Amado; [1]Roberto Kenji Nakamura Cuman
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Key words: sepsis, inflammatory response, natural products.

Introduction: Sepsis is a complex syndrome due to an uncontrolled systemic inflammatory response, resulting from a generalized infection, which can lead to dysfunction, failure and organs death. Sepsis is not only restricted to bacterial infection, but also by any microorganism and/or its products (toxins)[1]. Due to the immune response caused by sepsis, several clinical manifestations are observed, such as: changes in body temperature, changes in blood leukocyte counts, hypotension, changes in heart rate and breathing, among others[2]. **Aim:** In this work, we evaluated the effect of (-)-α-Bisabolol (BISA) on the leukocyte recruitment and nitric oxide (NO) production into peritoneal cavity of mice after sepsis induced by cecal ligation and puncture (CLP) model. **Methods:** Mice C57BL/6 female were treated by oral route with BISA at doses of 50, 100 and 200 mg/kg or vehicle 1 h before sepsis induction. Sepsis was induced by CLP model. Six hours after sepsis induction the animals were euthanatized and peritoneal cavity was washed with 1 mL of phosphate-buffered saline. The total and differential leukocytes count was performed and the supernatant obtained were utilized for NO mensuration. NO concentration was performed by nitrite concentration, through the Griess reaction. Data were expressed as the mean ± SEM. Results were statistically analyzed by using one-way variance analysis followed by Tukey's test ($p<0.05$). The experimental protocols were approved by the Ethical Committee on Animal Experimentation of the State University of Maringá (protocol: 8233230916). **Results:** The sepsis induction promoted an increase in leukocyte recruitment compared SHAM group. BISA treatment at dose of 100 mg/kg reduced leukocyte recruitment into peritoneal cavity in 41.28% compared to control group. However, in the doses of 50 and 200 mg/kg, the BISA treatment did not showed effect on leukocyte recruitment in this model. Additionally, we observed that BISA treatment at dose of 100 mg/kg reduced significantly the mononuclears and polymorphonuclears leukocytes number in peritoneal cavity. Our results showed that BISA treatment at doses of 50 and 100 mg/kg did not reduced NO concentration in peritoneal cavity. However, the BISA treatment in the dose of 200 mg/kg promoted an significantly increase of NO concentration in 292.83%. **Conclusion:** Our results showed that BISA treatment exhibited effect on the leukocyte recruitment and NO production in CLP-induced sepsis model.

Acknowledgments: CAPES and CNPp.

References:

[1]Araújo, C.V., Estato, V., Tibiriçá, E., Bozza, P.T., Castro-Faria-Neto, H.C., Silva, AR., 2012. PPAR gamma activation protects the brain against microvascular dysfunction in sepsis. Microvascular research. 84, 218–21.
[2]Kovach, M.A., Standiford, T.J., 2012. The function of neutrophils in sepsis. Current opinion in infectious diseases. 25, 321–327.
in acute inflammatory response. Chemico-Biological Interactions, 242:247-53, 2015.

EFFECTS OF ETHANOL WITHDRAWAL ON ANXIETY AND LOCOMOTOR ACTIVITY OF MICE.

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Key words: anxiety, mouse, ethanol, open field and light / dark box.

Introduction: Animal models of ethanol withdrawal-induced anxiety have been used to explore the neurobiology underlying withdrawal and to evaluate the utility of therapeutic agents aimed at reducing withdrawal severity. The elevated plus maze, light/dark box (LDB), and open field (OF) tests are the most commonly used tests. However, ethanol withdrawal effects, especially those dependent on spontaneous motor activity, are difficult to measure and frequently result in ambiguity in interpreting the data as being indicative of anxiety-like states or of non-specific effects of ethanol withdrawal on locomotion. The objective of the study was to evaluate behavioral changes induced by ethanol withdrawal in mice using the OF and the LDB tests**. Material and methods:** Male Swiss mice (25-30 g) received i.p. of saline or ethanol (2 g/kg) daily for 10 days. Seven, 21 or 35 h after ethanol withdrawal, each animal was individually placed in the OF (5 min) where it was evaluated for the time spent, the number of entries in the periphery and in the central area and the travelled distance. Subsequently, the animals were subjected to the LDB test (5 min), where they were evaluated for the time spent in the light side and the number of crossings between both sides of the box. All procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 031/2010). Data were expressed as mean ± S.E.M. and analyzed by one-way ANOVA followed by the Newman Keuls post hoc test. **Material and methods Results:** Twenty four hours after ethanol withdrawal, a significant decrease was detected in the time spent ($F_{5,67}$=3,94, P<0.01; saline=20.52±2.22; 24 h ethanol withdrawal=10.78±1.73) and in the number of entries ($F_{5,67=}$3,34, P<0.01; saline=12.64±1.52; 24 h ethanol withdrawal =7.31±1.02) in the central area of the OF. No significant difference was observed in the travelled distance in the OF at 24 h following ethanol withdrawal ($F_{5,67}$=1,38, P=0.24) in comparison to control group. A significant decrease was observed in the number of crossings in the LDB 24 h after ethanol withdrawal when compared with controls ($F_{5,67}$=9,62, P<0.001; saline= 10.79±1.25; 21 h ethanol withdrawal=6.16±1.44). There was no significant difference in any parameter analyzed in the OF or LDB tests 7 or 35 h after ethanol withdrawal when compared to controls (P>0.05). **Discussion:** Anxiogenic-like effects were detected at 24 h but not after 7 h or 35 h of ethanol withdrawal in mice, indicating that this period should be an opportune period to test pharmacological interventions aimed to decrease ethanol withdrawal-induced anxiety.

Acknowledgments: Universidade Estadual de Maringá (UEM).

References:

AMERICAN PSYCHIATRIC ASSOCIATION. Diagnostic and Statistical Manual of Mental Disorders (DSM-V), 5th edition, 2013. Washington DC, American Psychiatry Association, 2013.

BOTIA, B. et al. Basal anxiety negatively correlates with vulnerability to ethanol-induced behavioral sensitization in DBA/2J mice: modulation by diazepam. Alcoholism, Clinical and Experimental Research, v. 39, n.1, p.45-54, jan. 2015.

TANNIC ACID: ANTIOXIDANT AND ANTI-WRINKLE ACTIVITIES IN A CELL FREE SYSTEM AND PHOTOPROTECTIVE POTENTIAL IN L929 FIBROBLASTS UVB-IRRADIATED

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Keywords: Tannin, UVB irradiation, oxidative stress

Introduction: The over-exposure to ultraviolet (UV) radiation induces deleterious effects on human skin, mainly due to generation of reactive oxygen species (ROS), which causes oxidative stress and injury to cellular molecules. Long-term damage includes premature aging[1] and photocarcinogenesis, that eventually can progress to a skin cancer, depending on the genetic predisposition and frequency of exposure[2]. Therefore, one of the prevention strategies is through compounds with potential to contribute to the maintenance of the redox balance in cells and reduce the harmful effects caused by UV irradiation. **Aim:** To investigate the antioxidant and anti-wrinkle activities of tannic acid and the photoprotective effect against oxidative stress induced by UVB radiation in L929 cells. **Methods:** The antioxidant potential was evaluated by the DPPH and xanthine/luminol/xanthine oxidase (XO) assays and the anti-wrinkle potential was analyzed using the anti-collagenase and anti-elastase assays. The photoprotective activity investigation was performed in L-929 cells pre-treated for 1h and UVB-irradiated with 600 mJ/cm^2. The cell viability was assayed using neutral red method in cells treated with different concentrations of the compound for 24 h and in cells both treated for 1 h and then irradiated. The ROS levels were verified using the H_2DCF-DA probe, either in cells irradiated or exposed to H_2O_2. The activity of the enzyme NADPH oxidase was assayed by superoxide radical dependent lucigenin chemiluminescence. The evaluation of the catalase (CAT) and superoxide dismutase (SOD) antioxidant enzymes were performed through the decomposition of H_2O_2 and by self-oxidation of pyrogallol, respectively. And the endogenous antioxidant glutathione reduced (GSH) levels were measured using the fluorochrome o-phthalaldehyde. **Results:** Tannic acid showed a high antioxidant and anti-wrinkle potentials. The pre-treatment in UVB-irradiated cells partially recovered cell viability by decreasing the oxidative stress, including the decrease of ROS generation induced by both irradiation and H_2O_2, by decreasing NADPH oxidase activity, by increasing the activity of the antioxidant enzymes CAT and SOD and increasing GSH levels. **Conclusion:** Tannic acid presented a considerable antioxidant and anti-wrinkle activities and attenuated UVB-induced photodamage by decreasing the oxidative stress. These data suggest a potential use of tannic acid in UV-protective therapy.

Acknowledgments: CAPES, CNPq, FA

References:
[1]Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature. 408, 239-247.
[2]Mollho-Pessach, V., Lotem, M., 2007. Ultraviolet radiation and cutaneous carcinogenesis. Curr. Probl. Dermatol. 35, 14–27.

PRODUCTION OF β-CYCLODEXTRIN IN CONTINUOUS ULTRAFILTRATION SYSTEM

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Keywords: Cyclodextrin, CGTase, ultrafiltration.

Introduction: Cyclodextrins (CDs) are formed by the action of the enzyme cyclodextrin glycosyltransferase (CGTase) on the starch. The most common are α-CD, β-CD and γ-CD, composed of 6, 7 and 8 glucose units, respectively. They have the ability to encapsulate a great number of molecules, increasing their stability and solubility, for example. Due to the large increase in the use of CDs, several researchers have sought better technological advantages in the production of these molecules[1]. **Aim:** This research aimed to produce β-CD by means of a continuous ultrafiltration system, using the semi purified CGTase from *Bacillus firmus* strain 37. **Methods:** The β-CD production was performed from 5% (w/V) corn starch substrate, in the presence of 10% (V/V) ethanol in a jacketed reactor with a capacity to 500 mL of reaction medium. The reactor was coupled to a hollow fiber ultrafiltration module, equipped with a 50,000 MWCO exclusion limit column, capable of separating the CDs and other inhibitory products and, at the same time, recovering the CGTase to continue acting on the starch. The continuous production was maintained during 264 h (11 days), and ultrafiltrate aliquots were collected every 12 h to determine the β-CD produced. **Results:** In the first 12 h of production, that was carried out without ultrafiltration, the yield was 16.90 mmol/L and, in sequence, the continuous ultrafiltration system was put into operation, resulting in satisfactory yields, since the decrease in β-CD concentration occurred slowly. CGTase maintained partially its activity throughout the entire test, without the need for enzyme replacement. At the end of the 264 h the β-CD concentration was 5.85 mmol/L. The production of α and γ-CD was low throughout the production period. After the first 12 h, α and γ-CD concentrations were 0.24 mmol/L and 1.74 mmol/L, respectively. This concentration decreased gradually and, from the time 180 h, these CDs were no longer detected in the reaction medium. This condition can be considered very favorable, especially when considering the purification aspect to obtain the isolated CDs, which is mostly desirable. **Conclusion:** These results demonstrate the effectiveness of the continuous ultrafiltration system for a better utilization of CGTase capacity in the production of CDs. This research brings new perspectives to the production of CDs and may contribute to their obtaining on an industrial scale in Brazil.

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References:
[1]Gawande, B., Patkar, A., 2001. Alpha-Cyclodextrin Production using Cyclodextrin Glycosyltransferase from Klebsiella pneumonia AS-22. Starch. 53, 75-83.

ACARICIDAL, LARVICIDAL AND *ANTI-MYCOBACTERIUM TUBERCULOSIS* ACTIVITY OF ROOT EXTRACT AND ISOLATES FROM *PIPER CORCOVADENSIS* (MIQ.) C. DC.

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Key words: *Piper corcovadensis*, *Rhipicephalus microplus*, *Aedes aegypti*, tuberculosis

Introduction: *Piper corcovadensis* Miq. C. DC. (Piperaceae) is a plant native to Brazil known as João brandinho. It is popularly used in treatments such as rheumatism, pain, flu and cough. Some amides were isolated from roots extract (1). **Aim:** The aim of this work was to evaluate the acaricidal, larvicidal and antituberculosis activity of the root extract, obtained from Soxhlet (ES) of *P. corcovadensis*, piperovatine and piperlonguminine/ isopiperlonguminine (FRPI). **Methods:** In order to obtain the ES, the roots of *P. corcovadensis* were collected in Diamante do Norte, Paraná, Brazil. The plant material was dried, pulverized, and subjected to the Soxhlet extractor apparatus with dichloromethane. After that, the ES was concentrated, lyophilized and stored at 4°C. Piperovatine and FRPI (90% piperlonguminine and 10% isopiperlonguminine) were isolated by classical chromatography and identified by NMR. For the acaricidal and larvicidal activities, ES and the isolates were diluted in 2% ethanol in aqueous solutions at concentrations of 100 to 1 µg/mL, and for antituberculosis activity the ES and the isolates were diluted in DMSO at concentrations of 250 to 1.9 µg/mL. The acaricidal action on *Rhipicephalus microplus* was evaluated by the larval immersion test (2) and the test in semi-natural conditions (3), and larvicidal activity was evaluated in larvae of the 3rd and 4th stages of *Aedes aegypti* by the larval immersion test (4), and the lethal concentration (LC) of 50 and 99% was determined by the Probit analysis. Antituberculosis activity was investigated on *Mycobacterium tuberculosis* by determination of minimum inhibitory concentration (MIC) using the technique of Resazurin Microtiter Assay Plate (5). **Results:** Piperovatine presented LC_{50} and LC_{99} of 5.2 and 25.4 µg/mL, respectively to tick larvae and showed LC_{50} and LC_{99} of 17.8 and 48.5 µg/mL, respectively to mosquito larvae. Piperovatine and ES showed efficacy of 95.52 and 96.63%, respectively, in semi-natural conditions. For the antituberculosis activity, piperovatine and FRPI presented MIC of 3.9 and 7.8 µg/mL, respectively. **Conclusion:** In this way, ES, piperovatine and FRPI presented potential biological activities.

Acknowledgments: CAPES

References: (1)Costa SS, Mors WB. Phytochemistry 20(6) (1981), 1305-1307. (2)Drummond RO, Ernest SE, Trevino JL, Gladney WJ, Graham OH. Journal of Economic Entomology 66 (1973), 130–133. (3)Araújo LX, Novato TPL, Zeringota V, Matos RS, Senra TOS, Maturano F, Prata MCA, Daemon E, Monteiro CMO. Parasitology Research 114 (2015), 3271-3276. (4)Costa JGM, Rodrigues FFG, Angélico EC, Silva MR, Mota ML, Santos NKA, Cardoso ALH, Lemos TLG. Revista Brasileira de Farmacognosia 15 (2005), 304-309. (5)Palomino JC, Martin A, Camacho HG, Swings J, Portaels F. Antimicrobial Agents and Chemotherapy 46 (2002), 2720-2722.

PHOTOCHEMIOPROTECTIVE EFECTS OF *Campomanesia guaviroba* AGAINST UVB RAYS

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Introduction: Solar radiation is responsible for lesions, mediated by oxidative stress, which alter the skin metabolism, leading to photoaging and even the development of cancers. Plants are considerable sources of bioactive molecules that can act in the prevention/treatment of oxidative damage to the skin[1]. In this context, it is expected to demonstrate the medicinal potential of *Campomanesia guaviroba*, belonging to Myrtaceae family rich in species with antioxidant and antiinflammatory activity, against UVB solar radiation. **Aim:** Evaluate the cytotoxicity and photochemical potential of ethyl acetate fraction (AF) of *Campomanesia guaviroba* against damage caused by UVB radiation on L-929 fibroblasts. **Methods:** The plant material (leaves) was dried (40 °C) in a circulating air oven, ground in a knife mill (1.6 mm diameter mesh) and characterized. The ethanolic extract was obtained by percolation and, after removal of the extractive solvent in a rotary evaporator, lyophilized and adequately stored. The extract was subjected to liquid-liquid partition resulting in hexane, ethyl acetate and hydromethanol fractions. Cytotoxicity was determined by the the incubation of murine fibroblasts lineage L-929 treated for 24 h at 5.66 µg/mL(DPPH IC_{50} previously determined), after cells were subjected to the neutral red assay. Photochemioprotection was evaluated by the percentage of cell viability on irradiated L-929 fibroblasts (UVB 500 mJ/cm^2) and treated 1 h before, during and 1 h after irradiation (5.66 µg/mL)[2]. Quercetin was used as a positive control. **Results:** On citotoxicity assay cell viability was maintained at 95.47%, not significantly differing from the negative control (p-value 0.05). On the cells irradiated, during-treatment (50.23%) and post-treatment (61.61%) the cell viability was lower than negative control (67.08 and 63.71%, respectively). However, the pre-treatment inhibited UVB-damage on fibroblast (74.44%), better than positive control quercetin (69.86%). **Conclusion:** The results obtained show that *Campomanesia guaviroba* has active ingredients with potential activity for the prevention of photo-oxidative skin damage, justifying the continuity of the studies.

Acknowledgments: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

References:
[1]Ratz-Lyko, A., Arct, J., Majewski, S., Pytkowska, K. 2015. Influence of polyphenols on the physiological process in the skin. Phytotherapy Research. 29, 509-517.
[2]Borenfreund, E., Puerner, J.A. 1984. A simple quantitative procedure using monolayer culture for toxicity assays. Journal of Tissue Culture Methods. 9, 7–9.

PREPARATION AND RHEOLOGICAL PROPERTIES OF EMULGEL CONTAINING CURCUMIN

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Key words: emulgel, curcumin, rheology

Introduction: Emulgels are oil-water systems, where the water phase is gelled by polymers, such as acrylic acid derivatives. These preparations provide increased availability and solubilization of hydrophobic drugs such as curcumin (CUR), increasing their stability and modified release. **Aim:** This study aimed to prepare and evaluate the rheological properties of the formulation that did not change their properties after thermal stress and centrifugation for buccal application. **Methods:** Four emulgels were prepared containing 15% (w/w) poloxamer 407 (P407), 0.50% (w/w) bioadhesive polymer (BP), 0.75% (w/w) oil phase (OP) and 0.08% (w/w) CUR. The preliminary stability studies were performed by six ice-defrost cycles. In each cycle, emulgels were kept for 24 hours at -5 ± 2 ℃, and, then, 24 hours at 40 ± 2 ℃. In the beginning of the studies and in the end of the sixth cycle, the emulgels were evaluated for organoleptic properties (color, odor and phase separation), centrifugation, which provides the instability index and drug content. The rheological properties of emulgel without and containing CUR were evaluated. Flow and oscillatory rheology were performed using a controlled stress rheometer at 5 ℃, 25 ℃ and 37 ℃ with parallel cone-plate geometry. Gelation temperature was determined by oscillatory mode with temperature sweep. **Results:** All formulations showed similar drug content. But, in the end, the preliminary stability studies evidenced that formulations composed by oil phase of sesame oil and the BP, Carbopol 974P®, showed absence of phase separation and lower instability index values, which indicates that the thermal stress and centrifugation was not enough to change de original properties of systems. Consequently, this formulation was selected for rheological analysis, which displayed plastic behavior with higher yield stress and thixotropy area than the same formulation in the absence of the drug. On the other hand, the effect of CUR presence decreased the consistency index. Moreover, the increase of temperature increased the consistency index, yield stress and thixotropy area. The oscillatory rheometry showed those formulations without or containing CUR were viscoelastic. Gelation temperature of emulgels composed by CUR was 33.27 ± 0.06 °C. **Conclusion:** Formulations composed by P407, Carbopol 974P®, sesame oil and CUR were stable for the evaluated characteristics and demonstrated suitable rheological characteristics for buccal application.

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References:

[1]Sultana SS, Parveen P, Rekha MS, Deepthi K, Sowjanya C, Devi AS. 2014. Emulgel – a novel surrogate approach for transdermal drug delivery system. Indo Am J Pharm Res. 4(11):5250–300.

[2]Shen Y, Ling X, Jiang W, Du S, Lu Y, Tu J. 2015 Formulation and evaluation of Cyclosporin A emulgel for ocular delivery. Drug Deliv .22(7):911–917.

EVALUATION OF RADICAL SCAVENGING ACTIVITY AND INTESTINAL CELL VIABILITY OF BRAZILIAN PROPOLIS BY-PRODUCT

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Keywords: Propolis by-product; Antioxidant activity; Caco-2; HT29-MTX.

Introduction: Propolis is a natural adhesive resinous compound produced by honeybees. During propolis extract production, a resinous by-product is formed[1]. This resinous waste is currently undervalued and underexploited. **Aim:** The aim was to evaluate the physicochemical characteristics as well as the antioxidant activities, cell viability of by-product (WPE) and compare to propolis (PE) in order to stimulate the re-use and valorisation of WPE, based on three Rs concept. **Methods:** The methods chosen for the physical-chemical evaluation of the extracts were: determination of dry residue content, density, alcohol content and pH[2]. Total phenolic content (TPC) was determined spectrophotometrically according to the Folin–Ciocalteu procedure. Total flavonoid content (TFC) was determined by a colorimetric assay based on the formation of flavonoid-aluminium compound. The reduction of the DPPH radical was determined by measuring the absorbance at 517 nm. Extracts aliquots were added to FRAP reagent and the reaction mixture incubated. The increase in absorbance at 592 nm was measured[3]. The reduction of the ABTS radical was determined by measuring the absorbance at 750 nm[2]. The scavenging activity against reactive oxygen (ROS) and nitrogen species (RNS) assays were performed using a microplate reader for fluorescence, UV/Vis and chemiluminescence measurements, in the scavenging assays of superoxide anion radical (O2•−), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), nitric oxide (•NO) and peroxyl radical (ROO•). PE and WPE were assessed in Caco-2 and HT29-MTX cell lines using the MTS reagent. Cell grown separately in tissue culture flasks in a complete medium. Cells were seeded into wells of 96-well plates and incubated overnight at standard conditions to reach exponential growth prior to the assay test. The cultured cells were incubated for 24 h in the presence of different samples concentrations. The plates were read at 490 nm with background subtraction at 630 nm[2]. **Results:** The results revealed that the WPE meets the physical and chemical quality standards expected and showed that the propolis waste contains similar amounts of TPC and TFC to propolis. Also, a good scavenging activity against ROS and RNS determined by the assays. Linear positive correlations were established between the TPC of both samples and the antioxidant activity evaluated by three different methods (DPPH, ABTS and FRAP assays). The extracts were also screened for cell viability assays in HT29-MTX and Caco-2, showing a viability concentration-dependent. **Conclusion:** These results suggest that propolis by-product can be used as a new rich source of bioactive compounds for different areas.

Reference:

[1] Lenardão, E. J., et al. (2003). "Green chemistry": os 12 princípios da química verde e sua inserção nas atividades de ensino e pesquisa. p. 123-129.

[2] Bruschi, M. L. et al. (2002). Contribution to the quality control protocol of propolis and its extracts. p. 289 -306.

[3] Rodrigues, F. et al. (2013). Medicago spp. extracts as promising ingredients for skin care products. p.634-644.

[4] De Francisco, L. M. B. et al. (2016). Nanoparticles of waste material of propolis and gelatin as a novel system for delivery of L-ascorbic acid. p.1-12.

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EFFECTS OF MICROENCAPSULATED QUERCETIN IN INTERSTITIAL CELLS OF CAJAL, NNOS AND M2 MACROPHAGES DENSITY OF DIABETIC MICE

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Key-words: Interstitial cells of Cajal; Diabetes Mellitus; Microencapsulated quercetin

Introduction: the chronic hyperglycemia on Diabetes Mellitus causes an oxidative stress by reducing the activity of the antioxidant enzymes, increasing the production of free radicals, which can induce apoptosis of Interstitial Cells of Cajal (ICC)1, alterations in the enteric innervation that express nNOS and release inflammatory cascades that activate macrophages (M2), also involved in the intensification of smooth muscle contraction2. Quercetin is capable of protecting the tissues of the damage caused by free radicals and lipid peroxidation. Objective: evaluate the ICC, nNOS and macrophages density of diabetic rats treated with microencapsulated quercetin (10mg/kg and 100mg/kg). **Methods:** thirty-six male ninety days Wistar rats were used (CEUA 073/2014), divided in six groups: normoglycemic (N), normoglycemic treated with 100mg/kg microencapsulated quercetin (QM100), normoglycemic treated with 10mg/kg (QM10), diabetic (D), diabetic treated with 100mg/kg microencapsulated quercetin (DQM100) and in the dose of 100mg/kg (DQM10). Animals of D and DQ groups suffered DM induction by Streptozootocin endovenous injection (35mg/kg). QM and DQM groups were submitted, daily, to gavage for tratament. At 150 days old, the rats were anesthetized (thiopental 40mg/kg) and the jejunum was collected for immunohistochemical techniques. The results were submitted to the One-way Blocked Analysis of Variance (ANOVA) test with Fischer post-test. Significance level was 5%. **Results:** there was an ICC-MY density reduction on diabetic animals comparing to the N group. Microencapsulated quercetin administration (DQ group) reestablished the density in 49%. In QM100 group, there was an ICC-MY density reduction related to N group. In the myenteric plexus, nNOS quantitative analysis showed a 14.5% reduction in D related to N, 67% reduction in D related to DQ10, and a 62.9% and 7.7% rise in NQ10 and NQ100 groups ($p<0.0001$ to all). In contrast, there was an expressive raise in the macrophages density in diabetics related to N ($p<0.0001$) and reductions of 61% and 89% comparing diabetic treated groups with D. In addition, there was a reduction of the same parameter in NQ100 related to N ($p<0.0001$). **Conclusion:** treatment with 10mg/kg microencapsulated quercetin suggests a Cajal density protection mechanism, but not nNOS and M2 macrophages. However, recent studies have demonstrated that nNOS and macrophages work on the Cajal networks maintenance minimizing the damage caused by Diabetes.

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References

[1] Kostitska, I. O. et al. Morphological Aspects of Diabetic Gastroparesis.**Galician Medical Journal**, v. 22, n. 4, p. 8-12, 2015.

[2] Neshatian, L., Simon G. J., Farrugia G. Macrophages in diabetic gastroparesis – The missing link? **Neurogastroenterology and Motility**, doi: 10-1111/nmo- 12418, p. 1-12, 2014

MORPHO-ANATOMICAL STUDY OF *Croton floribundus* BARK

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Key words: Euphorbiaceae; ethnobotanical; pharmacognosy

Introduction: *Croton floribundus* Spreng., Euphorbiaceae, which is popularly known in Brazil as "capixingui", is a native and non-endemic tree that can reach 6-10 m tall[1]. This plant is common in Atlantic Rainforest and popularly used to treat leukemia, tumors and syphilis. **Aim:** The aim of this study was to describe the morpho-anatomical and histochemical characteristics of the *C. floribundus* bark. **Methods:** The bark of *C. floribundus* were collected in 2016 at State University of Maringá (51°56'S, 23°24'W). The specimens with inflorescences were deposited in the Herbarium of State University of Maringá (HUEM 30778). Analysis of optical microscopy, scanning electron microscopy (SEM), histochemical tests[2] and qualitative X-ray microanalyses were performed. **Results:** The *C. floribundus* bark was classified as "curved" and the external surface is gray, with lichens and presence of horizontal striae. The internal surface is pink, rough, with perpendicular striae in the largest axis of the bark. The fracture is fibrous. The bark has a bitter taste and is astringent and slightly spicy. The suber of *C. floribundus* is constituted by flat cells strata, with reddish content and that react positively with the ferric chloride, as a resulted of its composition by polyphenols. The cortical parenchyma was divided in to two regions. The first region has about 6-10 of cells layers with reduced diameter and circular shape, thin cell walls and reduced intercellular spaces in cross section. The second region is characterized by the formation of cortical parenchyma cells with larger diameter, thin cell walls and very few intercellular spaces. Even in this second region there are idioblasts and druse crystal that react positively in the presence of ferric chloride, Sudan IV glycerin and 60% chloral hydrate with 25% sulfuric acid, confirming the presence of polyphenols (more abundant), lipophilic substances and calcium oxalate crystals, respectively. The crystals were analyzed for their elemental composition and the spectra showed peaks for calcium (20.3%), carbono (27.4%) and oxygen (41.3%). In the bark cortical region there are typical gelatinous fibers which have internal layers of malleable appearance and reduced lumen. Groups of macrosclereids and gelatinous fibers were observed in the pericyclic region. The laticifers were observed near the phloem region and the histochemical tests confirmed the presence of starch, calcium oxalate crystals and polyphenols near the vascular system. **Conclusion:** The analysis revealed that the main pharmacognostic characteristics of the *C. floribundus* bark are related to distribution of gelatinous fibers, idioblasts and calcium oxalate crystals.

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References

[1]*Croton.* Accessed Jul. 2017. *In:* Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro, http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB17512.
[2]Farmacopeia Brasileira. 2010. Agência Nacional de Vigilância Sanitária. Brasília, DF. Brasil.

MOLECULAR CLONING OF THE CYCLODEXTRIN-GLYCOSYLTRANSFERASE GENE FROM *Bacillus firmus* STRAIN 37 IN *Bacillus subtilis* WB800 AND PRODUCTION OF CYCLODEXTRINS BY RECOMBINANT CGTASE

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Key words: cyclodextrin-glycosyltransferase; cyclodextrins; cloning.

Introduction: The enzyme cyclomaltodextrin glucanotransferase (CGTase) catalyzes the degradation of the starch, resulting in α-, β- and γ-cyclodextrins (CDs), which are capable to form inclusion complexes and stabilizing a broad spectrum of substances [1]. The strategy of cloning and expression of recombinant CGTase may be a viable alternative to obtain an enzyme production with sufficient yield to be economically feasible its application in industrial processes. **Aim:** This work aimed to improve the CD production by the study of enzyme CGTase, in which the CGTase gene from *Bacillus firmus* strain 37 was isolated, cloned and expressed in *Bacillus subtilis* WB800. **Methods:** *Bacillus firmus* strain 37 was used as CGTase-producing bacteria, *Bacillus subtilis* WB800 as host bacteria and plasmid pWB980 as expression vector. The analysis *in silico* was performed and a cloning strategy was determined. PCR amplification of CGTase was accomplished from the genomic DNA extracted from *B. firmus* strain 37, followed by TOPO-TA® binding and transformation into *Escherichia coli* DH5α [2;3]. The cloning was confirmed by CGTase sequencing and subcloning was performed using plasmid pWB980, restriction enzymes SmaI and NheI, and transformation into *B. subtilis* WB800. After cloning, recombinant CGTase was expressed, purified and its activity compared to CGTase from *B. firmus* strain 37. **Results:** Cloning of the CGTase gene in *E. coli* DH5α was performed and sequencing of the ligated gene confirmed the CGTase sequence of *B. firmus* strain 37, that is composed of 2022 base pairs. Subcloning was successfully performed, employing plasmid pWB980 and transforming into *B. subtilis* WB800. The most suitable medium for the production of recombinant CGTase was 2xYT, showing significantly higher enzymatic activity compared to the other evaluated media. The enzymatic activity of recombinant CGTase was 1.33 µmol β-CD/min/mL. This value of activity represents an increase of 7.4 times compared to the enzymatic activity of crude extract of CGTase obtained from the wild strain. **Conclusion:** The recombinant CGTase cloning and expression strategy was efficient and the results obtained provide essential data for the large scale production of the recombinant enzyme, with the possibility of obtaining high yield. Therefore, this recombinant CGTase is economically viable to application in industrial processes.

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References:
[1]COSTA H, et al. Carbohydrate Research, v. 344 (2009), p. 74–79.
[2]SAMBROOK J, RUSSEL DW. Molecular Cloning. 3ª ed., v. 3. (2001), Cold Spring Harbor Laboratory Press, New York.
[3]ZAHA A, et al. Técnicas de DNA Recombinante. Biologia Molecular Básica, ed., Capítulo 15, Porto Alegre, Mercado Aberto .

ETHYL-ACETATE FRACTION OF *TRICHILIA CATIGUA* PROTECTS AGAINST OXIDATIVE STRESS AND NEUROINFLAMMATION AFTER CEREBRAL ISCHEMIA/REPERFUSION

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Key words: Global cerebral ischemia; Oxidative stress; Neuroinflammation.

Introduction: *Trichilia catigua* (“catuaba”) preparations have been used in folk medicine as physical and mental tonics, especially as a sexual stimulant. Antinociceptive, antiinflammatory, and *in vitro* neuroprotection has been observed in animals. Cerebral ischemia/reperfusion (I/R) is associated with oxidative stress, inflammation, neurodegeneration, and neuropsychological déficits. We repoted that an ethyl-acetate fraction (EAF) of *T. catigua* reduced the learning/memory impairments caused by I/R, in the absence of sustained histological protection. **Aim:** Here we investigated the antioxidant and anti-inflammatory properties of *T. catigua* in an *in vivo* model of I/R. **Methods:** Male Wistar rats were subject to 15 min of I/R (4-VO model). Vehicle was given by gavage 30 min prior to and 1 h after I/R. On day 1 post-ischemia the effects of *T. catigua* (400 mg/kg, p.o.) in reduced glutahione (GSH), oxidized glutahione (GSSG), superoxide dismutase (SOD), catalase (CAT), malondialdeyde (MDA), and protein carbonyl groups (PCG) were measured as oxidative stress markers. In a second experiment the expression of glial cells (micróglia and astrocytes) was measured immunohistochemically as neuro-inflammation markers. Finally the effect of *T. catigua* on the activity of myeloperoxidase (MPO) was also evaluated as a marker of neutrophils infiltration. The generalized linear model (GLM) with a normal distribution was used for between-group comparisons of the variable-responses.This protocol had the approval of internal Ethical Committee (CEUA N 4952280814/2014). **Results:** The levels of GSH, GSSG, the GSH/GSSG ratio, as well as the SOD activity and the content of PCG were normalized to the control level after treatment with the EAF of *T. catigua*. The loss in CAT activity and the formation of MDA elicited by I/R were not prevented by *T. catigua.* Ischemia-induced activation of glial cells and MPO was also prevented by *T. catigua*. **Conclusion:** The results demostrate that *T. catigua* possess both antioxidant and anti-inflammatory activities after TGCI in rats, which may have contributed to the memory protective effect *T. catigua* reported previously.

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References:
[1]Truiti, M.T. *et al*. 2015. Trichilia catigua ethyl-acetate fraction protects against cognitive impairments and hippocampal cell death induced by bilateral common carotid occlusion in mice. J. Ethnopharmacol. 172, 232–237.

Limonium brasiliense: A CITOTOXIC EVALUATION IN VERO CELLS

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Key words: *Limonium brasiliense*, citotoxicity, *Herpes simplex*.

Introduction: *Limonium brasiliense* (Boiss.) Kuntze (Plumbaginaceae), popularly known as baicuru, is a plant native to southern Brazil[1]. The rhizomes of *L. brasiliense* are popular used for the treatment of premenstrual tension, menstrual disorders and genitourinary tract infections[2,3]. The *Herpes simplex* virus type 1 (HSV-1) may remain latent in the body and causes orofacial and ocular infections in 90% of the population[4]. The drug of choice for treatment of HSV-1 is Acyclovir, but there are already some drug-resistant strains, requiring the search for new compounds for treatment that are no toxic to healthy cells. The MTT [3-(4,5 Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] is a rapid and sensitive method that evaluates cell viability by reducing MTT by dehydrogenases in the cell mitochondria[5]. **Aim:** The aim of this study was to evaluate the cytotoxicity of the extract, semi-purified fractions and substances isolated from *L. brasiliense*. **Methods:** The crude extract (CE), aqueous fraction (AQF), ethyl acetate fraction (EAF), another fraction containing the compounds samarangenin A, samarangenin B and epigallocatechin-3-*O*-gallate together (F7) and isolated compounds from *L. brasiliense* were tested to evaluate cytotoxicity against Vero cells, by MTT methodology. **Results:** The results were concentração citotoxica 50% (CC_{50}) values of 85 ± 5 µg/mL for CE, 56.67 ± 11.55 µg/mL for the AQF, 41.67 ± 12.58 µg/mL for EAF, 43.33 ± 5.7 µg/mL for F7, 66.67 ± 15.28 µg/mL for epigallocatechin-3-*O*-gallate, 33 ± 7 µg/mL for samarangenin A and 45 ± 5.77 µg/mL for samarangenin B. **Conclusion:**The compounds are considered non-cytotoxic and can be used for future studies of antiviral activity analysis against HSV-1 and semi-solid product development for treatment of orals vesicles caused by the virus.

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[1]CORREA, M.; PENNA, L. D. A. Dicionario das plantas uteis do Brasil e das exoticas cultivadas: volume 5. MR. **Rio de Janeiro: Instituto Brasiliero de Desenvolvimento Florestal 687p.-illus.. Por Icones. Geog,** v. 4, 1974.
[2] MOURA, T. F. A. et al. Estudos farmacológicos preliminares das raízes do *Limonium brasiliense* (Boiss.) Kuntze-Plumbaginaceae (Baicuru). **Caderno de farmácia. Porto Alegre, RS**, 1985.
[3] CARDOSO, M. L. *Limonium brasiliensis* (boiss.) kuntze, plumbaginaceae (baicuru): desenvolvimento galenico e extratos. 1990.
[4] VILLARREAL, E. C. Current and potential therapies for the treatment of herpes virus infections. In: (Ed.). **Progress in drug research**: Springer, 2003. p.263-307.
[5] FREIMOSER, F. M. et al. The MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay is a fast and reliable method for colorimetric determination of fungal cell densities. **Appl Environ Microbiol,** v. 65, n. 8, p. 3727-3729, 1999.

Limonium brasiliense: STRUCTURAL ANALYSIS AND EVALUATION OF CYTOTOXICITY IN VERO CELLS FOR TREATMENT OF HUMAN HERPES SIMPLEX

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Key words: *Limonium brasiliense*, tannins, *Herpes simplex*.

Introduction: *Limonium brasiliense* (Boiss.) Kuntze (Plumbaginaceae), popularly known as baicuru, is a plant native to southern Brazil[1]. Its popular use is by decoction or infusion of the rhizomes, which are used for the treatment of premenstrual tension, menstrual disorders and genitourinary tract infections[2,3]. It is known the existence of phenolic compounds in the genus *Limonium*[4] as it is also known the biological activities of this genus relating to the substances present[5]. The *Herpes simplex* virus type 1 (HSV-1) causes orofacial and ocular infections, and may remain latent in the body. Acyclovir is the drug of choice for HSV-1 treatment, but there are already some drug-resistant strains. **Aim:** The aim of this study was to find a new treatment against HSV-1 from extracts, fractions and isolated compounds from *L. brasiliense*. **Methods:** For the isolation of the compounds was carried out a classic column chromatography containing Sephadex LH20 and as mobile phase ethanol. For structural elucidation, the mass spectrometry method was used. The crude extract (CE), aqueous fraction (AQF), ethyl acetate fraction (EAF), another fraction containing the compounds samarangenin A, samarangenin B and epigallocatechin-3-*O*-gallate together (F7) and isolated compounds from *L. brasiliense* were tested to evaluate cytotoxicity against Vero cells, by MTT methodology. **Results:** The results of chromatographic isolation and structural analysis suggested molecules of (epi)galocatechin-(epi)galocatechin, myricetin galactosidegalate, (epi)galocatequinagalate-(epi)galocatequinagalate, samarangenin A and samarangenin B. The cytotoxicity of the compounds in Vero cells showed CC_{50} values of 85.00 ± 5.00 µg/mL for CE, 56.67 ± 11.55 µg/mL for the AQF, 41.67 ± 12.58 µg/mL for EAF, 43.33 ± 5.70 µg/mL for F7, 66.67 ± 15.28 µg/mL for epigallocatechin-3-*O*-gallate, 33.00 ± 7 µg/mL for samarangenin A and 45.00 ± 5.77 µg/mL for samarangenin B. **Conclusion:** The compounds are considered non-cytotoxic and can be used for studies of antiviral activity against HSV-1 and development of lip cream to treat vesicles caused by the virus. The isolation allowed the separation of 5 compounds, which will be elucidated by nuclear magnetic resonance.

Acknowledgments: CAPES, CNPq, INCT_if, Fundação Araucária.

[1]CORREA, M.; PENNA, L. D. A. Dicionario das plantas uteis do Brasil e das exoticas cultivadas: volume 5. MR. **Rio de Janeiro: Instituto Brasiliero de Desenvolvimento Florestal 687p.-illus.. Por Icones. Geog,** v. 4, 1974.
[2] MOURA, T. F. A. et al. Estudos farmacológicos preliminares das raízes do *Limonium brasiliense* (Boiss.) Kuntze-Plumbaginaceae (Baicuru). **Caderno de farmácia. Porto Alegre, RS**, 1985.
[3] CARDOSO, M. L. *Limonium brasiliensis* (boiss.) kuntze, plumbaginaceae (baicuru): desenvolvimento galenico e extratos. 1990.
[4] EREN, Y.; ÖZATA, A. Determination of mutagenic and cytotoxic effects of *Limonium globuliferum* aqueous extracts by Allium, Ames, and MTT tests. **Revista Brasileira de Farmacognosia,** v. 24, n. 1, p. 51-59, 2014.
[5] HASLAM, E. Polyphenol–protein interactions. **Biochemical Journal,** v. 139, n. 1, p. 285, 1974.

TOTAL PHENOLICS AND ANTIOXIDANT CAPACITY OF *Eugenia hiemalis* CAMBESS. AND *Eugenia blastantha* (O. BERG) D. LEGRAND.

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Keywords: oxidative stress; medicinal plants; Myrtaceae.

Introduction: Extracts of medicinal plants are considered strong antioxidant candidates in the prevention and/or treatment of the damages caused by reactive species of the cellular metabolism, mainly by the presence of phenolic compounds[1]. **Aim:** Evaluate the total phenolic content (TP) and antioxidant capacity (AC) of ethanolic extracts (EE) and fractions of *Eugenia hiemalis* (Eh) and *Eugenia blastantha* (Eb). **Methods:** The ethanolic extracts of *E. hiemalis* (EEEh) and *E. blastantha* (EEEb) were obtained by percolation from the dried and ground leaves. After being concentrated and lyophilized, they were dissolved in methanol:water (1:1, v/v) and submitted to the liquid-liquid partition, resulting in the hexane (EhHF and EbHF), ethyl acetate (EhAF and EbAF) and hydromethanolic (EhMF and EbMF) fractions. The TP content (mg GAE/g) was determined by Folin-Ciocalteau[2] method and AC by DPPH•[3] (IC_{50} - µg/mL), ABTS•+[4] (mM Trolox/g) and FRAP[5] (mM Trolox/g) methods. **Results:** For extract and fractions of *E. hiemalis*, EEEh presented the highest TP content (510.55 ± 5.62), followed by EhAF (486.46 ± 3.79), EhMF (415.86 ± 7.47) and EhHF (195.68 ± 1.92). EhAF demonstrated high AC in DPPH•, ABTS•+ and FRAP methods (4.00 ± 0.00; 5.29 ± 0.04 and 2.86 ± 0.01, respectively), followed by EEEh (5.06 ± 0.02; 4.83 ± 0.00 and 0.89 ± 0.00, respectively), EhMF (9.41 ± 0.01; 3.25 ± 0.03 and 0.80 ± 0.00, respectively) and EhHF (23.92 ± 0.06; 1.44 ± 0.01 and 0.32 ± 0.01, respectively). For extract and fractions of *E. blastantha*, EEEb presented high TP content (520.52 ± 7.81), followed by EbMF (491.62 ± 6.53) and EbAF (428.31 ± 5.70). The TP content was not detected for EbHF under the conditions tested. EbAF showed high AC in DPPH•, ABTS•+ and FRAP methods (6.44 ± 0.01; 4.37 ± 0.01 and 1.11 ± 0.00, respectively), followed by EEEb (10.08 ± 0.02; 3.12 ± 0.02 and 0.71 ± 0.01, respectively), EbMF (10.56 ± 0.08; 3.00 ± 0.01, and 0.40 ± 0.00, respectively) and EbHF (191.24 ± 0.09; 0.92 ± 0.00 and 0.27 ± 0.01, respectively). **Conclusion:** The results indicate that *Eugenia* species are promising objects in the search for natural antioxidants.

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References:
[1]BARBOSA, K. B. F. COSTA, N. M. B.; ALFENAS, R. C. G.; BRESSAN, J. 2010. Oxidative stress: concept, implications and modulating factors. Revista de Nutrição, 23, 629-643.
[2]SINGLETON, V. L.; ROSSI, J. A. 1999. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal Enology Viticulture, 16, 144-158.
[3]EL-MASSRY, K. F.; EL-GHORAD, A. H.; FAROUK, A. 2002. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chemistry, 79, 331-336.
[4]RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F. D. 2007. Determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS•+. Comunicado Técnico, 128, 1-4.
[5]RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F. D. 2006. Determinação da atividade antioxidante total em frutas pelo método de redução do ferro (FRAP). Comunicado Técnico, 125, 1-4.

PREPARATION AND CHARACTERIZATION OF MICROSPONGES CONTAINING DRUGS WITH DIFFERENT WATER SOLUBILITY

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Keywords: microsponges, characterization, drug delivery

Introduction: Drug delivery systems offers several advantages when compared to conventional dosage forms. Among the benefits can be mentioned the modulation of the release process, reduction of toxicity, improvement of drug availability into a specific site, which leads to better adherence to treatment by patients[1]. Among the microparticles, polymeric microsponges (MS) are rigid and porous structure capable to incorporating a relatively large amounts of drug into their interconnect channels[2]. MS are a relatively new strategy, thus has a few studies using them. **Aim:** Thus, the aim of this work was to obtain and characterize MS containing different drugs. **Methods:** MS was prepared by quasi-emulsion technique[2]. Solution of ethylcellulose (0.5%, w/w), HPMCphtalate (0.03%, w/w) and metronidazole (MTZ), methylene blue (MB), propolis (PPL) or curcumin (CUR) was prepared in dichloromethane (organic phase). Aqueous solution (1%, w/v) of porogen was dripped in polymeric solution. This dispersion was dripped into an aqueous poloxamer 188 dispersion (aqueous phase) was prepared. This dispersion was magnetically stirred for 24 h. MS were dried at 60 ℃ in the hot air oven. It was evaluated the morphology by SEM, product yield (PY), drug content (DC), entrapment efficiency (EE), particle size (PS) by DLS, and ATR. All MS presented spherical form and pores in the surface. **Results** PY was 44.82, 38.17, 56.62 and 79.44% to MB, PPL, MTZ and CUR, respectively. Moreover, the DC and EE was not possible to confirm the presence of PPL into the MS by polyphenols content, for others drug was obtained to DC 0.027, 2.24 and 0.69%, and to EE 10.78, 11.40 and 74.35% to MB, MTZ and CUR, respectively. The particle mean diameter ranged from 0.370 to 1.10 µm. It was not possible to see the characteristic bands of the drugs in the ATR spectra of the MS. **Conclusion** Therefore, it was possible to prepare MS containing drugs of different water solubility, the ATR results suggesting that active agents were incorporated into the systems, which can be confirmed with the EE.
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References:
Restani, R.B.; Correia, V.G.; Bonifacio, V.D.B., Aguiar-Ricardo, A.J. *SupercritFluids.* 55 (2010), 333-339.
Arya, P.; Pathak, K.*Int J Pharm*. 460 (2014), 1-12.

AQUEOUS FRACTION OF *Stryphnodendron adstringens* INDUCES ULTRASTRUCTURE ALTERATIONS IN HUMAN CERVICAL CANCER CELLS

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Key words: Cervical cancer, *Stryphnodendron adstringens*, ultrastructure alterations.

Introduction: Cervical cancer is the fourth most common cancer, often associated with human papillomavirus (HPV)[1]. Medicinal plants are promising source of efficient anti-cancer drugs [2]. **Aim:** Investigate the ultrastructure alterations induced by aqueous fraction of *Stryphnodendron adstringens* (F2) in human cervical cancer cell lines transformed by HPV 18 (HeLa), HPV 16 (SiHa) and non-immortalized (C33A). **Methods:** All three cell lines were treated with F2 fraction (IC_{50} and IC_{90}) combined with or without N-acetylcysteine (NAC; 5 mM) for 24 h. Cells were processed by transmission electron microscopy; where cells were fixed with glutaraldehyde, post-fixed, dehydrated with acetone, embedded in Epon resin and observed in JEM 1400 JEOL microscope. **Results:** The treatment with IC_{50} of F2 fraction resulted in ultrastructure alterations in HeLa cells, included mitochondrial swelling and autolysosome; in SiHa cells were observed the same alterations with addition of loss of mitochondrial cristae. In the HeLa and SiHa cells treated with IC_{90} of F2 were also observed plasma membrane disruption and nuclear membrane alteration. In the C33A cells treated with IC_{50} and IC_{90} of F2 were observed mitochondrial swelling, loss of mitochondrial cristae, plasma membrane disruption and nuclear membrane alteration. However, cells preincubated with NAC for 2 h before treatment with IC_{50} of F2 showed preserved ultrastructure of mitochondria, plasma membrane and nuclear membrane, and autolysosome. On the other hand, in the treatment with IC_{90} of F2 after preincubation with NAC there were mitochondrial swelling, loss of mitochondrial cristae and, increase in the number or size of autolysosome. The control groups of HeLa, SiHa and C33A cells showed no ultrastructural alterations. Morphologic analysis by transmission electron microscopy is considered a “gold standard” for cell death classification [3]. Generally, in apoptosis cell death were observed fragmentation and condensation of nucleus, and organelles swelling, such as mitochondria; however apoptotic cells undergo a late process of secondary necrosis, characterized by plasma membrane disruption [4]. **Conclusion:** Thus, after treatment with IC_{50} of F2, HeLa and SiHa cells showed intense nuclear and mitochondrial changes indicative of apoptosis death. Treatment with IC_{90} also revealed plasma membrane disruption, characteristic of secondary necrosis. After treatment, all these alterations were observed in C33A cells.

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References

[1] Graham S. V. The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. Clinical Science. 131 (2017), 2201-2221.

[2] Fridlender M., Kapulnik Y., Koltai H. Planta derived substances with anti-cancer activity: from folklore to practice. Frontiers in Plant Science. 6 (2015),799.

[3] Berghe T. V., Grootjans S., Goossens V., Dondelinger Y., Krysko D. V., Takahashi N., Vandenabeele P. Determination of apoptotic and necrotic cell death *in vitro* and *in vivo*. Methods. 61 (2013), 117-129.

[4] Wong, R. S. Y. Apoptosis in cancer: from pathogenesis to treatment. Journal of Experimental & Clinical Cancer Research. 30 (2011), 87.

EVALUATION OF THE IN VITRO ACTIVITY OF *Matricaria chamomilla* L. ESSENTIAL OIL AGAINST *Leishmania amazonensis* L.

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Keywords: Essential oil. *Matricaria chamomilla* L. Leishmaniasis.

Introduction: Treatments used for leishmaniasis are commonly effective, but they are limited due to high cost and toxicity (1). Thus, there is a need to develop new treatments for leishmaniasis, and in this context the natural products are targets of these researches (2). *Matricaria chamomilla* L., known popularly as chamomile, has been used for centuries as anti-inflammatory, hepatoprotective, for cicatrization and other applications (3). **Aim:** The aim of this study was evaluated the activity of *M. chamomilla* essential oil in promastigotes and amastigostes forms of *Leishmania amazonensis* L. **Methods:** The in vitro antiproliferative activity against promastigotes forms was performed using $1x10^6$ parasites/mL at 25 ℃ in Warren's medium containing 10% FBS, that were grown in 96-well culture, various concentrations of *M. chamomilla* essential oil were tested and incubated for 72 h. Leishmanicidal activity was determined by direct counting of the free-living parasites in Neubauer chamber. The activity against intracellular amastigotes the amount of $5x10^5$ cells/mL of macrophages J774 A1 and $5x10^6$ parasites/mL promastigotes were plated on cover slips in the wells of the 24-well microplate, in RPMI 1640 medium supplemented with 10% FBS and incubated for 24 h at 36 ℃. After 24 h the infected macrophages were treated with different concentrations of *M. chamomilla* essential oil and incubated for 48 h. The monolayers were then fixed with methanol and stained with 10% Giemsa stain. The results are expressed as the number of parasites/100 macrophages. The cytotoxicity was evaluated by MTT in macrophages and VERO cells, for determined the cytotoxic concentration. **Results:** With the treatment of the parasites with the *M. chamomilla* essential oil was calculated the inhibition percentage of the parasites, and the concentration corresponding to 50% and 90% inhibition of the parasites. The IC_{50} concentrations in promastigotes and in amastigotes forms were 3.33 µg/mL and 14.56 µg/mL, respectively. The IC_{90} concentration in promastigotes forms was 30.83 µg/mL and for amastigotes forms was > 100 µg/mL. In macrophages and in Vero cells was evaluated the cytotoxic concentration 50% (CC_{50}). The CC_{50} in macrophages and in Vero cells were 19.71 µg/mL and 181.73 µg/mL, respectively. **Conclusion:** With these results was possible observed that the *M. chamomilla* essential oil showed relevant activity against promastigotes and amastigotes forms, because of that more experiments will be perform.

Acknowledgments

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References

(1)GARCIA, F. P.; BIDÓIA, D. L.; UEDA-NAKAMURA, T.; SILVA, S. O.; NAKAMURA, C. V. **Evidence-Based Complementary and Alternative Medicine**, v. 2013 (2013), p.1-11.
(2)CAMACHO, M.D.R., PHILLIPSON, J.D., CROFT, S.L., SOLIS, P.N., MARSHALL, S.J., GHAZANFAR, S.A., **Journal of Ethnopharmacol.** v. 89 (2003), p. 185–191.
(3)BABENKO, N.A.; SHAKHOVA, E. G. **Experimental Gerontology**, v.14 (2006), p. 32-39.

STABILITY STUDY OF HYALURONIC ACID BASED NANOEMULSIONS CONTAINING *P. pubescens* FRUITS OILS

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Key words: *P. pubescens*, nanoemulsions, stability.

Introduction: *Pterodon pubescens* Benth species, commonly known as "sucupira", is a Brazilian native specie used in folk medicine as anti-rheumatic and anti-inflammatory (Carvalho et al. 1999). Pharmaceutical forms based on nanoemulsions have attracted great attention in different areas of the research, due to the stability conferred to these systems (Bruxel et al. 2012). **Aim:** The aim of this work was to evaluate the physicochemical stability of nanoemulsions containing *P. pubescens* fruits oils in the presence and absence of vitamin E, in order to determine in which environmental conditions the developed systems are more stable and to avaluate the effect of addition of vitamin E, as antioxidant, on the chemical stability of the same**. Methods:** The formulations were prepared by mixing water and PEG 40H (10%, w/w), followed by the addition of oil *P. pubencens* (3%, w/w) and soy lecithin (Lipoid S100) (1%, w/w) in the high-speed shear apparatus (IKA® T25 basic, Germany) at 18000 rpm for 15 min. Thereafter, hyaluronic acid was added under magnetic stirring. F3a and F3b were prepared without and with Vitamin E in the oil phase, respectively. The stability study was performed for 180 days under the following storage conditions: 5 ℃ ± 2 ℃, 30 ℃ ± 2 ℃ and 40 ± 2 ℃ with 75% relative humidity. Size distribution, polydispersity index, pH and chemical recovery were monitored during this study period at predetermined intervals**. Results:** The addition of vitamin E as antioxidant promoted an improvement in the recovery of active from carrier systems stored at 30℃. The formulations stored at 40 ℃ (75% UR), presented significant change in their physicochemical characteristics, with a chemical degradation of the constituents of oils of approximately 50%. The formulations evaluated the 5℃ and 30℃ showed better stability during the period analyzed. The droplet size and polydispersity index presented a slight increase, with exception to the polydispersity index of the formulation F3b stored at 30 ℃, which decreased during this period. The pH of formulations remained in around 6.0 at the end of the study and the chemical recovery obtained was around 100% for both systems analyzed. **Conclusion**: The data obtained during this study demonstrated the physicochemical stability of the analyzed systems, proving to be satisfactory in the use as carrier systems of the oil of *P. pubescens*.

Acknowledgments: FINEP, Fundação Araucária, CAPES, CNPq and COMCAP-UEM.

References:

Bruxel F, Laux M, Wild LB, Fraga M, Koester LS, Teixeira HF. Quím. Nova. v. 35 (2012), p. 1827-1840.
Carvalho JC, Sertié AJ, Barbosa MV, Patrício KC, Caputo LR, Sarti SJ, Ferreira LP, Bastos JK. J. Ethnopharmacol. v. 64 (1999), p. 127–133.

MORPHO-ANATOMICAL STUDY OF *Croton floribundus* LEAVES

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Key words: Euphorbiaceae; quality control, trichomes.

Introduction: *Croton floribundus* Spreng. belongs to the Euphorbiaceae family and the Crotoneae tribe is a native and non-endemic tree from Brazil[1]. This specie is popularly known as “capixingui”, “capexingui”, depending the Brazilian region that this plant is found. According to ethnobotanical data, tea from *C. floribundus* bark is used to treat leukemia, tumors and syphilis. **Aim:** The aim of this study was to describe the morpho-anatomical characteristics of *C. floribundus* leaves for providing quality control data of this specie**. Methods:** Shade and sun leaves of *C. floribundus* were collected in 2016 at State University of Maringá (51°56’S, 23°24’W and 51°56’15”S, 23°24’17”W, respectively). The plant material with inflorescences was deposited in the Herbarium of State University of Maringá (HUEM) (registration number 30778 and 30726, respectively). Analysis of optical microscopy and scanning electron microscopy (SEM) as well histochemical tests were performed[2]. **Results:** The leaves of *C. floribundus* are simple, whole, with interpeciolar stipule, alternate phyllotaxy and peninérvea venation. The surface of the leaf blade is rough, and the petiole is wrinkled. Both, leaf blade and petiole have pleasant smell and slightly sweet. The petiole measured 7 cm in length and central insertion in the leaf blade. On the abaxial side of *C. floribundus* leaves there are stellate non-glandular trichomes that ranging between stellate-rotate and stellate-lepidote. On the adaxial side there are as "cat's claw" trichomes present one elongated cell and a pedestal that elevates it above the level of the other epidermal cells as well stellate trichome with erect central radius, designated as "porrect", located of the midrib. The liphophilic character of the thin cuticle was confirmed by Sudan IV glycerin. In cross-section, the leaf blade shows a uniserate epidermis, formed by small cells and has isodiametric cells in the midrib. Hypoestomatic leaf and paracytic stomata can be observed. The mesophyll is dorsiventral with a stratum of palisade parenchyma. The layers of spongy parenchyma vary of 3-5 cells layers, this cell has irregular shape, allowing the formation of large intercellular spaces. Druse are common in the mesophyll of *C. floribundus*. In cross section, the midrib shape is slightly convex on the adaxial side and very prominent concave on the abaxial side. Subepidermal colenquimic are present on both leaf sides, followed by parenchyma tissue, that is formed by rounded cells of irregular sizes containing starch grains, as well idioblasts with calcium oxalate crystal. The vascular system is represented by collateral vascular bundles, that have single, continuous and biconcave shape; laticifers were observed. The petiole has stellate trichomes predominantly multiradiate. There are thick angular collenchyma strata and two concentric anficrival vascular bundles smaller. The vascular system is collateral, unique, in the shape of arch, discontinuous in the mid and continuous at the base of the petiole. **Conclusion:** The analysis revealed few anatomical differences between the shade and sun specimens. The main morpho-anatomical characteristics were related to leaf trichomes.

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References

[1]*Croton.* Accessed Jul. 2017. *In:* Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro, http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB17512.

[2]Farmacopeia Brasileira. 2010. Agência Nacional de Vigilância Sanitária. Brasília, DF. Brasil.

ANTIMICROBIAL ACTIVITY OF EXTRACTS FROM *P. CERNUM, P. RIVINÓIDES*, *P. ARBOREUM* AND *P. MIKANIANUM*

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Key words: dermatophytes, *Piper arboreum* and microorganism

Introduction: Plants which have been used as medicines over hundreds of years, constitute an obvious choice for study. The advent of synthetic antimicrobials in the mid of the last century lead to lack of interest in plants as a natural source for antimicrobial drugs[1,2]. The dermatophytes belonging to three genera, Trichophyton, Microsporum and Epidermophyton, have the ability to invade keratinized tissues, such as hair, skin or nails, of humans and other animals. **Aim:** Showed antimicrobial activity of extracts belonging to general Piper[3]**. Methods:** Extract of *P. cernum, P. rivinoides*, *P. arboreum* and *P. mikanianum* were obtained using liquid extraction with soxhlext and dichloromethane as solvent. Thus, the extracts were stored in a glass container under freezer (-20 ° C). Later, *P. arboreum* extract was submitted to fractionation using the eluent grade p.a. in increasing order of polarity, and silica gel 60 (70-230 mesh) in a column. After evaporation of the solvents, the yields were calculated and the vials were stored in a freezer at -10 ℃. For the microbiological assays, the minimum inhibitory concentrations of Piper extracts against different microorganisms (*C. albicans, C. parapsilosis and C. tropicalis, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus, Tricophuton mentagrophytes, Tricophuton rubrum and Microsporum gypseum*)[4]. Fluorescence microscopy by Calcofluor White Stain and Checkerboard tests by broth microdilution method were performance to determine *in vitro* interactions between drugs and dermatophyte species. *Piper arboreum* was tested in association with antifungal agents fluconazole or nistatine. **Results:** The plants extracts were obtained in desirable amounts. The *Piper arboreum* extract was fraction in six diferents parts, and this fraction shower activity againt dermatophytes. The MIC were determined for the four plant extracts. Both *Pipers* showed activity against dermatophytes with MIC values ranginig from 62,5µg/mL to 1000µg/mL; but the best were *Piper arboreum*. For another microorganism *P. cernum, P. arboreum, P. rivinóides* and *P. mikanianum* extract was not active at higher concentration tested (1000 µg/mL). Only *P.arboreum* showed activity against *Bacillus subtilis* (MIC 125µg/mL). *P.arboreum* extracts presented the best activity against *T. rubrum*, so the effects were evaluated against this dermatophytes under fluorescence microscopy. The microscopy images show intense fluorescence in hyphal growth, with abundant, continuous and healthy hyphae in control cells.**Conclusion:** The present study reports the effect of different extracts of *Piper* against dermatophytes, bacteria and fungi; with strong fungal inhibition and causing morphological alterations in their hyphae.

Acknowledgments: CAPES, CNPQ and UEM.

Reference

[1]Sen A, Batra A. 2012. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. Int J Curr Pharm Res;4:67—73.
[2]Rinaldi MG. 2000.Dermatophytosis: epidemiological and microbiological update. J Am Acad Dermatol;43:120—4.
[3]Macura, A.B., Macura-Biegun, A., Pawlik, B., 2003. Susceptibility of fungal infections of nails in patients with primary antibody deficiency. Comparative Immunology, Microbiology & Infectious Diseases 26, 223–232
[4]Clinical Laboratory Standards Institute. Reference method for broth dilution antifungals susceptibility testing of conidiumforming filamentous fungi: approved standard, . 2nd ed., Wayne, PA: Clinical and Laboratory Standard Institute; 2008 , M38-A2.

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

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Key words: *Tetradenia riparia* (Hochst). Codd, *Staphylococcus aureus*, biofilm, essential oil, nanoparticles.

Introduction: Biofilm is a microbial community that bacteria live in an extracellular matrix composed of proteins, extracellular DNA and polysaccharides[1]. *Staphylococcus aureus* is an important microorganism that has the ability to form biofilm on a various range of surfaces. 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[2]. Antimicrobial agents of natural origin are effective and economical alternatives, as essential oils (EO). However, with the disadvantage of rapid oxidation, nanoencapsulation is an alternative that improves stability, reduces toxicity and controls the release of oil[3]. **Aim:** Preparation, characterization and evaluation of antibiofilm activity of free and nanoencapsulated essential oil of *Tetradenia riparia* (Hochst). Codd against *S. aureus*. **Methods:** The antibiofilm effect of EO was observed by broth microdilution method according to CLSI[4]. Nanoprecipitation with PLA (Poly-lactide) was used to obtain nanoparticles containing EO. The nanoparticles (NP) was characterized by DLS and SEM for morphology and size distribution. Thermal analysis was realized by DSC. **Results:** 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. Nanoparticles were found to be nanometric, round with regular structures. ΔHm values decreased with the incorporation of *T. riparia* EO, that suggest the encapsulation of EO in the PLA matrix. **Conclusion:** EO and NP could be effective as an antibiofilm alternative treatment.

Acknowledgments: CNPq, CAPES.

References:

[1]Raffaella, C.; Casettari, L.; Fagioli, L.; Cespi, M.; Bonacucina , G.; Baffone, W**.,** Activity of essential oil based microemulsions against *Staphylococcus aureus* biofilms developed on stainless steel surface in different culture media and growth conditions. **International Journal of Food Microbiology**, v. 241, p. 132-140, 2017.

[2]Shin, K.; Yun, Y.; Yi, S.; Lee, H. G.; Cho, J.; Suh, K.; Lee, J.; Park, J. Biofilm-forming ability of *S. aureus* strains isolated from human skin. **Journal of Dermatological Science**, v. 71, p. 130-137, 2013.

[3]SÃO-PEDRO, A. *et al*., The use of nanotechnology as an approach for essential oil-based formulations with antimicrobial activity. **Microbial pathogens and strategies for combating them; science technology and education (A. Mendez-Vilas. Ed.)** v. 1, p. 1364-1374, 2013.

[4]Clinical and Laboratory Standards Institute**.** 2012: Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard, 9th ed. M07-A9, CLSI, Wayne, PA.

OPIORPHIN FACILITATES, BUT DOES NOT ANTICIPATE THE ANTIPANIC-LIKE EFFECT OF FLUOXETINE

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Keywords: Opiorphin, fluoxetine, panic disorder.

Introduction: Opiorphin inhibits the catabolism of endogenous opioid peptides[1] and produces antipanic-like effect mediated by μ-opioid receptor activation in the dorsal periaqueductal grey (dPAG)[2].

Aim: This study intended to show if intra-dPAG microinjection of opiorphin could anticipate and/or facilitate the antipanic-like effect produced by fluoxetine, an antidepressant used to treat panic disorder, in rats submitted to the panic animal model, the elevated T-maze (ETM).

Methods: Male Wistar rats were submitted to the ETM (UEM Ethics Committee 1121010415) 7 days after stereotactic surgery for implantation of a cannula in the dPAG. Rats were treated chronically by 21 days with an ineffective dose (5 mg/kg; *n*=7-8) of fluoxetine or subchronically by 7 and 14 days with an effective dose (10 mg/kg; *n*=6-10 and *n*=5-8, respectively) of fluoxetine. At the test day, rats were treated intraperitoneally with imipramine 20 min before the intra-dPAG microinjection of opiorphin (2.5 nmol/0.2 μl). Ten min after opiorphin microinjection, inhibitory avoidance (s), escape latencies (s) and locomotion (m) were assessed. For all experiments, four independent groups were formed: vehicle+vehicle, vehicle+opiorphin, fluoxetine+vehicle and fluoxetine+opiorphin. Avoidance was analysed by two-way repeated measures analyses of variance (RMANOVA). The escape latencies were merged and analysed as well as locomotion, by two-way ANOVA. Duncan's *post-hoc* test was used to compare group differences.

Results: Synergistically, combination of ineffective doses of acute opiorphin and chronic fluoxetine produced an increase in escape latency compared to all other groups ($p<0.05$) (pre x treatment interaction - $F_{(1,27)}=8.29$; $p<0.01$). Opiorphin did not anticipate the antipanic-like effect of fluoxetine (7 days; pre x treatment interaction - $F_{(1,26)}=0.72$; *N.S.*) nor (14 days; pre x treatment interaction - $F_{(1,22)}=2.17$; *N.S.*). None of the experiments changed inhibitory avoidance latencies nor the locomotion.

Conclusion: Our results show that opiorphin facilitates, but does not anticipate the antipanic-like effect of fluoxetine. Further studies are needed to investigate the feasibility of using chronic treatment of an association between fluoxetine and opiorphin-like compounds in the therapy of panic.

Reference:

[1]Wisner et al., 2006, *PNAS*, 103, 17979–17984.

[2]Maraschin et al., 2016, *Neuropharmacology*, 101, 264-270.

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EVALUATION OF THE PRODUCTION OF SHORT CHAIN FATTY ACIDS BY STRAINS OF LACTOBACILLI AND BIFIDOBACTERIA GROWN ON THE MEDIUM CONTAINING FRUCTO-OLIGOSACCHARIDES FROM *CICHORIUM ENDIVIA* ROOTS.

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Key words: Fructo-oligosaccharide, *Cichorium endivia*, roots
Introduction: Fructo-oligosaccharides (FOS) are a group of nondigestible carbohydrates that have a linear structure comprising β-D-fructofuranosyl units (2 → 1). They are non-digestible in the human gastrointestinal tract but selectively fermented by intestinal microbiota. The main fermentation products of FOS are lactic acid and acetic acid.[1] *Cichorium endivia* also known as "Escarole" is a vegetable belonging to the family Asteraceae, where the aerial parts are used as salads and raw material for the production of fructose.[2] Objetive: In the present study, FOS present in the roots of *C. endivia* were tested and their ability to produce short chain fatty acids by bifidobacteria and lactobacilli was evaluated. **Material and methods:** Concentrations of lactic and acetic acids, as the main fermentation products of lactobacilli and bifidobacteria, were measured using the isotachophoretic (ITP) method. After fermentation, the samples were subjected to isotachophoretic separations using IONOSEP 2003 device (Recman, Czech Republic). Prior to analysis, the samples (positive control - Orafti® P95, *C. endivia* FOS, negative control - basal medium) were diluted with 150 volumes of deionized water, and then purified using the Puradisc FP 30 filter with a pore size 0.2 μm. Solution containing 10 mM HCl, 22 mM ε-aminocaproic acid and 0.1% 2-hydroxy-ethylcellulose (pH 4.5) as leading electrolyte (LE) was used. As trailing electrolyte (TE), 5 mM caproic acid was used. The microorganisms employed were *Bif. breve* **CCDM 562,** *Bif. animalis* subsp.*lactis* **Bb12,** *Lbc. fermentum* **RL25,** *Lbc. animalis* **CCDM 382. Results and discussion:** The production of acetic acid was the highest for strains *Bif breve* CCDM 562, *Bif. animalis* subsp.*lactis* Bb12 when compared to the basal medium, however was lower than medium containing Orafti® P95. The production of lactic acid was the highest for strains *Lbc. fermentum* RL25 and *Lbc. animalis* CCDM 382 when compared to the basal medium but these values did not exceed the production of lactic acid in the medium containing Orafti® P95**. Conclusion: The production of acetic and latic acids increased significantly in the presence of FOS of *C. endivia* when compared to the basal medium (negative control).**

Acknowledgments: PCF, CAPES, CNPq.

References:
[1]Verspreet, J., Dornez, E., Van den Ende, W., Delcour, J.A., Courtin, C.M., 2000. Cereal grain fructans: Structure, variability and potential health effects. Trends in Food Science & Technology,43, 32-42.
[2]Sinkovič, L., Demšar, L., Žnidarčič, D., Vidrih, R., Hribar, J., Treutter, D., 2015. Phenolic profiles in leaves of chicory cultivars (*Cichorium intybus* L.) as influenced by organic and mineral fertilizers. Food Chemistry, 166, 507–513.

PREPARATION AND CHARACTERIZATION OF MUCOADHESIVE MICROSTRUCTURED SYSTEM CONTAINING SEMIPURIFIED EXTRACT OF *Limonium brasiliense* AGAINST *Helicobacter pylori*

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Key words: X-ray diffraction, FTIR, microparticles.

Introduction: *Helicobacter pylori* is a bacterium that infects the digestive tract and tends to attack the lining of the stomach. The infection caused by this bacterium is one of the main causes of peptic ulcer and increase the risk of gastric cancer. The plant species *Limonium brasiliense* (Boiss.) Kuntze (Plumbaginaceae) is popularly known as baicuru and is found in South America, mainly in the coast. Its rhizome is popularly used for treatment of premenstrual tension, menstrual disorders and genitourinary infections[1]. Previous studies reported a large concentration of the phenolic compounds in the *Limonium brasiliense*[2]. Mucoadhesive polymers have been used to develop microstructured systems for improving drug delivery by promoting a much more intimate contact with the mucus layer for an extended period of time. Natural products containing phenolic compounds have shown good activity against *H. pylori*. **Aim:** The aim of this study was to develop microparticles containing semipurified extract of *Limonium brasiliense* for treatment of *H. pylori* and characterize these particles by X-ray diffraction (DRX) and Fourier transform infrared (FTIR) analysis. **Methods:** The crude extract (CE) of baicuru was obtained by turbo extraction using acetone: water (7: 3) as the extractive liquid. The CE was partitioned with ethyl acetate and water obtaining the aqueous fraction that was used in the present work. The microparticles were produced by spray drying technique, using carbopol or polycarbophil as mucoadhesive polymer. The X-ray diffraction and FTIR analyzes were performed to characterize the particles. **Results:** DRX analysis showed that all samples tested were amorphous. The FTIR results suggest that there is an interaction between the polymers and the extract in the microparticles. **Conclusion:** The results suggest interaction between the components of the particles. Other tests will be performed to confirm whether this interaction can influence positively in the therapeutic efficacy of formulations against *H. pylori*.

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References:

[1]Fenner, R., Betti, A.H., Mentz, L.A., Rates, S.M.K., 2006. Plantas utilizadas na medicina popular brasileira com potencial atividade antifúngica. Rev. Bras. Cienc. Farm. 42, 269–394.
[2]Ragonese, A.E.; Milano,V.A. *Vegetales y sustâncias tóxicas de La flora Argentina*. In: *Enciclopedia Argentina de agricultura y Jardinería*. ACME, Buenos Aires, 1984, p. 17, 234.

TRANSIENT CEREBRAL GLOBAL ISCHEMIA IN RATS INDUCES MEMORY DEFICITS AND ACTIVATION OF A mTOR-INDEPENDENT AUTOPHAGY PATHWAY

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Keywords: brain ischemia, autophagy, rats.

Introduction: Transient and Global Cerebral Ischemia (TGCI) is an immediate and severe outcome of reversible cardiac arrest, characterized by a global reduction of cerebral blood flow. Ischemic patients who survived long after cardiac arrest may develop cognitive and executive dysfunction and sensory and motor impairments, which lead to difficulty in psychosocial or vocational reintegration[1]. Autophagy is an essential process that promotes selective degradation of cellular components and regulates cellular functions such as survival, death and metabolism[2]. Autophagy is also activated as an adaptive response to nutrient deprivation, hypoxia and oxidative stress. Experimental evidence indicates an increase in the autophagy under ischemic cerebral injury conditions. However, there is controversy as to whether this process plays a neuroprotective or neurotoxic role on the cells[3]. **Aim:** characterize the autophagic mechanisms at different time points following TGCI in order to better understand the pathophysiology of brain ischemia. **Methods:** Rats were submitted to the TGCI model through permanent occlusion of the vertebral arteries with subsequent transient occlusion of the carotid arteries for 15'. Sixteen days after reperfusion, the animals were exposed to the Open Field Test (OF) followed by the Object Location Test (OLT) for evaluation of locomotor activity and spatial memory, respectively. The animals were sacrificed at different time points after reperfusion and the brains were removed for analysis of autophagic pathway-related proteins in the hippocampus by means of Western Blot analysis. **Results:** Rats submitted to TGCI model showed memory deficits reflected by decreased discrimination index in the OLT when compared to sham group ($t_{(17)}$=3,920, p<0,05). Locomotor activity was not significantly different between groups ($t_{(19)}$=0,18, p>0,05). Enhanced levels of phosphorilated AKT were observed 3 hours, 72 hours and 7 days after reperfusion of ischemic rats when compared with sham group ($X^2_{(4)}$=13,88, p<0,05). Similarly, enhanced phosphorylation of mTOR was observed 3 hours, 7 days and 16 days after reperfusion when compared with sham group ($X^2_{(4)}$=13,91, p<0.05). Increased expression of Beclin-1 was revealed by ANOVA 72 hours after reperfusion when compared with sham group ($F_{(5,22)}$=2,72, p<0,05). ANOVA also shown enhanced expression of lipidated LC3-II ($F_{(5,22)}$=1,96, p<0,05) and Bax ($F_{(4,22)}$=3,07, p<0,05) 7 days after reperfusion in the hippocampus of ischemic rats when compared with sham group. **Conclusion:** TCGI induces memory impairments sixteen days after reperfusion without affecting locomotor activity. The dynamics of the autophagic processes seem to occur in a time-dependent manner, with concomitant increase in the levels of pro-apoptotic and autophagic proteins. The observed increase in the related proteins appears to be independent of mTOR pathway.

Acknowledgments: CAPES, UEM and USP.

References:

[1]Bacarin C.C. et al., 2016. Postischemic fish oil treatment restores long-term retrograde memory and dendritic density: An analysis of the time window of efficacy. Behavioral Brain Research. 311, 425-439.
[2]Hochfeld W.E. et al., 2013. Therapeutic induction of autophagy to modulate neurodegenerative disease progression. Acta pharmacologica sinica. 34(5), 600-604.

[3]Mariño G. et al., 2011. Longevity-relevant regulation of autophagy at the level of the acetylproteome. Autophagy. 7(6), 647-649.

THE USE OF GOLGI-COX STAINING TO INVESTIGATE THE EFFECTS OF FISH AFTER CEREBRAL ISCHEMIA

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Key words: Golgi-cox staining,brain ischemia,neuroplasticity.

Introduction: Based on the principle of metallic impregnation of neurons, the Golgi-Cox staining method allows to visualize, with unsurpassed sharpness, the fine cytoarchitecture of neurons, including the cell soma, axons, dendrites, and dentritic spines. We previously reported that treatment with fish oil (FO) facilitated the recovery (or preservation) of memory that otherwise is severely lost after cerebral ischemia. Fish oil did not prevent, however, ischemia-induced neuronal death. On the other hand, FO prevented the loss of immunorreactivity to the microtubule-associated protein 2 (MAP 2), a cytoskeletal protein that is highly compartmentalized in dendrites. Our hypothesis is that FO-mediated dendritic neuroplasticity contributed to the memory protective effect of FO. **Objectives**: To investigate this hypothesis in more details by using the Golgi-Cox technique, among others. Here, we present only the results regarding the implantation of the Golg.i-Cox technique in animals that were subjected to transient, global cerebral ischemia (TGCI). **Methods:** Young male Wistar rats (250-300g) were subjected to TGCI or sham surgery. Seven days later the brain was removed and stored in the Golgi-Cox solution for 24 hours at 37 °C. The brain remained in a new impregnation solution for additional 19 days in the dark, after which it was sectioned in cryostat (100 µm thickness). Random cuts were placed in a humid chamber for approximately 30h in the dark. Finally, the material was fixed in two steps: (i) dark phase, where the sections were immersed in ammonium hydroxide solution and Kodak Fix, and (ii) clear phase, where tissue dehydration and diaphanization were performed. This protocol had the approval of internal Ethical Committee (CEUA N 2879100816)**. Results:** The implantation of the Golgi-Cox technique was achieved successufully, as revealed by detailed visualization of the neuronal structures such as neuronal body, dendrites, and dendritic spines in the pre-frontal region of the cerebral cortex. **Conclusion:** The Golgi-Cox technique was standardized in our laboratory, which will allow us to investigate the effects of TGCI on the neuronal morphology and the effects of FO thereon.

Acknowledgments: CNPq, CAPES

References:

[1]Bacarin CC,et al. Postischemic fish oil treatment restores long-term retrograde memory and dendritic density: An analysis of the time window of efficacy. Behav Brain Res. 2016;311:425-39.
[2]R. Gibb, B. Kolb. A method for vibratome sectioning of Golgi–Cox stained whole rat brain Journal of Neuroscience Methods 79 (1998) 1–4.
[3]Zaqout S, Kaindl AM. Golgi-Cox Staining Step by Step. Front Neuroanat. 2016; 31;10:38.

TECHNOLOGICAL DEVELOPMENT, CHARACTERIZATION AND IN VITRO EVALUATION OF LSPN331-LOADED LIPOSOMES AS NANOCARRIERS TO TREATMENT OF CUTANEOUS LEISHMANIASIS

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Keywords: liposomes, macrophage, *Leishmania amazonensis*

Introduction: Liposomes-macrophage interactions have been studied with different therapeutic goals (1). The use of liposomes represents a targeting strategy of antileishmanial agents to treat parasitic diseases more efficiently (2). **Aim:** The aim of researh was the development of LSPN331-loaded liposomes to improve the treatment efficacy against *Leishmania amazonensis*. **Methods:** Were developed liposomes (LUVs) with and without surface modifications: LUVs (control particle), LUVs-HA (hyaluronic acid) and LUVs-HA/Thiochol (hyaluronic acid-thiocholesterol). Encapsulation efficiency (%EE) of LSPN331 was directly mensured by HPLC-PDA method previously validated. The average particle diameter, polydispersity index (PDI) and zeta potential (ZP) of the aqueous suspensions of LUVs were analyzed by Zetasizer system (Malvern). 30-days stability was analysed. The morphological analysis were performed by Transmission Electron Microscopy (TEM). Phospholipid content was determined by Stewart assay (3). Evaluation of *in vitro* cytotoxicity assay on macrophages J774.A1 and vero cells by MTT assay; and of *in vitro* antiproliferative activity against promastigotes and intracellular amastigotes forms, determined by direct counting of the free-living parasites and parasites/100 macrophages. Biodistribution studies *in vivo* and *ex vivo* were used to investigate the targeting efficiency of dye-labeled LUVs intravenously injected in mice model. **Results**: The LUVs showed an average diameter 212.5-238.3 nm and monodisperse size distribuition 0f 0.054-0.172. The %EE were between 35.6-64.5% and remained stable after 30 days. The success of surface modification was confirmed by TEM micrographs and ZP difference between control LUVs and LUVs-HA or LUVs-HA/Thiochol, from -14.7 mV to -46.5 mV and -37.3 mV, respectively. The %phospholipids of all formulations were 30.9-36.6%. LSPN331-loaded LUVs were more effective againt amastigotes forms than free drug. The cytotoxicity assay revealed LUVs were less toxic to macrophages and vero cells than the free drug. The biodistribution study showed the targeting efficacy of LUVs, confirmed by e*x vivo* fluorescence imaging of major organs. **Conclusion:** The LUVs developed remained stable after the hydrophobic drug encapsulation and surface modifications. The activity against *Leishmania amazonensis* was improved, and them were able to target the site of action. Our data suggests the LSPN331-loaded LUVs will contribute for cutaneous leishmaniasis treatment.

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References

1. Glucksam-Galnoy Y., Zor T., Margalit R. **Journal of controlled release.** 160 (2012), 388–393.
2. Borborema, S. E. T., Schwendener R. A., Osso Junior J. A., Andrade Junior H. F., Nascimento N. **International journal of antimicrobial agents.** 38 (2011), 341–347.
3**.** Stewart, J.C. M. **Analytical biochemistry**, 104 (1980), 10-14.

COMPARISON BETWEEN INTRINSIC SOLUBILITIES OF CLOPIDOGREL BISULFATE IN TWO POLYMORPHIC FORMS

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Key words: Clopidogrel bisulfate; Solubility; Polymorphism.

Introduction: Clopidogrel bisulfate (CLB) is an antiplatelet drug used for treatment of atherosclerotic events[1]. This drug presents crystalline polymorphism, appearing in six different polymorphic forms and an amorphous form. However, only I and II forms are used by the pharmaceutical companies[2]. Drug polymorphism may attribute distinct physical and chemical properties for one compound and this situation could be a barrier for manufacturing and regulatory control[3]. The knowledge about physical and chemical properties, as solubility characteristics, could guide a rational development, minimizing costs and failings on development phase, when more than one polymorph type is used. Solubility changings can modify the release and absorption of the drug for different polymorphs. **Objective:** To determine the solubility of CLB I and II forms in aqueous media with different pH. **Methods:** 37.5 mg of CLB I and II forms were dissolved in 50 mL of three aqueous media (distilled water, phosphate buffer pH 6.8 or acid buffer pH 1.2), in a closed system with controlled agitation and temperature (37ºC). After 30 minutes a sample was analyzed by HPLC system to get the final concentration. All experiments were performed in triplicate. **Results:** The theoretical concentration (0.75 mg/ml) was compared to observed for each CLB form and media. The dissolution percentage of CLB I and II forms was higher for acid buffer, followed by water and buffer pH 6.8. This behavior showed that solubility of I and II forms of CLB was pH dependent. But percentage solubility seems not differ between CLB I and II forms. However, more experiments to increase replicates are needed to confirm this postulation. **Conclusion:** The solubilization of CLB is pH-dependency and it can affect the absorption site *in vivo.* That is an important topic to be consider on drug development phase.

References:

[1]JACOBSON, A. K. Platelet ADP receptor antagonists: Ticlopidine and clopidogrel. Best Practice and Research: Clinical Haematology, v. 17, n. 1, p. 55–64, 2004.
[2]KORADIA, V.; CHAWLA, G.; BANSAL, A. K. Qualitative and quantitative analysis of clopidogrel bisulfate polymorphs. Acta pharmaceutica, v. 54, n. 3, p. 193–204, 2004.
[3]YU, L.; REUTZEL, S. M.; STEPHENSON, G. A. Physical characterization of polymorphic drugs: An integrated characterization strategy. Pharmaceutical Science and Technology Today, v. 1, n. 3, p. 118–127, 1998.

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DIHYDROCAFFEIC ACID PREVENTED UVB PHOTODAMAGE ON L929 FIBROBLASTS BY DECREASING OXIDATIVE STRESS AND SUPPRESSING THE MAP KINASES PATHWAY

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Keywords: phenolic acid; photochemoprotection; reactive oxygen species.

Introduction: UVB irradiation induce oxidative stress, which is involved with a set of detrimental outcomes in human skin[1]. Dihydrocaffeic acid (DHCA) have demonstrated antioxidant activity[2], and could protect skin against UVB damages. **Aim:** Evaluate photochemoprotective effect of DHCA against UVB radiation on L929 fibroblasts and clarify molecular mechanisms. **Methods:** Cell viability was evaluated by neutral red (NR) assay after incubation of the cells for 24 h with different concentrations (35 - 280 µM) of DHCA or UVB (100 – 800 mJ/cm^2). In order to evaluate the protection of DHCA against UVB induced cell death, cells were treated with DHCA (35 and 70 µM) for 1 h before UVB radiation (600 mJ/cm^2), incubated for 24 h and then submitted to NR assay. For next experiments, cells were treated with 35 µM of DHCA for 1 h before UVB radiation. H_2DCFDA (2',7'-dichlorodihydrofluorescein diacetate), DPPP (diphenyl-1-pyrenylphosphine) and Hoechst microscopy assays were used to evaluated intracellular reactive oxygen species (ROS) production, lipid peroxidation and DNA damage, respectively. Antioxidant capacity were evaluated by superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) analysis. To evaluate DHCA effect on expression of UVB induced p38 and JNK phosphorylation in the mitogen-activated protein (MAP) kinase pathway, which can increase metalloproteinases expression, western blot assay was performed. **Results:** Concentrations of 35 and 70 µM of DHCA were not toxic to cells (98.3 ± 2.6 and 95.5 ± 4.5% of cell viability, respectively), while 600 mJ/cm^2 of UVB kill 48.2 ± 0.7% of cells, being chosen to perform the next analysis. DHCA at 35 µM concentration significantly decreased UVB induced cell death (18.0 ± 1.7% of protection), and this concentration was selected to performed next analysis. DHCA significantly reduced ROS production (45.5 ± 10.7% of inhibition), lipid peroxidation (38.6 ± 6.9% of inhibition) and DNA damage (decreased DNA condensation on microscopy assay) on cells irradiated with UVB. DHCA also increasing CAT and SOD activities (62.0 ± 1.3 and 44.2 ± 4.9% of increase, respectively) and GSH content (71.8 ± 0.2% of increase). Furthermore, DHCA reduced p38 and JNK expression (38.7 ± 5.4 and 25.0 ± 6.7% of reduction, respectively). **Conclusion:** The present study revealed that DHCA prevent UVB induced L929 fibroblasts death by decreasing oxidative stress and underlying damages.

References

[1] LEPHART, E. D. Skin aging and oxidative stress: Equol's anti-aging effects via biochemical and molecular mechanisms. *Ageing Research Reviews*, v. 31, 2016.
[2] HUANG, J. et al. Antioxidant effects of dihydrocaffeic acid in human EA.hy926 endothelial cells. *Journal of Nutritional Biochemistry*, v. 15, 2004.

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INFLUENCE OF VITAMIN E ON THE STABILITY OF SOLID LIPID NANOPARTICLES LOADED WITH *Pterodon pubescens* OIL

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Key words: Sucupira; physicochemical stability; antioxidant.

Introduction: In product development process, in addition to complete physicochemical characterization, is very important to ensure stability during the shelf life of this[1]. **Aim:** The aim of this work was to evaluate the influence of the addition of vitamin E on physicochemical and microbiological stability study of the solid lipid nanoparticles (SLNs) loaded with *P. pubescens* oil (PpO) for 180 days. **Methods:** The SLNs-1 and SLNs-2, without and with vitamin E, respectively, were prepared by the fusion-emulsification method. 5 ml of each formulation were conditioned in glass bottles, and incubated in three different climatic conditions, being at 5 ℃ ± 2 ℃; 30 ℃ ± 2 ℃ and 40 ℃ ± 2 ℃ (75 % RU ± 5 % RU). In intervals of 0, 30, 60, 90 and 180 days, the particle size, polydispersity index (PDI), pH, total content and encapsulation efficiency (EE %) were evaluated. The accelerated physical stability of SLNs was also evaluated using the LUMiSizer® 611 dispersion analyzer. In addition, the microbiological stability of the SLNs was evaluated on freshly prepared SLNs samples and after 180 days of incubation, according to the Brazilian Pharmacopoeia V[2]. **Results:** In the stability study, the addition of vitamin E only influenced the protection conferred to PpO when SLNs were stored at 40℃, where the total content of vouacapans was higher in SLNs-2 than SLNs-1. In the rest of parameters, as well as in other storage conditions, the adjuvant showed no interference. At 30℃/75 % of RU, the results of granulometric analysis, zeta potential, total content and EE % of SLNs remained unchanged when compared to the freshly prepared formulations. Only pH values change significantly, which could be related to the oxidation of formulations components due to the presence of residual oxygen in the bottles. At 5℃ and 40℃, destabilization was observed after 180 and 30 days of analysis, respectively. The accelerated physical stability confirmed the results obtained by shelf stability study. Microbiological stability was confirmed by the absence of microorganisms growth both in the freshly samples and after 180 days storage at 30℃. **Conclusion** Among tested conditions, a climatic condition of 30℃/75% of RU proved to be more appropriate for transport and storage of SLNs.

Acknowledgments: CAPES, FINEP, CNPq, Gattefossé, Lipoid.

References:

[1]Coelho, L.P. et al., 2005. Antinociceptive properties of ethanolic extract and fractions of *Pterodon pubescens* Benth. seeds. Journal of Ethnopharmacology, 98, 109–116.

[2]Brasil. Farmacopéia Brasileira V, 2010. Diário Oficial da União, 1, 236-253.

CHEMICAL CHARACTERIZATION OF SEMI-PURIFIED EXTRACTS OF *Maytenus ilicifolia* BY UHPLC-HRMS

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Key words: *Maytenus ilicifolia**, espinheira santa***, chemical characterization

Introduction: *Maytenus ilicifolia* Mart. ex. Reissek is a member of the Celastraceae family, is known as *espinheira santa*, *cancorosa*, *espinheira de deus*, *espinheira divina*, *quebrachilho*, *salva vidas*, among others. Indigenous and rural communities use it due to their analgesic property, antitumor, aphrodisiac, antispasmodic, contraceptive, anti-ulcer, diuretic and curative properties. *M. ilicifolia* presents a complex composition in terms of its chemical compounds , among which are terpenes, triterpenes, essential oils, tannins, glycolipids and alkaloids[1,2,3]. **Aim:** In this work were analyzed semi purified fractions of *M. ilicifolia*, by UHPLC-HRMS to identify the compounds present in these fractions. **Methods:** To obtain the crude extracts, extraction of the dry and ground *M. ilicifolia* leaves by turbo extraction was carried out, using as extractor liquid mixtures of ethanol: water and acetone: water. The extracts were partitioned with ethyl acetate and *n*-butanol. The ethyl acetate fraction (EAF) and the *n*-butanol fraction (*n*BF) were analyzed using a reverse phase C18 analytical column with mass spectrometric detection in negative ion mode by a Q-TOF Impact II (Bruker, Germany). **Results:** To EAF was possible identified 3 compounds, epicatechin, procyanidin B2 and kaempferol-3-Galactoside-6-Rhamnoside-3-Rhamnoside. To *n*BF was identified 2 compounds, glycosylated flavonoids, quercetin-3-O-α-L-Rhamnopyranosyl(1-2)-β-D-glucopyranoside-7-O-α-L-Rhamnopyranoside and kaempferol-3-Galactoside-6-Rhamnoside-3-Rhamnoside. **Conclusion:** This assay allowed the confirmation of the presence of several polyphenols in the semi-purified extracts of *M. ilicifolia*.

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References

[1] MOSSI, A. et al. Chemical variation of tannins and triterpenes in Brazilian populations of *Maytenus ilicifolia* Mart. Ex Reiss. **Brazilian Journal of Biology,** v. 69, n. 2, p. 339-345, 2009. ISSN 1519-6984.
[2] PESSUTO, M. B. et al. Atividade antioxidante de extratos e taninos condensados das folhas de *Maytenus ilicifolia* Mart. ex Reiss. **Química Nova,** v. 32, p. 412-416, 2009. ISSN 0100-4042. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-40422009000200027&nrm=iso >.
[3] QUEIROGA, C. L. et al. Evaluation of the antiulcerogenic activity of friedelan-3beta-ol and friedelin isolated from *Maytenus ilicifolia* (Celastraceae). **J Ethnopharmacol,** v. 72, n. 3, p. 465-8, Oct 2000. ISSN 0378-8741 (Print) 0378-8741 (Linking).

ANTIOXIDANT CAPACITY EVALUATION OF *Maytenus ilicifolia* **EXTRACTS**

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Key words: *Maytenus ilicifolia*, espinheira-santa, antioxidant capacity

Introduction: *Maytenus ilicifolia* Mart. ex. Reissek, a member of the Celastraceae family, popularly known as *espinheira santa, cancorosa, espinheira de deus, espinheira divina, quebrachilho, salva vidas,* among others. In Brazil it is widely used to treat gastric ulcers, but in indigenous and rural communities is used for its analgesic properties, antitumor, aphrodisiac, antispasmodic, contraceptive, anti-ulcer, diuretic and healing[2,3]. **Aim:** The objective of this work was prepared crude extracts and fractions, then evaluate the antioxidant capacity of the extracts and fractions**. Methods:** Extraction process was carried out from the dry leaves of *M. ilicifolia* by turbo extraction to obtain the aqueous, hydro alcoholic and acetone: water extracts. The extracts were partitioned with ethyl acetate and *n*-butanol. The choice of the fractions tested was made based on previous studies of the antimicrobial activity of the extracts and fractions. The antioxidant potential was measured by the ability of the extract to sequester DPPH radical and by ferric reducing antioxidant power (FRAP) method, both spectrophotometrically[1,4]. **Results:** The half maximal inhibitory concentration (IC_{50}) on DPPH varied from 14.51 a 98.35 µg/mL, while the standard (Trolox) had an IC_{50}= 7.25±0.11. The results obtained for the FRAP assay ranged from 0.77 to 5.40 mM trolox/g extract. The ethyl acetate fraction of the hydro alcoholic extract showed the best capacity antioxidant in the DPPH and FRAP assay. **Conclusion:** These results suggest that *M. ilicifolia* is an interesting source of active constituents with a great antioxidant capacity.

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References

[1]BRAND-WILLIAMS, W.; CUVELIER, M.-E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **LWT-Food science and Technology,** v. 28, n. 1, p. 25-30, 1995. ISSN 0023-6438.
[2]MOSSI, A. et al. Chemical variation of tannins and triterpenes in Brazilian populations of Maytenus ilicifolia Mart. Ex Reiss. **Brazilian Journal of Biology,** v. 69, n. 2, p. 339-345, 2009. ISSN 1519-6984.
[3]QUEIROGA, C. L. et al. Evaluation of the antiulcerogenic activity of friedelan-3beta-ol and friedelin isolated from Maytenus ilicifolia (Celastraceae). **J Ethnopharmacol,** v. 72, n. 3, p. 465-8, Oct 2000. ISSN 0378-8741 (Print)0378-8741 (Linking).
[4]RUFINO, M. S. M. et al. **Metodologia científica: determinação da atividade antioxidante total em frutas pelo método de redução do ferro (FRAP) (in Portuguese).** . In Comunicado técnico online ed.;. Fortaleza: EMBRAPA 2006.

DYNAMIC INTERFACIAL TENSION AND DILATATIONAL RHEOLOGY OF SAPONINS FROM *Sapindus saponaria* L.

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Key words: dynamic interfacial tension; dilatational rheology; saponins.

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ntroduction: The use of biosurfactants (like triterpenic saponins) to replace classic surfactants is revealed as a healthy and environmentally friendly alternative to stabilize emulsions and form drug release systems. Although several articles address the surface properties of the saponins of *Quillaja saponaria* Molina and *Yucca schidigera* [1], these particularities of the metabolites from western soapberry *Sapindus saponaria* L. were not investigated. The micelles organization characteristic is dependent on the botanical origin of the material. Thus, this research is indeed important for the understanding of surface tensioactive properties and potential as interface stabilizer between immiscible liquids. **Aim:** In order to investigate the potential of saponins extracted from *S. saponaria* L. pericarp to stabilize nanoemulsions and other emulsified systems, its interfacial properties were studied. **Methods:** In order to obtain enriched fraction of saponins, hydroethanolic extract of pericarp were subject to a solid phase extraction (SPE) in a reverse phase cartridge with octadecylsilane (ODS LC-18), eluted in increasing gradient of acetonitrile and water. Dynamic interfacial tension and dilatation viscoelastic properties were measured with pendant drop tensiometer (Tracker, Teclis France). **Results:** The complex dilatation and dilatation storage modules were analyzed, as well as the interfacial tension values, for different concentration values of saponin solutions in PDMS, whose results clearly showed the tensoactive potential of these secondary metabolites. The higher the concentration of saponin, the lower the interfacial tension between water and oil. At the concentration of 0.01 g/ml saponins, the value was 7.01 mN/m. Furthermore, the formation of an elastic interfacial film was observed macroscopically by the adsorption of the saponin molecules. This phenomenon corroborates the understanding of the stabilization of the systems emulsified by saponins, which are responsible for structuring a gel.**Conclusion:** The use of these compounds supports the development of a sustainable product with promising technological applications.

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References:
[1]Faria, J. T., Oliveira, E. B., Minim, V. P. R., Minim, L. A. 2017. Perfomance of *Quillaja* bark saponin and β-lactoglobulin mixtures on emulsion formation and stability. Food Hydrocolloids. 67, 178-188.

CERUM OXIDE NANOPARTICLES PROTECT NEUTROPHILS FROM UVB-INDUCED DAMAGE BY DECREASING NEUTROPHILS OXIDATIVE ACTIVITY

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Key words: Neutrophils, Cerium Oxide, Antioxidants.

Introduction: Oxidative stress results from the redox imbalance between the increase of free radicals production and the decrease of enzymatic and non-enzymatic antioxidants[1]. A series of changes in proteins, lipids and DNA are caused when oxidative stress is initiated. However, it is known that antioxidant substances have the ability to neutralize, retard or even inhibit the action of free radicals[2]. Nanoparticles of cerium oxide (CNP) showed in a previous studies pro-oxidant and selective antioxidant properties[3], and catalytic mimetic antioxidant potential to catalase[4] and superoxide dismutase[5]. **Aim:** To investigate the effect of CNP on neutrophils (PMN) exposed to UVB radiation. **Methods:** We evaluated the effect of CNP and CNP2 pre-treatment for 1 h before irradiation with 500 mJ/cm^2 on UVB-induced reactive oxygen species (ROS) and hypochlorose acid (HOCl) production in PMN (2.0×10^6/mL) and also the effect of CNP and CNP2 pre-treatment on UVB-induced catalase, superoxide dismutase (SOD), NADPH oxidase activity in PMN cells. **Results:** We observed that the production of total ROS in PMN irradiated and treated with CNP and CNP2 were significantly lower when compared to the irradiated and untreated PMN group ($P < 0.0001$). The HOCl production also significantly decreased in PMN irradiated and treated with CNP and CNP2 compared to the irradiated and untreated PMN group ($p<0.0001$ and $p<0.001$, respectively). We also observed an increase in catalase and SOD activity in PMN irradiated and treated with CNP and CNP2 compared to the irradiated and untreated PMN group ($p<0.001$ and $p<0.0001$, respectively). The NADPH oxidase activity were also significantly reduced in PMN irradiated and treated with CNP and CNP2 compared to the irradiated and untreated PMN group ($p<0.01$). **Conclusion:** This study suggests that pre-treatment with cerium oxide nanoparticles might protect neutrophils against the damage induced by UVB radiation by reducing the UVB-induced oxidative activity.

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References

1. Machlin, L. J.; Bendich, A. Free radical tissue damage: protective role of antioxidant nutrientes. **The FASEB Journal**. 1 (1987), v.1, n.6, p.441-445, 1987.
2. Finkel, T.; Holbrook, N. J. Oxidants, odidative stress and the biology of ageing. **Nature**, v. 408, n. 6809, p. 239-247, 2000.
3. Alili, L.; Maren, S.; Montfort, C. V.; Giri, S, Das, S.; Carroll, K. S.; Zanger, K.; Seal, S.; Brenneisen, P. **Antioxidants & redox signaling**, v.19, n. 8, p.765-778, 2013.
4. Pirmohamed, T.; Dowding, J. M; Singh, S.; Wasserman, B.; Heckert, E.; Karakoti, A. S., King, J. E. S.; Seal, S.; Self, W. T. **Chemical Communications**, v. 46, n.16, p. 2736-2738, 2010.
5. Heckert, E. G.; Karakoti, A. S.; Seal, S.; Self, W. T. **Biomaterials**, v. 29, p.2705-2709, 2008.

BARBATIMÃO AQUEOUS FRACTION: CHEMICAL AND BIOLOGICAL EVALUATION IN *in vitro* ALZHEIMER DISEASE MODEL

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Key words: Alzheimer disease*, barbatimão*, polyphenols

Stryphnodendron adstringens, popularly known as *barbatimão* is a typical plant of Brazilian cerrado that presents in its barks high levels of polyphenols, represented mainly by condensed tannins of the type prodelfinidines and prorobinetinidines that were identified from the ethyl acetate fraction originated from an acetone: water extract[1], however 78% of the crude extract is represented by aqueous fraction which does not have chemical characterization studies. Several studies show that the consumption of plants rich in Polyphenols decrease neurodegenerative disease in the population, among them the Alzheimer disease (AD)[2], which is the leading cause of dementia in the world, accounting for about 60 to 80% of cases[3], and so far, remains unhealed, so the discovery of new drugs that treat or prevent AD is necessary. The aim of this work is the chemical characterization of the aqueous fraction and the *in vitro* evaluation of its protective activity against the βA25-35 peptide in human neuroblastome line (SH-SY5Y), as also its effect in the expression modulation of genes relative to AD. To aqueous fraction compounds identification it was necessary the development of an HPLC analytical method to monitor the subfractions originated by the Sephadex LH20 column chromatography fractionation technique, and the identification of the substances present in the subfractions will be done by the analysis of LC-ESI MS and MALDI. Through the cellular viability analysis by the MTT technique[4], the cytotoxicity was evaluated, as well as the protection of the aqueous fraction in human neuroblastome line, against the drug inducing βA25-35 damage. The effects of the aqueous fraction on the modulation of the expression of the AD related genes will be performed by RT-qPCR[5]. As result, the best HPLC analytical development was provided by the use of C18 column as the stationary phase, and acidified methanol, water and isopropanol as the mobile phase. The MTT assay shows that the concentration above 7,81 µg/mL of aqueous fraction decreased the cell viability of the human neuroblastome line. Until now, it may be concluded that the aqueous fraction is a very complex matrix, which makes difficult substances isolation, and the data of mass analyze will be fundamental to identify the compounds, as well the protection and modulation evaluation of genic expression will be required to conclude on a possible protection in the *in vitro* model of AD.

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References:

[1]Mello, J.C.P., Petereit, F., Nahrstedt, A., 1996. Flavan-3-ols and prodelphinidins from *Stryphnodendron adstringens*. *Phytochemistry***, v.**41 (3), p. 807-813.
[2]Commenges et al., 2000. Intake of flavonoids and risk of dementia. *Eur. J. Epidemiol*. v.16, p.357–363.
[3]Alzheimer's Association., 2016. Alzheimer's disease facts and figures. *Alzheimers Dement*, v.10, p.47–92.
[4]Mosmann, J., 1983. Rapid colorimetric assay for cellular growth and survival; application to proliferation cytotoxic assays. *J Immunol Methods*, v.65, n.1-2, p.55-63.
[5]Pfaffl, M.W., Horgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real time PCR. *Nucleic Acids Res*, v.30, n.9, p.1-19.

EVALUATION OF *Trichilia catigua* EXTRACTS AGAINST *Helicobacter pylori* BY RT-PCR

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Key words: *Trichilia catigua*, *Helicobacter pylori*, Real time PCR.

Introduction: *Trichilia catigua* A. Juss. (Meliaceae) is a tree known as 'catuaba' and is widely distributed in Brazil, being largely used in folk medicine. Studies carried out with *T. catigua* barks suggest this plant has antimicrobial, antinociceptive, aphrodisiac, antioxidant, antidepressant, and preventive action against brain damage[1,2,3]. *Helicobacter pylori* is a Gram-negative bacterium that is present in about 50% of the stomach mucosa of the world population, being associated with several gastric disorders, among them cancer[4,5]. **Aim:** The aim of this work was evaluate the action of 'catuaba' extracts against *H. pylori* on cellular and molecular levels. **Methods:** The dry 'catuaba' barks were crushed and submitted to turboextraction with acetone:water [7:3 w/v; crude extract (CE)], which was partitioned with ethyl acetate and water, yielding the ethyl-acetate fraction (EAF) and aqueous fraction (AF). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *H. pylori* ATCC 43629 was determined by broth microdilution for the extracts and inhibition assay of the urease enzyme. Cytotoxicity to AGS cell line was also performed by MTT. Finally, the extracts activity against *H. pylori* at the molecular level was evaluated by RT-PCR, by the expression of the genes encoding the virulence factors CagA, VacA, and UreA. **Results:** The EAF had MIC value at 512 and 1024 µg/ml for MBC. CE and AF presented values above 1024 µg/ml. CE, EAF, and AF showed percentages of urease inhibition of 44.60; 25.83, and 42.34%, respectively, at the 1024 µg/ml concentration. The extracts were non-toxic to AGS cells at the 62.5 µg/ml, which concentration was used for RT-PCR. The genes were normalized with the 23s reference gene, which EAF showed a statistically significant decrease for CagA gene expression and CE for VacA. **Conclusion:** CE and EAF have potential coadjuvant activity against *H. pylori* treatment.

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References:

[1]Pizzolatti, M.G.; Koga, A.H; Grisar, E.C.; Steindel, M. Trypanocidal activity of extracts from Brazilian Atlantic Rain Forest plant species. **Phytomedicine**, v.9, p.422-426, 2002.

[2]Oliveira, K.P.; Sofiat, F.T; Mourao, K.S.M.; Marques, L.C. Análise farmacognóstica comparativa de dois lotes de cascas de *Trichilia catigua* Adr. Juss. (Meliaceae), a catuaba da Bahia. **Revista de Pesquisa e Inovação Farmacêutica,** v.3, n.1, p.2-8, 2011.

[3]Longhini, R.; Lonni, A.S.G.; Sereia, A.L.; Krzyzaniak, L.M.; Lopes, G.C.; Mello, J.C.P. *Trichilia catigua*: therapeutic and cosmetic values. **Brazilian Journal of Pharmacognosy**, v.27, p.254-271, 2017.

[4]Blaser, M. J. Berg, D. E. *Helicobacter pylori* genetic diversity and risk of human disease. **Journal of Clinical Investigation**. v.107, p.767-773, 2001.

[5]Wen, S.; Moss, S. F. *Helicobacter pylori* virulence factors in gastric carcinogenesis. **The Cancer letter.** v.282(1), p.1-8, 2009.

PROPOLIS FILM-FORMING SYSTEMS

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Key words: propolis; film-forming systems; factorial design

Introduction: Together with the numerous benefits of the life expectancy raise, there are also direct impacts to the elders' health. Due to the natural human aging, associated to great levels of ultraviolet light exposition, there is a growth to the skin cancer incidence[1]. The chosen therapy for this disease is the surgical removal of lesions. The topical chemotherapy combined to an increase on the healing speed of the affected site, may guarantee complete tissue recover, drastically reducing the local relapses. Many studies verify the propolis (PRP) anticancer and healing activities[2]. However, the existent dermic formulations, as creams, gels and lotions, do not ensure the PRP release in a safe and efficacious way. The available preparations need various daily applications[3] and, in most cases, they are applied in form of wound dressing, to stay in long contact to the surgical excision site, causing discomfort and less adhesion to the treatment. **Objective:** To enhance the efficacy of this natural product, it is suggested the development of film-forming systems, capable of carrying the drug to the target during adequate time, reducing its toxicity and diminishing the number of daily applications, taking to a higher therapy adhesion. **Methods:** To control the PRP and its byproduct (BP) quality and as well as of its extract, these tests were performed: loss on dry, extractive content, soluble fraction in ethanol 96º GL, dryness residue, pH, total polyphenols content (TPC) and contents of waxes, ashes and alcohol[4]. To prepare the film-forming binary polymeric systems (Poloxamer 407 and Carbopol 971P, Carbopol 974P or polycarbophil), in combinations with three concentrations of extract, it was made a factorial design with 3 levels and 2 factors to evaluate the effects on the gelation temperature (Tgel) and texture profile analysis (TPA) of the formulations (F1-F10). **Results** The PRP, the BP and its extracts showed quality compatible with PRP of the same region, the TPC value of BPE shows that many active substances are kept on the PRP residue. For the TPA, the presence of C971, was the factor that contributed the most to compressibility, hardness and adhesiveness. The cohesiveness and springiness were highly similar between the formulations. The analysis of the response surface for the Tgel, indicated that the polymer is the main contributing factor. As the formulations containing C971 presented the lowest Tgel and those with PC the highest Tgel. Still, the extract concentration increase lowers the Tgel. In view of the objectives of these formulations, those with Tgel in range of 27-33 ºC were selected (F1, F7 and F10). **Conclusion:** According to these preliminary tests, those three formulations were chosen to the continuity of the development of a film-forming system to the controlled release of PRP and BP.

Acknowledgments: CNPq; CAPES; FINEP.

References:

[1]VERISSIMO, P.; BARBOSA, M. V. J. 2009. Tratamento cirúrgico de tumores de pele nasal em idosos. Ver. Bras. Cir. Plast., v. 24, n. 2
[2]SILVA-CARVALHO, R.; BALTAZAR, F.; ALMEIDA-AGUIAR. 2015. Propolis: A Complex Natural Product with a Plethora of Biological Activities That Can Be Explored for Drug Development. Evid. Based Complement. Alternat. Med.p. 1 – 29.
[3]ADEWUMI, A. A.; OGUNJINMI, A. A. The healing potential of honey and propolis lotion on septic wounds. Asian Pac. J. Tropical Biomed., v.1, n.1, p. 55 – 57, 2011.
[4]FARMACOPEIA Brasileira. 5. ed. São Paulo: Atheneu, 2010.

CANNABIDIOL PREVENTS MEMORY IMPAIRMENT AFTER CHRONIC CEREBRAL HYPOPERFUSION COMBINED WITH DIABETES IN MIDDLE-AGED RATS

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Key words: chronic cerebral hypoperfusion, diabetes, cannabidiol

Introduction: Experimental and clinical evidence show that chronic cerebral hypoperfusion (CCH) precedes the cognitive decline in dementia. Among several morbidities (e.g., hypertension, atherosclerosis, dyslipidemia) diabetes is a major risk factor for age-related dementia (1). This picture worsens when CCH is combined with diabetes, mainly in the elderly. In the broad spectrum of action of canabidiol (CBD), evidence indicate that this major secondary metabolites of Cannabis sativa has therapeutic potential for treatment of age-related dementia (2). **Aim:** To evaluate the effects of CBD on the memory deficit caused by CCH + diabetes in middle-aged rats. **Methods**: Male Wistar rats (12 months old) were made diabetic by a single dose of streptozotocin (35 mg/Kg, i.v.). Animals with glycemia values > 250mg/dl were considered hyperglycemic. The animals were trained for 15 days in the aversive radial maze (AvRM) in order to learn the task. Learning performance was expressed by three parameters: (i) latency to find the goal box, (ii) number of reference memory errors and (iii) number of working memory errors. After that, the animals were subjected to sham operation or chronic cerebral hypoperfusion (4–VO/ICA model). CBD (10 mg/Kg) or vehicle was given at the first step of the 4-VO/ICA surgery and continued until the end of behavioral testing. Four experimental groups were generated: sham+vehicle; sham+CBD; 4VO/ICA+vehicle and 4VO/ICA+CBD. Retrograde memory performance was evaluated 7, 14, and 21 days after surgery. Behavioral data were analyzed by two-way ANOVA followed by the *post hoc* Bonferroni test. **Results:** Significant differences in latency, reference memory errors and working memory errors (t_{10} = 4, 93 a 10, 02, p < 0, 0001) were detected when comparing 4-VO/ICA+vehicle *versus* sham+vehicle group. Significant difference was found between the 4-VO/ICA+vehicle group and 4-VO/ICA+CBD group (t_{37}= -10, 02 a -4, 92 p < 0, 0001). No difference was detected between sham and 4-VO/ICA+CBD groups (t_{37}= 0, 13 a 0, 75 p > 0, 05). **Conclusion:** CBD prevents cognitive impairments in middle-aged animals subjected to CCH + diabetes.

Acknowledgments: CNPq e CAPES.

References:

1. DE LA TORRE, J.C. Alzheimer disease as a vascular disorder: nosological evidence. **Stroke**. V. 33, P. 1152-1162, 2002.

2. MORI, M.A.; MEYER, E.; SOARES, L.M.; MILANI, H.; GUIMARÃES, F.S.; DE OLIVEIRA, R.M. Cannabidiol reduces neuroinflammation and promotes neuroplasticity and functional recovery after brain ischemia. **Prog Neuropsychopharmacol Biol Psychiatry**. V. 75, p. 64-105, 2017.

PREPARATION AND PHYSICOCHEMICAL CARACTERIZATION OF MUCOADHESIVE THERMORESPONSIVE SYSTEMS

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Key words: propolis, quality control, drug delivery.

Introduction: Bees (*Apis mellifera* L.) produce the propolis (PRP) from plants sources. PRP has complex chemical composition, consisting mainly in resinous compounds, waxes, volatile oils and aromatic acids, pollen grains and several others [(1,2)]. As biological properties, the antibacterial, fungicidal, antioxidant, antiviral, anti-inflammatory and immunostimulant activities stand out [**(1, 2)**]. PRP extracts are usually prepared with ethanol and/or water, and it can be added in mucoadhesive thermoresponsive systems. These polymer platforms can increase the availability of the active agent, as well as the contact time with the mucosa membrane, improving the therapeutic efficacy [(4)]. **Aim:** Therefore, the aim of this work was to prepare thermoresponsive mucoadhesive systems containing propolis and to evaluate their physicochemical characteristics. **Methods:** A sample of PRP was evaluated as loss of drying, wax content, ash content, extractive content, and determination of fraction extractable in 96 °GL ethanol. The extract was prepared by turboextraction. To evaluate the quality of the PRP extract, the following tests were performed: pH, relative density, dryness residue, the alcohol content, and total phenol content[**(3)**]. The mucoadhesive thermoresponsive system containing 20% (w/w) poloxamer 407 (P407) and 0.15% (w/w) carbomer 934P (C934P) with different concentrations of PRP extract (4, 8, 12, 14, 16%, w/w), and performed its physicochemical characterization by preliminary sol-gel transition temperature, relative density, and pH[(3)]. **Results:** The PRP sample, as well as its extract presented good quality. The systems containing the combination of thermoresponsive and mucoadhesive polymers P407 and C934P, respectively, presented good physicochemical characteristics. The preliminary gelation temperature showed $T_{sol/gel}$ of 17.33; 18.33; 15.00; 17.33 and 15.67 ℃ for the preparations containing 4, 8, 12, 14 and 16% (w/w) of PRP extract, respectively. In addition, it was observed that the higher the extract concentration, the lower the gelation temperature. The relative density values of systems were close to 1 g/ml, close to the water density, because they are mainly composed of water. The values of pH were dependent on the pH of the extract, close to 7.0. **Conclusion:** PRP sample and its extract presented good quality and they could be used in the preparation of the formulations. Likewise, the polymer systems with PRP extract in different concentrations presented characteristics in agreement with the quality parameters. The content of PRP in the formulation modified the sol-gel transition temperature.

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References

[(1)]Bruschi, M.L.; et al. Contribuição ao protocolo de controle de qualidade da própolis e de seus derivados. Rev. Ciênc. Farm.; 23 (2002), 289 -306.
[(2)]De Francisco, L.M.B. Obtenção e caracterização de nanopartículas contendo ácido ascórbico utilizando o subproduto da extração de própolis. Maringá-PR. UEM, 2013. 130p. Dissertação.
[(3)]Farmacopéia Brasileira. 5. ed. São Paulo: Atheneu, 2010
[(4)]Ferreira, S.B.D.S., et al. Rheological, mucoadhesive and textural properties of thermoresponsive polymer blends for biomedical applications. Journal of the mechanical behavior of biomedical materials 55 (2015) 164–178, 2015.

HEADSPACE-GC/MS ANALYSIS IN THE ASYMMETRIC REDUCTION OF (4*S*)-CARVONE CATALYZED BY *Phoma* sp

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Introduction: Aside from the development in instrumentation and methodologies, which are necessary for improvements in the quality of chemical analyses, efforts are being made to reduce the negative impact of chemical analyses on the environment and to enable implementation of sustainable development principles to analytical laboratories.[1,2] Therefore, the use of the Headspace extraction method represents a tool with a great positive impact on the environment, mainly related to waste reduction, elimination of the use of reagents and solvents, use of integrated analytical systems for improvement analytical, efficiency and miniaturization of methods to decrease the risk to the operator and environmental hazard.[3] **Aim:** Optimize the use of the Headspace-GC/MS methodology as an analytical tool for biocatalytic reactions. **Methods:** Headspace methodology as an analytical tool for monitoring a biocatalytic reaction.[4,5] The parameters evaluated were fungus biomass (*Phoma* sp), substrate mass ((+)-carvone) and pH. **Results:** It was evidenced that the parameter that most influenced the conversion rate and diastereosimeric excess (*d.e.*) was pH. That is, when the reaction was carried out at pH 5 it was possible to obtain 100% conversion rate and *d.e*. > 80%. **Conclusion:** It has been shown that the Headspace method represents a useful and sensitive analytical tool to monitor biocatalytic reactions, as well as the fungus Phoma sp, an efficient biocatalyst to promote hydrogenation reactions of activated alkenes.

Keywords: Biocatalysis; Response surface methodology; Headspace method.

Acknowledgments

Cnpq; Capes; Unicesumar.

References

[1]GAŁUSZKA A., MIGASZEWSKI Z., NAMIEŚNIK J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices, TrAC - **Trends Anal. Chem.** 50 (2013) 78–84.

[2]NAMIESNIK J. Trends in environmental analytics and monitoring, **Crit. Rev. Anal. Chem.** 30 (2000) 221–269.

[3]ARMENTA S., GARRIGUES S., de la GUARDIA M. Green analytical chemistry, **Trends Anal. Chem.** 27 (2008) 497–511.

THE USE OF THE CELL WALL OF *Saccharomyces cerevisiae* TO VECTOR THE SUGIOL DITERPENE AGAINST INFECTION CAUSED BY *Leishmania infantum*

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Keywords: Yeast cell wall particle, leishmaniasis, sugiol

Introduction: Incidence of leishmaniasis has been increasing at the last years, emphasizing the need to develop new approaches to treat neglected diseases[1]. The use of biocompatible particles to improve the selective delivery of drugs is an interesting strategy to use poor water soluble molecules, like the sugiol diterpene. Yeast cell wall particle (YCWP) present a rich constitution of β-1,3-D-glucan able to be recognized by dectin-1 receptor exposed on membrane surface of macrophages – the main cells infected by the *Leishmania* spp[2]. **Aim:** The aim of this study was to entrap sugiol in the inner of YCWP obtained from *Saccharomyces cerevisiae*, and to evaluate its activity against *Leishmania infantum.* **Methods:** YCWP were obtained from basic and hot extraction of the intracellular material, ensuring the conservation of β-1,3 glucan. The contact of acetone sugiol solution and YCWP, during 2 h, at -20 ℃ promoted sugiol´s entrapment. *L. infantum* promastigotes and peritoneal macrophage were treated with sugiol, YCWP+sugiol and empty YCWP. Peritoneal macrophages were harvested from BALB/c mice, infected with *L. infantum* promastigote, and treated according described above to evaluate the amastigote growth inhibition. These samples were fixed on glutaraldehyde and embedded in Epon to be analyzed the interaction among the host cell, amastigote and YCWP by TEM. **Results:** The eficiency of sugiol encapsulation was 31.5%, corresponding to 7 µg of sugiol/mg YCWP. Sugiol was active against promastigotes and amastigotes, showing IC_{50} of 4.1±0.2 and 5.7±0.4 µg/mL, respectively and slightly toxic on peritoneal macrophage (CC_{50}= 80.5±7.8 µg/mL). Empty YCWP and YCWP+sugiol at 10 mg/mL were not cytotoxic. YCWP and YCWP+sugiol did not present activity on promastigotes, even after 168 h of incubation. YCWP+sugiol were active against intracellular amastigotes and 1 mg of YCWP+sugiol was able to inhibit 53.1% of the amastigote growth. TEM micrographs could show the presence of amastigotes and YCWP inside the same parasitophorous vacuole and consequent changes in the nucleus and mitochondria of amastigotes. **Conclusion:** The results endorsed the YCWP like a promising strategy to vector insoluble drugs using cheap raw material. YCWP+sugiol were no significant cytotoxicity, but the presence of YCWP and amastigote in the same cellular compartment justifies the verified antileishmania activity.
Acknowledgments: CAPES, CNPq, Fundação Araucária/PRONEX and FINEP.

References:
[1] Kaye, P., & Scott, P. (2011). Leishmaniasis: complexity at the host–pathogen interface. *Nature Reviews Microbiology*, *9*(8), 604–615.
[2] Brown, G. D., Herre, J., Williams, D. L., Willment, J. A., Marshall, A. S. J., & Gordon, S. (2003). Dectin-1 Mediates the Biological Effects of β-Glucans. *The Journal of Experimental Medicine*, *197*(9), 1119 LP-1124.

ENCAPSULATION EFFICIENCY OF MUCOADESIVE MICROPARTICLES CONTAINING SEMIPURIFIED EXTRACT OF *Limonium brasiliense* FOR TREATMENT OF *Helicobacter pylori*

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Key words: *Limonium brasiliense*, microparticles, encapsulation efficiency.

Introduction: *Limonium brasiliense* (Boiss.) Kuntze (Plumbaginaceae), known as baicuru or guaicuru, is a native plant of the south coast of Brazil. Its rhizome is popularly used for treatment of premenstrual tension, menstrual disorders and genitourinary infections[1]. Previous studies have reported a large concentration of the phenolic compounds in the *Limonium brasiliense*[2]. Mucoadhesive microparticles have been developed for increase the contact time of the dosage form with the mucous membranes, promoting drug release at the site for an extended period of time. Natural products containing phenolic compounds have shown good activity against *H. pylori*. **Aim:** The aim of this study was to develop microparticles containing semipurified extract of *Limonium brasiliense* for treatment of *H. pylori* and to verify the encapsulation efficiency of the microparticles. **Methods:** The crude extract (CE) of baicuru was obtained by turbo extraction using acetone: water (7:3) as the extractive liquid. The CE was partitioned with ethyl acetate and water obtaining the aqueous fraction that was used in the present work. The microparticles were produced by the spray drying technique. The 2^3 factorial design was used to evaluate the influence of formulation parameters, such as extract concentration, amount of ethylcellulose and type of mucoadhesive polymer (polycarbophil or carbopol), in the characteristics of the particles. **Results:** The values obtained for encapsulation efficiency ranged from 80.56% to 104.25%. Particles prepared with carbopol presented higher encapsulation efficiency. **Conclusion:** The microparticles obtained showed good encapsulation efficiency. Other tests will be performed to confirm the effectiveness of formulations against *H. pylori*.
Acknowledgments: CAPES, CNPq, ICNT_if, FINEP/Comcap/UEM, Fundação Araucária.

References:
[1]Fenner, R., Betti, A.H., Mentz, L.A., Rates, S.M.K., 2006. Plantas utilizadas na medicina popular brasileira com potencial atividade antifúngica. Rev. Bras. Cienc. Farm. 42, 269–394.
[2]Ragonese, A.E.; Milano,V.A. *Vegetales y sustâncias tóxicas de La flora Argentina*. In: *Enciclopedia Argentina de agricultura y Jardinería*. ACME, Buenos Aires, 1984, p. 17, 234.

IMPACT OF SUPPLEMENTATION WITH QUERCETIN MICROCAPSULES ON ENTERIC NERVOUS SYSTEM AND OXIDATIVE STATE IN THE ILEUM OF DIABETICS RATS

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Key words: Diabetic neuropathy, Oxidative state, Quercetin microcapsules.

Introduction: The oxidative damage generated by hyperglycemia in diabetes mellitus causes injury and / or cell death. Among the most affected cells are nerve cells. Diabetic neuropathy in the enteric nervous system is one of the most common complications among patients where the enteric neurons the most affected cells. One way to mitigate the damage caused by oxidative stress could be antioxidant supplementation to reestablish redox cellular balance. In this context, quercetin is a substance that has antioxidant properties since quercetin removes free radicals increasing antioxidant capacity against oxidative damage. **Main:** The objective of this study was to evaluate the antioxidant effect of microencapsulated quercetin supplementation in the enteric innervation and oxidative state in the ileus of diabetic rats. **Methods:** Adult Wistar rats (Rattus norvegicus) was randomly divided into four groups (n = 6 animals): normoglycemic rats (N group), normoglycemic rats supplemented with microencapsulated 100 mg/kg quercetin (NQ100 group), diabetic rats (D group) and diabetic rats supplemented with microencapsulated 100 mg / kg quercetin (DQ100 group). The blood (for analysis of oxidative status) and ileum (for immunohistochemical evaluation of innervation and analysis of oxidative status) were collected. The results were submitted to statistical analyzes. Level of significance was 5%. The experimental protocol was approved by the animal ethics committee: 073/2014. **Results:** D group showed decreased ($p<0.05$) neuronal and glial density and increased ($p<0.05$) morphometry of both populations. NQ100 group showed decreased ($p <0.05$) neuronal density. DQ100 group presented increased ($p <0.05$) total glial cell population and decreased ($p <0.05$) glial fibrillar acid protein expression and glial cell populations on ganglionic area in comparison with D group ($p <0.05$). **Conclusion:** The supplementation with quercetin promoted antioxidant effects. However, did not prevent neuronal and glial loss.

References:

CHANDRASEKHARAN, B.; SRINIVASAN, S. Diabetes and the enteric nervous system. Neurogastroenterology e Motility, v. 19, n. 12, p. 951-960, 2007.

AYEPOLA, O. R., BROOKS, N. L., OGUNTIBEJU, O. O. Oxidative stress and diabetic complications: the role of antioxidant vitamins and flavonoids. In: Antioxidant-Antidiabetic Agents and Human Health. InTech, 2014.

SEMIPURIFIED FRACTION OF *Stryphnodendron adstringens* PROTECTS AGAINST Aβ PEPTIDE CYTOTOXICITY IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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Key words: Alzheimer's disease, Barbatimão, Polyphenols.

Introduction: Alzheimer's disease (AD) is the commonest form of dementia and is a social problem worldwide and, to date, there is no cure. Pathologically, is characterized by amyloid-beta (Aβ) peptide plaques deposits, intracellular neurofibrillary tangle, hyperphosphorylation of tau protein and neuronal cell death, being that the Aβ aggregation and the oxidative stress are the main responsible for the development and progress of AD. A previous study carried out by our research group showed that the ethyl-acetate fraction (EAF) of *Stryphnodendron adstringens* (Barbatimão) may have a promising potential against the AD due the polyphenol contents, antioxidant and anti-acetylcholinesterase activities[1]. However, to date, there are no reports available regarding neuroprotective effects of *S. adstringens*, *in vitro* or *in vivo*. **Aim:** Investigate the effect of the EAF of *S. adstringens* against $A\beta_{25-35}$ peptide cytotoxicity in SH-SY5Y cells and evaluate the expression of ten genes related to AD to help elucidating his role in the neuroprotection. **Methods:** Cell viability was assessed using the methyl thiazol tetrazolium (MTT) assay[2]. Briefly, SH-SY5Y cells were pretreated with different concentrations of EAF of *S. adstringens* (7.81-31.25 µg/ml) for 2 hours and treated with 10 µM $A\beta_{25-35}$ for 24 h. The cell viability was measured and expressed as percentage relative to control. The Real-Time Quantitative Reverse Transcription (RT-qPCR) was carried out using the CFX96™ Real-Time System[3] to determine the expression of the genes *A2M*, *ACHE*, *ADAM10*, *APOE*, *APP*, *GSK3β*, *LRP1*, *MAPT*, *PSEN1* and *PSEN2*. The validation of reference genes (*HPRT1* and *GAPDH*) and the relative gene expression were determined by the software REST 2009 (Relative Expression Software Tool/Qiagen). **Results:** The MTT assay has shown that the EAF of *S. adstringens* significantly attenuated the $A\beta_{25-35}$-induced cell death. Increased levels of the EAF from 7.81 to 15.62 µg/ml exerted an additive protection ($p<0.05$), which was not observed at the concentration of 31.25 µg/ml. The treatment also attenuated the expression of the gene *MAPT*. **Conclusion:** The EAF of *S. adstringens* may protects neuroblastoma cells against Aβ-induced oxidative damage, at least in part, by increasing the cellular redox potential and inhibiting the expression of the gene *MAPT*, related to the tau protein hyperphosphorylation.

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References:

1. Sereia, A. L. *et al. Anais do VII Seminário Anual do PCF*, p.20, 2016.

2, Mosmann, T. *J immunol Methods*, v.65, p. 55-63, 1983.

3, Pfaffl, M. W.; Horgan, G. W.; Dempfle, L. *Nucleic Acids Res*, v. 30, n. 9, p. e36, 2002.

B2-KININ RECEPTORS IN THE DORSAL PERIAQUEDUCTAL GRAY ARE IMPLICATED IN THE PANICOLYTIC-LIKE EFFECT OF OPIORPHIN

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Key words: opiorphin, bradykinin, panic model.

Introduction: Opiorphin, an inhibitor of the enzymes neutral endopeptidase and aminopeptidase N, is responsible for degradation of neuropeptides. Intra-dorsal periaqueductal gray (dPAG) injection of opiorphin had a panicolytic-like effect mediated by μ-opioid receptor (MOR) activation, possibly, caused by the increase of enkephalins[1]. In previous studies, it was observed that the panicolytic-like effect of opioids on dPAG was mediated by the synergistic activation of MOR and serotonin 5-HT_{1A} receptor (5-HT_{1A}R). However, the enzymes inhibited by opiorphin are also responsible for degrading other peptides, such as bradykinin, that has a panicolytic-like effect mediated by bradykinin type 2 receptor (B2R) and MOR in the dPAG[2]. **Aim:** This study investigates the participation of 5-HT_{1A}R and B2R in the panicolytic-like effect of opiorphin, using the dPAG electrical stimulation test (EST). **Methods:** Male Wistar rats were submitted to dPAG EST seven days after stereotactic surgery for implantation of the chemitrode in dPAG. The Δ escape threshold (μA) was defined by the difference between the lower intensity of electric current capable of evoking escape behavior, before and after drug administration. All drugs were injected intra-dPAG (0.2μL/120s)[Ethics Committee-1121010415-CEUA/UEM]. Data were analyzed by two-way ANOVA followed by Tukey's *post hoc* test. **Results:** *Experiment 1* shows that opiorphin (5.0 nmol) increased the escape threshold, and this panicolytic-like effect was not blocked by pretreatment with the selective 5-HT_{1A}R antagonist, WAY-100635 (0.74 nmol) [$F_{(1,17)}$= 0.02, *N.S*]. *Experiment 2* shows that a combination of ineffective doses of the 5-HT_{1A}R agonist 8-OH-DPAT (0.8 nmol) and opiorphin (2.5 nmol) did not increase the escape threshold [$F_{(1,20)}$ = 0.01, *N.S*]. *Experiment 3* shows that opiorphin (5.0 nmol) increased the escape threshold, and this panicolytic-like effect was blocked by pretreatment with the selective B2R antagonist HOE-140 (0.04 nmol) [$F_{(1,21)}$ = 15,34, p<0.001]. *Experiment 4* shows that a combination of ineffective doses of the opiorphin (2.5 nmol) and BK (1.0 nmol) significantly increased the escape threshold [$F_{(1,19)}$= 30.53, p<0.0001]. *Experiment 5* shows that BK (4.0 nmol) increased the escape threshold, and this panicolytic-like effect was not blocked by pretreatment with the selective 5-HT_{1A}R antagonist WAY-100635 (0.74 nmol) [$F_{(1,20)=}$ 0.87, *N.S.*]. **Conclusion:** The present and previous results showed that opiorphin has a panicolytic-like effect in the dPAG mediated by B2R and MOR activation.

Reference:

[1]Maraschin et al., Neuropharmacology. 101, 264-70. 2016;
[2]Sestile et al., Neuropharmacology. 123, 80-87, 2017.

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ISOLATION AND IDENTIFICATION OF SEMIPURIFIED FRACTION POLYPHENOLS FROM CATUABA BARKS (*TRICHILIA CATIGUA*)

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Key words: Neoclorogenic acid, cinchonains, tannins

Introduction: *Trichilia catigua* A. Juss. (Meliaceae) is popularly known as 'catuaba' and 'catiguá', the species is widely found between South America and Central America. Its barks are used in folk medicine as a tonic for the treatment of fatigue, stress, impotence, and memory deficits[1]. The species presents antioxidant, analgesic, vasodilator, anti-inflammatory, and antidepressant activities and is also used as a stimulant. Chemically, *T. catigua* stands out for its high content of phenolic compounds, mainly flavonoids and tannins[2]. **Aim:** The aim of this work was the isolation and structural identification of substances present in the semipurified fraction. **Methods:** In this study, the ethyl-acetate fraction obtained from the crude acetone: water (7: 3 v/v) extract of *T. catigua* barks[3], which was subjected to various chromatographic methods, such as column chromatography, high speed counter-current chromatography (HSCCC), and thin layer chromatography with the objective of isolating phenolic compounds. Identification was performed by 1D (^{1}H and ^{13}C) spectroscopic methods of NMR, 2D NMR ($^{1}H/^{1}H$-COSY), and mass spectrometry. **Results:** According to the isolation, the following compounds were identified as follow: neochlorogenic acid (**1**), epicatechin, and cinchonains Ia, Ib, and IIb. **Conclusions:** To the best of our knowledge, the compound **1** is described here for the first time in the genus *Trichilia*. The employed methods demonstrated to be good to isolate tannins.

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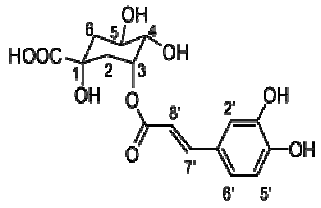

(**1**)

[1] Godinho, J.; Oliveira, R.M.W.; Sa-Nakanishi, A.B.; Bacarin, C.C.; Huzita, C.H.; Longhini, R.; Mello, J.C.P.; Nakamura, C.V.; Previdelli, I.S.; Dal Molin Ribeiro, M.H.; Milani, H. Ethyl-acetate fraction of *Trichilia catigua* restores long-term retrograde memory and reduces oxidative stress and inflammation after global cerebral ischemia in rats. Behavorial Brain Research, v. 337, p. 173-182, 2017.

[2] Longhini, R.; Lonni, A.A.S.G.; Sereia, A.L.; Krzyzaniak, L.M.; Lopes, G.C.; Mello, J.C.P. *Trichilia catigua*: therapeutic and cosmetic values. Revista Brasileira de Farmacognosia, v. 27, p. 254-271, 2017.

[3] Resende, F.O.; Rodrigues-Filho, E.; Luftmann, H.; Petereit, F.; Mello, J.C.P. Phenylpropanoid substituted flavan-3-ols from *Trichilia catigua* and their in vitro antioxidative activity. Journal of the Brazilian Chemical Society, v. 22, p. 2087-2093, 2011.

Acknowledgments: CAPES, CNPq

OBTAINING AND CHARACTERIZATION OF LIPOSOMES OBTAINED FROM THE SEMIPURIFIED FRACTION OF *Trichilia catigua*

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Key words: *Trichilia catigua*, liposomes, plant extract.

Introduction: The application of active in modified release systems emerged as an alternative for the development of liposomes containing plant derivatives. In industry, the incorporation of essential oils or plant extracts into cosmetic products has been increasingly motivated by researchers in the field. The study with liposomes became interesting because they are structures with size and composition similar to the cells of the human body, have low toxicity, good biocompatibility, allow size adjustment for different applications, can carry different hydrophilicities and are sensitive to stimulus such as changes in temperature, pH and magnetic field. **Aim:** Obtain and characterize liposomes containing semi-purified fraction of *Trichilia catigua* A. Juss (catuaba). **Methods:** A desing of mixtures was applied to evaluate the activity of each component (phospholipids, cholesterol and active) of the liposomes in the methodologies tested. Liposomes were obtained using the ethanol injection method followed by extrusion using polycarbonate membranes with 0,2 µm controlled pores. After obtaining the liposomal vesicles, analyzes were performed on DLS of medium size, zeta potential and polydispersion index of the vesicles. The encapsulation efficiency was evaluated by HPLC. **Results:** All suspensions had vesicles smaller than 0,234µm after extrusion, polydispersity index less than 0.372 and absolute value of the zeta potential relatively high. The encapsulation efficiency was approximately 100%. **Conclusion:** Thus, the ethanol injection method followed by extrusion was effective for the formation of nanovesicle with relative homogeneity and good encapsulation efficiency. **Acknowledgments:** CNPq, Capes, FINEP, UEM.

References:

BRIUGLIA, M.-L. et al. Influence of cholesterol on liposome stability and on in vitro drug release. **Drug delivery and translational research,** v. 5, n. 3, p. 231-242, 2015. ISSN 2190-393X.

FRÉZARD, F. et al. Lipossomas: propriedades físico-químicas e farmacológicas, aplicações na quimioterapia à base de antimônio. **Quimica Nova,** v. 28, n. 3, p. 511-518, 2005.

MUFAMADI, M. S. et al. A review on composite liposomal technologies for specialized drug delivery. **Journal of drug delivery,** v. 2011, 2011. ISSN 2090-3014.

ROGERIO, L. C.; MACHADO, L. Q. Eficácia in vitro de lipossomas furtivos contendo antimoniato de meglumina e miltefosina para o tratamento da leishmaniose tegumentar americana. 2017

EVALUATION OF THE MORPHOLOGY AND THERMOTROPIC PROFILE OF LIPOSOMES CONTAINING TRICHILIA CATIGUA EXTRACT

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Key words: *Trichilia catigua*, liposomes, plant extract.
Introduction: Modified release systems have emerged as an alternative for the release of drugs or active agents in a given environment, with specific time and speed. Among these, liposomes deserve prominence due to their characteristics such as low toxicity, versatility of composition and size, biodegradability and biocompatibility. These structures allow the increase of formulation stability and reduction of drug toxicity, as well as the possibility of increasing biological activity as a modified release system. **Aim:** The objective of this work was to obtain and characterize liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC), cholesterol and ethyl acetate fraction of *Trichilia catigua* A. Juss. **Methods:** Liposomes containing the DPPC and DMPC phospholipids, cholesterol and the semi-purified fraction of *Trichilia catigua* were obtained by the ethanol injection method followed by extrusion with polycarbonate membrane with 0.2 µm controlled pores. The vesicle morphology was analyzed by transmission electron microscopy and the thermotropic behavior of each sample by differential scanning micro calorimetry (µ-DSC). **Results:** Electronic microscopic analysis confirmed the lamellar structure and amorphous spherical geometry of all samples. The results of µ-DSC showed the thermotropic behavior of the different components of each sample. It was possible to observe the transition of subgel (Ts) in sample C_1 and pre-transition (Tp) in sample C_3, besides the liquid-crystalline transition (Tm) observed in the three samples (C_1, C_2 and C_3). **Conclusion:** Therefore, the method used allowed to form spherical nanometric vesicles that present different thermotropic behaviors due to their different compositions.
Acknowledgments: CNPq, Capes, FINEP, UEM.

References:

ADEOTI, I. A.; HAWBOLDT, K.; SANTOS, M. R. Thermal and flow properties of fish oil blends with bunker fuel oil. Fuel, v. 158, p. 641-649, 2015. ISSN 0016-2361.
BRIUGLIA, M.-L. et al. Influence of cholesterol on liposome stability and on in vitro drug release. **Drug delivery and translational research,** v. 5, n. 3, p. 231-242, 2015. ISSN 2190-393X.
FRÉZARD, F. et al. Lipossomas: propriedades físico-químicas e farmacológicas, aplicações na quimioterapia à base de antimônio. **Quim. Nova,** v. 28, n. 3, p. 511-518, 2005.
MOTHÉ, C. G.; AZEVEDO, A. D. D. Análise térmica de materiais. São Paulo: iEditora, 2002.
SINKO, P. J. Martin: físico-farmácia e ciências farmacêuticas. **São Paulo: Editora Artmed**, 2008.

DEVELOPMENT AND PARTIAL CHARACTERIZATION OF LIPOSOMES CONTAINING ESSENTIAL OIL OF *Rosmarinus officinalis* L.

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Key words: Liposomes, nanoparticles, *Rosmarinus officinalis* L.
Introduction: Medicinal plants and their essential oils have been the source of various studies based on ethnobotany, chemical composition and therapeutic activities. As an alternative for the incorporation of these natural substances in pharmaceutical formulations, industries aim to associate these bioactives with nanoparticle systems, in order to obtain more efficient and active formulations. The encapsulation of *Rosmarinus officinalis* essential oil, known as rosemary, represents a great interest due of its activity in the prevention of oxidative stress, by eliminating free radicals, acting as antioxidants; which confers applications in the pharmaceutical, food and cosmetics fields[1]. Liposomes are vesicular systems, composed of amphiphilic phospholipids organized in bilayers, which surround aqueous compartments. These nanoparticles allow the incorporation of vegetable oils, facilitating the interaction of liposomes-cells, increasing the permeation of substances in the epidermis and intensifying the release at the desired location[2]. **Aim:** Develop nanoparticle systems to encapsulate rosemary oil. **Methods:** It was developed a factorial study using the method of mixing design by Statistical Software to establish the optimal concentrations of dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), cholesterol and rosemary oil [3]. Formulations were prepared by the ethanolic injection method, in which the components were solubilized in ethanol and injected into aqueous medium under constant stirring and heating. The vesicles characterization was performed in terms of mean size, polydispersity index and zeta potential using the Nanoplus Zeta/Nano Particle Analyzer[2]. **Results:** Nanoparticles obtained presented an average size smaller than 216.4 nm ± 5.4 after extrusion, with polydispersity index 0.253 ± 0.023, and zeta potential ranging from -12.03 to 2.98 mV. **Conclusion:** The development of systems containing phospholipids and cholesterol was capable to form nanoparticles and encapsulate rosemary oil.

References:

[1]RIBEIRO, Andreia et al. Microencapsulation of Rosmarinus officinalis L.(rosemary) aqueous extract for application in functional foods. In: **6th Workshop on Green Chemistry and Nanotechnologies in Polymer Chemistry**. 2015
[2]JAAFAR-MAALEJ, C.; DIAB, R., ANDRIEU, V.; ELAISSARI, A., FESSI, H. Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation. **Journal of Liposome Research**, v.20(3), p.228–243, 2010.
[3] PERALTA-ZAMORA, P.; MORAIS, J. L.; NAGATA, N. Por que otimização multivariada?**Engenharia Sanitária e Ambiental**. v.10, n.2, p.106-110, 2005.

POPULATION PHARMACOKINETICS OF BROMOPRIDE

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Key words: Bromopride, population pharmacokinetic, modeling.
Introduction: Bromopride (BRO) is an antiemetic and prokinetic agent used to treat motility dysfunction at gastrointestinal tract and vomiting. This drug is used by pregnant, patients on chemotherapy and is also an option, to treat gastroesophageal reflux disease in adults and children. Available since early seventies, we have little knowledge about its pharmacokinetic. The literature presents only a paper, that studies the pharmacokinetics of BRO, using compartmental approach with data from 18 volunteers[1]. **Aim:** The aim of this study was to develop the pharmacokinetic profile of BRO using the population approach. **Methods:** The data for the modeling was obtained from BRO bioequivalence studies presented to ANVISA as a part of requirements to register a generic drug. All BRO registrations were analyzed to collect anthropometric data, plasma concentration per time and laboratory exams from volunteers involved in these studies. These data were initially tabulated in Excel® for later population modeling using the Monolix® software. **Results:** It was found six bioequivalence studies in ANVISA dataset, which resulted in the collection of information from 139 subjects. The sample was characterized by 69 male and 70 healthy adult female. The age range 18-50 years and body weight range 47-91 Kg. The model with first order absorption, two compartments and lag time was the most suitable for this dataset, although the study that first describe the pharmacokinetic profile of BRO could fit their data in a one-compartment model. The population pharmacokinetic parameters calculated were lag time (Tlag) = 0.45 ± 0.019 h, absorption constant (ka) = 1.63 ± 0.13 h^{-1}, clearance (Cl) = 44.7 ± 1.8 L/h, volume of distribution in central compartment (V1) = 234 ± 13 L, intercompartmental clearance (Q) = 40.6 ± 5.7 L/h and volume of distribution in peripheral compartment (V2) = 113 ± 6.6 L. **Conclusion:** The population approach is a more modern tool that considers inter and intraindividual variability during the modeling process, which not happen when we use only the compartmental approach. This can explain the differences between the population parameters found in this work from the pharmacokinetic described previously. Moreover, due to the greater number of individuals in this study, it was possible to fit a two-compartmental model.
Acknowledgments: ANVISA, PK/PD Lab, CAPES.

References:

[1]Brodie, R. R., Chasseaud, L.F., Darragh, A., Lambe, R.F., Rooney, L., Taylor, T., 1986. Pharmacokinetics and bioavailability of the anti-emetic agent bromopride. Biopharmaceutics & Drug Disposition.7, 215-222.

LABIPROS & STEVIA SOUL: THE INTERACTION BETWEEN ACADEMIA AND INDUSTRY AT THE SERVICE OF THE CONSUMER

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Key words: sweetener industry, *Stevia rebaudiana*, Stevita.
Introduction: Universities and industry have been collaborating for over a century. While business sector, in order to supply the market demand, commonly promotes investments in research, technological development and innovation, universities are usually prepared to offer scientific knowledge and specialized researchers groups.[1] The partnership between both sectors results in many cases in a generation of competitive differentials, able to attend the needs of contemporary society.[2] **Aim:** The aim of this study was establish a methodology for the *in vitro* cultivation and development of the "ST 4001" plant from Stevia Soul for acclimatization in the field. **Methods:** The collaboration between LABIPROS (Laboratório de Biotecnologia de Produtos Naturais e Sintéticos) and Stevia Soul was through a service contract, process number 7408/2017 registered in the CSD (Coordenadoria de Serviço e Desenvolvimento Regional) of State University of Maringá, which describes the services that will be provided, the goals to be achieved and the financial resources. The explants used in the experiments were obtained from "ST 4001" plant provided by commercial field Stevia Soul and transferred to semi-solid medium MS (Murashige and Skoog, 1962)[3] in aseptic environment, and maintained in photoperiod, temperature and humidity control. After the third *in vitro* subculture, the plants returned to the industry and were acclimated in a suitable substrate. **Results:** The results showed that acclimatization was successful, with the production of plants free of pathogens and pesticides, allow to obtain plants with uniformity in a short time, as required by the industry. **Conclusion:** In conclusion, the work showed that there was a collaboration between the university and the private sector in the development of efficient biotechnological methodologies contributing to the consolidation of the partnership between academia and industry to serve the consumer. **Acknowledgments:** Stevia Soul, CAPES and CNPq.

References:

[1]Mancebo, et al. 2015. Política de expansão da educação superior no Brasil 1995-2010. Revista Brasileira de Educação, 20, 60, 31-49
[2]Berni, et al. 2015. Interação Universidade-Empresa para a inovação e a transferência de tecnologia. Revista Gual, Florianópolis. 8, 2, 258-277
[3]Murashige T. & Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15, 473-497

HIGH-CARBOHYDRATE AND HIGH-FAT DIETS MODULATE BRAIN FATTY ACID COMPOSITION AND INFLAMMATORY GENE EXPRESSION IN MICE

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Key words: Brain fatty acids; Brain inflammation; Fatty acid metabolism.

Introduction: It is well established that fatty acid (FA) accumulation is modulated by composition of the diet. Nevertheless, dynamics of the FA deposition appear different from tissue to tissue. It has previously been reported that mice fed with a high-fat diet (HFD) for 56 days have increased inguinal FA deposition[1]. It has also been verified that FA deposition, in particular saturated FA (SFA) and monounsaturated FA (MUFA), are exacerbated in livers from mice fed a high-carbohydrate diet (HCD) associated with a pro-inflammatory state [2]. The brain is rich in FA that represent more than half of the brain dry weight . Brain FA and its metabolites have several functions, including the regulation of liver glucose production and food intake [3]. Moreover, the high intake of simple sugars and saturated FA (SFA), have been associated with a reduction in the brain cognitive function [4]. However, few studies have evaluated the changes in brain FA composition that are induced by different diets. **Aim:** To investigate the effect of a HCD or HFD on brain FA profile and inflammatory gene expression in mice. **Methods:** Mice fed with a HCD or HFD for 0, 7, 14, 28 or 56 days were compared. FA composition was measured by using gas chromatography and gene expression through quantitative polymerase chain reaction. All experiments were approved by Scientific Advisory Committee on Animal Care of the State University of Maringá (protocol 002/2014). Data was analyzed by a Student's t-test or ANOVA (one-way) and post-test of Tukey. $P < 0.05$ were considered significant. **Results:** The HFD group showed faster deposition of SFA, MUFA and PUFA. However, after 56 days, the amount of FA, the ratios of PUFA/SFA, MUFA/SFA, n-6/n-3 and activities of stearoyl-CoA desaturase-1 and elongases, were similar (HCD vs. HFD). HCD group had higher activities of Δ-6 desaturase and *de novo* lipogenesis (HCD vs. HFD). On day 56, HFD group had an increased ($p<0.05$) inflammatory marker index in the total brain and hippocampus. **Conclusion:** Both HCD and HFD modulate the speed of FA deposition, the activities of lipid metabolism enzymes, and inflammatory gene expression in the brain. The higher inflammatory state was associated with the faster FA deposition in the brain of HFD mice.

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References:

1. Obici, S., Tavoni, T. M., Barrena, H. C., Curi, R., Bazotte, R. B. *Cell Biochem. Funct.* 2012, *30*, 335–339.
2. Silva-Santi, L. *et al. Nutrients* 2016, *8*, 682.
3. Lam, T. K. T., Schwartz, G. J., Rossetti, L. *Nat. Neurosci.* 2005, *8*, 579–584.
4. Beilharz, J. E.; Maniam, J.; Morris, M. J. *Nutrients* 2015, *7*, 6719–6738.

SYNERGISTIC INTERACTION OF BERBERINE AND FLUCONAZOLE AGAINST *CANDIDA ALBICANS* AND *CANDIDA TROPICALIS*

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Key words: Berberine, *Candida albicans*, *Candida tropicalis*, Fluconazole
Introduction: Berberine is an alkaloid, used in the treatment of bacterial diarrhea, intestinal parasite infections and ocular trachoma infections, with weak activity against *C. albicans* and *C. glabrata*. However, the combination of berberine with fluconazole resulted in better effect (1). The objective of this study is to verify the synergistic activity of berberine with fluconazole against *Candida albicans* and *Candida tropicalis*, microencapsulate them, aiming at production of an oral ointment for patients who have recurrences of oral candidiasis. **Methods:** Berberine and fluconazole were obtained industrially pure, from Sigma Aldrich and Pfizer Pharmaceuticals, respectively. Minimal inhibitory concentrations (MICs) against *C. albicans* and *C. tropicalis* were determined according to CLSI reference procedure (2). Synergic interaction of berberine and fluconazole was assessed by Checkerboard test, and the effect of berberine against *Candida* biofilm formation by MTT reduction assay. Polymeric microparticles containing berberine alone and in combination with fluconazole were produced with sodium alginate by Spray- drier in an LM MSD 1.0(3). **Results:** Berberine alone had a fungicidal effect at the concentration of 125µg/ml for *C. albicans* ATCC 10231 and *C. tropicallis* ATCC 28707 31.2µg/ml. A Fractional Inhibitory Concentration (FICI) of 0.04 and 0.48 respectively showed synergistic interaction. The FICI is the sum of the MIC of each drug in combination divided by the MIC of the drug used alone and An FIC index < 0.5 is considered synergism. Berberine had also inhibitory effect against biofilm formation. BIC50 (Biofilm Inhibitory Concentration) was 15µg/mL for *C albicans* ATCC 10231 and 12µg/mL for *C. tropicalis* ATCC 28707. **Conclusion:** Berberine has good antifungal effect against *C. albicans* and *C. tropicalis*, including biofilm formation. And when combined with fluconazole, it is synergistic.
Acknowledgments: CAPES
References:
(1) Iwazaki RS, et al. (2010). In vitro antifungal activity of the berberine and its synergism with fluconazole. *Antonie Leeuwenhoek* 97 201–205.

(2)Clinical and Laboratory Standards Institute (CLSI). Reference Method for Broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A3. Clinical and Laboratory Standards Institute, Villanova, Pa, 2008[b]

(3)Brambilla, LZS, et al. (2017). "*Piper regnellii* extract biopolymer-based microparticles: production, characterization and antifungal activity." *Journal of Applied Microbiology*.

TISSUE REPAIR ACTIVITY OF AROEIRA CRUDE EXTRACT

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Key words: Herpes simplex type 1, Aroeira, tissue repair activity.

Introduction: Herpes simplex virus type 1 (HSV-1) is responsible for eye and orofacial infections. Usually they are transmitted by direct mucosal contact and discontinuity of the skin, and after the primary infection, the virus remains in the peripheral neurons. Recurrent infection can occur and it can be more severe in immunocompromised patients, resulting from invisible lesions to the naked eye to debilitating lesions[1]. Acyclovir is the drug of choice for treatment of herpetic infections, but several cases of resistance, especially in immunocompromised patients have been reported[2]. Therefore, finding new drugs for the HSV-1 treatment is needed. Preparations made with *Schinus terebinthifolia* Raddi, popularly known as Aroeira, are traditionally used for topical treatment of skin and mucosal injuries in general[3]. Previously, *in vitro*[4] and *in vivo*[5] assays showed the anti-HSV-1 activity of the hydroethanolic extract of stem bark from *S. terebinthifolia.* So, it can contribute for the decrease of viral dissemination, mainly the acyclovir-resistant strains. **Aim:** The aim of this study was investigate the effectiveness of Aroeira crude extract on healing of fibroblasts culture lesions *in vitro*. **Methods:** The methodology used was the *in vitro* scratch wound assay using L-929 cell culture. **Results:** The wound closure in culture of fibroblast L-929 was observed within 48 h of incubation with 4 µg/mL of crude ėxtract of Aroeira, confirming the potential healing described in the literature. **Conclusion:** The healing activity is beneficial in the topical application against HSV-1 infection as it could contribute to improve the remission of herpetic lesions while preventing the virus from infecting new cells. This set of activities is possible due to the variety of compounds present in the crude extract of Aroeira, and suggesting the benefits of using crude extracts.

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References:

[1]Trabulsi, L. R.; Alterthum, F., 2008. Microbiologia. 5ª Ed., São Paulo, Ateneu.
[2] Lorenzi, H.; Matos, F. J. A., 2002. Plantas Medicinais No Brasil: Nativas E Exóticas. Nova Odessa, Sp: Instituto Plantarum ·
[3] Morfin, F, Thouvenot, D., 2003. Herpes Simplex Virus Resistance To Antiviral Drugs. J Clin Virol. 26:29–37.
[4] Nocchi, S. R.; Moura-Costa, G. F.; Novello, C.R.; Rodrigues, J.; Longhini, R.; Mello, J. C. P.; Dias Filho, B. P.; Nakamura, C.V.; Ueda-Nakamura, T. 2016. In Vitro Cytotoxicity And Anti-Herpes Simplex Virus Type 1 Activity Of Hydroethanolic Extract, Fractions, And Isolated Compounds From Stem Bark Of *Schinus Terebinthifolius* Raddi. Pharmacog Mag, 12, 160-164.
[5]Nocchi, S.R., Companhoni, M.V., De Mello, J.C., Dias Filho, B.P., Nakamura, C.V., Carollo, C.A., Silva, D.B., Ueda-Nakamura, T., 2017. Antiviral Activity Of Crude Hydroethanolic Extract From *Schinus Terebinthifolia* Against Herpes Simplex Virus Type 1. Planta Med. 83(6):509-518.

PREPARATION AND CHARACTERIZATION OF THERMO-RESPONSIVE BIOADHESIVE SYSTEM CONTAINING METRONIDAZOLE AND PROPOLIS MICROPARTICLES

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Key Words: bioadhesion, metronidazole, microparticles, propolis, development.

Periodontal disease is characterized by loss of attachment of the periodontal ligament and destruction of adjacent bone tissue. This is an infectious and inflammatory disease that affects the supporting and tissues of the teeth(1). Some studies have confirmed the activity of propolis in the prevention and treatment of periodontal disease(2). Propolis has several pharmacological activities, for example, anti-inflammatory, antimicrobial, antiviral, antitumor, antioxidant, antiprotozoal and cicatrizant(3). Metronidazole has antiprotozoal and antibiotic activities and is used in dental treatments, such as periodontitis(4). The technology of micronencapsulation is a strategy that allows developing new formulations, as it increases the therapeutic efficacy, being able to mask unpleasant taste and odor, and protecting the drug itself(2). The objective of this work was to prepare and characterize a thermoresponsive bioadhesive system containing microparticles of propolis and metronidazole for administration in the periodontal pocket, aiming the treatment of periodontitis. The microparticles were prepared by the methodology previously developed and optimized(4). Morphological analysis, determination of mean particle size distribution and metronidazole release were also performed. The formulation was composed of 20% (w/w) poloxamer 407 (P407) and 0.20% (w/w) Carbopol 971P® (C971P). The analysis of texture, mucoadhesive strength, seringueability and softness were performed by the TA-XTplus texture analyzer (Stable Micro Systems®) and the rheological analysis of continuous shear, oscillatory and solid / gel transition temperature by the MARS II rheometer (Haake®). The total polyphenol and metronidazole contents were 1.63 ± 0.0696% (w/w) and 2.00 ± 0.0539% (w/w), respectively, and the encapsulation efficiency for propolis was 70.02 ± 0.3813% and 12.03 ± 0.0539% for metronidazole. In vitro release studies have demonstrated that the microparticles provided a modified drug release. The texture profile analysis allowed evaluating the properties of the preparation, hardness, compressibility, adhesiveness, elasticity and cohesiveness, which were all satisfactory. The work required to expel the syringe formulation (seringueability) was 29.75966 ± 0.33068 N.mm. The binary polymer blend containing P407 and C971P presented plastic flow behavior and exhibited properties dependent on temperature and oscillatory frequency.
Thus the preparation process of ethylcellulose microparticles containing propolis and metronidazole showed to be a viable method and the drug release profile from the obtained structures has been shown to be modified (prolonged) and controlled.

References

(1) Seymour, R. A.; Heasman, P. A. Drugs, diseases, and the periodontum. New York: Oxford Medical Publications, Oxford University Press, 1992.
(2) Bruschi, M. L. Desenvolvimento e caracterização de sistemas de liberação de própolis intrabolsa periodontal. 2006. 318 f. Tese (Doutorado em Ciências Farmacêuticas)–Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto: 2006.
(3) Burdock, G.A Review of the biological properties and toxicity of bee própolis (Propolis). Food Chem. Toxicol., v.36, p. 347-363, 1998.
(4) Ferreira, Sabrina Barbosa De Souuza et al. Microparticles containing propolis and metronidazole: in vitro characterization, release study and antimicrobial activity against periodontal pathogens. Pharmaceutical development and technology, v. 19, n. 2, p. 173-180, 2013.

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ORAL THERAPEUTIC EFFICACY OF HYDROETHANOLIC EXTRACT FROM *Tanacetum parthenium* ON HERPETIC LESIONS

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Keywords: Chlorogenic acid; Genotoxicity; HSV-1; *Tanacetum parthenium*.

Introduction: Herpes simplex virus type 1 (HSV-1) is an enveloped, double-stranded DNA virus, widely distributed in the world population, causing mild orofacial lesion, which could become severe. Standard treatment is performed with nucleoside analogs, highly specific to infected cells. However, the selection of resistant strains hinders the treatment of patients with recurrent lesions. *Tanacetumparthenium (*L.) Schultz-Bip is an herbaceous plant used in folk medicine for numerous diseases. Our research group had already demonstrated *in vitro* anti-HSV-1 activity of the hydroethanolic extract of *Tanacetum parthenium (*L.) Schultz-Bip (CHE), as well as its *in vivo* oral safety. **Aim:** The objective of this study was to determine the major constituents of this extract, the oral therapeutic efficacy *in vivo* and the genotoxicity as well. **Methods:** CHE was analyzed by high performance liquid chromatography, coupled to mass spectrometer ESI IT, equipped with an electrospray ionization source and an ion trap analyzer. In order to quantify the major constituents, a chromatographic method by HPLC DAD, equipped with LC 30AD and DAD SPD M30Awas developed. For the genotoxicity test, adult Swiss mice were treated by gavage with vehicle, 2 doses of CHE, and cyclophosphamide. After 24h the bone marrow was collected and processed. Slides were prepared, stained with Giemsa, followed by cell counting. The oral therapeutic efficacy[(1)] test was performed with BALB/c mice that were previously infected with HSV-1. Animals were treated once a day for 10 days. The lesions were photographed, and the lesion score was determined. On the 10th day the animals were euthanized. **Results:** The analysis of the CHE by LC-MS resulted in the identification of 23 distinct substances, among them chlorogenic acid, caffeic acid, chlorogenic acid derivatives and parthenolide. The extract did not induce genotoxic alterations, when compared with positive control (cyclophosphamide), corroborating with the safety evaluated in the previous tests. The analysis of the lesions of oral therapeutic efficacy assay confirmed the anti-HSV-1 activity of the extract. The weight evolution of the animals confirmed the safety of this treatment in the infected animals. **Conclusion:** The hydroethanolic extract of *Tanacetum parthenium* (L.) Schultz-Bip. is composed by phenolic acids and sesquiterpene lactones, active against HSV-1 infection and non-genotoxic *in vivo*.

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References

[(1)] CARDOZO, F. T. G. S.; LARSEN, I. V.; CARBALLO, E. V.; JOSE, G.; STERN, R. A.; BRUMMEL, R. C.; CAMELINI, C. M.; ROSSI, M. J.; SIMÕES, C. M. O.; BRANDT, C. R. In vivo anti-herpes simplex virus activity of a sulfated derivate of *Agaricus brasiliensis*mycelial polysaccharide Antimicrobial Agents and Chemotherapy, v. 57, n. 6, 2013.

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REGIONAL MARINGÁ