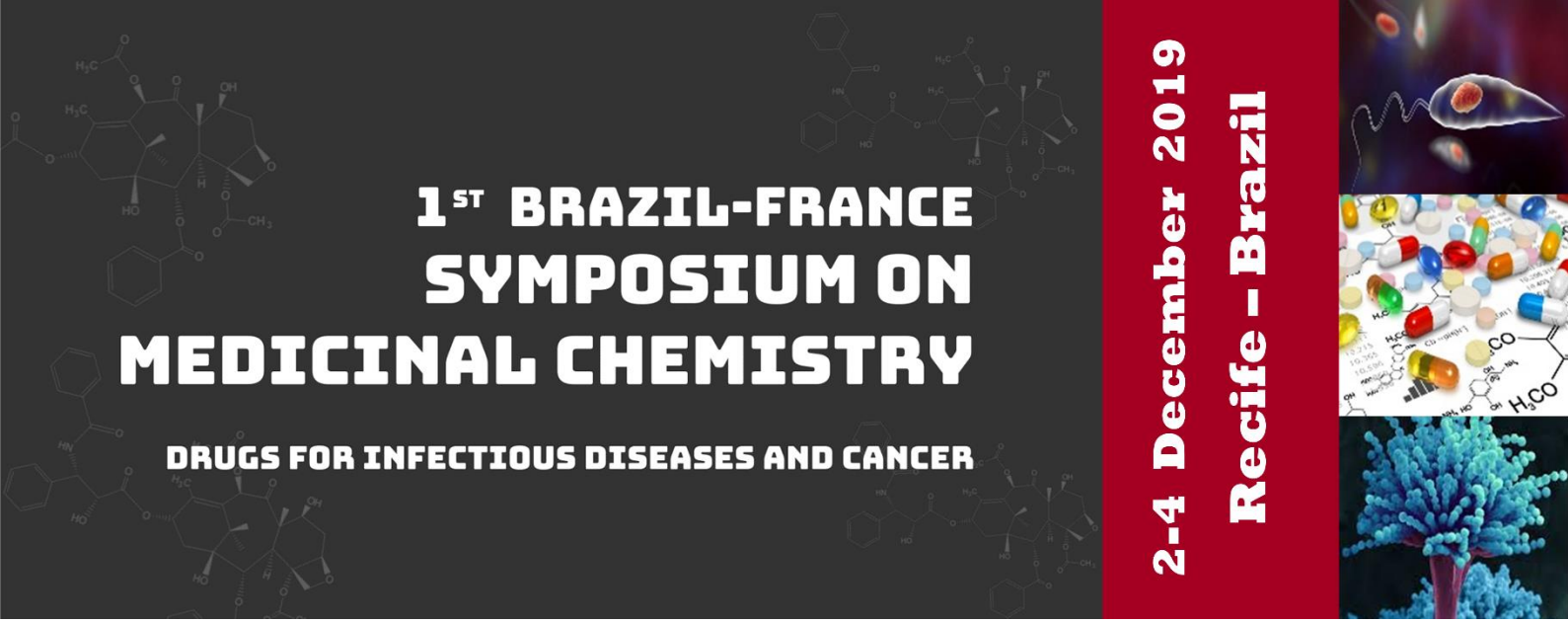

ANNALS OF THE

1ST BRAZIL-FRANCE SYMPOSIUM ON MEDICINAL CHEMISTRY:

DRUGS FOR INFECTIOUS DISEASES AND CANCER

SUPPORT:

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Katharina Porto – UFPE

SCIENTIFIC PROGRAM

02/12/2019 – **Monday**

08h00 – **Registration**

09h00 – **Minicourse 1**: Avaliação da atividade antimicrobiana de produtos sintéticos e de origem natural.
Profa. Gláucia Manoella de Souza Lima – UFPE
Prof. Reginaldo Gonçalves de Lima Neto – UFPE

09h00 – **Minicourse 2**: Avaliação *in vitro* e *in vivo* da atividade anticâncer de produtos sintéticos, biotecnológicos e de origem natural.
Profa. Jeyce Kelle Ferreira de Andrade – UFRPE
Prof. Moacyr Jesus Barreto de Melo Rego – UFPE

09h00 – **Minicourse 3**: Planejamento e síntese de fármacos antiparasitários.
Prof. Jefferson Luiz Princival – UFPE
Dr. Luiz Felipe Gomes Rabello Ferreira

09h00 – **Minicourse 4**: Avaliação da atividade biológica de moléculas candidatas a fármacos antiparasitários.
Dra. Valéria Pereira Hernandes - Instituto Aggeu Magalhães/Fiocruz
Dra. Aline Caroline da Silva Santos - Instituto Aggeu Magalhães/Fiocruz

19h00 – **Opening Lecture**
President: Prof. Dra. Teresinha Gonçalves da Silva- UFPE

19h30 – **Plenary Lecture**
"France-Brazil collaborative Project for the Discovery of azaheterocyclic compounds as anit-Trypanosoma cruzi agentes to fight against Chagas disease"
Prof. Dr. Pascal Marchand – Université de Nantes, France

20h30 – **Welcome Reception**

03/12/2019 – **Tuesday**

08h00 – **Registration**

09h00 – **Plenary Lecture**
"Pesquisa e Desenvolvimento de fármacos antimicrobianos: presente, passado e futuro"
Prof. Dr. Henrique Douglas Melo Coutinho – URCA

10h00 – **Session 1**: Epidemiology, mechanisms of resistance, and medicinal chemistry of antibacterial drugs.
"Contribuição da química medicina na obtenção de fármacos antimicrobianos"
Prof. José Gildo de Lima – UFPE

"Microwave-assisted vc conventional synthesis: compounds with antituvercular and anticancer properties"
Prof. Eugenio Hernández Fernández – Universidad Autónoma de Nuevo León (México).
"Resistência microbiana no ambiente: situação atual desafios"
Profa. Maria Amélia Vieira Maciel – UFPE

11h30 – **Oral Presentations**

12h30 – **Lunch**

14h00 – **Plenary Lecture**
"Metabólitos secundários bioativos de fontes terrestres e marinhas"
Profa. Otília Deusdênia Loiola Pessoa – UFC

15h00 – **Session 2**: Recent advances in R&D of antineoplastic agentes derived from or inspired in natural products.
"Hidrogel à base de copaíba para prevenção e tratamento da mucosite oral quimioinduzida e radioinduzida: estudo clínico randomizado"
Profa. Aurora Karla de Lacerda Vidal – UFPE
"Bioprospecção de compostos heterociclicos nitrogenados com ação antitumoral"
Prof. Ricardo Olímpio de Moura – Universidade Estadual da Paraíba
"Plantas do Nordeste e atividade antitumoral: experiências com metabólitos secundários"
Profa. Gardênia Carmem Gadelha Militão – UFPE.

16h30 – **Oral Presentations**

17h30 – **Poster Session**

04/12/2019 – **Wednesday**

08h00 – **Registration**

09h00 – **Plenary Lecture**
"Study of ergosteral biosynthesis in fungi"
Dra. Isabelle Ourliac-Garnier – Université de Nantes, France

10h00 – **Session 3**: Epidemiology, mechanisms of resistance, and development of antifungal drugs.
"Aminotiofenos: seus papéis no combate às infecções microbianas"
Prof. Francisco Jaime Mendonça Júnior – Universidade Estadual da Paraíba.
"Mecanismos de resistência fúngica"
Prof. Reginaldo Gonçalves Lima Neto – UFPE
"Epidemiologia e diagnóstico dos fungos dimórficos emergentes em Pernambuco"
Prof. Armando Marsden Lacerda Filho – UFPE

11h30 – **Oral Presentations**

12h00 – **Lunch**

14h00 – **Plenary Lecture**
"Triagem Virtual de fármacos"
Prof. Marcelo Zaldini Hernandes – UFPE

15h00 – **Session 4**: Modern approachs in drug discovery for the treatment of neglected diseases.
"Synthesis and biological evaluation of guanylhydrazone and thiaosemicarbazone derivatives against Leishmania chagasi amastigotes"
Prof. Thiago Mendonça de Aquino – Universidade Federal de Alagoas.
"Cell biology approachs to study parasite-drug interactions"
Dra. Regina Célia Bressan Queiroz de Figueiredo – Instituto Aggeu Magalhães/Fiocruz.
"Terapêutica experimental na esquistossomose: o estado da arte"
Dra. Sheilla Oliveira de Andrade – Instituto Aggeu Magalhães/Fiocruz.
"Avanços da nanotecnologia no desenvolvimento de formulações para esquistossomose"
Dr. Fábio Formiga - Instituto Aggeu Magalhães/Fiocruz.

17h00 – **Closing Cerimony**

SUMMARY

WELCOME LETTER

The **1st Brazil-France Symposium on Medicinal Chemistry** was planned to get together Brazilian and French researchers on the field of medicinal chemistry, emphasizing on design, synthesis, and biological evaluation of drugs for the treatment of infectious diseases and cancer.

The 1st BFSMC is the result of a Capes-Cofecub project between the UFPE and the Université de Nantes. Supported by the Institut Français du Brésil, the 1st BFSMC aims to strengthen the existing collaborations between these two countries and provide a favorable environment to new collaborations, mainly in the Northeast region, with the participation of young researchers and the involvement of Pharmaceutical Sciences and Biotechnology post-graduate programs.

It will be a unique opportunity to the scientific divulgation on medicinal chemistry in Northeast region with the participation of national and international researchers, which will benefit Brazilian research groups and post-graduate programs.

Teresinha Gonçalves da Silva
President

PRELIMINARY ASSESSMENT OF THE ANTIMICROBIAL ACTIVITY AND PHARMACKINETIC PARAMETERS OF PIPERINE AND ITS DERIVATIVES

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ABSTRACT

INTRODUCTION: Piperine is a natural amide belonging to the alkaloid class, being one of the major constituents of plants of the genus Piper, in which the species *Piper nigrum* L. is one of the plants that presents the highest content of this compound. According to the ethnobotanical surveys found in the literature, this species has several uses as a condiment and in traditional medicine, mainly for the treatment of infections and inflammation. Also, the increase in bacterial resistance makes necessary the development of new therapeutic alternatives to fight clinically relevant pathogenic microorganisms. **AIMS:** This work aimed to carry out a preliminary evaluation of the antimicrobial activity of piperine and some of its derivatives, to determine the possible chemical groups related to their antimicrobial activity, as well as to calculate some pharmacokinetic parameters *in silico*. **METHODS:** Piperine was extracted using the innovative method for simultaneous extraction and purification named Góes Apparatus (patient registration: BR 10 2017 021099 5) (GÓES, 2018). The derivatives were obtained by the basic hydrolysis of piperine to piperic acid, which was further esterified to ethyl piperate. The antimicrobial activity of these compounds was evaluated by the microdilution method to determine the minimum inhibitory (MIC) and bactericidal (MBC) concentrations in 96-well plates using Mueller-Hinton broth for bacteria and RPMI 1640 medium for yeast. The following strains from the Culture Collection of the Department of Antibiotics/UFPE were used: *Bacillus subtilis* (UFPEDA 16), *Candida albicans* (UFPEDA 1007), *Enterococcus faecalis* (UFPEDA 138), *Escherichia coli* (UFPEDA 224), *Micrococcus luteos* (UFPEDA 06), *Micrococcus smegmatis* (UFPEDA 71), *Serratia marcescens* (UFPEDA 398), and *Pseudomonas aeruginosa*. (UFPEDA 39). After the addition of the substances to the wells, they were inoculated with 1 x 10^5 CFU and then incubated at 37 °C for 24 h for bacteria and 48 h for yeast. Resazurin was used as an indicator of growth. For the determination of MBC, 2 µL aliquotes from the wells, in which resazurin was not metabolized, were transferred to Petri dishes with Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for yeast. The pharmacokinetic parameters were evaluated using the online tool SwissADME (DAINA; MICHIELIN; ZOETE, 2017). **RESULTS AND DISCUSSION:** The simultaneous extraction and purification method optimized to isolate piperine from black pepper, using hexane/ethyl acetate (6:4) as elution system, yielded 0.5 % (w:w). Both hydrolysis and esterification reactions to obtained piperic acid and ethyl piperate showed yields above 85%. Regarding the

antimicrobial activity, the compounds were active against 6 of the 9 tested microorganisms. Piperine showed MIC values of 4 μg/mL for *B. subtilis*, 8 μg mL for *S. aureus* and *M. luteos*, 16 μg/mL for *M. smegmatis* and *C. albicans*, and 128 μg/mL for *E. faecalis*. On the other hand, piperic acid showed MIC values of 8 μg/mL for *S. aureus*, 16 μg/mL for *B. subtilis* and *C. albicans*, 32 μg/mL for *M. smegmatis*, and 128 μg/mL for *E. faecalis*. The ethyl piperate derivate was the least active, with MIC values of 16 μg/mL only for M. smegmatis, 32 μg/mL for *B. subtilis*, 64 μg/mL for *S. aureus*, *M. luteos* and *C. albicans*, and 128 μg/mL for *E. faecalis*. None of the compounds were able to inhibit the growth of Gram-negative bacteria (*E. coli*, *S. marcescens*, and *P. aeruginosa*). Evaluating the three compounds, the statistical results showed that piperine and piperic acid presented higher activities compared to ethyl piperate, exhibiting lower inhibitory concentrations. In addition, the evaluation of the pharmacokinetic parameters showed that piperine has the highest lipophilic profile compared to the other two derivatives, with a mean LogP of 3.04, whereas piperic acid and ethyl piperate had a mean LogP of 2.23 and 2.96, respectively, which possibly justifies greater activity of the original molecule. These results showed that the antimicrobial activity of these compounds are not only related to the benzodiaxole group, which is a well-known pharmacophore for antimicrobial and anti-inflammatory activities, but it is also influenced by the amide/carboxylic acid groups in piperine and piperic acid, respectively (LIMA, 2015). **CONCLUSION:** It is possible to conclude that the terminal portion of these compounds may have an influence on antimicrobial activity since the changes made in the piperine molecule generated compounds with significant differences in antimicrobial activity and pharmacokinetic properties. Therefore, further studies are necessary to determine the possible contributions made by the groups present in the molecules used in our study.

Keywords: Medicinal plants, antibacterial activity, antifungical activity, *Piper nigrum*, infection.

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IN VITRO ANTICANCER EFFECT OF NEW THIAZOLIDINIC DERIVATIVES

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ABSTRACT

INTRODUCTION: Cancer is considered a complex genetic disease, represented by the disordered growth of its cells. Cancer development is related to exposure to exogenous and endogenous carcinogenic factors. Exogenous carcinogenic factors account for approximately 80% of cases and include eating habits, lifestyle, physical agents, chemical agents and biological agents; while endogenous carcinogenic factors include an individual's genetic makeup, age, immune system damage, physiological condition, and inflammation such as ulcerative colitis and pancreatitis (INCA, 2017). According to INCA (2017), the main cancers that cause the most death are: lung, prostate, female breast, uterine colon, straight stomach, esophagus, and liver. Given the above, there is a commitment to seek new anticancer medicines, including thiazolidinedione-derived compounds, known to have some biological activities, such as antimicrobial, hypoglycemic, and antitumor (KHAN et al., 2018). **AIMS:** To evaluate the in vitro anticancer effect of thiazolidinedione derivatives. **METHODS:** Thiazolidinonic derivatives (NW series) were supplied by the Laboratory of Chemistry and Therapeutic Innovation (LQIT) da Universidade Federal de Pernambuco (UFPE). Initially a cytotoxic screening of NW series derivatives was performed on tumor cell lines (HL- 60, NCI-H292, HCT-116, HT29 e MCF7) and normal (L929), where the percentage of cell growth inhibition (IC%) at 50 μg / mL was evaluated by the MTT assay, 3- (4,5- dimethyl-2-thiazolyl) -bromide 2,5-diphenyl-2H-tetrazolium (ALLEY et al., 1988). An intensity scale was used to evaluate the cytotoxic potential of the compounds tested, being classified as: high cytotoxic activity (ranging from 75 to 100% inhibition), moderate activity (cell growth inhibition ranging from 50 to 75%) and low activity. (growth inhibition less than 50%) (RODRIGUES et al., 2014). Subsequently, 50% inhibitory concentrations (IC_{50}) of the samples were determined by the same method. **RESULTS AND DISCUSSION:** The cytotoxic activities of compounds expressed as percent cell growth inhibition values (IC%) are described in Table 1. Of the derivatives tested only three substances (NW05, NW06 and NW17) presented cytotoxic activity greater than 75% inhibition in adherent cell lines and one in suspension, which were chosen for the determination of IC50. In the IC50 test, only the NW05 presented IC50 ≤ 4.0 μg/mL. *In vitro* cytotoxicity tests are important to verify the toxicity of new compounds in the early stages of drug development (PUTNAM et al. 2002). According to the parameters defined by the United States National Cancer Institute (NCI), a substance is considered cytotoxic when it has an $IC_{50} \leq 4.0$ μg/mL (ITHARAT et al., 2004). These criteria reinforce that the thiazolidinediones derivatives evaluated present considerable cytotoxic potential in human tumor cells.

Table 1. Percentage of cell growth inhibition (IC%) of thiazolidinonic derivatives (NW series) in tumor.

Samples	Cell Lines (IC%)				
	HL-60	**NCI-H292**	**HCT116**	**MCF7**	**L929**
NW05	67.79 ± 2.59	81.67 ± 0.05	81.97 ± 0.12	60.92 ± 0.41	25.49 ± 0.48
NW06	32.74 ± 1.52	63.65 ± 0.46	69.99 ± 3.42	84.62 ± 0.12	15.03 ± 6.41
NW07	40.61 ± 1.44	28.23 ± 3.69	61.49 ± 5.40	58.24 ± 0.75	21.08 ± 8.80
NW10	63.30 ± 5.80	27.34 ± 3.88	59.98 ± 4.53	56.10 ± 0.18	6.11 ± 3.13
NW17	77.84 ± 0.45	31.40 ± 9.41	52.43 ± 4.39	45.22 ± 2.47	15.83 ± 5.26
NW18	67.62 ± 6.03	37.39 ± 1.49	56.23 ± 4.19	43.08 ± 1.06	15.10 ± 3.13
NW19	63.15 ± 3.58	36.19 ± 3.86	55.36 ± 4.38	38.85 ± 1.28	9.20 ± 0.75
NW21	64.53 ± 1.01	27.95 ± 9.67	68.49 ± 0.45	42.15 ± 3.22	19.58 ± 3.82
DOXO	91.26 ± 0.30	92.74 ± 0.05	86.75 ± 0.36	86.95 ± 0.12	92.94 ± 0.41

Regarding thiazolidinediones, Rodrigues et al. (2018) conducted studies and found that 5-(2-bromo-5-methoxybenzylidene)-thiazolidine-2,4-dione showed high cytotoxicity against the NCI- H292 lung cancer cell line. IC_{50} of 1.26 µg/mL. The results of this study indicate that the derivatives are promising molecules for the development of new antitumor drugs since cytotoxicity ranged from moderate to high. **CONCLUSION:** The results obtained in this study allowed us to conclude that the derivatives NW05, NW06, NW17 showed potent cytotoxic activity for tumor cell lines, highlighting among them the compound NW05.

Keywords: Antitumor, cytotoxicity, thiazolidinediones.

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EVALUATION OF THE PHOTOPROTECTIVE ACTIVITY OF ISOLATES OF *Waltheria viscosissima*

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ABSTRACT

INTRODUCTION: There is a great demand for the use of medicinal plants in the cure and prevention of pathologies. Such benefits are conferred through the therapeutic potential of the secondary metabolites present in these products. The marketing of medicinal plants is quite common in street fairs and are also cultivated in homes, where there is greater exchange of knowledge and experience regarding the practice and consumption of popular formulas that seek healing effects. Therefore, popular knowledge about the use and efficacy of plants is of great importance for research and discovery of new biologically active substances[1,2]. Brazil stands out for its biodiversity, presenting the largest diversity of plants and living organisms in nature. Many plants synthesize various bioactive compounds during secondary metabolism, which have a variety of biological activities in the body, such as antioxidant, anti-inflammatory, hypoglycemic, among others. Therefore, the phytochemical study of medicinal plants for the identification of multifunctional compounds is of utmost importance[3,4]. The major interest in these biologically active substances of plant origin is the search for new inhibitors, mainly in the identification and isolation of metabolites capable of retarding the action of key enzymes in diseases and/or activating other molecules that act to overcome diseases[5]. In Brazil, *Waltheria viscosissima* A. St. Hil. (family *Malvaceae*), popularly known as "malva viscosa", is a plant rich in essential oils and mucilage. It is native to the country and commonly found in the Amazonas, Caatinga, Cerrado and Atlantic Forest. This plant has in its extracts the flavonoid substances as major compounds, which display many biological activities including antioxidant, antibacterial, anti-inflammatory and protection of free radicals produced by exposed to ultraviolet light[6,7]. **AIMS:** This study aimed to evaluate the photoprotective activity in the extract and fraction of substances isolated from *W. viscosissima*, a plant native to the northern and northeastern regions of Brazil **METHODS:** The technique used to verify the photoprotective effect of the test sample (extract and chloroform fraction of *W. viscosissima*) employed different concentrations of the natural product (10, 50, 100 and 500 µg/mL). Using spectra absorption spectrophotometry on the ultraviolet radiation spectrum as proposed by Mansur et al. (1986)[8], 290 to 320nm (at 5 nm intervals) scans lasting 5 minutes were performed, after which the absorbance measurements were performed and read using a digital spectrophotometer (Biospectro®) with 1cm quartz

cuvette. Subsequently, the data were submitted to the equation of Mansur et al. (1986)[8] to measure the sun protection factor (SPF) *in vitro*. This method lists the erythematogenic effect and radiation intensity (EE X I) that were measured by Sayre et al. (1979)[9] and determined by applying the following formula: Spectrophotometric SPF = HR. Σ EE (λ). I (λ). Abs (λ). **RESULTS AND DISCUSSION:** The analysis of the photoprotective activity of the extract and fraction isolated from *W. viscosissima*, performed on the UVB radiation spectrum, showed a photoprotective potential of 25.01 at the 1000 µg/mL, which is considered the highest photoprotection concentration for the tested spectrum range. The concentration of 500 µg/mL showed photoprotective potential, with SPF of 17.14 (extract) and 25.01 (fraction). The concentrations of 50 µg/mL and 100 µg/mL obtained results less than/equal to 6, an inactive/low value for photoprotection. In Resolution of Directors Collegiate - RDC No. 30 (June 1, 2012) of the National Health Surveillance Agency (ANVISA), the technical regulation on sunscreens establishes 6 protective factors as the minimum SPF[10] **CONCLUSION:** The concentrations highlighted in the SPF assay are possible candidates for use in phytocosmetics. Therefore, it can be considered that the phytochemical process of isolation of the *W. viscosissima* compounds point to a better display of UVB photoprotection activity.

Keywords: Phytotherapy, Ultraviolet Radiation, SPF, Chelating Effect

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EVALUATION OF THE ANTIFUNGAL ACTIVITY OF DRIED EXTRACTS FROM *Syzygium cumini* (L.)

Manuela Carine Cavalcante Erhardt[1]; Caio César de Andrade Rodrigues Silva[2]; Camila Luíz Gomes[2]; Emerson de Oliveira Silva[3], Victor de Albuquerque Wanderley Sales[3], Camila Gomes de Melo[3], Williana Tôrres Vilela[2], Pedro José Rolim Neto[4]

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ABSTRACT

INTRODUCTION: The antibiotic-resistant fungi and bacteria are currently a recognized problem, and thus, a new alternative for the treatment of resistant strains is of common interest. Research centers around the world have reported antimicrobial activities of plant secondary metabolites aiming the development of a new antimicrobial agent (1,2). *Syzygium cumini* (L.) Skeels belonging to the Myrtaceae family is popularly known as *jambolana, jamelão* ou *azeitona preta* in Brazil. *S cumini* is widely known to have many therapeutic properties. Among them, antimicrobial activity has been recently reported, mainly associated with its content of polyphenols and tannins as secondary metabolites (3,4). **AIMS:** To execute *in vitro* antifungal sensitivity tests using *Syzygium cumini* (L.) dried extracts. **METHODS:** The methodology used was in accordance with the protocol described in M27-A3 (5). The antifungal response was evaluated in water, absolute ethanol, ethanol:water (1:1, v/v) and methanol:water (2:10, v/v) extracts, obtained by extraction using a 200W microwave reactor, with extraction time of 2 minutes and maximum temperature of 30 °C. The alcoholic solvents were removed by rotary evaporation, and then the extracts were freeze-dried at negative pressure parameters (29 x 10^{-6} mmHg and - 60ºC for 100 hours). In the *in vitro* antifungal sensitivity test, two yeast strains were used as standard with sensitive profile: *Candida krusei* 6528 ATCC and *C. parapsilosis* ATCC 22019 enriched in sterile RPMI 1640 culture medium sterilized using 0,22 µm pore filter membranes (Millipore®). Aqueous extracts and ethanol:water (1:1, v/v) were diluted with water and the concentrations tested were 4092 µg/mL, 2046 µg/mL, 1023 µg/mL, 511.5 µg/mL, 256 µg/mL, 128 µg/mL, 64 µg/mL, 32 µg/mL, 16 µg/mL and 8 µg/mL. The ethanolic and methanol:water extracts (2:10, v/v) were tested at 64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.25 µg/mL and 0.125 µg/mL. The determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) tests were conducted in a 96-well microtiter plates. Tests were also performed on clinical isolates from blood cultures of patients in the intensive care unit (ICU) of the Hospital of Clinics of Pernambuco, with antifungal resistance against *Candida albicans, C. glabrata, C. guilliermondii, C. parapsilosis, C. orthopsilosis* and *C. tropicalis*. Isolates suspensions were prepared in saline, and the inoculum volume was adjusted to 5.0 mL for dilution in RPMI. **RESULTS AND DISCUSSION:** In order to direct the study and standardize the best extractive solution

obtained from *S. cumini* leaves, the antifungal activity of four extracts from different solvents was evaluated. Among the dried extracts from *S. cumini* only the hydroethanolic (1:1, v/v) solution showed antifungal activity, especially against *C. glabrata* and *C. orthopsilosis* isolates with MIC and MFC of 128 µg/mL. The MIC and MFC for the other isolated strains were: 512 µg/mL for *Candida albicans*, *C. guilliermondii* and *C. tropicalis*, and 1024 µg/mL for *C. parapsilosis*. These results are in accordance with those presented by Pereira and collaborators (2016), since they verified that the 70% *S. cumini* hydroethanolic extract obtained MIC of 125 µg/mL against *C. albicans* isolates. The authors reported that this action is possibly associated with the disruption of cell membrane permeability promoted by ergosterol complexation (6). The aqueous, ethanolic and methanolic extracts (20%) did not inhibit the growth of the isolated species. However, ethanolic and methanolic (20%) extracts, although not showing results in the inhibition of fungal growth, this action cannot be disregarded, since they were evaluated with very low concentrations when compared to hydroethanolic extract 50%, thus future studies with higher concentrations are required. These results from alcoholic extracts (20%) showed that the removal of organic solvents occurred effectively in the drying process, thus avoiding its interference with the antifungal properties of the extracts. **CONCLUSION:** Since toxicity and drug resistance has been increasingly reported for antifungal medicines, the detection of new bioactive substances with antifungal activity is extremely relevant. This work demonstrated the antifungal potential of *S. cumini* extract, which can be promising for a future development of pharmaceutical forms for the treatment of candidiasis.

Keywords: *Syzygium cumini* (L.); Medicinal plants; Therapeutic alternative; Antifungal medicines, Extraction process.

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STRUCTURE-ACTIVITY RELATIONSHIP OF INDOLE DERIVATIVES IN *Aedes aegypti* (Diptera: Culicidae) and *Artemia* sp. (Artemidae)

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ABSTRACT

INTRODUCTION: Considered as a major reemergence viral disease in the world, dengue, chikungunya and zika has as its the main vector the *Aedes aegypti* (L.) (Diptera: Culicidae)[1]. The interventions on this vector includes control measures, particularly the use of larvicides as a way to control it[2]. However, its excessive and indiscriminate use causes the insect to become resistant, and there is a constant need to exchange the larvicide[3]. An alternative to avoid problems caused by the use of these products is the search for new compounds with less environmental impact and better benefits to human health. Indole is a derivative of the amino acid tryptophan, belonging to the class of alkaloids and has a range of biological activities[4.] An alternative to avoid problems caused by the use of these products is the search for new compounds with less environmental impact and better benefits to human health. Indole is a derivative of the amino acid tryptophan, belonging to the class of alkaloids and has a range of biological activities[5]. Its chemical structure has a pyrrole heterocyclic ring, the C3 of this ring is susceptible to chemical reaction, making it possible to obtain its derivatives by Friedel-Crafts regioselective reaction. **AIMS:** In view of these facts, indole derivatives have been synthesized and evaluated against a target organism, *Ae. aegypti*, and to a non-target organism, *Artemia* sp., aiming to find potent and selective compounds against *Ae. aegypti*. **METHODS:** Thus, 12 indole analogues were synthesized as potential larvicidal agents against *Ae. aegypti* in its 3rd larval stage followed by the evaluation of the toxicity in nauplii of *Artemia* sp. The compounds were identified by thin layer chromatography, purified on a silica gel 60 chromatographic column and characterized by melting point, 13C and 1H NMR, mass spectrum and infrared. **RESULTADOS E DISCUSSÃO:** Branched aliphatic chain derivatives were more potent than the others, and the linear ones exhibited potency oscillation as the addition of the methylene chains. Toxicity tests indicated that (3-chlorophenyl)1-(1*H*-indol-3-yl)methanone (2l), with high larvicidal potency (LC_{50} = 50.59 ppm), showed the highest selectivity index, being less toxic to *Artemia* sp. **CONCLUSION:** In summary the 1-(1*H*-indol-3-yl)hexan-1-one (2e), exhibited the best larvicidal potency. However, this derivative showed low selectivity index and high toxicity to brine shrimp. The (3-chloropheny)1-(1*H*-indol-3- yl) methanone (2l), besides showing a reasonable larvicidal activity relative to indole, it also showed to be >19.77 times less toxic to *Artemia* sp. The high selectivity of this chlorinated derivative reveals the need for a more in-depth study of halogenated aromatic derivatives of indole in C3 with the aim of obtaining greater selectivity between the two species.

Keywords: Indole, Dengue, Chicungunya, Zika, Larvicidal activity.

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EVALUATION OF ANTIBACTERIAL AND MODIFYING ACTIVITY OF ANTIBIOTIC ACTION OF FRUCTANS OF *Agave tequilana* WEBER VAR. *blue* AGAINST MULTIRESISTENT *Escherichia coli* STRAINS

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ABSTRACT

INTRODUCTION: The use of medicinal plants is considered a therapeutic alternative for cases of antibacterial resistance, since the antibacterial agents currently used in clinical practice are not effective against these multiresistant microorganisms. The species *Escherichia coli* is considered a bacterium that has a resistance profile with high developmental indices and is characterized as a gram-negative bacterium responsible mainly for cases of gastrointestinal infections. Therefore, as *Agave* plants, mainly *Agave tequilana*, a species commonly used as a tequila product, are great sources of food ingredients caused by the presence of fruits. Fructans are considered as fructose polymers synthesized by sucrose and can be highlighted as the main carbohydrate found in some plant species, so they can use some pharmacological activity. **AIMS:** The aim of the present study was to evaluate the antibacterial activity and modifying the antibiotic action of fructans against *Escherichia coli* strains. **METHODS:** For the extraction of plant material, it was obtained from the stem collection of *Agave tequilana* plants present in Nayarit, Mexico and donated by a local company. The native fruit fraction (NAF) was obtained from the ground stem and suspended in water to obtain juice (18 to 22 ° Brix) and was subsequently filtered and spray dried. The high polymerization fraction (HPF) and intermediate polymerization fraction were obtained by tangential NF ultrafiltration at the Tepic Technological Institute, Nayarit, Mexico. To perform the tests, initially the products were diluted to a concentration of 1024µg/mL. The microorganism used before the test was passed to a Petri dish and incubated at ± 37°C for 24 hours. After this period, bacterial inocula were performed in triplicate from the transfer of the microorganism to tubes containing sterile saline to a concentration of 0.5 on the *Mc Farland* scale. To perform the Minimum Inhibitory Concentration (MIC), *eppendorfs* were filled with 100µL of bacterial inoculum and 900µL of 10% BHI, transferred 100µL of each *eppendorf* to 96-well sterile microplates. Subsequently, the products were microdiluted to the penultimate well of each column, incubated at ± 37°C for 24 hours. Antibiotic activity was modulated by filling the *eppendorfs* with 150µL of inoculum, volume related to the minimum subinhibitory concentration (MIC / 8), and from this, the volume of 10% BHI. *Eppendorfs* with 1350µL of medium and 150µL

used as modulation control were also prepared. Immediately afterwards, the microdilution plates were filled with 100µL of each *eppendorf* and then microdiluted 100µL of antibiotic and incubated at ± 37°C for 24 hours. To observe the results of both tests sodium resazurin is added after this time period. The antibiotics used were Gentamicin, Cephalotin and Benzylpenicillin. And the products tested were native fructans, high grade and intermediate polymerization fructans. **RESULTS AND DISCUSSION:** From the MIC, it was observed that the fructans did not obtain clinically relevant results, ≥1024µg/mL. However, when performing the modifying activity of antibiotic action, it was possible to observe that in relation to the NAF, an antagonism was observed when associated with Gentamicin, which, on the other hand, no significant results were observed when associated with the other antibiotics tested. Regarding HPF and IPF, synergism was observed when associated with gentamicin, and antagonism when associated with other antibiotics. Not many studies related to fructan antibacterial activity are described. Some studies show activity of inulin, a fructan present in some vegetables, against strains of *E. coli* and *Staphylococcus aureus*. **CONCLUSION:** Thus, it was observed that fructans with high and intermediate degree of polymerization present synergistic antibacterial activity when associated with gentamicin. On the other hand, they have antagonism when used together with cephalothin and Benzylpenicillin against the *E. coli* strain used. Thus, these fructans may be useful in treating infections caused by *Escherichia coli*. However, further studies are needed to help prove the antibacterial activity of fructans.

Keywords: Fructans. Antibacterial. *Escherichia coli*. *Aquila tequilana*.

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ANTI-*Candida* ACTIVITY OF *Portulaca elatior* ROOT LECTIN

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ABSTRACT

INTRODUCTION: The genus *Candida* has several species associated with the development of human infections, either superficial or invasive [1]. *Candida* species are cosmopolite, being widely distributed. They are known to compose the normal microbiota of animals and can be found in the vaginal mucosa, skin and mouth. [2]. The species of greatest medical importance are: *Candida albicans, Candida parapsilosis, Candida tropicalis, Candida glabrata, Candida krusei, Candida guilliermondii e Candida lusitaniae* [3] However, the treatment available for fungal diseases is the use antifungals, that can be responsible for causing various side effects in patients. In addition, the resistance developed by fungi to these drugs represents a major challenge [4]. Thus, it is important to seek alternative ways of controlling fungal diseases. Lectins are proteins that bind reversibly and selectively to carbohydrates. The genus *Portulaca* has about 120 species worldwide and, in Brazil, 13 species have been reported [5]. *Portulaca elatior* is a species found in Bahia, Pernambuco and Paraíba. From its root, it has already been isolated a lectin named PeRoL. **AIMS:** This work aimed to evaluate the activity of PeRoL against *C. tropicalis*, *C. albicans*, *C. krusei* and *C. parapsilosis* isolates. **METHODS:** *P. elatior* roots were ground and homogenized in saline solution (0.15 M NaCl) to obtain the crude extract. The extract was chromatographed on a chitin column, equilibrated with saline solution (0.15 M NaCl) and the lectin was eluted with 1.0 M acetic acid. The minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations were determined by the broth microdilution assay. **RESULTS AND DISCUSSION:** PeRoL presented MIC and MFC of 16 μg/mL respectively for all the isolates tested. The antifungal activity of lectins can their ability to bind to carbohydrates present on the surface of microorganisms, which may trigger different responses. **CONCLUSION:** The lectins of *P. elatior* root has antimicrobial activity against *Candida* spp.

Keywords: Lectin. Candida. Antimicrobial activity. Antifungal activity.

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DEVELOPMENT AND CHARACTERIZATION OF MULTI-COMPONENT SYSTEMS FOR IMPROVEMENT OF PRAZIQUANTEL SOLUBILITY

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ABSTRACT

INTRODUCTION: Schistosomiasis is a disease caused by the *Schistosoma* trematode, especially the *S. mansoni* species. The acute phase of schistosomiasis is usually asymptomatic but may result into extremely severe clinical forms and lead the patient to death. The parasites can evolve to severe or fatal clinical forms, turning schistosomiasis a key of great relevance as a public health problem (1). Praziquantel (PZQ) is the drug of choice for treatment, but it has high permeation and low solubility, thus hindering its oral administration (2). **AIMS:** In this context, the objective of this work was to obtain and characterize a multicomponent system containing praziquantel, layered double hydroxides (LDH) and pilivinylpyrrolidone (PVP) aiming to increase drug solubility to optimize therapy. **METHODS:** The PZQ: LDH: PVP multicomponent system was obtained by two methods: co-evaporation by oven drying and co-evaporation by spray- dryer. The obtained materials were characterized by the following methods: Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (DR-X) and thermal analysis. Solubility assays were also performed. **RESULTS AND DISCUSSION:** The infrared absorption spectrum showed expected results from the compounds. It was noticed a displacement of the drug characteristic curve in the thermal analysis, beyond the increase of the necessary temperature to reach its decomposition reaction, suggesting a thermal protection of praziquantel. In DR-X diffractograms, the systems presented amorphous characteristics, and the expected peaks from LDH in the multicomponent system had a reduction in their intensity, especially in the system obtained by spray-dryer, suggesting a possible interaction between the drug, LDH and PVP. In the solubility evaluated (24 hours), the multicomponent obtained by drying oven was equivalent to 0.34mg / mL, while the multicomponent obtained by spray-dryer was 0.21mg / mL. An increase in solubility in the multicomponent obtained by co-evaporation with subsequent drying in oven was observed, when compared to the drug alone (which corresponds to 0.22mg / mL). In contrast, the spray-dryer co-evaporation method, during 24 hours showed a smaller release of drug when compared to the drug alone, suggesting an encapsulation of compounds, leading to the prolonged release of praziquantel, which exceeded the 24 hours of study. **CONCLUSION:** The synthesis of the LDH was successfully obtained and succeed at increasing praziquantel

solubility. The PZQ: HDL: PVP obtaining as multicomponent systems was confirmed by the characterization techniques used when compared to the PZQ and LDH alone. Through the solubility assays, it was noted that the multicomponent obtained by co-evaporation with subsequent oven drying was able to generate an increase of solubility when compared to the drug alone. The spray drying co- evaporation method, during 24 hours, had a smaller release when compared to the isolated drug, suggesting an encapsulation of praziquantel and resulting in a prolonged release of the drug.

Keywords: Neglected diseases; Multicomponent; Praziquantel; Spray Dryer.

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EX SITU OPTIMIZATION FOR OBTAINING BENZNIDAZOLE: ZIF SYSTEM AS AN ALTERNATIVE TREATMENT OF CHAGAS DISEASE

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ABSTRACT

INTRODUCTION: *Trypanosoma cruzi* is the etiological agent of Chagas disease, a serious public health problem in developing countries. Benznidazole (BNZ), a broad spectrum 2-nitroimidazole derivative, presents antiparasitic activity and is the only anti- chagasic drug available in Brazil (1). However, BNZ has high toxicity and low water solubility, which leads in a reduced absorption in the gastrointestinal tract, and therefore, in a low bioavailability. To address these problems, increasing solubility and modulation of drug release, Metal Organic Framework (MOF) -based Drug Delivery System (DDS) has been developed, coordinating polymers that enable a controlled drug release kinetics. Zeolitic Imidazolate Framework-8 (ZIF-8), a subclass of MOF, has garnered considerable attention due to their large surface area, permanent porosity, high thermal stability, and the capability of presenting different topologies and the controlled transport of drugs (2). **AIMS:** The present work aims the development of method to obtain and optimize a drug delivery system based on benznidazole and ZIF-8, as an example of MOF. **METHODS:** The methodology was based on the co-precipitation method. The systems were produced at 1:1 molar ratio BNZ:ZIF-8 in two different solvents in order to optimize the maximum efficiency of drug incorporation in the Metal Organic Framework, varying the days of agitation. The molar ratio was based on the molecular weights of BNZ and ZIF-8, which are 260.25 and 229.61 $g.mol^{-1}$, respectively. The dosing method was previous developed by Soares-Sobrinho (2007), which used the absorption spectrophotometry in the Ultraviolet-Visible region to measure the drug incorporation efficiency (3). **RESULTS AND DISCUSSION:** It was observed in the incorporation curves that after four days of agitation, the incorporation efficiency of BNZ into ZIF-8 was 0 (zero) for the aqueous system. In contrast, the obtained system using acetone had a lower concentration point on day 4, resulting in approximately 38% of incorporation. **CONCLUSION:** Thus, a DDS with incorporation efficiency of 38% was obtained. This could be obtained through extensive optimization of an *ex situ* method, varying days of agitation and solubilization solvents. Thus, the 1:1 (mol/mol) BNZ: ZIF-8 system obtained in acetone was selected as the best DDS developed in this work.

Keywords: American Trypanosomiasis; BNZ; Drug Delivery System; Metal Organic Framework; Drug incorporation.

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ANTIBACTERIAL ACTIVITY AND POTENTIALIZATION OF THE ANTIBIOTIC ACTION OF FRUITS OF *Agave tequilana* AGAINST *Pseudomonas aeruginosa* STRAINS

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ABSTRACT

INTRODUCTION: Bacterial infections are caused for a long time or by many deaths in various sectors of society. Since Alexandrer Fleming's discovery of penicillin in 1928, the use of antibiotics to fight off diseases caused by these microorganisms has grown. Bacterial resistance is currently one of the most relevant public health problems globally. Noting this, the Ministry of Health made it mandatory for the Hospital Infection Control Commissions (ICC) to develop programs to rationalize the use of antimicrobials, aiming at a better quality of care and prevention of infections, according to the criteria established by the World Health Organization, in order to reduce the selection / induction process of multiresistant strains. Among the resistant strains, some stand out as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter baumannii*, which stand out in hospital environments. As an alternative to be tested is fructan which are fructose polymers synthesized from sucrose and occur as the main reserve carbohydrate of some plants and one of them we can highlight *Agave tequilana*. **AIMS:** Evaluate an antibacterial activity and check for a possible interaction of *Agave tequilana* stem fructan with antibiotics used in the clinic against *Pseudomonas aeruginosa* strains. **METHODS:** For extraction of plant material, it was possible after collecting the Agave tequilana plant capsule present in Nayarat, Mexico and donated by a local company. The native capture fraction (NAF) was applied after leaving the milled capsule and suspended in water to obtain juice (18 to 22 Brix) and was subsequently filtered and spray dried. The high polymerization fraction (HPF) fraction and the intermediate polymerization fraction were used by tangential NAF ultrafiltration. To perform the tests, the products were diluted to a concentration of 1024µg / mL. Regarding the microorganism used, before the test, it was passed to Petri dish and incubated at ± 37 ° C for 24 hours. After this period, bacterial inocula were performed in triplicate from the transfer of the microorganism to tubes containing sterile saline to a concentration of 0.5 on the Mc Farland scale. To perform the Minimum Inhibitory Concentration (MIC), eppendorfs were filled with 100µL of bacterial inoculum and 900µL of 10% BHI, transferred 100µL of each eppendorf to 96- well sterile microplates. Subsequently, the products were microdiluted to the penultimate well of each

column, incubated at ± 37 ° C for 24 hours. Antibiotic activity was modulated by filling the eppendorfs with 150 µL of inoculum, volume related to the minimum subinhibitory concentration (MIC / 8), and from this, the volume of 10% BHI. Eppendorfs with 1350µL of medium and 150µL used as modulation control were also prepared. Immediately afterwards, the microdilution plates were filled with 100µL of each eppendorf and then microdiluted 100µL of antibiotic and incubated at ± 37 ° C for 24 hours. To observe the results of both tests sodium resazurin is added after this period. The antibiotics used were Gentamicin, Cephalotin and Benzylpenicillin. And the products tested were native fructans, high grade and intermediate polymerization fructans. **RESULTS AND DISCUSSION:** MIC did not present microbial activity containing results of 1024 µg / mL. The literature is still scarce regarding studies with microbiological activities of fruits (TF) of Agave tequilana stems. TF has activity with certain bacterial lines such as Escherichia coli and Salmonella, however, there was no association of TF bacterial activity with P. aeruginosa strains. High polymerization TF shows the potentiating activity of the antibiotic Gentamycin, while native fructan and the intermediate fructan pollutant show antagonism when associated with Gentamycin. Fructan showed no activity associated with the antibiotic Benzylpenicillin. In tests performed in association with cephalothin, native and intermediate polymerization TF, showed potentiation activity, however, a high polymerization fructan showed antagonism when associated with cephalothin**. CONCLUSION:** Therefore, the results shown or fruity do not contain bacterial activity, however, it was possible to verify the potentiation activity against associated fructan-dependent *P. aeruginosa* strains. native and intermediate with cephalothin containing potentiation activity, that is, synergism and should be investigated to evaluate how chemical interactions that promote this interaction can thus contribute to future research.

Keywords: Fructans. Antibacterial. Potentiation. Antibiotic. *Aquila tequilana.*

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EVALUATION OF CYTOTOXIC ACTIVITY OF THE EXTRACTS OF SOME MELASTOMATACEAE SPECIES AGAINST CANCER CELL LINES AND MURINE MACROPHAGES

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ABSTRACT

INTRODUCTION: Cancer is the second major cause of death in the world and its treatment still requiring the use of therapeutic modalities, like radiotherapy and chemotherapy, although majority of them is effective in terms of removal and attack of cancer cells, especially early in the disease. However, those treatments are not very selective (1). Consequently, the study of new selective target compounds with therapeutic potential against cancer has been intensified, specially using natural compounds. The cytotoxic potential of diferents Brazilian medicinal plants has been investigated in many studies; however, due to the country's great biodiversity, the therapeutic potentialities of most species remain unknown (2). Melastomataceae leaves crude extract have been reported to exhibit various pharmacological activities, such as antioxidant, anti- inflammatory, antimicrobial, anticancer, antiulcer, and wound healing. However, for having a high species richness, not all have been studied (3). **AIM:** The aim of this study was to analyze the cytotoxic activity of diferents species of Melastomataceae against cancer cells and murine macrophages. **METHODS:** The crude leaves extracts of species: *Miconia amacurensis, Miconia ciliata, Miconia holosericea, Miconia hypoleuca, Miconia minutiflora* were prepared using hexane, ethyl acetate and methanol as solvent. The evaluation of the cytotoxic effect was measured by the MTT (3-[4,5-dimethylthiazol- 2-yl] -2,5-diphenyltetrazolium) reduction assay. The MTT is a soluble yellow tetrazolium salt that when is captured by an endocytosis process, was converted by metabolically active cells through the activity of dehydrogenase enzymes to an insoluble blue formazan compound that will be dissolved and quantified spectrophotometrically at 560 nm. The absorbance value of formazan is relatively proportional to the number of viable cells. To this study, was evaluated the extracts inhibition percentage on cancer cells: NCI- H292 (lung mucoepidermoid carcinoma), HL-60 (acute promyelocytic leukemia), MCF-7 (breast carcinoma), HEp-2 (human larynx carcinoma); and murine macrophages cells line J774.A1 and RAW 264.1. Cells were treated with extracts at concentration of 50 µg/mL for 72 h. **RESULTS AND**

DISCUSSION: Three types of extracts with different polarities (hexane, ethyl acetate and methanol) were performed for the five species of the Melastomataceae family collected. The results showed that for the hexanic extracts, the specie *M. holosericea* presented a higher inhibition percentage for the HL-60 (95.43%), MCF-7 (76.17%) and HEP -2 (91.67 %) and the specie *M. hypoleuca*, for the HL-60 (99.54%) and NCI-H292 (75.67%). The other species presented high inhibition percentage (above 70%) for HEp-2 cell. All Ethyl acetate extracts showed a high inhibition percentage for cell lines HL-60 and HEp-2, with results ranging from 73.3- 100% inhibition of cell viability. Methanolic extracts showed no inhibition percentage above 70% for cancer cells, except for the species *M. holosericea*, which presented 100% inhibition for HL-60 cells and 82.39% for HEp-2 cells line. The results of murine macrophage cell lines showed a lower inhibition of the cell viability, less than 70% in the hexane and Methanolic extracts. These results showed selectivity of extracts to cancer cells. **CONCLUSION:** The date showed that the species of the Melastomataceae family studied are strong candidates for the discovery of new molecules with anticancer action, especially the species *M. holosericea* and *M. hypoleuca.* This species were able to inhibit the growth of leucemia and human larynx carcinoma by both hexane and ethyl acetate extracts.

Keywords: Natural products; MTT assay; Cancerology; *M. holosericea*; *M. hypoleuca*

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EVALUATION OF THE ANTITUMORAL ACTIVITY OF NOVEL MESOIONIC DERIVATIVE, MC-1

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ABSTRACT

INTRODUCTION: Cancer is the definition of a set of diseases in which cells of an organism divide abnormally and uncontrollably and can invade adjacent tissues. Therefore, cancer can be considered a syndrome since it has a total of 200 diseases that share some common characteristics but differ greatly in relation to their genetic and histopathological origin, prognosis, aggressivity and treatment. In 2018, the International Agency for Research on Cancer (IARC) estimated 18.1 million new cancer cases worldwide and 9.6 million related deaths. According to the survey, one in five men and one in six women develop cancer during their lifetime, and one in eight men and one in 11 women die of the disease. The increasing incidence of cancer in the world population is due to population growth, aging as well as the level of socio- economic development (1). Cancer has more severe consequences especially in low-to- middle development countries, and demographic and epidemiological transitions indicate a growing impact of the cancer burden in the coming decades (2). The treatment of cancer varies depending on the etiology of the disease as well as its stage. More than one approach can be used by generating a combination of treatments. Among the available approaches, we can highlight chemotherapy, which consists in the use of chemotherapy agents alone or in combination with the aim of treating malignant tumors. The purpose of chemotherapy is to prevent the multiplication of cancer cells, prevent metastasis and eliminate existing tumors. Drugs belonging to the chemotherapeutic class have different mechanisms of action and may act on the cell cycle in a specific way (microtubule formation inhibitors, glucocorticoids, folate pathway inhibitors, antimetabolites, and antitumor antibiotics) or nonspecific (alkylating agents and platinum complexes). However, there are several chemotherapeutic drugs and some cancers/patients resistance that justify an effort to study the anticancer activity of synthetic as well as natural products. In addition, many patients suffer from side effects due to the similarity between tumor cells and healthy cells. Thus, antineoplasic drugs become limited by acting in a systemic and nonspecific manner, affecting mainly cells that are in constant division such as capillary endothelial cells, leukocytes and gastrointestinal cells. As consequence, different adverse effects such as nausea and vomiting, alopecia, weakness and malaise, immunosuppression, fever and the development of depression are observed (3). The mesoionic compounds are synthetic substances classified as heterocyclic betaines with five-membered flat compounds with at least one side chain, alpha atom in the same plane of the ring and having dipole moment (5). These compounds are not aromatic despite being stabilized by the displacement of charges and electrons (6). These molecules have several biological activities, which can be justified

by their structural configuration: the relatively small size associated with variations in positive and negative electron densities gives them amphiphilic character (7), thus allowing interactions with biomolecules such as proteins and DNA (8). In previous studies, several mesoionic derivatives have demonstrated relevant biological activities such as antibacterial, leishmanicidal, antiparasitic and antitumor activity (7;8;9). Thus, because of the wide range of possibilities for therapeutic applications of mesoionic compounds and their derivatives, studies on their effectiveness and mechanisms of action against different non-clinical models are needed. **AIMS:** The aim of this study was to evaluate the antitumor activity of the novel mesoionic derivate compound 4-phenyl- 5- (4- trifluromethylphenyl) -1,3,4-thiadiazolium-2-phenylamine (MC-1) on Ehrlich Ascitic Tumor (TAE) murine model. **METHODS**: Swiss mice (n = 6) were intraperitoneally (ip) injected with $1.0x10^6$ / tumor cells / animal and one hour late the animals were treated with the MC-1 (6,25, 12,5 and 25 mg/kg intraperitoneally) over a period of nine days. During this period the weight of the animals was measured daily, and weight variation was determined by comparing the daily weight averages of each group with their weight averages prior to initiation of treatment. The evaluation of antitumor activity of compound MC-1 was determined from parameters such as waist circumference, tumor volume and animal weight, which are directly linked to tumor progression. Potential cytotoxic effect was analyzed by cell viability of ascites fluid using trypan blue stain. Histopathological parameters of the vital organs as liver, kidney, and spleen were also evaluated. The data were analyzed by the nonparametric analysis of variance (ANOVA) method with Tukey post-test for multiple comparisons. Graph Pad Prism version 6.0 software was used and values with $p < 0.05$ were considered significant (Graph Pad Software Inc., San Diego, U.S.A.). **RESULTS AND DISCUSSION:** As a result of our study, it was observed that the treatment with the mesoionic compound MC-1 (6,25mg/kg) had significant effect against the analyzed parameters ($p < 0.05$). The substance was able to inhibit weight gain, a typical feature of tumor development, reduce the tumor volume, waist circumference and the amount of cells in ascites fluid, indicating a potent cytotoxic effect of the MC-1 over the tumor cells. Besides, the MC-1 treatment promoted lymphocyte and neutrophil migration to the cell tumor injected site independently of macrophage migration. The up-regulation of inflammatory cell migration to the tumor site may be due to the inflammatory process developed by tumor cell death; however, more studies must be done to clarify the possible immunomodulatory property of the MC-1. Histological analysis revealed that MC-1 treatment was able to reverse histopathological changes caused by tumor growth in the liver and spleen and did not cause any damage to the kidneys. **CONCLUSION:** These preliminary results allowed us to infer that the MC-1 has a significant antitumor effect on Ehrlich Ascitic Tumor murine model, mainly by decreasing the Ehrlich cells viability into the injected site, opening new possibilities of studies to understand its mechanisms of action as an immunomodulatory and antineoplastic molecule.

Keywords: Mesoionic compounds; antitumor activity; cancer; Ehrlich Ascites Tumor; synthetic compounds.

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ANTIFUNGAL ACTIVITY OF *Moringa oleifera* SEED EXTRACT

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ABSTRACT

INTRODUCTION: *Candida* yeasts are commensal members of human microbiota. However, some of these fungal species may become opportunistic pathogens due to predisposing factors, such as immune system impairment. *Moringa oleifera* is a protein- rich medicinal plant widely grown around the world. Proteins isolated from *M. oleifera* seeds, including lectins (carbohydrate-binding proteins), showed antimicrobial activity (1). **AIMS:** To determine the antifungal activity of a seed extract of *M. oleifera* against *Candida* isolates. **METHODS:** Seed flour (10 g) was homogenized (4 h, 27 °C) with 0.1 M citrate phosphate pH 3.0 containing 0.15 M NaCl (100 mL) using a magnetic stirrer. Then, the mixture was filtered, centrifuged (9,000 *g*; 15 min; 4 ºC) and the collected supernatant corresponded to the extract. Protein concentration was determined according to Lowry et al. (2). The presence of lectins was evaluated by the hemagglutinating activity (HA) assay using rabbit erythrocytes (2.5% v/v) according to Paiva and Coelho (3). Specific HA was calculated by the ratio between HA and protein concentration. HA inhibition assay was performed by incubating the sample with carbohydrates before HA determination. The presence of trypsin inhibitory activity and proteolytic activity were also evaluated according to Pontual et al. (4) and Azeez et al. (5), respectively. Antifungal activity was evaluated against *Candida albicans* and *Candida krusei* isolates by the broth microdilution assay. The minimum inhibitory (MIC) and fungicidal (MFC) concentrations were determined. **RESULTS AND DISCUSSION:** The extract showed a protein concentration of 14.87 mg/mL and presented a specific HA of 8.61. The specific HA was reduced to 2.15 in the presence of mannose and N-acetyl D-glucosamine, confirming that the agglutination was due to lectin presence. The extract also contained trypsin inhibitor (10.8 U/mg) and protease (14.6 U) activities. The extract showed fungistatic effect on *C. albicans* (MIC = 1.86 mg/mL) and *C. krusei* (MIC = 7.43 mg/mL) and was also fungicide to both isolates (with MFC of 7.43 mg/mL and 14.87 mg/mL, respectively). **CONCLUSION:** The antifungal activity of a protein extract of *M. oleifera* seeds was demonstrated. Future studies should be performed aiming to determine whether this activity is linked to the presence of lectin, trypsin inhibitor and/or protease.

Keywords: *Candida*. Trypsin inhibitor. Lectin. Protease.

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ANTIBACTERIAL ACTIVITY OF *Moringa oleifera* SEED EXTRACTS

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ABSTRACT

INTRODUCTION: *Moringa oleifera* is a native species to India, Pakistan and Afghanistan that is able to adapt to various environmental conditions. *M. oleifera* seeds are used for water treatment because they contain natural coagulants and antibacterial compounds (1). An extract from moringa seeds prepared in citrate phosphate buffer (BE) showed the presence of lectins (specific hemagglutinating activity of 8.61), trypsin inhibitor (10.82 U/mg) and protease (14.6 U) activities. Also, seed extract in water (WE) contain lectins and antibacterial molecules (1). **AIMS**: This work investigated BE and WE for antibacterial activity against human pathogenic species. **METHODS**: The extracts were obtained by homogenizing (4 h, 27 ºC) the seed flour (10 g) in distilled water (WE) or 0.1. M citrate-phosphate buffer pH 3.0 (BE). The extract were obtained after filtration and centrifugation (9,000 *g*; 15 min; 4 ºC). Protein concentration was determined according to Lowry et al. (2). The presence of lectins was evaluated by the hemagglutinating activity (HA) assay using rabbit erythrocytes (2.5% v/v) according to Paiva and Coelho (3). Specific HA was calculated by the ratio between HA and protein concentration. HA inhibition assay was performed by incubating the sample with carbohydrates before HA determination. The presence of trypsin inhibitory activity and proteolytic activity were also evaluated according to Pontual et al. (4) and Azeez et al. (5), respectively. The antibacterial activity was evaluated against *Escherichia coli*, *Salmonella enterica* serovar. Enteritidis and *Staphylococcus saprophyticus* through the broth microdilution assay and determination of the minimum inhibitory (MIC) and bactericidal (MBC) concentrations. **RESULTS AND DISCUSSION**: WE showed protein concentration of 13.22 mg/mL and specific HA of 9.68, which was reduced to 2.42 in the presence of mannose, N-acetyl D-glucosamine and methyl D- mannopyranoside, confirming the presence of lectins. WE also showed trypsin inhibitor (11.6 U/mg) and protease (11.45 U) activities. WE was bacteriostatic for *S. enterica* (MIC = 1.65 mg/mL), *E. coli* and *S. saprophyticus* (MIC of 6.61 mg/mL for both). This extract was bactericidal for *S. saprophyticus* (MBC of 13.22 mg/mL) and the other isolates (MBC of 6.61 mg/mL). BE was bacteriostatic for *S. enterica* (MIC = 1.86 mg/mL), *E. coli* (MIC = 3.72 mg/mL) and *S. saprophyticus* (MIC = 7.43 mg/mL) and bactericidal (MBC = 7.43 mg/mL) for all isolates. **CONCLUSION**: WE, although containing lectin, trypsin inhibitor and protease in similar levels than BE, showed lower bacteriostatic efficiency against *E. coli* and bactericidal effect against *S. saprophyticus*.

Keywords: Trypsin inhibitor. Lectin. Protease.

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ANTIBACTERIAL ACTIVITY OF *Arrabidaia chica* LEAF EXTRACT AGAINST ISOLATES OF *Salmonella enteritidis* AND *Escherichia coli*

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ABSTRACT

INTRODUCTION: *Arrabidaea chica*, also known as "crajiru", is a tree plant belonging to the Bignoniaceae family, widely used in folk medicine to treat infections and inflammation. *A. chica* leaf extract, with high content of phenolic compounds, showed low acute and subacute toxicity in oral tests and absence of cytotoxicity in hamster ovarian cells (1). However, there are no reports on the identification of bioactive proteins in this species. **AIMS:** This work aimed to evaluate the presence of bioactive proteins (lectins, trypsin inhibitors and proteases) and antibacterial activity in an *A. chica* leaf extract against *Salmonella enteritidis* and *Escherichia coli*. **METHODS:** Leaf flour (7.5 g) was homogenized (4 h, 27 °C) with 0.1 M citrate phosphate buffer pH 3.0 containing 0.15 M NaCl (100 mL) using a magnetic stirrer. Then, the mixture was filtered, centrifuged (9,000 *g*; 15 min; 4 °C) and the collected supernatant corresponded to the extract. Protein concentration was determined according to Lowry et al. (2). The presence of lectins was evaluated by the hemagglutinating activity (HA) assay using rabbit erythrocytes (2.5% v/v) according to Paiva and Coelho (3). Specific HA was calculated by the ratio between HA and protein concentration. HA inhibition assay was performed by incubating the sample with carbohydrates before HA determination. The presence of trypsin inhibitory activity and proteolytic activity were also evaluated according to Pontual et al. (4) and Azeez et al. (5), respectively. Antibacterial activity was evaluated against *S. enteritidis* and *E. coli* isolates by the broth microdilution assay. The minimum inhibitory (MIC) and bactericidal (MBC) concentrations were determined. **RESULTS AND DISCUSSION:** The extract showed protein concentration of 7.22 mg/mL and specific HA of 2.21, which was reduced to 1.1 in the presence of fructose, methyl D-glycopyranoside and methyl D-mannopyranoside, confirming the presence of lectins. The extract also contained trypsin inhibitor (23.97 U/mg) and proteolytic activity (135.7 U). The extract was bacteriostatic for the both isolated (MIC = 7.22 mg/mL) but bactericidal activity was not detected at the tested concentrations. **CONCLUSION**: The *A. chica* leaves contain lectin, trypsin inhibitor and protease activities as well as molecules with antibacterial effect.

Keywords: Trypsin inhibitor. Lectin. Bacteriostatic.

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EVALUATION OF CELL DEATH INDUCTION PATHWAYS OF 1,4-NAPHTOQUINONE IN HL-60 CELL LINES

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ABSTRACT

INTRODUCTION: In the medical chemistry, the quinones are an important group of organic compounds due yours several benefits to the human health, like activities as antibacterial, antifungal, antiviral, and the most important, antitumoral. The class of quinones can be found widely in nature, being these compounds named from to the compounds they were originated, among them the naphthoquinones, derivate from naphthalene are the most important subgroup of quinones, due your biological activity anticancer (1). A example of naphthoquinone with a strong potential antitumoral it's the 1,4 – naphthoquinones, a molecule whose activity it's very described on literature. The 1,4-naphthoquinone contributes to the control of the amount of carcinogenic cells between the induction of the apoptosis cascade and between the elevation of production of reactive oxygen species, inducing the oxidative stress, leading the cell to apoptosis (2). Leukemias, carcinomas that affect blood tissue, are tumors that reach a large portion of the world population. Exist several types of leukemias, in which they are characterized by the type of affected cell and in the course of the disease (3) Acute promyelocytic leukemia (LPA), is a type of neoplastic disorder, characterized by intense proliferation and accumulation of myeloblasts in the bone marrow, resulting in various damage to patients, especially for adults and the elderly (4). Cytotoxicity tests contribute to the understanding of the mechanism of action of drugs that are under development, where it is possible to ascertain the possibility of unwanted cellular effects as well as the degree of effectiveness of a specific drug, in the most various types of cells, such as cancer cells. **AIMS:** The purpose of the present study was to evaluate the capacity to induce cellular death and the possible pathways involved in 1,4- naphthoquinone in hl-60 cells. **METHODS:** Cells were maintained in RPMI 1640, cells were seeded in 96-well plates (100mL of $3x10^6$ cells/mL for HL-60 and 100mL 1,4 naphthoquinone (MNT) (0.39–25mg/mL) dissolved in DMSO:Medium (1:99 v/v; 100mL) was added to each well and incubated for 72 h or 24 h. DMSO 1% was used as negative control. After 69 h or 21 h of treatment, 25mL of MTT (5 mg/mL) was added to each well and 3 h later, the MTT-formazan product was dissolved in 100mL of DMSO and the absorbance was measured at 595 nm in spectrophotometer. For the cell viability test the cells were incubated with

propidium iodide (10μg/mL). In the analysis of cell cycle and DNA content, triton (0.1%)/propide iodide (10μg/mL) was used, and for mitochondrial depolarization test a 123 rodamine solution (1μg/mL) was used. All analyses were done on the GuavaEasyCyteHT system (Merck-Millipore) equipment using Guava Soft™ version 2.7 software. Five thousand events were evaluated by experiments carried out in triplicate and two independent experiments. For cytotoxicity assays, the IC50 values and their 95% confidence intervals were obtained by nonlinear regression with the GraphPad Prism Program (San Diego, CA, USA). Mean differences were compared by one-way ANOVA followed by Tukey's test or Dunnett's test ($p < 0.05$). **RESULTS AND DISCUSSION:** HL-60 cell treated with MNT had cytotoxic activity against all tumour cell line tested (0.9 and 1.0 μg/mL) in 72 and 24 h Respectively. The concentrations of 1.0 and 2.0 μg/mL were chosen for tumor mechanism tests. MNT induced a reduction in viability by almost 100%, demonstrating only 6% and 3.5% of viable cells at concentrations of 1.0 and 2.0 μg/mL, respectively after 24 h. In the mitochondrial depolarization test, at the concentration of 1.0 μg/mL, NTM did not present mitochondrial depolarization (only 9% depolarized cells), compared with negative control (16%), while at the concentration of 2.0 μg/mL depolarization mitochondrial occurred in 44% of cells. In the DNA Fragmentation test to MNT at both tested concentrations caused fragmentation around 10% and 60% of cells. This shows that somehow MNT induced cell death (1.0 μg/mL) is not so related to mitochondria that is related to the intrinsic pathway of apoptosis, thus being related to other pathways such as the extrinsic pathway of apoptosis, or other pathways of (necrosis and autophagy). Recent studies have shown that a novel 1,4-naphthoquinone derivatives induce apoptosis via ROS-mediated p38/MAPK, Akt and STAT3 signaling in human hepatoma Hep3B cells. The authors related cell death induction with mitochondrial involvement in ROS production, which corroborates the results found in our tests (4). Yue Wang and collaborators, demonstrated in their work that two new derivatives of 1.4 naphthoquinone present potent antitumor action for inducing apoptosis in lung cancer cells, through increased oxidative stress, through hyperproduction of reactive species oxygen. The data in this study also corroborate the results of the present study (5). **CONCLUSION:** Based on the results obtained in the present study, we can conclude that 1.4- naphthoquinone has a potential inductor of apoptosis and can act on more than one route to accomplish this task.

Keywords: Apoptosis. Leukemia. Naphthoquinones. Cell death. Mechanism of action.

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EVALUATION OF ANTICANCER ACTIVITY OF EXTRACTS PRODUCED BY *Streptomyces* sp.

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ABSTRACT

INTRODUCTION: Cancer is considered a major public health problem in developed and developing countries and is the second leading cause of death in Brazil and worldwide. In spite of the diversity of technologies and research applied on this field, there is still no effective therapy for the treatment of all types of cancer and the currently used chemotherapeutic agents present high toxicity profile (1). It's is known that more than 60% of pharmaceutical approved drugs are derived from natural compounds, with 50% of this rate obtained from actinobacteria, making them a biologically important group in the discovery of new drugs. Actinobacteria are important microorganisms that produce bioactive compounds, which stand out for having different biological activities, such as antifungal and antitumor (2). **AIMS**: The work aimed to evaluate the anticancer potential of acetonic, ethanolic and methanolic extracts produced from *Streptomyces* UFPEDA 3407 (20G). **METHODS**: The bacterial strain *Streptomyces* sp. UFPEDA 3407 (20G), isolated in the region of Maué, State of Amazonas-Brazil, was provided by UFPEDA (UFPEDA Microorganism Collection of the Department of Antibiotics/ UFPE). For the preparation of the extracts, the biomass was obtained from fermentation in submerged culture of the microorganism. After this, the biomass extracts were obtained by extraction with water miscible solvents (ethanol, methanol and acetone) (3). The evaluation of cytotoxic activity of extracts against human cancer cells was performed by the 3- (4,5-dimethyl-2-thiazole) -2,5-diphenyl-2-H-tetrazolium bromide (MTT) test. This assay is based on the conversion of MTT to formazan by the action of the succinyl dehydrogenase enzyme present in the mitochondria of viable cells (4). Cell suspensions (10^5 cells/mL) of the cell lines NCI-H292 (lung cancer), HEp-2 (laryngeal cancer), HL- 60 (leukemia), HT-29 (colon cancer) and MCF-7 (breast cancer) were used. The extracts were initially screened against cell lines, distributed in 96 well plates and incubated with the extracts at a concentration of 50 μg / mL for 72h. After this time, cells were incubated again

with MTT (5 mg / mL) for 3 h, formazan crystals were dissolved in DMSO and absorbance was read at 540 nm using a microplate reader. The result was given in percent inhibition of cell growth (IC%). After initial screening, extracts that showed moderate activity (> 70% inhibition) in two cell lines were submitted to determination of the concentration that inhibits 50% of growth comparated to the negative control (IC_{50}) at concentrations 0.39 - 50 µg. / mL. Doxorubicin was used as standard for both tests. **RESULTS AND DISCUSSION**: On initial screening, acetone biomass extract (BM- Acet) showed high cytotoxic activity, with inhibition of cell growth ranging from 75 to 95% in the tested cell lines. Methanolic extract (BM-MetOH) demonstrated moderate cytotoxic activity, and its cell growth inhibition rate ranged from 68 to 71%. However, it was the ethanolic extract (BM-EtOH) that presented the highest inhibition values, reaching 100% for MCF-7, HL-60 and Hep-2 tumor lines. In the subsequent test, all extracts demonstrated IC_{50} <30 µg/mL, showing that these are promising extracts after purification and isolation of substances responsible for the anticancer effect. The MTT assay is a rapid and sensitive test used to determine possible detrimental effects on mitochondria and cellular metabolism. It is an advantageous method as it minimizes the need for experimental animals as well as provides data to determine specific cell or organ toxicity. **CONCLUSION**: The extracts obtained from *Streptomyces* sp. showed cytotoxic activity against human tumor lines HEp-2, HT-29, NCI-H292 and HL-60, with IC_{50} values less than or equal to doxorubicin. Thus, the extracts are promising alternatives in the search for new antineoplastic agents.

Keywords: Actinobacteria. Extracts. Cytotoxicity. Cancer.

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EVALUATION OF THE CYTOTOXICITY OF LECTIN FROM *Microgramma vacciniifolia* FRONDS (MvFL) TO HUMAN MESENCHYMAL STEM CELLS AND LEUKEMIA CELL LINES (K562 AND JUKART)

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ABSTRACT

INTRODUCTION: Stem cells have the capacity for self-renewal and differentiation in different tissue lines. Stem cell-based therapies that take advantage of these capabilities to treat diseases are receiving increasing attention. Among the various cell types, mesenchymal stem cells (MSCs) benefit from fewer ethical issues and numerous MSC- based therapies for tissue repair and immune disorders have been tested. Natural compounds have been studied as agents for induction of the differentiation of these cells. Also, many studies have been performed to investigate plant-derived compounds, such as lectins, with the ability to impair tumor formation or control cancer progression (1). Lectins are proteins capable of binding to carbohydrates in a specific and reversible manner, being able to interact with glycoconjugates present at cell surfaces (2). Lectins can induce different kinds of cell responses, including apoptosis, necrosis, cell growth inhibition and proliferation. Some lectins have higher affinity for carbohydrates found in cancer cells than for those present at the membranes of normal cells (3).The *Microgramma vacciniifolia* frond contains a lectin (MvFL) with immunomodulatory activity on human immune cells (peripheral blood mononuclear cells – PBMCs). MvFL was not cytotoxic to lymphocytes present among PBMCs and, induced the release of TNF-α, IFN-ɤ, IL-6, IL-10, and nitric oxide by them. MvFL also stimulated T lymphocytes from PBMCs to differentiate into CD8+ cells. The activation (indicated by CD28 expression) of these cells was also stimulated (4). Leukemia is a name for a cancer that affects white blood cells (leukocytes), compromising the body's defense system. Lectins have been reported to be cytotoxic to leukemia tumor lines, even when did not affect PBMCs viability (5). **AIMS:** To evaluate the cytotoxicity of MvFL to human mesenchymal stem cells (MSCs) and leukemia cell lines (K562 - chronic myelogenous leukemia; and JURKAT - leukemic T-cell lymphoblast). **METHODS:** The MSCs were obtained from umbilical cord collected after cesarean delivery and were processed up to 3 h after collection. The umbilical cord was cut into pieces and distributed into culture bottles containing complete DMEM medium. Over a period of 21 days (37 ºC, 5% CO_2, 80% moisture), the MSCs spontaneously

migrate to the surface of the culture bottle. Cytotoxicity assay was evaluated by MTT assay using MSCs (1×10^4/mL) treated for 24 h with different concentrations of MvFL (0.1–100 µg/mL) in 96-well microplates. The percentage of viable cells was calculated in comparison with the negative control. The viability of MSCs treated or not with the lectin at 50 µg/mL was also evaluated by flow cytometry using annexin V (AnnV) and propidium iodide (PI) for the detection of apoptosis and/or necrosis. The results were analyzed using the BD Accuri C6 Software. AnnV–/PI+ cells were considered necrotic and AnnV+/PI– cells were considered to be in the early stages of apoptosis. Double staining was recorded as necrosis or late apoptosis and double negatives were considered viable cells. Cytotoxicity of MvFL (1.56–200 µg/mL) to human leukemia cell lines was also evaluated by MTT assay for 24 h using RPMI 1640 medium with HEPES supplemented with 10% (w/v) fetal bovine serum in 96-well microplates. **RESULTS AND DISCUSSION:** MvFL was slightly cytotoxic to MSCs at the highest concentration (100 µg/mL), reducing viability to 80%, but did not present cytotoxicity to the MSCs at the other tested concentrations (0.1–50 µg/mL), in comparison with control. MvFL was not able to induce significantly apoptosis or necrosis, being the number of viable cells (98%) similar to that in control. Moreover, MvFL did not present cytotoxicity to the tumor cell line K562 at any tested concentration (1,56–200 µg/mL) in comparison with control. However, MvFL at the concentration of 100 µg/mL was cytotoxic to JURKAT (25% of mortality), but did not present cytotoxicity at the other tested concentrations. **CONCLUSION**: MvFL was slightly cytotoxic to MSCs obtained from umbilical cord but did not induce apoptosis or necrosis in these cells. This result stimulates detailed studies on the ability of this lectin in induce differentiation of stem cells at non-cytotoxic concentrations. MvFL did not show remarkable cytotoxic activity for human leukemia cell lines.

Keywords: Lectin. Umbilical cord cells. Leukemia. Anticancer. Cytotoxicity.

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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *Portulaca elatior* ROOT LECTIN

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ABSTRACT

INTRODUCTION: Infectious diseases caused by bacteria are responsible for high morbidity and mortality rates and the most effective way to treat these diseases is still the use of antibiotics. However, the overuse of these drugs has favored the emergence of resistant strains; in addition, most of them have several side effects [1]. Therefore, the search for new effective molecules in alternative control of microbial infections is needed. The use of medicinal plants has spread mainly in underdeveloped countries where health services are not accessible [2] Plants contain several bioactive compounds, such as lectins. Lectins are proteins that binds selectively and reversibly to carbohydrates. They can bind to glycoconjugates present on the surface of pathogen cells and trigger some cellular changes, such as pore formation and cell leakage [3]. PeRoL is a lectin present in the roots of *Portulaca elatior* [4]. **AIMS:** In this work, the antibacterial activity of PeRoL against isolates of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis* was evaluated. **METHODS:** *P. elatior* roots were ground and homogenized in saline solution (0.15 M NaCl) to obtain the crude extract. The extract was chromatographed onto a chitin column, equilibrated with 0.15 M NaCl and the lectin was eluted with 1.0 M acetic acid. The minimum inhibitory (MIC) and minimum bactericidal (CMB) concentrations were determined. **RESULTS AND DISCUSSION:** PeRoL showed bacteriostatic activity against all bacterial isolates tested, with MIC of 32.5, 4.06 and 8.12 µg/mL for *S. aureus*, *P. aeruginosa* and *E. faecalis* respectively. **CONCLUSION:** PeRoL is a lectin with potential to be evaluated as an alternative antibiotic for combating bacterial infections.

Key words: *Portulaca elatior*. Lectin. Pathogenic bacteria

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INCREASED CONCENTRATION OF TOTAL PHENOLICS BY INDUCING WATER DEFICIT IN PEPPER PLANTS

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ABSTRACT

INTRODUCTION: *Schinus terebinthifolius* is a small tree of the family Anacardiaceae, popularly called aroeira that has great therapeutic potential. The mastic contains several antiseptic, anti-inflammatory and bactericidal medicinal properties, which combat infections of the respiratory, urinary tract and feminine reproductive system. This medicinal action of aroeira is due to the phenolic compounds that have been exploited for bioremediation applications. These metabolites that are produced in the secondary metabolism of the plant can be increased by inducing water deficit. The effect of water deficit on the concentration of secondary compounds will depend on the intensity, duration and period of stress imposed on plants, as well as the characteristics of the species in producing phytochemicals as drought tolerance mechanisms. **AIMS:** The aim of this study was to determine the increase of total phenol concentration in different organs of young plants of *Schinus terebinthifolius* under water deficit. **METHODS:** Phytochemical analysis of total phenols was performed at the Chemical Laboratory of the Federal Rural University of Pernambuco, Recife-PE, Brazil. The experimental design was completely randomized by 4 water regimes (100% field capacity (FC), 75% FC, 50% FC and 25% FC) and 4 replications for phenol extraction. 0.500g of dried and ground samples of leaves, stems and roots of each plant were weighed and transferred to erlenmayer, in which 40 mL of 80% alcohol was added and stirred for 30 minutes. Then the extracts were filtered and transferred to tubes, supplemented with distilled water, homogenized and brought to the refrigerator for color development. For the analysis of total phenols, 0.4mL of extract was placed in tubes, adding 10mL of distilled water, 2.0mL of sodium carbonate and 1mL of Folin- Denis. The tubes were shaken and allowed to stand for 30 minutes to read at 760nm absorbance. Based on the standard tannic acid curve the results were calculated and expressed in mg.g. Data were subjected to analysis of variance and means compared by Tukey test at 5% probability, with the aid of the SISVAR 7.0 program. **RESULTS AND DISCUSSION:** Water deficit induced a significant increase of phenolic compounds in leaves of aroeira plants. Probably the plants have synthesized phenolic compounds of secondary metabolism in the leaves where the photosynthetic apparatus is found. Regarding the total phenol concentration of the treatments with lower water availability, a higher concentration in the leaves of the treatment (50% FC), followed by the

treatment (25% FC) was verified. This observation corroborates the literature that states that the accumulation of phenolic compounds is related to the plant's defense mechanism against stress. The main reason why water deficit induces the accumulation of phenolic compounds is related to modulation of the phenylpropanoid biosynthesis pathway. In the present research the organs (stems and roots) of the plants observed an increasing trend of phenol concentration, although there was no significant difference between soil water levels. Likewise, this tendency was also observed in plants to increase the concentration of total phenols as water regimes became more pronounced. The effect of drought on metabolite concentration is sometimes dependent on the degree of stress. **CONCLUSION:** The results indicated that the moderate water deficit induces increase in the concentration of total fanols in the leaves of young aroeira plants at the beginning of their development.

Keywords: Total phenolics. *Schinus terebinthifolius*. water deficit.

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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL PROFILE OF THE ETHY ACETATE EXTRACT FROM *Miconia caiuia* A. BARK. B.

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ABSTRACT

INTRODUCTION: The excessive and uncontrolled use of drugs, the difficulty of diagnosis and the poor hygiene conditions contribute to the antibiotic resistance index. Therefore, the search for new drugs to fight resistant pathogens has increased on the world, and that these new molecules can act by preventing signal transduction and bacterial gene plasticity, in order to eradicate these superbacteria (1,2,3). The species *Miconia caiuia* has been recently identified by the botanist Earl Celestino and and has been studied by our research group in its chemical and pharmacological aspects". **AIMS:** The objective of this work was to evaluate the antibacterial activity of the ethyl acetate leaves extract of *Miconia caiuia* and to determine its phytochemical profile. **METHODS:** The bacteria used for the antimicrobial test were *Staphylococcus aureus* (UFPEDA 02); *Bacillus subtilis* (UFPEDA 86); *Micrococcus luteus* (UFPEDA 100) and *Enterococcus faecalis* (UFPEDA 138), all from Microorganisms collection of the Antibiotics Department of the Federal University of Pernambuco-UFPEDA. To determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (CMB), the broth microdilution methodology was performed (4). Initially, 90 µL of the Muller Hinton Broth (MHB) medium was added to each well and thereafter, from the third column (A3), 90 µL of the extracts at a concentration of 1,880 µg/mL were added. This aliquot was homogenized and transferred to the fourth column (A4) and so on to the twelfth column (A12), which receives the extracts at a concentration of 2.9 µg/mL. In the last one, the aliquot (90 µL) after homogenization was discarded. Finally, a 10 µL aliquot a bacterial suspension correspond to 0.5 McFarland standard scale (approximately 1.5 x 10^8 CFU/mL) was added in each well. The plates were incubated at 37 °C for 24 h. Subsequently, 30 µL of resazurin was added for quantitative analysis of microbial growth in the wells. For the determination of CMB, a 5 µL aliquot of the concentrations that did not show visible activity to the naked eye on the microplate was picked on Petri dishes containing Mueller Hinton Agar (MHA). These plates were incubated at 37 °C for 24 hours. MBC was

considered the lowest concentration of the extract where there was no cell growth on the surface of MHA. The phytochemistry screening of ethyl acetate extraxt was performed by thin layer chromatography (TLC) (5). The *M. caiuia* extract was weighed and a solution at 5 mg/mL was prepared in athyl acetate solvent. Plates with silica gel were used as stationary phase and as mobile phase solvents at the following proportions: hexane: ethyl acetate (70:30 v/v), hexane: ethyl acetate (50:50 v/v) and hexane: chloroform : ethyl acetate: methanol (2: 3: 4: 1 v/v/v/v). After elution, the plate was dried and sprayed with specific developers and examined under ultraviolet light (254 and 365 nm). Some developers required heating at 100 ° C to view the ultraviolet or visible color bands. **RESULTS AND DISCUSSION:** The MBC MIC of *M. caiuia* AcEOt extract was 940 µg/mL for *M. luteus* and *E. faecalis*; 117 µg/mL for *S. aureus* and 235 µg/mL for *B. subtilis*. The MBC values for UFPEDA 02 and UFPEDA 86 were 940 µg/Ml, respectively. It was not possible to determine the BMI of *M. caiuia* AcOEt extract for UFPEDA 100 and UFPEDA 138, since it was higher than the initial concentration tested. Due the fact of *M. caiuia* is a new species of Miconia, to our knowledge, this is the first report of its antimicrobial activity. The phytochemical screening showed the presence of flavonoids and tannins. These results corroborate those described in the literature with the species *M. stenostachya*, *M. ligustroides* and *M. sellowiana* (7). **CONCLUSION:** Ethyl acetate from *Miconia caiuia* can be considered an antimicrobial potential to be studied, as it was able to inhibit the growth of the tested bacteria, emphasizing its microbial action against *S. aureus* bacteria. The inhibitory action can be attributed to the class of secondary metabolic identified in the extract.

Keywords: Secondary metabolites. Gram positive bacteria. Phytotherapy.

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ANNALS OF THE
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APPLICABILITY OF CURCUMIN AS AN ANTICANCER PHYTOCHEMICAL: A BRIEF REVIEW

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ABSTRACT

INTRODUCTION: Cancer is one of the leading causes of death worldwide, so developing research to find new forms of prevention, early detection and effective treatments for this disease is of utmost importance. The most commonly used cancer treatments available today, besides surgery, are chemotherapy and radiotherapy. Although chemotherapy and radiotherapy trigger several side effects, including nausea, vomiting, intestinal and urinary complications. The main preventive components of diet in cancer development are: lycopene, isoflavones and resveratrol, vegetables, fruits and spices such as turmeric, which is highlighted by its active principle: curcumin. Curcumin is the major component of C. longa rhizomes, accounting for about 2% of the dry weight of rhizomes. Curcumin can be obtained commercially as a mixture of three components: curcumin (~ 77%); demethoxycurcumin (~ 17%); and bisdesmethoxycurcumin (~ 3%). In India this mixture of curcuminoids can be found in the form of capsules and ointments for topical application, whether or not mixed with other components. The type of effect presented by curcumin depends partly on the route of administration.**AIMS:** Although there are many physiological mechanisms that can be explored as a way of intervention in the development of cancer, either through the direct interaction between certain exogenous substances with cancer cell structures causing a consequent inhibition of their growth, as well as through indirect interactions such as the expressiveness of cancer. Certain genes (more specifically, regarding their transcription) responsible for the carcinogenesis process, one of the processes that are most relevant to the understanding and application of these phenomena is the anticancer activity of curcumin. **METHODS:** The integrative review comprises five steps: establishment of the problem, i.e., definition of the theme of the review in the form of question or primary hypothesis; literature search (after definition of inclusion criteria); data evaluation (the characteristics or information to be collected from the studies are defined), analysis of the results (identifying similarities and conflicts); presentation and discussion of findings. The literary material consulted for the elaboration of this review consisted of numerous articles drawn from various electronic platforms, which provided not only content directly for the review but also other bibliographic references that made it, all with publication dates. The selected works should address the main theme (cancer and biochemical properties of curcumin). We also

used review articles, unconnected with the theme, which served only as a model for the construction of this review. **RESULTS AND DISCUSSION:** In HeLa, SiHa and CasKi cervical carcinoma strains, cell proliferation was dose-dependent, and a directly proportional relationship between dead cell percentage and increased decurcumin concentration was observed. Selective cytotoxicity of curcumin has also been reported in HPV positive strains, with low expression of HPV C33A negative cells. The decrease in E6 protein mRNA levels after incubation with curcumin was also documented, and in the CasKi and SiHa cell lines it was possible to observe a significant decrease in E6 expression with only 6 hours of treatment. This result was reflected in the restoration of p53 protein levels, known to act in the inhibition of carcinogenic processes[1,2]. However, although there are several characteristics of interest for biomedical studies, especially against cancer, the pharmacokinetics of curcumin confers to this compound some limitations, such as low bioavailability and rapid metabolization, with a consequent decrease in the time of action[3]. These limitations are a result of low solubility, rapid first- pass metabolism, low absorption and rapid systemic elimination. Although this herbal medicine is fat-soluble and thus able to cross the cell membrane freely, the low bioavailability causes its temporary minimum effective blood concentration to impair its therapeutic effects[4]. Several approaches have been applied to compensate for these disadvantageous features such as chemical modification and compound encapsulation. Due to the fact that different cell types respond differently to a compound, more effective curcumin derived compounds have been sought[5]. **CONCLUSION:** Thus, considering that cancer is one of the major causes of mortality and that the side effects of radiotherapy and chemotherapy are intense, several studies seek new therapeutic strategies. Administration of the curcumin compound has been demonstrated through numerous biochemical assays with various cancer cell lines over the past decades, a promising alternative for the development of new phytochemical-based anticancer therapies. Despite the limitations imposed by the pharmacokinetic properties of curcumin, its use may be favored by the use of alternative methodologies or compounds derived from it.

Keywords: Cancer. Curcumin. Review. Pharmacokinetics.

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SENSITIZER EFFECT OF β-LAPACHONE AND 1,4-NAPHTOQUINONE INCREASES CELL DEATH IN ACUTE PROMYELOCYTIC LEUKEMIA IN SUBLETHAL DOSES

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ABSTRACT

INTRODUCTION: Among the set of diseases that are currently characterized as cancer, leukemias are the most frequent, especially in children. Annually over 300,000 new cases are reported worldwide. However, chemotherapy currently used in the clinic has severe adverse effects due to the high doses administered in patients, causing immunosuppression that often leads the patient to death. β -lapachone is an o- naphthoquinone that demonstrates cytotoxic potential in several types of tumors in vitro and in vivo, including in micromolar concentrations, so that it was postulated as a new antitumor drug. Currently new cytotoxic combination strategies are being explored in preclinical and clinical studies to identify synergy mechanisms between agents. The literature has described the β-lapachone with a sensitizing agent for its reducing capacity, including at low concentrations, leaving cells likely to receive cytotoxic treatment. **AIMS:** The purpose of the present study was to evaluate the sensitizing and sensiitizer effect of β-lapachone with the 1,4-naphthoquinone compound in promyelocytic leukemia cells. **METHODS:** HL-60 cells were plated at concentration ($0.3x10^6$/mL), after β-lapachone (β-lap) at 3 concentrations (0.5, 1.0 and 1.5 μg/mL), the plate was added to the plate 30minutes and 1 hour before incubation with 1.4 naphthoquinone. After this time, the derivative 1.4 naphthoquinone (MNT) was added in concentrations 0.5 and 1.0 μg/mL, respectively. The choice of concentration was based on previous tests performed through the MTT test. For the cell viability test the cells were incubated with propidium iodide (10μg/mL). In the analysis of cell cycle and DNA content, triton (0.1%)/propide iodide (10μg/mL) was used, and for mitochondrial depolarization test a 123 rodamine solution (1μg/mL) was used. All analyses were done on the GuavaEasyCyteHT system (Merck-Millipore) equipment using Guava Soft™ version 2.7 software. Five thousand events were evaluated by experiments carried out in triplicate and two independent experiments. For cytotoxicity assays, the IC50 values and their 95% confidence intervals were obtained by nonlinear regression with the GraphPad Prism Program Demo (Intuitive Software for Science, San Diego, CA, USA). Mean differences were compared by one- way analysis of variance (ANOVA) followed by Tukey's test or Dunnett's test ($p < 0.05$). **RESULTS AND**

DISCUSSION: Morphological analysis of all concentrations tested showed that dead cells showed signs of death by apoptosis (formation of apoptotic bodies and DNA fragmentation) and autophagy (formation of autophagic vacuoles). Compounds that present the quinonic ring increase intracellular oxidative stress, leading tumor cells to death by several pathways. Therefore, this group of molecules has aroused the interest of research in cancer chemotherapy (4). In the cellular feasibility test, associative treatment with 30 minutes of β-lap sensitization at concentrations of 0.5 and 1.0μg/mL sensitized the substance MNT, drastically reducing cellular viability when compared to sensitization made in time of 1 hour. In the mitochondrial depolarization Test, 1-hour time sensitization increased depolarization at all tested concentrations. Regarding cell cycle analysis, the MNT substance sensitized with 0.5 μg/mL of β-lap for 30 minutes showed cycle stop in G1, already at the sensitizing concentration of 1.5μg/mL of β-lap there was a cycle stop in G1 at both awareness times. In the DNA fragmentation assay, at all sensitization concentrations tested, an increase in DNA fragmentation was observed, which reiterates cell death demonstrated in the feasibility test, highlighted in the 30- minute time in the three concentrations of β-lap sensitization of fragmentation levels were above 15% compared to negative control (2%). β -lapachone has high toxicity in therapeutic doses, which makes it impossible to use in the clinical routine. However, its potent antitumor action stimulates research of synergistic activity and sensitizing in order to decrease doses and maintain pharmacological action (5). In the present study, we proved that β-lapachone sensitizes leukemia cells (HL-60) to receive treatment with another drug at sublethal concentrations. **CONCLUSION:** Our results have shown that β-lapachone at low concentrations is a potent sensitizer in leukemia cells, reducing its side effects and increasing the effectiveness of other substances. MNT caused increased cell death in sublethal doses when sensitized with β-lapachone, where the concentration of 0.5μg/mL (1/2 of the value of CI50) sensitized with 0.5μg/mL of β-lapachone caused cell death and cycle stop, showing that it is possible to decrease doses, with the maintenance of therapeutic activity. These results are unheard of for these molecules together.

Keywords: Sensitizer. 1,4-naphthoquinone. β-Lapachone. Cell death. Leukemia.

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EVALUATION OF ANTIBACTERIAL ANTIBIOFILM ACTIVITIES OF *Bixa orellana* LEAF LECTIN

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ABSTRACT

INTRODUCTION: *Bixa orellana* L. is a plant from the tropical regions of America, whose main application is concentrated in the food industry, where its seeds are used as a dye. In addition, extracts of this plant showed insecticide and antibacterial activities. Lectins are proteins that bind to carbohydrates and exhibit various biological activities, including antimicrobial and antibiofilm actions (1). Biofilms are complex and structured communities of microorganisms enclosed in a self-produced polymeric matrix. The biofilms give to the microorganisms protection against environmental adversities and a higher tolerance to antibiotics as compared to planktonic forms (1). **AIM:** This work aimed to evaluate the antibacterial and antibiofilm activities of a lectin isolated from the leaves of *B. orellana* (BoLL). **METHODOLOGY:** BoLL was isolated following a previous established protocol that include protein extraction in 0.15 M NaCl, precipitation with ammonium sulphate (60% saturation) and chromatographies on CM-Sephadex and Sephadex G-75 columns. Antibacterial activity was evaluated against Bacillus megaterium (ATCC 14945), *Escherichia coli* (UFPEDA 224), *Pseudomonas aeruginosa* (UFPEDA 416), *Salmonella enterica* serovar. Enteritidis (UFPEDA 414), *Staphylococcus aureus* (UFPEDA 02) and *Streptococcus pyogenes* (ATCC 16642) by the broth microdilution assay (2) aiming to determine the minimal inhibitory (MIC) and bactericidal (MBC) concentrations. Antibiofilm activity was evaluated against *B. megaterium* by the crystal violet method (3). **RESULTS AND DISCUSSION:** BoLL was only able to inhibit the growth of *B. megaterium* (MIC of 18.2 µg/mL). The lectin did not show bactericidal effect. The antibiofilm activity of the lectin against this bacterium was evaluated at sub- and supra-inhibitory concentrations (1.12–80.0 µg/mL). In all the cases, there was a stimulatory effect on biofilm production (>400% in regard to untreated control). The overproduction of biofilm matrix has been associated to a defensive mechanism activate by bacterial cells in response to foreign substances in their environment. **CONCLUSION:** BoLL did not show expressive antibacterial and antibiofilm activities against the isolates of human pathogens that were evaluated. However, this lectin showed a good bacteriostatic activity against *B. megaterium* that can be evaluated more deeply in the future.

Keywords: *Bixa orellana*. Lectin. *Bacillus megaterium*.

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SYNTHESIS AND TEST OF ANTIMICROBIAN SUSCEPTIBILITY OF NEW 2-METHYL-3-AMINO-1,4-NAPHTOKINONIC ANALOGS

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ABSTRACT

INTRODUCTION: The easy access to the irrational use of drugs, mainly from the antimicrobial class, caused bacteria to develop defense mechanisms distinct from those already known. In recent years, several antimicrobial treatments have been classified as ineffective due to mechanisms developed to circumvent drug function at the active site. Medicinal chemistry involves several steps in the production of new active molecules essential in the production of new medicines. Naphthoquinones are natural and synthetic compounds that have multiple biological activities, among them, antibacterial action. **AIMS**: Five analogous 2-methyl-3-amino-1,4-naphthoquinones analogs were synthesized and then susceptible tested in 96-well microtiter plates. **METHODS:** They were synthesized under the SNAr method, at the end they were purified and characterized by 1 H NMR and 13 C NMR. Subsequently, the minimum inhibitory concentration (MIC and CMB) was standardized from the 2000 µM concentration for the strains: Staphylococcus aureus (ATCC2913), Klebsiella pneumoniae (ATCC 700603), Pseudomonas aeruginosa (ATCC 27853) and Escherichia coli (ATCC). 25992) based on the 96 well plate microdilution method. Dilutions of 1:10 were made in Mueller Hinton (MH) broth thus consisting only 10% of DMSO, and consequently serial dilution was performed throughout the plate, to control the test were used ciprofloxacin, broth MH and DMSO. Bacterial suspensions were standardized on the Mc Farland scale to 0.5 equivalent between 1-2 x 108 colony forming units (CFU / mL). After the plate preparation was completed, they were incubated for 24 h and the result was observed by microdilution plate reader, following international standards for MIC results. **RESULTS AND DISCUSSION:** Of the 2-methyl-3-amino-1,4- naphthoquinones tested, one obtained a MIC of 31.5 µM for S. aureus and in addition to this series obtained around 250-2000 µM for the same strain. While for other strains no satisfactory results were found (> 2000 µM). Subsequently, the bactericidal minimum concentration (CMB) tests were performed. Of all compounds synthesized for the S. aureus strain obtained CMB ≥125 µM. **CONCLUSION:** The 1,4-naphthoquinones tested showed antimicrobial activity varying significantly the MIC, and among these variations obtained concentrations considered good to prototypes to drugs. Thus, with the results we can improve the action of the new development test molecules to the active pharmaceutical ingredients presenting this nucleus as a base. FINANCIAL SUPPORT: UFPE, DcFar and LABSINFA.

Keywords: Naphthoquinones. Antimicrobial. *Staphylococcus aureus.*

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EFFECT OF ORTHO-EUGENOL IN *Staphylococcus aureus* WITH MsrA EFFLUX PUMP

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ABSTRACT

INTRODUCTION: *Staphylococcus aureus* is a causative agent of serious infectious diseases, some examples of these conditions range from simpler infections such as skin related to more severe dysfunctions such as pneumonia and toxic shock syndrome, the pathogenic potential of *S. aureus* is often aggravated. due to the acquisition of resistance to multiple drugs and other compounds with antimicrobial activity. This resistance is mainly promoted by mechanisms such as efflux pumps, which act as the primary response to harmful compounds through proteins that expel toxic substances outside the bacterial membrane. However promising research has identified substances that when associated with antibiotics for clinical use, are able to potentiate their action, inhibiting efflux activity and enabling the restoration of the susceptibility of the bacteria to. Ortho-eugenol is a poorly studied phenylpropanoid, but it has already shown effective antinociceptive and anti-inflammatory activity. However, its activity against strains with efflux mechanisms has not been evaluated. **AIMS:** Given these assumptions, the present study aimed to evaluate the antimicrobial activity and the ability of the antibiotic MIC to modify and inhibit the efflux mechanism of the Ortho-eugenol synthetic compound on the *S. aureus* strain bearing the MsrA efflux pump. **METHODS:** Compounds used in the experiments were purchased from *Sigma Aldrich*-Brasil and diluted to a standard concentration of 1024 µg/mL. The *S. aureus* strain used was: RN4220, which expresses MsrA efflux protein, which expels antimicrobial drugs and DNA intercalating dyes. The strain was replicated in a Petri dish and incubated in *Heart Infusion Agar* (HIA, Difco) at ± 37 °C for 24 hours. After this period, triplicate bacterial inoculate were made from the transfer of the microorganism to tubes containing 6 mL of sterile saline to a concentration of 0.5 on the McFarland scale. To determine the Minimum Inhibitory Concentration (MIC), *eppendorf*s® were filled with 160 µL of bacterial inoculum and 1440 µL of 10 % *Brain Heart Infusion* (BHI) and then 100 µL of each *eppendorf*® were transferred to 96-well sterile microplates. Subsequently, the products were micro diluted and the plate incubated at ± 37 °C for 24 hours. To evaluate the modification of the antibiotic activity, *eppendorf*s® was filled with 160 µL of inoculum, volume related to the Minimum Subinhibitory Concentration (MIC/8) of Ortho-eugenol and inhibitors, and from this, the volume of BHI a 10 %. *Eppendorf*s® with 1440 µL of medium and 160 µL of inoculum used as modulation control were also prepared. Immediately afterwards the microdilution plates

were filled with 100 µL of the solution of each *eppendorf*® and subsequently micro diluted with 100 µL of antibiotic and incubated the plate at ± 37 °C for 24 hours. To evaluate the modification of Ethidium Bromide (EtBr) MIC, *eppendorfs*® was filled with 160 µL of inoculum, volume referring to the Minimum Subinhibitory Concentration (MIC/8) of Ortho-eugenol and inhibitors, and from this, the volume of BHI at 10 %. Eppendorfs® with 1440 µL of medium and 160 µL of inoculum were prepared and used as modulation control. Immediately afterwards, the microdilution plates were filled with 100 µL of each *eppendorf*® solution and subsequently micro diluted with 100 µL antibiotic and incubated the plate at ± 37 °C for 24 hours. To observe the test results, 20 µL of sodium resazurin was added after the 24-hour interval. Erythromycin was used as a standard antibiotic and the inhibitors were Chlorpromazine and Carbonyl Cyanide M- chlorophenylhydrazone (CCCP). Test results were expressed as the geometric mean. Statistical hypothesis analysis was applied using *Two-Way* ANOVA, followed by Bonferroni *post hoc* test using GraphPad Prism 7.0 software. **RESULTS AND DISCUSSION:** It was observed in the present study that Ortho-eugenol was not directly active against *S. aureus* RN4220 strain, which carries the MsrA efflux mechanism, conferring MIC ≥ 1024 µg/mL. However, when combined with the erythromycin antibiotic Ortho-eugenol significantly reduced its MIC from 512 µg/mL to 40.50 µg/mL, these results suggest a possible synergistic relationship between ortho-eugenol and the antibiotic used, indicating that this terpene potentiated effectiveness, reversing the bacterial resistance of the strain tested. According to Coutinho and collaborators, the combination of multiple drugs has been effective in clinical use for treatment against resistant pathogenic bacteria. This is done by combining one or more antibiotics. Therefore, therapy by combining antibiotics with animal, plant or microorganism products may become the best strategy to inhibit the various mechanisms of bacterial resistance, since, when associated, their action may occur on multiple cellular targets. Regarding the modification of EtBr MIC, an increase of 32 µg/mL to 40.32 µg/mL was observed. These results indicate a likely propensity to antagonism, proposing that ortho- eugenol does not act against the MsrA efflux mechanism. **CONCLUSION:** Although Ortho-eugenol had no clinically relevant direct activity against the *S. aureus* RN4220 strain, which carries the MsrA efflux mechanism, it expressed synergism when associated with erythromycin, enhancing the efficacy of the antibiotic by reducing MIC. However, in association with EtBr Ortho-eugenol was prone to antagonism, increasing EtBr MIC and the resistance of the bacterial strains used, so it is assumed that ortho-eugenol is not effective against the efflux mechanism. However, it is possible that it may act against other resistance mechanisms and efflux proteins, which can be observed by reducing the MIC of the antibiotic. However, further studies will be needed to elucidate these mechanisms and the effect of ortho-eugenol on them.

Keywords: Terpenes. Synthetic Derivatives. Efflux inhibitors. Bacterial Resistances.

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ANTI-STAPHYLOCOCCUS AUREUS ACTIVITY AND AGAINST OTHER BACTERIAL SPECIES OF NEW 2-AMINO-1,4-NAPHTHOQUINONES DERIVATIVES

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ABSTRACT

INTRODUCTION: Bacterial resistance is a phenomenon of natural evolution in which treatments are becoming ineffective and the most persistent and severe infections. In this light, pharmaceutical chemistry has been seeking alternatives with the discovery of potentially promising new molecules that combat bacterial disorders and a possible non-implementation of pharmacological treatment. The naphthoquinonic nucleus is a phenolic derivative, of which it has antiparasitic, antineoplastic, antifungal and antimicrobial activity. **AIMS:** Thus, five 2-amino-1,4-naphthoquinonic analogs were synthesized for the purpose of susceptibility testing in 96-well microtiter plates and determination of the minimum bactericidal concentration (CMB). **METHODS:** First, the compounds were synthesized via rSNA, and after purification the structures were confirmed by1 C-NMR and 13 C-NMR. For susceptibility, an initial concentration of M in DMSO (2000 µM) based on international susceptibility plate methodologies was standardized and tested at an initial concentration of 0.02 M in strains for the purpose of determining the minimum inhibitory concentration. as: Staphylococcus aureus (ATCC2913), Klebsiella pneumoniae (ATCC 700603), Pseudomonas aeruginosa (ATCC 27853) and Escherichia coli (ATCC 25992). Being subjected to 1:10 dilutions under Mueller Hinton Broth (MH) resulting in 10% DMSO. After decreasing DMSO concentration, serial dilution was performed throughout the plate. As control, ciprofloxacin, broth MH and DMSO were used. Bacterial suspensions were standardized on the McFarland scale from 1 to 2 x 108 CFU / mL and incubated for 24 hours, then observed. Subsequently, aliquots of the last four concentrations were determined for BMC determination, added to MH Agar and incubated again for a further 24 hours. **RESULTS AND DISCUSSION:** Of the 2-amino-1,4- naphthoquinones tested, one obtained a MIC of 62.5 µM for S. aureus and in addition to this series obtained around 125 to ≥2000 µM for the same strain. Subsequently, the bactericidal minimum concentration (CMB) tests were performed. Among them a compound, obtained CMB of 2000 µM and in addition molecules obtained ≥ 125 µM, this result for S. aureus. However, for E. coli there was a variation from 1000 to ≥ 2000 µM. *P. aeruginosa* from 2000 to ≥ 2000 µM and finally K. pneumoniae ranged from 2000 to ≥ 2000 µM. **CONCLUSION:** Of the 1,4-naphthoquinones tested, antimicrobial activity varied widely, and among these variations, a compound obtained concentration with moderate activity, facilitating our perspective to improve activity with the addition of new substituents that optimize new prototype perspectives for *Staphylococcus* drugs. aureus obtaining as base nucleus 1,4-naphthoquinone.

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Keywords: Naphthoquinones. Antimicrobial. Microdilution.

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2,2-DIBROMO-3-NITRILOPROPIONAMIDE: SYNERGISTIC EFFECT WITH MEROPENEM AND INHIBITION OF TWITCHING MOTILITY ON MULTIDRUG-RESISTANT *Pseudomonas aeruginosa* STRAINS

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ABSTRACT

INTRODUCTION: Bacterial resistance represents a public health problem, as well as the ineffectiveness of antibiotics has encouraged the search for new antimicrobial molecules (1). *Pseudomonas aeruginosa* is a Gram-negative ubiquitous microorganism and is often associated with severe nosocomial infections with high capacity to infect immunocompromised hosts.It is a microorganism capable of forming biofilms, this feature complicates the infectious process as it decreases bacterial exposure to antimicrobial agents and protects it from the host immune response (2).The first step in biofilm formation is bacterial cell adhesion to a surface, especially the one mediated by type IV pili (twitching) (3, 4). 2,2-dibromo-3-nitrilopropionamide (DBNPA) is known as an industrial slimecide used in several applications (5). Therefore, the search for molecules able to inhibit bacterial adhesion and reverse the resistance of *Pseudomonas aeruginosa* is studied by several researchers worldwide. **AIMS:** The aim of this study was to determine the 2,2-dibromo-3-nitrilopropionamide activity (DBNPA) on twitching mobility and to evaluate its interaction with meropenem against multiresistant *Pseudomonas aeruginosa* strains. **METHODS:** Initially, the antimicrobial activity of DBNPA and meropenem against 12 strains of *Pseudomonas aeruginosa* was determinated by microdilution broth at concentrations ranging from 1024 to 2 $\mu g.mL^{-1}$(6). The study of the interaction between DBNPA and meropenem was performed against the most resistant strains (LFBM-H03, LFBM-H07, LFBM-H09, LFBM-C03, SPM-1) by checkerboard method (7). The criteria used to evaluate the synergistic effect were defined by Fractional Inhibitory Concentration Index (FIC index). The twitching assay were performed on medium whose composition is (g/L): Pancreatic digest of casein-17.0; peptic digest of soybean-3.0; glucose-2.5; sodium chloride-5.0; dipotassium phosphate-2.5 and agar-10. The DBNPA activity on twitching motility of *Pseudomonas aeruginosa* LFBM-H16, LFBM-H17, LFBM-H21, PAO1 and SPM-1 strains was determinated at concentrations equivalent to 128 µg/mL = MIC; 64 µg/mL = ½ x MIC and 32 µg/mL = ¼ x MIC. Plates without DBNPA were used as control. The zones of growth at the interface between agar and the bottom of the plate were measured in milimetres. **RESULTS AND DISCUSSION:** All *P. aeruginosa* strains showed meropenem MICs ranging from 4 to 1024 $\mu g.mL^{-1}$ and DBNPA showed MICs ranging from 128 to 512 $\mu g.mL^{-1}$. A synergistic effect was observed between DBNPA and meropenem against all strains (FICi values ranging from 0.14 to 0.5). The reduction of meropenem MIC was equal to 93.75 %. The DBNPA combinated with meropenem acts synergistically by inhibiting

multidrug-resistant *Pseudomonas aeruginosa* strains. DBNPA at 128 µg/mL inhibited the twitching motility of all *P. aeruginosa* strains. When compared to control, the DBNPA sub-inhibitory concentrations (64 and 32 µg/mL) were able to reduce by half the growth zone *P. aeruginosa*. Sub-inhibitory concentrations of DBNPA affect directly the *P. aeruginosa* twitching motility. Research on DBNPA activity in combination with carbapenems is currently lacking. Only patents describing synergism between DBNPA and compounds with activity on slime-producing microorganisms in industrial waters have been published (8,9,10 and 11). According to Ishikawa and collaborators (12), Paullus and collaborators (13) and Williams and collaborators (14), the mechanism of action of DBNPA seems to be related to changes in plasmatic membrane fluidity. DBNPA is a molecule of electrophilic character that probably reacts with nucleophilic sulfur-containing amino acids and amine-containing amino acids in membrane proteins. These modified proteins present in membrane affect its fluidity. In fact, our results confirm this mechanism of action. By destabilizing the *P. aeruginosa* cytoplasmic membrane, DBNPA compromises all necessary biochemical processes cell viability (i.e. carrier proteins, constitutive and hydrolytic enzymes, such as beta-lactamase). In this way, meropenem will easily penetrate the bacterial cell and exert its activity, observed by the synergism found and by the reversal of meropenem resistance. DBNPA activity can also be observed by inhibiting twitching motilit for the same reasons described above. **CONCLUSION:** DBNPA demonstrated antibacterial ctivity in combination with meropenem, both acting synergistically, and having high potential for reducing their MIC against multidrug resistant *Pseudomonas aeruginosa* strains. compound was able to inhibit twitching motility

Keywords: *Pseudomonas aeruginosa*. Twitching motility. Drugs combination. DBNPA.

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2,2-DIBROMO-3-NITRILOPROPIONAMIDE ACTIVITY IN SYNERGY WITH BETA-LACTAM AGAINST METHICILLIN RESISTANT S*taphylococcus aureus* STRAINS

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ABSTRACT

INTRODUCTION: Bacterial resistance to antimicrobials mainly from nosocomial isolates represents a serious public health problem. Part of this resistance is due to indiscriminate use of antimicrobials agents (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) presents resistance to all β-lactam and the therapy is limited to the use of glycopeptides, oxazolidine and quinupristin–dalfopristin (2). Therefore, the search for new bioactive molecules is a subject of study of many researchers around the world. 2,2-dibromo-3-nitrilopropionamide (DBNPA) is a biocide known for its high microbicidal effect against several microorganisms (3,4). **AIMS:** To determine the antibacterial activity of DBNPA against MRSA strains and to evaluate *in vitro* interaction of DBNPA with beta-lactam antibiotics against MRSA strains. **METHODS:** MRSA strains (n=9) were obtained from the Laboratório de Fisiologia e Biquímica de Micro-organismos (LFBM/UFPE) collection. A standard strain from the *American Type Culture Collection* (ATCC) *S. aureus* ATCC 33591 was included. Initially, the Minimum Inhibitory Concentrations (MIC) of DBNPA and selected beta-lactam (oxacillin, cefepime and ceftriaxone) were determined by plate microdilution method (5). Then, *in vitro* interaction was performed by checkerboard method. Drugs were distributed on microplates to obtain a final concentration equal to six or nine lower dilutions than MIC for DBNPA and beta-lactam antibiotics respectively. A bacterial suspension standardized (10^4 CFC/well) was added to each well. The plates were incubated at 37°C for 24 hours. Data were interpreted after the calculating of the fractional inhibitory concentration index (FICI) values as follows: (DBNPA MIC in combination with beta-lactam ÷ DBNPA MIC) + (beta-lactam MIC in combination with DBNPA ÷ beta-lactam MIC). The combination was considered synergistic when FICI≤0.5, indifferent when 0.5≤FICI≤4.0 and antagonist when FICI≥4.0 (6). The tests were performed in triplicate of independent experiments. **RESULTS AND DISCUSSION:** DBNPA showed activity against all MRSA strains, whose MIC values ranged from 32 to 128 $\mu g.mL^{-1}$. Wolf and Sterner (1972) (3) observed DBNPA activity against a methicillin sensitive *S. aureus* strain whose MIC value were 250 $\mu g.mL^{-1}$. In this study, all MRSA strains showed resistance profile to cefepime (MIC from 32 to 1024 $\mu g.mL^{-1}$), ceftriaxone (MIC from 16 to 1024 $\mu g.mL^{-1}$) and oxacillin (MIC from 16 to 1024 $\mu g.mL^{-1}$). The interaction evaluated by the checkerboard method demonstrated synergistic effect between antibiotics and DBNPA in all strains tested. FICI values ranged from 0.140 to 0.5 for DBNPA+cefepime combination; 0.129 to 0.5 DBNPA+ceftriaxone, while for the DBNPA+oxacillin combination, the FICI values ranged from 0.126 to 0.375. In the majority of combinations, DBNPA (0.125×MIC) with beta-lactam antibiotics were able to reduce MIC

values against MRSA strains to values ≥75.0%. DBNPA was able to reverse the resistance of all strains to beta-lactam FICI≤0.5, focus on oxacillin+DBNPA combination against the LFBM OXA3 strain (FICI=0.126), which was the combination presented the lowest FICI value compared to the other strains tested. In fact, our results confirm the DBNPA mechanism of action by destabilizing *Staphylococcus aureus* cytoplasmic membrane compromises all necessary biochemical processes to cell viability. According to the *Brazilian Surveillance and Control of Pathogens of Epidemiological Study* (BrSCOPE), 14% of microorganism infections are caused by *S. aureus* and of these 43.7% correspond to MRSA strains (7), whereas in patients with HIV virus, MRSA co-infections represent 36.8% (8). Data like these found in this study these are currently encouraged as there are few therapeutic options for treating MRSA infections and their increasing isolation. **CONCLUSION:** The DBNPA+cefepime, DBNPA+ceftriaxone and DBNPA+oxacillin combinations acts synergistically and present potential for reversal *in vitro* resistance of MRSA strains.

Keywords: DBNPA. MRSA. Synergism. β-lactams.

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ANTIMICROBIAL ACTIVITY OF *M. amacurensis, M. ciliata, M. holosericea* AND *M. hypoleuca* (MELASTOMATACEAE)

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ABSTRACT

INTRODUCTION: *Miconia* is popularly known for old cinnamon, this specie is used in folk medicine for the treatment of arthritis. Studies on biological activities with species of the *Miconia* genus have shown that they have analgesic, antimalarial and anticancer properties (1,2,3). Currently, bacterial resistance to synthetic antibiotics is widespread, so the search for new natural substances with similar action has grown. Studies with ethanolic extracts of species such as *Miconia albicans* and *Miconia rubiginosa* showed very significant antifungal and antibacterial activities (4). However, research on antimicrobial action of *Miconia* species is still scarce. **AIMS:** This study aimed to evaluate the antimicrobial potential of hexane, ethyl acetate and methanol extracts of *Miconia amacurensis, Miconia ciliata, Miconia holosericea* and *Miconia hypoleuca.* **METHODS:** The plants species were collected from Atlantic forest of Alagoas state - Brazil. The species were identified by Dr. Earl Celestino and a voucher of each species was deposited at the Instituto do Meio Ambiente de Alagoas (IMA-AL). The collected materials were kept in an incubator for 72 h at 50 º C and were subsequently ground and their metabolites were extracted by maceration in apolar and polar solvents, kept for 7 days at room temperature and protected from light. After this period, the residues were successively filtered and concentrated in a rotary evaporator. For antimicrobial activity four Gram-positive bacteria were used: *Staphylococcus aureus*, *Micrococcus luteus, Bacillus subtilis* and *Enterococcus faecalis*; three Gram- negative bacteria: *Escherichia coli, Serratia marcescens* and *Pseudomonas aeruginos*a; an acid-resistant alcohol: *Mycobacterium smegmatis* and the yeast *Candida albicans,* obtained from the Microorganisms Collection of the Department of Antibiotics from Federal University of Pernambuco. Then, the antimicrobial activity profile was evaluated according to the agar diffusion methodology, using 6 mm diameter discs of filter paper, according to the *Clinical and Laboratory Standards Institute* (CLSI, 2012). For this, 10 µL of the extracts were impregnated at a concentration of 300 mg/mL in the discs and then placed on the Petri dishes (15 x 90 mm) inoculated in MHA solid medium (Müeller – Hinton Agar) and incubated at 37 ° C by 24 h

for bacteria and at 28 °C by 48 h for yeast. After incubation microbial, growth inhibition halos were measured and quantified in mm. The positive control was erythromycin for bacteria and nystatin for yeast. In order to quantify the Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC), the susceptibility test by the broth microdilution methodology was performed according to the *National Committee for Clinical Laboratory Standards* - NCCLS (2002; 2003). For the determination of MIC, 96-well sterile plates were used, adding 90 µL of the Müller Hinton Broth (MHB) medium to each well, and from the third column, 90 µL of the extract was added at a concentration of 16 mg/mL, this mixture was homogenized and transferred to the fourth column and so on to the twelfth column, which received the extract at a concentration of mg/mL. Then, 10 µL of the microorganism in suspension were added and the plates with medium, extract and microorganism were incubated. Finally, 30 µL of rezasurin (0.1 mg/mL) was added in each wells to perform quantitative microbial growth analysis and to determine the antimicrobial activity of each sample dilution. For the determination of MMC, an aliquot of 5 µL from the concentrations that showed activity in the MIC plate, was placed in Petri dishes containing MHA, these were incubated, and the lowest extract concentration of MMC, where there was no cell growth on the surface of the medium, was considered. **RESULTS AND DISCUSSION:** In the disk diffusion test, hexane extracts showed no activity against any of the pathogens used, while those in ethyl acetate inhibited the growth of all Gram- positive bacteria and the yeast, except for *M. mirabilis* extract, which did not exhibit activity against the yeast growth. In contrast, methanolic extracts were active against Gram-positives bacteria (*S. aureus, M. luteus and B. subtilis*), Gram-negative (*P. aeruginosa*), acid-resistant-alcohol (*M. smegmatis)* and yeast (*C. albicans*), except *M. mirabilis* and *M. prasina* which showed no activity on *P. aeruginosa, M. smegmatis* and *C. Albicans,* respectively. Although hexane extracts did not show activity in the disk diffusion method, on the MIC and MMC tests all extracts were active against the exploited microorganisms, which may be related to the limitations of the diffusion test, since the medium (MHA) and this solvent have opposite polarities, making it impossible to move around the surface of the medium and, consequently, its action. According to several researchers, an extract is able to become conducive for future researches if it exhibits activity at a concentration of less than 0.1 mg/mL (5), therefore, can be considerated condusive the ethyl acetate extracts of *M. amacurensis* and *M. ciliata*, active at a concentration of 0.06 mg/mL against *M. luteus*, as well as the methanolic extracts of *M. ciliata* and *M. holosericea*. **CONCLUSION:** Based on these results, it can be concluded that the extracts of the botanical species tested in this study showed antimicrobial potentials, especially the ethyl acetate extracts of *M. amacurensis* and *M. ciliata* such as the methanolic extracts of *M. ciliata* and *M. holosericea*, placing them as future targets of antibiotic therapy, so further investigation into their efficacy and possible toxicities *in vivo* is required.

Keywords: Antibiotic. Microorganisms. Phytotherapy.

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ANNALS OF THE
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EVALUATION OF THE CYTOTOXIC ACTIVITY OF NEW NAPHTHOQUINONES DERIVATIVES (AMINO-1,4-NAPHTHOQUINONE)

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ABSTRACT

INTRODUCTION: Cancer is a group of more than 100 diseases which are characterized by the uncontrolled growth of transformed cells that invade tissues and organs and possibly metastasize in other regions of the body (INCA, 2019). In the treatment of malignant neoplasia not only a strong binding inhibiting the target is required but also acting specifically and reliably on neoplastic cells (LETAI, 2015). Ravichandiran e cols (2019) described the use of naphthoquinones that are secondary metabolites produced by algae and plants, having a wide spectrum of therapeutic actions such as antiparasitic, antifungal and mainly antitumor in cell lines such as A549 (lung), MCF-7 (breast) and HeLa (cervical). **AIMS:** The aim of this work was to synthesize unpublished 1.4 amino naphthoquinone derivatives and to analyze their cytotoxic activity against cancer cells and non-cancer cells, as well as their morphology by optical microscopy. **METHODS:** In cell culture, NCI-H292 cell lines (human lung mucoepidermoid carcinoma); HT-29 (human colon adenocarcinoma); HCT 116 (human colorectal carcinoma) and Vero (monkey kidney epithelial cells) were used. The strains were obtained from the Rio de Janeiro Cell Bank and were kept at the Cell Culture Laboratory of the Antibiotics Department - UFPE. Cytotoxic activity was determined by reduction of salt MTT 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide resulting in a purple-coloured formazan salt (LIU et al, 1997). The molecules (10 mg) were dissolved in dimethyl sulfoxide (1mL) and added to a 96-wells plate at a final concentration of 50 µM. The doxorubicin drug (10 µM) was used as a standard. Absorbance was measured on a microplate reader at a wavelength of 560 nm. Molecules that presented inhibition above 70 % were submitted to concentrations ranging from 0.39 to 50 µM, to determine the concentration that inhibits 50 % of growth in relation to the negative control (IC_{50}). Doxorubicin (0.078 - 10 µM) was used as a standard. In the morphological analysis, the rapid panotic staining was performed following the manufacturer's instructions. The concentrations of the molecules used were based on the IC_{50} values found in the MTT assay. The slides were analyzed under an optical microscope and the changes recorded by photography. **RESULTS AND DISCUSSION:** Seven new 1.4 amino naphthoquinone derivatives were synthesized. Only the DS13 derivative (50 µM) presented inhibition values greater than 70 % (85.93 - 96.96 %) in the HT-29, NCI-H292 and HCT-116 cancer strains but in the Vero strain, the inhibition was

50.76 %. Naphthoquinone (50 μM) showed cytotoxicity against HT- 29, NCI-H292, HCT-116 cell lines with inhibition percentages from 94.41 to 98.00 % and 35.35% for Vero. Posteriorly, the IC_{50} of naphthoquinone and DS13 were evaluated, where values were observed between 8.88 and 15.53 μM in relation to tumor lines HT-29; NCI-H292 and HCT-116. When analyzed at different incubation times in HCT-116, both substances were shown to have time-dependent action, decreasing values of IC_{50} are displayed. For morphological analysis, the HCT-116 strain treated with DS13 and naphthoquinones at concentrations of 3 and 6 μM in 72 h was chosen. It was possible to corroborate the previous findings that DS13 and naphthoquinone are capable of inducing cell death, being predominant the formation of pyknotic nuclei, characteristic of death by necrosis, however, it is necessary to perform more specific assays to determine the type of cell death. **CONCLUSION:** The new 1.4 amino naphthoquinone derivative (DS13) has been shown to be cytotoxic against cancer cell lines. Still, it exhibited time-dependent action, due to the proportionality between its effectiveness and the exposure time. Given this, the substance DS13 presents itself as a potential prototype for use in the treatment of cancer, and further trials are needed to prove the safety and mechanism of action *in vivo*.

Keywords: Cytotoxicity. Morphology. Naphthoquinones. HCT-116.

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ANTIBACTERIAL ACTIVITY OF EXTRACT AND PROTEIN FRACTION FROM *Punica granatum* FRUIT AGAINST HUMAN PATHOGENS

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ABSTRACT

INTRODUCTION: Currently, the search for new agents with antimicrobial action has become necessary because of the increase in the number of resistant microorganisms and the high toxicity of drugs. Lectins are carbohydrate- binding proteins that have been reported as antibacterial agents. *Escherichia coli* can form smooth, convex and circular colonies and is part of the normal microbiota; however, it can cause urinary tract infection, diarrhoea, meningitis and septicaemia (1). *Salmonella* is a genus of potentially pathogenic bacteria that commonly inhabit the intestinal tract of animals such as poultry and cattle but are responsible for severe food poisoning (2). *Staphylococcus saprophyticus* is associated with urinary tract infection (UTI) in humans. It has special urotropic and ecologic features that are distinctly different from other staphylococci and *E. coli* (3). *Punica granatum*, whose fruit is popularly known as pomegranate, belongs to the Lythraceae family. It is used in food, as therapeutic agent, and ingredients of cosmetics. In addition, there are studies that prove that pomegranate has antibacterial, anti-inflammatory, antioxidant, and antitumor action (4). **AIMS:** The objective of this work was to evaluate the antibacterial activity of saline extract from *Punica granatum* juice and its protein fraction against *Escherichia coli*, *Salmonella enterica* serovar. Enteritidis and *Staphylococcus saprophyticus*. **METHODS:** Saline extract was obtained by mixing the pomegranate juice with 0.15 M NaCl at a proportion of 9:1 (v/v) for 6 h. The lectin-rich fraction was obtained as described by Silva et al. (5) after treatment of the extract with 30% ammonium sulphate saturation for 4 h. The fraction corresponded to the supernatant obtained after centrifugation and dialysis. In order to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), the isolates of each species were cultivated in Mueller Hinton Broth (MHB) and the colony density was adjusted to 1.5 x 10^8 colony forming unit (CFU) per mL. The MIC was determined by the broth microdilution assay and corresponded to the lowest concentration capable of promoting growth reduction of 50% or more compared to the 100% growth control. For the determination of MBC, the content of wells containing the sample at concentrations equal or

higher than the MIC were inoculated in plates containing Mueller Hinton Agar (MHA) and incubated for 24 h. The MBC corresponded to the lowest concentration able to reduce the number of CFU by 99.9% compared to the initial inoculum. **RESULTS AND DISCUSSION:** The extract presented MIC of 4.875, 0.305 and 4.875 mg/mL for *Escherichia coli*, *Salmonella enteritidis* and *Staphylococcus saprophyticus*, respectively. MBC was detected only for *Salmonella enteritidis* (9.750 mg/mL). The protein fraction also presented MIC for the three species, with values of 3.570, 0.223 and 3.570 mg/mL in the same order. However, MBC was not detected in any of the species. **CONCLUSION:** Saline extract and protein fraction of *P. granatum* juice showed bacteriostatic action for all species tested; in addition, the extract showed bactericidal action for *S. enterica*.

Keywords: Pomegranate, pathogenic bacteria, antibacterial activity.

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PHYCHOCHEMISTRY AND ANTIFUNGAL (INTRINSIC AND COMBINED) ACTIVITY OF *Mesosphaerum suaveolens* (L.) KUNTZE

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ABSTRACT

INTRODUCTION: *Candida* yeasts reside in humans as commensals and are part of the normal microbiota in healthy subjects. However, when there is an imbalance between the microbiota and the immune system of the host, these fungi can become pathogens, causing the call candidiasis (SHINOBU et al., 2007). According to MORAIS-BRAGA et al. (2013), the microbiological resistance mechanisms usually occur during treatment, and this is why prolonged exposure to drugs favors fungal resistance. One important aspect to consider in this sense has been the folk use of plant extracts by local communities to cure or relief of symptoms associated with infectious diseases. The plant specie *Mesosphaerum suaveolens* (L.) Kuantze (family Lamiaceae), native to the Americas and commonly known in Brazil as "bamburral". It is used in traditional Brazilian medicine as it presents a number of therapeutic effects on the population (JESUS et al., 2013). **AIMS:** In this context, this study aimed to evaluate the antifungal activity and combinatorial action of aqueou extract from the leafs of *M. suaveolen* against yeast of the *Candida* genus. In addition, we investigated using HPLC, the phytochemicals of extract. **METHODS:** The botanical material, *M. suaveolens* was collected in at region in the south-central Ceará-Brazil, municipality of Quixelô. Frozen extract were lyophilized and yield calculated income (2.65%). Phytochemical prospecting was performed by High performance liquid chromatography (HPLC-DAD). Two yeasts of standard strains were used: *Candida albicans* (CA 77), *C. tropicalis* (CT 23) and clinical isolates resistant to multiple drugs: *C. albicans* (CA 40006) and *C. tropicalis* (CT 40042). Fluconazole was used as the reference antifungal drug. The Inhibitory Concentration of 50% of mortality the microorganisms (IC_{50}). Minimum Fungicidal Concentration (MFC) was determined by subculture in Sabourad Dextrose Agar. The effect of the combination extract/fluconazole was verified by microdilution with the extract in subinhibitory concentrations (MFC/16). The microbiological results were analyzed by one-way ANOVA followed by Bonferroni post hoc

test to evaluate the difference between the experimental groups. **RESULTS AND DISCUSSION:** The method for chemical characterization of *M. suaveolens* leaf extract revealed the presence of gallic acid (time Retention - tR = 9.73 min), catecin (tR= 7,33 min); caffeic acid (tR = 24.07 min), ellagic acid (tR = 31.49 min), rutin (tR= 25 min) and quercetin (tR = 46.93 min). In the analysis of antifungal activity data (MFC) the extract presented moderate data, since most of the tested fractions presented CFM <1024 µg/mL, that is, they present data below the cutoff value (NCCLS, 2002). With respect of the combination of natural product over fluconazole, a significance for both strains tested is remarkable since, compared to the control drug (fluconazole). Data observed with CA 77 (IC_{50} 4.7µg / mL) e CA40006 (IC_{50} 18.26 µg/mL). It is noted that the strains of *C. albicans* strains were effective mainly with the standard strains, causing a significant reduction in MIC when compared with fluconazole (IC_{50} 7.8 µg/mL and 28.82 µg/mL). Similar data are observed both strains of *C. tropicalis* (CT23 17.05µg/mL and CT40042 52.14µg/mL). Razo-Hernández et al. (2014) states that natural products have IC50 values between 1 and 500 µg / mL, which is in accordance with our values. **CONCLUSION:** This study showed for the first time the antifungal activity and combinatory effect of *M. suaveolens* extract. The study was relevant, since extract against from strains (*C. albicans* and *C. tropicalis*), exhibited significant synergistic effect in combination with fluconazole.

Keywords: *Candida.* HPLC. $IC_{50.}$

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EVALUATION OF ANTIBACTERIAL ACTIVITY AND ACTION INHIBITOR OF EFFLUX PUMPS IN *Staphylococcus aureus* STRAINS BY METHANOLIC EXTRACTS OBTAINED FROM FERMENTATION OF *Streptomyces* sp.

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ABSTRACT

INTRODUCTION: This bacteria is a commensal microorganism that can cause various types of infections and the number of infections caused by multidrug-resistant bacteria has proved to be one of the greatest threats to human health **(**Wertheim et al., 2005). One of the ways bacteria are resistant to antibiotics is due to the presence of efflux proteins. Efflux pumps are transmembrane proteins that act through the extrusion mechanism where in bacteria they act by removing harmful substances from within, such as the NorA, TetK and MrsA pumps present in *Staphylcoccus aureus* species (Blair et al., 2015). **AIMS:** The present study aims to elucidate the antibacterial effect and possible actions of the inhibition of efflux resistance mechanisms against strains *Staphylcoccus aureus* carrying the NorA, TetK and MrsA efflux pumps, by extracts obtained from the cultivation and fermentation of Streptomyces sp. **METHODS:** Actinobacteria *Streptomyces* sp. isolated from *Paullinia cupana* rhizosphere and its extracts obtained by cultivation and fermentation in rice-containing solid medium, generating the 4T (*Streptomyces* sp. UFPE 3413) and 12H (*Streptomyces* sp. UFPE 3405) extracts. In the evaluation of the antibacterial activity and action inhibitor of efflux pumps, the strains of *Sthaphylococcus aureus* 1199B (carrier of NorA and resistant to fluoroquinolones) and its wild strain 1199 were used; strain IS-58 (TetK carrier, tertacycline resistant) and RN4220 (MsrA carrier, macrolides resistant). The minimum inhibitory concentration (MIC) of extracts, ethidium bromide and antibiotics erythromycin, tetracycline and norfloxacin was evaluated to confirm the resistance level. An inoculum-containing eppendorf delivery medium was prepared in BHI liquid culture medium, which will be transferred to a 96-well microdilution plate and the substances microdiluted (1:1). In the efflux pump inhibition evaluation assays will be the substances will be tested at a subinhibitory concentration of MIC/8 and verified the possible reduction of MIC of antibiotics and ethidium bromide, the latter used as a marker of the inhibition effect of efflux. **RESULTS AND DISCUSSION:** The extracts did not show clinically relevant antibacterial activity, with all MICs ≥ 1024μg/mL. However, significant results were obtained with respect to its potential inhibitory pump by reducing the minimum inhibitory concentration of antibiotic and ethidium bromide. In the present study the 4T and 12H extracts showed an ability to reduce the MICs, when associated with both the antibiotic and ethidium bromide. Regarding the association with the antibiotic, the substances 4T and 12

H showed synergistic effect against the strain SA 1199 and RN 4220, reducing the norfloxacin and erythromycin MIC respectively. Compared to the SA 1199B strain only the extract 4T showed effect, as well as against IS-58, 12H showed significant results. The association with ethidium bromide resulted in synergistic effects with the extracts against all strains tested. Actinomycetes produce secondary metabolites with several proven biological activities including as antibiotics agents (Rajeswari et al. 2015). As an example of actinomycetes, we can mention species of the genus Streptomyces, which represent an important source for the production of already marketed antibiotics (Singh et al., 2014; Subramani and Aalbersberg 2012;). Thus, the significant results obtained in the present work may be related to the properties presented by this group of microrganism that acted as raw material for the extracts used in this study. **CONCLUSION:** The sum of the results found that extracted from fermentation of Streptomyces sp. has a good profile with possible inhibitory action of the NorA, TetK and MrsA efflux pump. All had several characteristics that indicate that they are potential drug-like antibacterial derivations. However, in vivo studies are required to verify the safety of these compounds.

Keywords: Antibacterial activity. Bacterial resistance. Synergism. Ethidium bromide. Drug targed.

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EVALUATION OF EUCALYPTOL *IN VITRO* CYTOTOXICITY IN TUMOR CELL LINES

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ABSTRACT

INTRODUCTION: Eucalyptol (1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane) is a monoterpene which can be found in essencial oleos of several plants. This chemical compound has a series of pharmacological properties that allow it to act on the human organism, bringing benefits such as antispasmoditc, hypoglycemic and antimicrobial activity (BHOWAL; GOPAL, 2015). Most chemicals agents used for cancer treatment have a narrow therapeutic window and they are not adequately effective because they are not selective for cancer cells. Mucoepidermoid carcinoma of the human lung is an aggressive disease of rare occurrence and mainly affects young people. It can be divided into two histological grades (high and low), which are related to the clinical presentation of the disease. The first degree shows a better survival (5 to 10 years), while in the second most patients languish in face of the disease (GUIMARÃES et al., 2016). Although there are a large number of antitumor drugs, they can cause side effects, which can aggravate the patient's clinical condition (KHAZIR *et al*., 2014). In view of this, it is necessary to develop research aimed at new drugs that have selectivity against tumor cells and without modify to normal ones. **AIMS:** This study aimed to evaluate the cytotoxicity of Eucalyptol in tumor cells (Hl-60, NCI-H292, HCT116, MCF7) and control (L929). **METHODS:** Cytotoxic activity was assessed by the MTT method of 3- (4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2H-tetrazolium bromide. And the human tumor cell lines used were NCI-H292 (human lung mucoepidermoid carcinoma) and HCT-116 (human colon cancer) maintained in culture medium DMEM and MCF-7 (human breast cancer), HL-60 (acute promyelocytic leukemia) kept in RPMI 1640 growth medium. The RPMI 1640 were supplemented with 10% fetal bovine serum and 1 % antibiotic solution with streptomycin and penicillin. The cells were kept in an oven at 37 °C in a humidified atmosphere anriched with 5 % CO_2. Cells were plated in 96- well plates and incubated for 24 h. The sample was added to the wells with final concentration of 50 µg/mL. Doxorubicin (5 µg/mL) was used as standard. After 72 h of reincubation 25 µl MTT (5 mg/ml) was added

and after 3 h incubation growth medium was aspirated with MTT and 100 µl 1 % DMSO was added to each well. Absorbance was measured on a microplate reader at a wavelength of 560 nm. The analysis was made from the comparison, where the sample with growth inhibition between 100 and 75 % has high activity, between 75 and 50 % moderate activity and ≤ 50 % low activity. The tests were done in quadruplicate and the percentage inhibition was calculated using GraphPad Prism 7.0. **RESULTS AND DISCUSSION:** The result of the cytotoxic activity of the compound was expressed by the percentage of cell growth inhibition (IC%). Eucalyptol showed the following results for NCI-H292 tumor cells (90.64% ± 0.56); HL-60 (88.80% ± 2.69); HCT116 (82.05% ± 4.69) and MCF7 (75.18% ± 0.53). Regarding the control L929 (22,89), it did not present significant cytotoxicity. According to a study by Kankeshani et al (2017), Eucalyptol is shown to have antitumor activity against cancer strains, one of them being MCF-7. This chemical compound can cause the depolarization of the mitochondrial membrane, by decreasing the action potential generating permanent mitochondrial membrane damage and increasing ROS production to a toxic level, leading the cell to apoptosis (SAMPATH, 2018). The IC_{50} test, which consists in verifying the value that inhibits 50% of cell growth, indicated that Eucaliptol inhibits the tumor growth of the tested cells and did not present significant cytotoxicity in the control. **CONCLUSION:** In this work it was observed that Eucalyptol has significant cytotoxic activity, in the cancer cell line NCI-H292 which was the one that showed the best result in relation to cancer cell proliferation, this substance also has low control toxicity (L292). Therefore, Eucalyptol may present itself as a substance with pharmacological potential to be evaluated by other bioassays.

Keywords: Antitumor Activity, Cancer, Citotoxic Ativity, Eucalyptol, Terpenes.

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EVALUATION OF ANTIBACTERIAL AND MODIFYING ACTIVITY OF ANTIBIOTIC ACTION OF FRUCTANS OF *Agave tequilana* WEBER VAR. *blue* AGAINST MULTIRESISTENT *Escherichia coli* STRAINS

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ABSTRACT

INTRODUCTION: The use of medicinal plants is considered a therapeutic alternative for cases of antibacterial resistance, since the antibacterial agents currently used in clinical practice are not effective against these multiresistant microorganisms. The species *Escherichia coli* is considered a bacterium that has a resistance profile with high developmental indices and is characterized as a gram-negative bacterium responsible mainly for cases of gastrointestinal infections. Therefore, as *Agave* plants, mainly *Agave tequilana*, a species commonly used as a tequila product, are great sources of food ingredients caused by the presence of fruits. Fructans are considered as fructose polymers synthesized by sucrose and can be highlighted as the main carbohydrate found in some plant species, so they can use some pharmacological activity. **AIMS:** The aim of the present study was to evaluate the antibacterial activity and modifying the antibiotic action of fructans against *Escherichia coli* strains. **METHODS:** For the extraction of plant material, it was obtained from the stem collection of *Agave tequilana* plants present in Nayarit, Mexico and donated by a local company. The native fruit fraction (NAF) was obtained from the ground stem and suspended in water to obtain juice (18 to 22 ° Brix) and was subsequently filtered and spray dried. The high polymerization fraction (HPF) and intermediate polymerization fraction were obtained by tangential NF ultrafiltration at the Tepic Technological Institute, Nayarit, Mexico. To perform the tests, initially the products were diluted to a concentration of 1024µg/mL. The microorganism used before the test was passed to a Petri dish and incubated at ± 37°C for 24 hours. After this period, bacterial inocula were performed in triplicate from the transfer of the microorganism to tubes containing sterile saline to a concentration of 0.5 on the *Mc Farland* scale. To perform the Minimum Inhibitory Concentration (MIC), *eppendorfs* were filled with 100µL of bacterial inoculum and 900µL of 10% BHI, transferred 100µL of each *eppendorf* to 96-well sterile microplates. Subsequently, the products were microdiluted to the penultimate well of each column, incubated at ± 37°C for 24 hours. Antibiotic activity was modulated by filling the *eppendorfs* with 150µL of inoculum, volume related to the minimum subinhibitory concentration (MIC / 8), and from this, the volume of 10% BHI. *Eppendorfs* with 1350µL of medium and 150µL used as modulation control were

also prepared. Immediately afterwards, the microdilution plates were filled with 100µL of each *eppendorf* and then microdiluted 100µL of antibiotic and incubated at ± 37°C for 24 hours. To observe the results of both tests sodium resazurin is added after this time period. The antibiotics used were Gentamicin, Cephalotin and Benzylpenicillin. And the products tested were native fructans, high grade and intermediate polymerization fructans. **RESULTS AND DISCUSSION:** From the MIC, it was observed that the fructans did not obtain clinically relevant results, ≥1024µg/mL. However, when performing the modifying activity of antibiotic action, it was possible to observe that in relation to the NAF, an antagonism was observed when associated with Gentamicin, which, on the other hand, no significant results were observed when associated with the other antibiotics tested. Regarding HPF and IPF, synergism was observed when associated with gentamicin, and antagonism when associated with other antibiotics. Not many studies related to fructan antibacterial activity are described. Some studies show activity of inulin, a fructan present in some vegetables, against strains of *E. coli* and *Staphylococcus aureus*. **CONCLUSION:** Thus, it was observed that fructans with high and intermediate degree of polymerization present synergistic antibacterial activity when associated with gentamicin. On the other hand, they have antagonism when used together with cephalothin and Benzylpenicillin against the *E. coli* strain used. Thus, these fructans may be useful in treating infections caused by *Escherichia coli*. However, further studies are needed to help prove the antibacterial activity of fructans.

Keywords: Fructans. Antibacterial. *Escherichia coli*. *Aquila tequilana*.

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EVALUATION OF THE ANTITUMOR POTENTIAL OF 3-METHYL-1,4-NAPHTHOQUINONE IN ACUTE PROMIELOCYTIC LEUKEMIA LINE (HL- 60)

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ABSTRACT

INTRODUCTION: 1,4-naphthoquinone compounds is as naphthalene organic derivatives and have been extensively investigated for their potential biological benefits, including their anti-inflammatory and anti-bacterial activities (1). Previous studies have already demonstrated the antitumor potential of new 1.4 naphthoquinone derivatives in some tumor cell lines, thus proving its efficacy in eliminating cancer cells in vitro (2). In addition, several studies have demonstrated that naphthoquinic derivatives are potent inhibitors of cyclin-dependent kinase proteins, resulting in cell cycle stop in several phases, which demonstrates the therapeutic importance of these molecules in the antitumor clinic (2). Naphtoquinones are described in the literature as potent ROS inducers, which explains much of their biological activity, where, the increase in the production of reactive oxygen species leads cells to trigger the process of cell death, by increasing damage these molecules cause to genomic and mitochondrial DNA. ROS have also been implicated in the metabolic of cancer cells, inducing metabolic reprogramming serving important roles in tumour initiation, progression and metastasis (1). In addition, based on the different redox status of normal and cancer cells, a novel therapeutic strategy based on drugs that increase ROS generation and promote apoptosis in cancer cells has arisen cancer therapy (3). **AIMS:** The purpose of the present study was to evaluate the capacity to induce cellular death and the possible pathways involved in 3-metyl-1,4- naphthoquinone in hl-60 cells. **METHODS:** Cells were maintained in RPMI 1640, cells were seeded in 96-well plates (100mL of $3x10^6$ cells/mL for HL-60 and 100mL 3-metil- 1,4 naphthoquinone (MNTq) (0.39–25mg/mL) dissolved in DMSO:Medium (1:99 v/v; 100mL) was added to each well and incubated for 72 h or 24 h. DMSO 1% was used as negative control. After 69 h or 21 h of treatment, 25mL of MTT (5 mg/mL) was added to each well and 3 h later, the MTT-formazan product was dissolved in 100mL of DMSO and the absorbance was measured at 595 nm in a plate spectrophotometer. For the cell viability test the cells were incubated with propidium iodide (10µg/mL). In the analysis bof cell cycle and DNA content, triton (0.1%)/propide iodide (10µg/mL) was used, and for mitochondrial depolarization test a 123 rodamine solution (1µg/mL) was used. All analyses were done on the GuavaEasyCyteHT system (Merck-Millipore) equipment using Guava Soft™ version 2.7 software. Five thousand events were evaluated by experiments carried out in triplicate and two independent experiments. For cytotoxicity assays, the IC50 values and their 95% confidence intervals were obtained by nonlinear regression with the GraphPad Prism Program Demo (Intuitive

Software for Science, San Diego, CA, USA). Mean differences were compared by one-way analysis of variance (ANOVA) followed by Tukey's test or Dunnett's test ($p < 0.05$). **RESULTS AND DISCUSSION:** Hl-60 cell treated with MNTq had cytotoxic activity against all tumour cell line tested (2.0 and 2.2 μg/mL) in 72 and 24 h Respectively. The concentrations of and 4.0 μg/mL were chosen for tumor mechanism tests. In the cell viability test, the naphthoquinone derivative decreased the number of viable cells compared to negative control. MNTq reduced cellular viability by 65% to 16% at concentrations of 2.0 and 4.0 μg/mL, respectively. In the research of (4) one of the compounds worked (para-quinone) can be calculated IC50 in some cell cancer strains such as HL-60. However, in the mitochondrial depolarization test, the opposite was observed, there was a greater depolarization of mitochondria at the concentration of 2.0μg/mL (42%) in relation to the concentration of 4.0μg/mL (20%), demonstrating that this substance also alters its mechanism of induction of death at different concentrations. In the cycle analysis MNTq (2.0 μg/mL) caused a stop of the cycle in G1 of the interphase suggesting a probable inhibition of cyclins. However, the higher concentrations did not cause cycle stop, since with the increase in the number of cells with fragmented DNA caused the cells to die independently of the phase, which demonstrates that the substance MNTq caused cell death in a way regardless of the phase. In the DNA Fragmentation test, the substance MNTq at concentrations of 2.0 and 4.0μg/mL had fragmentation around 10% and 17% of the cells, respectively. (5) noted in his research that A549 cells when exposed to a combination of a naphthoquinone exhibit better antiproliferative action. **CONCLUSION:** The results show that the naphthoquinone derivative MNTq cause cell death by different means of induction, however, the apoptotic and autophagic pathway were the most evidenced in our results. These data are unpublished for the substance MNTq, thus allowing further testing to be carried out for the confirmation of other death routes and pharmacological safety tests.

Keywords: Mechanism of action. Naphthoquinonic derivatives. Naphthoquinones. Leukemia. Cell Death.

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PHYTOCHEMICAL PROFILE BASED ON UPLC-QTOF-MS/MS AND CYTOTOXIC ACTIVITY OF *Anacardium microcarpum* DUCKE

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ABSTRACT

The plant species *Anacardium microcarpum* is widely used by popular medicine to treat various diseases, however, there are no literary reports proving its medicinal potential, especially in cytotoxic terms with cancer cells. In this context, ethanolic bark extract was evaluated to determine its phytochemical compounds by the UPLC-QTOF technique, as well as its cytotoxic potential with tumor and healthy cells. The chromatographic technique approached detected six such compounds, one of which was indeterminate. Regarding cytotoxicity, the results showed a moderate activity, presenting greater cytotoxicity for cancer cells. However, more in-depth studies should be conducted to elucidate the mechanism of action and evaluation of its pharmacological potential. **INTRODUCTION:** *A. microcarpum* Ducke (Anacardiaceae Family) is a plant native to the National Forest of Araripe, Ceará-Brazil, popularly known as "cajuí", widely cited in studies of ethnobotanical nature for the therapy of various diseases in folk medicine, highlighting the relevance of citations of the same for cancer treatments (RIBEIRO et al., 2014), which is considered a major public health problem worldwide (LUZ et al., 2018). One factor of paramount importance in the search for new bioactive molecules in the treatment against cancer is the selectivity for tumor cells. In this context, phytochemical-rich products such as plant extracts are viable alternatives. **AIMS:** The aim of this study was to perform a chromatographic survey using the UPLC-QTOF-MS system in negative ionization mode, in addition to evaluating the in vitro cytotoxic effect of *A. microcarpum* bark extract. **METHODS:** The phytochemical analysis was performed in an Acquity UPLC system (Waters), coupled to a Quadrupolo/Tempo de Voo (QtoF, Waters) system belonging to the Brazilian Agricultural Research Company - EMBRAPA (Fortaleza-Brazil). In the cytotoxic assays, after the 72 hour incubation period, the MTT chlorimetric method was performed, where there is a process of conversion of the 3-(4,5-dimethyl-2-thiazol)- 2,5diphenyl-bromide tetrazoluim (MTT) salt into formazan crystals, using five cell lines: human mucoepidermoid carcinoma of the lung (NCI-H292), human colon cancer (HCT116), mastocystoma (P815), acute promyelocitic leukemia (HL-60) and murine fibroblasts (L929). **RESULTS AND DISCUSSION:** High-performance liquid

chromatography coupled to high resolution mass spectrometry (UPLC-QTOF-MSE) allowed the partial identification of 6 compounds and 1 was not identified in the literature (m/z 635.0964). The products tested at a concentration of 50 µg/mL showed cytotoxicity in relation to the different strains tested, highlighting a moderate activity, data based on the percentage of cell viability, except for healthy fibroblasts, which did not present relevant cytotoxicity, it is emphasized that both strains tested present different sensitivities and consequently different results. These compounds of natural origin can be considered promising for the development of new drugs, because they can present relevant pharmacological actions. Satisfactory results are seen with quercetin, a typical flavonoid, which develops its mechanisms of action directly with the DNA of the cell (KUMAR et al., 2015). This, in turn, is a precursor fragment in the formation of various compounds present in the extract tested, in addition to galic acid and its derivatives. Both compounds are validated in the literature as potential antitumor compounds (LUZ et al., 2018) because they may be directly related to the cellular epigenetic mechanisms. However, extracts are complex mixtures, where there is interaction of compounds present in the sample, in constant modifications depending on the environmental conditions in which the plant is found, and this can enhance or delay the action of certain compounds (GALVÃO et al., 2018), because there may be correlations which alter its structural conformation, considerably changing its bioactivity (CUNHA et al., 2017). **CONCLUSION:** It was possible to identify 6 compounds in the extract, with predominance of flavonoids and most of the compounds present quercetin and/or gallic acid as fragments. The extract presented higher IC_{50} for fibroblasts compared to cancer cells. However, more in-depth studies should be conducted to elucidate the mechanism of action and evaluation of its pharmacological safety.

Keywords: Cajuí, UPLC-QTOF, flavonoids, tumor cells.

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OVERVIEW OF STRUCTURE-ACTIVITY OF DERIVATIVES OF 4-THIOXOTIAZOLIDIN-2-ONE SYNTHESIZED IN UFPE

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ABSTRACT

INTRODUCTION: Heterocyclic compounds have multiple uses in medicinal chemistry because they are present in drugs, of natural or synthetic origin. One of the most common rings is the thiazolidinic, which presents with a wide variety of derivatives and pharmacological actions, such as antimicrobial, for example (1). Thiazolidin-2,4-diones derive from the thiazolidine nucleus (5-limb ring, being sulfur at position 1 and nitrogen in position 3 of the heterocyclic ring) containing two carboniles in positions 2 and 4. An important derivative of this group is 4-thioxotiazolidin-2-one, which is obtained through thiazolidine tionation reaction, with Lawesson reagent or phosphorus pentasulfide (2). As in thiazolidinediones, 4-thioxothiazolidin-2-one are replaceable, and condensation with aldehydes in position C-5, one of the most significant modifications, happening in the presence of sodium acetate (3). The formation of 5-arylidene as a rational modification of the increase in antimicrobial activity (4). This demonstrates their importance for the development of new drugs, such as the advancement of beta-lactams antibiotic resistance (especially in healthcare environments), which is reflected in multiresistant strains that hinder the rational use of medicines and endanger human health. A carbon 5 alkylation can generate Z and E configuration in the molecule, and Z configuration is prevalent for its greater stability in condensation reactions (5). For these there is wide use of aldehydes, which reflect in the activity when present elements with important biological effects recognized by bioisosterism studies and structure-activity relationship. **AIMS:** This abstract seeks to compare molecular modifications made to 4-thioxotiazolidin-2-one, in doctoral thesis and master's dissertations by graduate students of UFPE and their results against microorganisms, determining the effects of their respective modifications. **METHODS:** The dissertations and thesis analyzed comparatively were obtained directly from the site "Repositório UFPE" (<https://repositorio.ufpe.br/>), searching for "4-thioxotiazolidin- 2-ona", 29 files were found, with one that was repeated. In the analysis, inclusion criteria were considered: thesis or dissertation of UFPE, in the period 2000-2019; for exclusion, those who only mentioned 4-thioxotiazolidin-2-ona were considered unfit, and did not have microbiological tests for such derivatives. After the analysis based on title, abstract and objectives of each study, there were 3 productions (2 dissertations and 1 thesis), which were analyzed and compared, regarding the microbiological tests presented and the chemical structures developed. The productions found that met the prerequisites were: (I) SILVA, I.M. Synthesis and Evaluation of Antimicrobial and Cytotoxic Activities of Thiazolidinic and Thiazolic Derivatives. 2013. Thesis (Doctorate); (P) SANTIAGO, P. B. G. da S. Synthesis, microbiological and cytotoxic evaluation of new thiazolidine-2,4-dionas and 4-thioxo-thiazolidine-2-onas. 2014. Dissertation (Master's degree); (J) da Silva Filho, J. Chemical and biological study of

thiazolidine-2,4-diona and 4-Thioxo-tiazolidine-2-ona derivatives. 2004. Dissertation (Master's degree). **RESULTS AND DISCUSSION:** Fifteen 5-arylidene-4- thioxotiazolidin-2-ona derivatives were described in the studies, and disc diffusion tests, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were performed, besides its physicochemical characterization and structural elucidation by techniques like H^1NMR, C^{13}NMR, IR, MS. The compounds are indicated following the author's first letter: I, P and J, followed by the molecule number in the order of their original work (adapted to avoid confusion). The derivatives had the following substitutions: R = 3-OH-4-OCH3 (Ia); 3-OCH3-4-OH (Ib); 2-OH (Ic); 4-OH (Id); 2- OH-5-Cl (Ie); 4-Br (If); 6-Br-4-Cl-2-oxo-2-chromine (Pa); 3-Br-4-CH3 (Pb); 5-Br- piridin (Pc); 2-Br-3-OH-4-OCH3 (Pd); 2-Br-6-F (Pe); 2-Br-5-OCH3 (Pf); 2,4-dCl (Ja); 6-CH3-4-oxo-4-chromine (Jb); 10-Cl-antracen (Jc). For positive pattern, the compounds with kanamycin, cefalexin and ketoconazole (I), kanamycin, cefotaxime and ampicillin (P), and benzoxazone (J) were analyzed. The compounds "I" were: for Gram-positive, Ia,b,e > kanamycin only (*M. luteus*); Ie > cefalexin (*E. fecalis*); all I > kanamycin (*B. subtilis*); none "I" was advantageous for Gram-negative; Ia,d > kanamycin (*M. smegmatis*); Id > ketoconazole (*C. albicans*) without significant action in filamentous. The "P" compounds were: for Gram-positive, Pd,e > kanamycin (*B. subtilis*); G- negatives, yeasts and BAAR. No "J" compounds were significant. The significant compounds followed for MIC and MBC determination. The most promising compounds were Ie,f for *E. faecalis*. P compounds exhibited MIC/CBM values between 4-16 μg/mL, not as significant. The results compared allow us to evaluate that the alteration of 5-arylidene is microbiologically effective for action in Gram-positive, but not so much for negative ones, requiring another substitution (N-alkylation), according to literature. The most significant changes were 2-OH-5-Cl (Ie) and 4-Br (If), both composed of halogens, important known bactericides. None of the studies performed molecular docking to verify intermolecular and drug-receptor complex interactions. But observing the radicals it was found that those with large volume in the molecule did not show good results and may indicate steric hindrance. Those with the best results were those with the most polar characteristics, with halogenated and oxygenated groups. **CONCLUSION:** The microbiological inhibition data reported in the theses and dissertations confirm that 5-arylidene-4-thioxothiazolidin-2-ones can be considered as promising drugs due to the advance of microbial resistance, and allowed to verify possibilities of substitutions and their effects relating the chemical structure with biological activity, allowing research to be streamlined to reduce resources (time, money) to continue the testing line that a drug candidate must follow to be marketed. The results allow us to evaluate that this type of substitution is more effective against Gram-positive, corroborating the literature and directing testing in more resistant strains that have their known epidemiological importance, especially in health-related infections (HAI).

Keywords: 4-thioxothiazolidin-2-one. Structure-activity relationship. Antimicrobial activity. Bioactive synthetic compounds. Bioisosterism.

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TERPINEN-4-OL ANTIBACTERIAL ACTIVITY AGAINST *Staphylococcus aureus.*

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ABSTRACT

INTRODUCTION: Plants have long been recognized as a valuable source of new drugs. Secondary plant metabolites, such as essential oils and their phytoconstituents, have been used throughout history for therapeutic purposes and are widely investigated for their antimicrobial potential. Terpninen-4-ol is a phytoconstituent present in the essential oils of several plants such as *Melaleuca alternifolia* (Tea Tree), which has its traditional use associated with combating superficial infections caused by Gram- positive bacteria (1). **AIMS:** Based on this, the objective of this study was to evaluate the antibacterial potential of Terpinen-4-ol phytoconstituent against clinical strains of *Staphylococcus aureus*. **METHODS:** For this study we used the clinical strains of *Staphylococcus aureus* LM-349 and LM-419, obtained from skin injury secretion and leg ulcer secretion, respectively, and belonging to the MICOTECA collection from the "Research Laboratory of Antibacterial and Antifungal Activity of Natural and Synthetic Bioactive Products"/ UFPB. The antimicrobial activity of Terpinen-4-ol (Sigma- Aldrich/Merck®) was evaluated by broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines for determination of Minimum Inhibitory Concentration (MIC) (2) by preparing an emulsion with 5% dimethylsulfoxide, 2% Tween 80 and sterile distilled water. The plates were incubated at 35±2°C for 24 hours and then bacterial growth was observed. MIC was defined as the lowest concentration of an antimicrobial that inhibited visible growth of a microorganism after 24h incubation. The Minimum Bactericidal Concentration (MBC) assay was also performed (3). After MIC, 10µL aliquots of the supernatants were removed from the wells of the microdilution plates at the concentrations corresponding to MIC, MICx2, MICx4 and MICx8 for each strain and inoculated into new microdilution plates containing only BHI medium. The plates were incubated at 35±2°C for 24 hours and then bacterial growth was observed. MBC was defined as the lowest concentration capable of causing complete inhibition of bacterial growth. All assays were performed in triplicate. **RESULTS AND**

DISCUSSION: The MIC of Terpinen-4- ol against both strains of *S. aureus* was 0.0625% (v/v) and this result is similar to results found by other authors using other strains of *S. aureus*, where Terpinen-4-ol has good activity even against strains resistant to conventional antibacterials (1,4). Through the MBC determination assay it was observed that Terpinen-4-ol was bactericidal against both strains of *S. aureus* used, from the concentration 0.125% (v/v), which corresponds to MICx2 and is reported that Terpinen-4-ol may possibly act by destabilizing the bacterial cell surface (1), although further studies are needed to confirm such action. **CONCLUSION:** Phytoconstituent Terpinen-4-ol has strong antibacterial activity against *S. aureus* strains, acting in a bactericidal manner and thus having potential for application in clinical practice. Further studies are needed to better investigate its mechanism of action and toxicity in order to provide more data on its efficacy and safety and thus enable the viability of a possible new drug against infections caused by *S. aureus*.

Keywords: *Staphylococcus aureus*. Terpinen-4-ol. MIC. MBC. Antibacterial.

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ANTIFUNGAL EVALUATION OF 1,3-THIAZOLIUM-5-THIOLATE MESOIONICS AGAINST CANDIDA AND ASPERGILLUS STRAINS

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ABSTRACT

INTRODUCTION: Fungal infections are a worldwide problem for which there is an urgent need to develop new therapeutic alternatives due to the increasing emergence of resistance to conventional antifungals (1). Mesoionic compounds are among the substances with antifungal activity and are composed of a five membered heterocyclic ring in which positive and negative charges are delocalized within an π electron system (2). **AIMS**: Therefore, taking into consideration the potential of mesoionics in structuring new drug candidates, two 1,3-thiazolium-5-thiolate mesoionic compounds were prepared and evaluated for antifungal potential against *Candida* and *Aspergillus* strains. **METHODS:** The 2-(4-chlorophenyl)-3-methyl-4-phenyl-1,3-thiazolium-5- thiolate **(2a)** and 2-(4-chlorophenyl)-4-(4-isopropylphenyl)-3-mesoionic compounds methyl-1,3-thiazolium-5-thiolate **(2b)** were synthesized according to the procedure described in the literature using the amido acids N-(4-chlorobenzoyl)-N-methyl-C- phenylglycine **(1a)** and N-(4-chlorobenzoyl)-N-methyl-C-(4-isopropylphenyl) glycine **(1b)** respectively (2). N-(4-chlorobenzoyl)-N-methyl-C-arylglycine (8.23 mmol) was solubilized in acetic anhydride (10mL), which was heated to a temperature of 55°C for 1 hour. The reaction mixture was cooled to room temperature, 10mL of carbon disulfide was added, resulting in the formation of a red solution which was heated at a temperature of 65°C for 1 hour. At the end of the reaction, the reaction mixture was cooled and remained at rest for 48h. Excess CS_2 was evaporated, 10% NaOH solution was added to the reaction mixture to pH 6 providing a red precipitate. The solid was filtered, washed several times with water, dried and recrystallized using ethanol as solvent. The structures of the compounds were confirmed by Infrared and Hydrogen NMR spectroscopic

techniques. Mesoionics 2a and 2b were submitted to biological assays for antifungal activity against the strains of *Candida parapsilosis* ATCC-22019, *Candida krusei* LM-65 and *Aspergillus fumigatus* ATCC-40640. For use in the assays the compounds were properly solubilized in dimethyl sulfoxide (5%), tween 80 (2%) and sterile distilled water. As positive control, amphotericin B (32 μg/mL) was used. **RESULTS AND DISCUSSION:** Mesoionic compounds **2a** and **2b** were obtained by cyclohydration 1a and 1b, respectively using acetic anhydride as a dehydrating agent followed by a 1,3-dipolar cycloaddition and cycloreversion with CS_2. In the infrared spectra of compounds **2a** and **2b**, the main characteristic observed is the 1290-1280 cm^{-1} stretch for C-S^-. In the range of 1602-1480cm^{-1} corresponds to the axial deformation of C=C and C=N of the mesoionic and aromatic rings. The ^1H NMR spectra showed the singlet for the three methyl hydrogens (CH_3-N) at δ 3.59 ppm for both mesoionics. For compound **2b** two additional signals were observed: one doublet for six hydrogens at δ
1.26 ppm and one septet at δ 2.96 ppm for the isopropyl group. The in vitro antifungal evaluation of compounds **2a** and **2b** was verified by the microdilution method using three strains of *C. parapsilosis* ATCC-22019, *C. krusei* LM-65 and *A. fumigatus* ATCC-40640 pathogenic fungi. The antifungal activity of the products was interpreted and considered as active or inactive according to the following criteria: up to 600μg.mL^{-1} = strong activity; 600-1500μg.mL^{-1} = moderate activity; above 1500μg.mL^{-1} = weak activity or inactive product (3). The substances showed 100% inhibition against the fungal strains. Compounds **2a** and **2b** had a minimum inhibitory concentration (MIC) of 1024μg/mL against all strains tested. **CONCLUSION:** The synthetic route used for mesoionic synthesis proved to be simple, effective and yielded 61% and 59% for compounds **2a** and **2b**, respectively. In the antifungal evaluation, the investigated compounds have antifungal activities with MIC of 1024 μg/mL and are classified with a moderate potential for the strains analyzed in this study.

Keywords: Mesoionic. Synthesis. *Candida*. *Aspergillus.*

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PHENOLICS DOSAGE AND CYTOTOXIC ACTIVITY OF *Miconia affinis* DC. (Melastomataceae)

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ABSTRACT

INTRODUCTION: The genus Miconia represents ¼ of the species of the family Melastomataceae. This genus has been the target of pharmacological and chemical studies due to the representative number of species identified and little studied from the biological point of view (1). In the literature we can find some activities described as antimicrobial (2), antitumor (3), genotoxic and mutagenic (4) of some of these species, besides chemical identification with the presence of terpenes and flavonoids (5). *Miconia affinis*, popularly known in Pernambuco as "*casquinho*" and can be found throughout the Atlantic Forest region (6). **AIMS**: The objective of this work was to quantify the presence of total and residual phenols of Miconia affinis extracts and to evaluate cytotoxic activity in tumor cells. **METHODS:** The spectrophotometric determination of the phenolic compounds was made by the colorimetric method using a Folin-Ciocalteu reagent, where tannic acid was used as the reference for calibration curve. The residual phenol content was also quantified with the Folin-Ciocalteu reagent and the method used was casein precipitation (7). 0.2 mL aliquots of the *M. affinis* hex, AcOEt and MeOH extracts were diluted in methanol (1 mg/mL, w/v). The samples remained in the dark for 30 minutes at room temperature and absorbance was measured by spectrophotometer at 760 nm against a blank prepared with distilled water. The tannic acid calibration curve was obtained using eight dilutions (0,5 – 10 µg/mL). To calculate the residual phenolic content, 6 mL of the diluted extracts (1 mg/mL, w/v) were stirred for 3 hours with 1 g of casein and 12 mL of distilled water. The results were expressed as milligrams of tannic acid equivalents per gram of extract (mg EAT/g). The samples were evaluated with six repetitions. Then the cytotoxic activity of the extracts was performed by the MTT [3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide] method. The human tumor lines used were: HEp- 2 (laryngeal carcinoma), HT-29 (colon cancer), MCF-7 (breast adenocarcinoma), and NCI-H292 (lung carcinoma), all cultured in culture medium DMEM - Dulbecco's Modified Eagle's Medium, supplemented with 10% Fetal Bovine Serum (SFB) and added 100 µg / mL penicillin and streptomycin. The strains came from the Rio de

Janeiro Cell Bank and were kept at the Cell Culture Laboratory of the Antibiotics Department of the Federal University of Pernambuco in a 5% CO2 atmospheric greenhouse. Cells were plated in 96-well plates at concentrations 0.3x10 5 cells/mL, after 24 hours, the extracts were added at a concentration of 50 µg/mL. After 72 hours of treatment, 25 µl MTT (5 mg / ml) was added to each well and after 3 h incubation, the MTT culture medium was aspirated and 100 µl dimethylsufoxide (DMSO) was added. Doxorubicin (5 µg / mL) was used as the standard drug. The absorbance was measured on an automatic microplate reader at 560 nm wavelength. The experiments were performed in quadruplicate and the inhibition percentage was calculated with the *GraphPad* Prism 7.0 Demo software. The concentration capable of reducing cell viability compared to control (IC50) by 50% was calculated to define the degree of cytotoxicity by nonlinear regression using the *GraphPad* Prisma 7.0 Demo software. **RESULTS AND DISCUSSION:** The quantitative analysis of total phenols expressed in milligrams equivalent of tannic acid per gram of extract (mg EAT / g) of extracts in AcOEt and MeOH of M. affinis were (154.84 ± 15.9 and 146.6 ± 12, 5) respectively. Regarding the residual phenols, the AcOEt extract presented the highest amount of total tannins (99, ± 9.5), no hexane extract was quantified only 21.1 ± 2.3 mg EAT/g. Phenolic and tannic acid derivatives have also been found in M. cabucu and M. rubiginosa species (8), these metabolites have pharmacological properties and promote antitumor activity (9). The EtOAc extract showed 100% inhibition of cell growth of MCF-7 and 88.66 ± 2.17 for HEp-2. Hex and MeOH extracts inhibited approximately 90% of colon cancer cells. The M. affinis extract tested for the MCF-7 strain presented an IC50 value of 16.33 µg / mL, this value, which characterized the extract as active according to the values established by the United States Cancer Institute, which considers the active extract with IC50 values below 30 µg / mL. None of the extracts tested for the HEp-2 strain had IC50 values below 30 µg / mL. **CONCLUSION:** The extracts of the M. affinis species presented phenols and tannins in their constitution. However, the AcOEt extract has a higher amount of these constituents than the others. In addition, this extract was the most promising in the cytotoxic test, these data infer that these compounds may be directly related to the activity presented by the highlighted extract.

Keywords: Secondary metabolites. Cancer. Phytotherapy.

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EVALUATION OF CYTOTOXIC ACTIVITY OF A PROTEASE IMMOBILIZED IN MAGNETIC NANOPARTICLES FRONT J774

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ABSTRACT

INTRODUCTION: Proteases are a group of complex enzymes that perform proteolysis functions, a common mechanism for activating or disabling enzymes, mainly involved in digestion and blood clotting. A vast amount of proteases are usually derived from the microbial fermentation process (fungi, bacteria). They have many industrial applications and are able to catalyze peptide bond hydrolysis. The industrial application applicable as a detergent constituent, food processing, leather industry, biotechnological products and pharmaceuticals. This broad set of use and requirements in industries motivated research on new proteases with different properties, which can favor the development of their products, preparation, storage and employment. Magnetic nanoparticles have been used for separation of proteases by means of a magnetic field replacing filtration and centrifugation, as well as for immobilization, with the aim of increasing the stability of protease, targeted application and adverse effects.**AIMS:** The objective of this work was to evaluate cytotoxicity against macrophages (J774. A1) of free and immobilized magnetic nanoparticles with a protease produced by a filamentous fungus. **METHODS:** The enzyme was obtained by solid fermentation of *Mucor subtilissimus* UCP 1262 using wheat bran as substrate. The extract obtained after 3 days of fermentation was initially precipitated with ammonium sulfate and then purified by ionic exchange chromatography DEAE-Sephadex A50 (25 x 12 x 2.0 cm), obtaining purified protease for magnetic nanoparticles were 1,1 mol L-$^{1}FeCl_3.6H_2O$ and 0.6 mol L^{-1} $FeCl_2.4H_2O$ (5mL) solutions were added to 50 mL of distilled water in magnetic agitation, and mols/L^{-1} NaOH was added drop by drop until reaching pH 10, at which point the particles were precipitated. The mixture was heated to 50 ºC for 30 min with vigorous agitation. Magnetic nanoparticles were completely washed with distilled water up to neutral pH. The material was dried and maintained at 25 ºC. Then the nanoparticles were coated with 2% glutaraldehyde. For enzyme immobilization, 10 mg of nanoparticles were added to 1mL of the phybrinolytic enzyme (5mg/mL) getting under mechanical agitation for 2 hours for immobilization. For the toxicity test, the MTT assay (bromide of 3- (4,5-dimetiltiazol-2- il) -2,5-diphenilthetrazolium) was used to determine cellular viability. Macrophages (J774. A1) used were obtained through the Rio de Janeiro Cell Bank (BCRJ) and were grown in Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with bovine fetal serum (10%) and penicillin-streptomycin (1%) 37ºC and 5% CO2. A density of 1x105 cells/mL were

placed in plates of 96 wells and after 24 h of incubation, the nanoparticles were free and immobilized for 24 hours, at final concentrations of 12.5; 25; 50; 100 and 200 μg/mL of the nanoparticle. After the treatment period, 20 μL of MTT solution (5mg/mL) was added, and the plates were incubated for 3h. After incubation the supernatant was removed and 100 μL of DMSO (Dimethylsulfoxide) were added. Absorbance was measured in Microplate Reader (BioteK Elx808) at length of 630nm. Cytotoxicity was expressed in cellular viability: (Absorbance of the treated cell population X 100 / Absorbance of the untreated cell population). **RESULTS AND DISCUSSION:** Magnetic nanoparticles had cellular viability slightly above 100% (125, 110, 115, 95 and 112%) respectively at the concentrations of 12.5; 25; 50; 100 and 200 μg/mL, while immobilized protease had cellular viability of 109, 93, 107, 91 and 134 % respectively at concentrations of 12.5; 25; 50; 100 and 200 μg/mL. The results indicate an increase in viability, but it cannot be determined as a cell proliferation, since the test used measures mitochondrial activity of cells. The vast majority of protease-related studies are based only on purification and characterization of them few studies show the immobilization of these enzymes, especially in magnetic nanoparticles. In addition, some highlight the conduct of cytotoxicity studies, since it is important to test the toxicity of the support used, mainly with the purpose of biotechnological applications, but there are few who continue to develop the research. **CONCLUSION:** It can be concluded that nanoparticles and immobilized protease in nanoparticles are not toxic to the J774 macrophage lineage. A1. Further cells proliferative assays will be performed against undifferentiated cancer cell lines using the flow cytometer for better confirmation of these results.

Keywords: Cytotoxicity. Nanotechnology. Magnetic field.

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CHEMICAL IDENTIFICATION OF ISOLATED HEXANIC FRACTION OF *Miconia caiuia* (Melastomataceae) AND DETERMINATION OF MIC IN CLINICAL ISOLATE OF *Candida glabrata*

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ABSTRACT

INTRODUCTION: Fungal infections caused by Candida yeasts have been increasingly frequent in patients with low immunity. The occurrence of these infections is directly related to the frequent use of broad spectrum antibiotics linked to other factors such as the use of central venous catheter and hematologic diseases (1, 2). The indiscriminate and nonspecific antibiotic therapy for Candida species has contributed to the increase in the resistance profile of yeasts to antifungal agents available (3). That way, the search for new fungicides has been intensified as an alternative to help fight these pathogens. **AIMS**: The objective of this study was to identify the chemical compound of the fraction obtained from the hexane extract *Miconia caiuia* and determine the antifungal activity of the extract and fraction clinical isolate of *Candida glabrata*. **METHODS:** The hexane extract of *M. caiuia*, obtained by the exhaustive maceration method, was fractionated in open column with silica gel 60 G (70-230 mesh) and mobile phase: hexane:ethyl acetate: acetone:methanol in gradient mode of increasing polarity. Subsequently, the fractions obtained were subjected to Thin Layer Chromatography (TLC) (4), using hexane as mobile phase (1:1 v/v); hexane:ethyl acetate (8:2 v/v); hexane:ethyl acetate (7:3 v/v) hexane:ethyl acetate (6:4 v/v); hexane:ethyl acetate (5:5 v / v); ethyl acetate (1:1 v/v); acetone:methanol (8:2 v/v); acetone:methanol (5:5 v/v) and methanol (1:1 v/v). In addition to column chromatography, it was employed in the isolation of the compounds, the recrystallization of fractions that were semi-purified and monitoring by TLC. The identification of the fraction was performed on gas chromatography-mass spectrometry (GC-MS) in CarbolWAX column and 5% phenyl methyl silicone. Mass spectra were recorded at 30-450 m/z. The individual components were identified by their mass spectrum corresponding to 70 eV. After obtaining the fraction, the extract and the fraction were submitted to the RPMI broth microdilution test at 0.5 *Mcfarland* scale (5) with initial fraction concentration of 1,250 µg/mL to determine Minimum Inhibitory Concentration (MIC)

value. For this trial, the clinical strain isolated from a leukemia patient was obtained from the Micoteca Culture Collection of the Federal University of Pernambuco under the registration number URM 4247. **RESULTS AND DISCUSSION:** The final yield in mg (milligrams) obtained by fractionation was 255.6 mg. The chromatogram used was based on the NIST 11 spectra library and the elucidation of the sample was performed based on the similarity index between the compound compared with data available in the library and in the literature. For the identification of the peak were considered characteristic fragments and intensity m/z, retention time and the area (%). The peak has an unsaturated triterpene profile, with a similarity rate of 83%, according to the library used. Chemical investigations of other species of *Miconia*: *M. rubiginosa, M. albicans* (6)*; M. cabucu, M. pepericarpa e M. sellowiana* (7) corroborates the results obtained. The MIC of *M. caiuia* hex extract and triterpene against URM 4247 isolate were 70 µg/mL and 156.2 µg/mL, respectively. Steroids and triterpenes are generally studied because they are active for various activities of biological interest, such as antimicrobial activity, in the literature it is possible to find MIC values of 2,500 µg / mL for C. albicans (ATCC 1023) and C. tropicalis from terpenes isolated from plant extracts (8), higher concentrations than those found in this work. **CONCLUSION:** The apolar extract of *Miconia caiuia* fell investigated in the present study was active for the tested URM 4247 yeast. The inhibitory action presented can be attributed to the identified triterpene present in the tested and fractionated extract. Although the MIC extract was better than the fraction, both results are promising to investigate its mechanism of death in *C. glabrata* (URM 4247).

Keywords: Melastomataceae. Antimicrobial. Non-albicans Candida.

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IN SILICO STUDY, SYNTHESIS AND EVALUATION OF THE ANTIFUNGAL POTENTIAL OF NEW 2-AMINO-THIOPHENE DERIVATIVES

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ABSTRACT

INTRODUCTION: 2-Aminothiophene derivatives are important synthetic intermediates for medicinal chemistry, allowing to obtaining several bioactive compounds, with emphasis on antifungal activities[1,2]. Previous studies carried out by the Laboratory of Synthesis and Vectorization of Molecules have identified promising antimicrobial activity of cycloalquil[*b*]thiophene derivatives, yet theoretical studies have demonstrated that their high LogP values are one of the main factors limiting their biological potential[3,4]. **AIMS:** Carry out a rational design of new 2-amino thiophenes obtained from 1,4-dithiane-2,5-diol in order to obtain new molecules with more suitable pharmacokinetic characteristics, and determine the *in vitro* antifungal activity of these new drug candidates. **METHODS:** To performe the *in silico* studies and analyse the pharmacokinetic and toxicological properties was used the method "Predicting Small- Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures (pkCSM)". The synthesis was conducted through the fourth version of Gewald reaction[5]. After structural confirmation, all compounds had their antifungal activities determined against dermatophyte fungi by determination of the minimum inhibitory concentrations (MICs) which were conducted by the broth microdilution method according to the Clinical and Laboratory Standard Institute protocols M27-A3 and M38- A2. The cellular cytotoxicity demonstrated by IC_{50} concentration was performed against non-tumor monkey kidney epithelial cell lines (VERO), human fibroblasts (MRC-05) and murine fibroblasts (3T3). **RESULTS AND DISCUSSION:** 13 new thiophene derivatives were obtained through microwave irradiation, resulting in compounds with yields ranged from 35 to 89%. *In silico* evaluation shows good bioavailability, the calculated Log*P* values, number of hydrogen bond acceptors (HBA), number of hydrogen bond donnors (HBD) and molecular weight (MW) were agreed with Lipinski's rule, having its values within the parameters. Furthermore, Topological Surface Area (TPSA) and number of rotatable bonds (nrotb), felt within the limit ranges. Together, these results indicate that the proposed compounds present good bioavailability. In the antifungal activity, the bets results were with compounds: CN05 (32μg/mL against *Trichophyton rubrum* 6753), CN13 (64μg/mL against *T. rubrum* 6753), CN17 (64μg/mL against *T. rubrum* 6753), CN19 (16μg/mL against *Epidermophyton*

floccosum 69999, *T. tonsurans* 700, *T. tonsurans* 2922) and CN21 (32μg/mL against *T. tonsurans* 700, *T. rubrum* 6753), some even better than fluconazole, used with reference. Toxicity analysis against Vero, MRC-05 and 3T3 cells revealed no differences in cell viability at any of the concentrations evaluated after 72h of incubation. **CONCLUSION:** Given the above, 2-amino-thiophene derivatives without C4-C5 cycloalkyl chains are promising in the development of antifungal compounds, demonstrating that the proposed modifications resulted in a decrease of Log*P*, and in compounds with better pharmacokinetic profile and antifungal activity. Thereby, *in vivo* study is essential for future medical applications.

Keywords: Gewald reaction. Dithiane. *In silico*. Druglikeness. Dermatophyte fungi.

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ANTIBACTERIAL ACTIVITIES OF NEW SUBSTITUTED N-ACYLIDRAZONIC DERIVATIVES

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ABSTRACT

INTRODUCTION: Bacterial infections remain a public health problem given the increasing bacterial resistance due to the indiscriminate use of antibiotics. In this context, the search for new leader compounds, strategies of molecular modification of antimicrobial agents has been, for many years, one of the main techniques approached, consisting of the accomplishment of small chemical modifications in a prototype matrix compound, with chemical structure and well-known biological activities. In addition, as bacterial resistance consists of microorganism recognizing drug structure, performing substitutions on existing drugs can ensure greater potential, overcoming resistance mechanisms and decreasing their toxic effects (1,2). *N*-acylhydrazones are organic compounds with chemical structure $R_1R_2C = NNH_2$ and have been extensively studied for their promising properties. The synthesis of these molecules is relatively simple, which attracts the attention of researchers, since the simplicity of synthesis will translate, in the long run, into cheaper drugs and therefore accessible to the population. Among the many biological applications performed by *N*-acylhydrazones, it can be highlighted antimicrobial and antiparasitic activities (3). **AIMS:** The objective of this work was to evaluate the antibacterial activity of new substituted *N*-acylhydrazonic derivatives JR-13, JR-15, JR-17 and JR-18 by determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). **METHODS:** The MIC and MBC determination of the new substituted *N*-acylhydrazonic derivatives was performed according to the Clinical and Laboratory Standards Institute (CLSI) (4) against strains *Acinetobacter baaumannil* (UFPEDA1025B), *Bacilus subtilis* (UFPEDA86), *Enterobacter aerogines* (UFPEDA348), *Micrococcus luteos* (UFPEDA06), *Pseudomonas aeruginosa* (UFPEDA416) and *Stahylococcus aureus* oxacillin resistant (UFPEDA709) from the collection of microorganisms from the Department of Antibiotics of the Federal University of Pernambuco. For bacterial reactivation, inoculum preparation and antimicrobial activity tests, Brain Heat Infusion (BHI) – HIMEDIA®, broth culture medium were used plus 5% mutton blood. Each of the new substituted N-acylhydrazones JR13, JR15, JR17, JR18 at initial concentration of 1000 µg/mL was added to the 96-well plate containing medium and successive dilutions to 0.98 µg/mL, followed by addition of microbial suspension at a concentration 1.5 x 10^8 colony forming units (CFU/mL), corresponding to 0.5 McFarland. The plate was incubated for 24 h to determine the MIC. For quantitative bacterial growth analysis, resazurin (0.01 m/ mL) was added to all wells, followed by incubation for 1 h and further reading of the plates. For the determination of the

CMB, an aliquot of the concentrations that showed activity on the MIC plate was chosen. These plates were incubated at 37 °C for 24 h and the lowest concentration of each of the new substituted N-acylhydrazones was considered as a bactericide where there was no growth on the surface of Mueller Hinton Agar. **RESULTS AND DISCUSSION:** The MIC of JR-13, JR-15, JR-17 and JR-18 for *Acinetobacter baaumannil* was 120 µg/mL in the four compounds, for *Bacilus subtilis* was respectively 120 µg/mL, 250 µg/mL, 120 µg/m and 180 µg/mL, for *Enterobacter aerogines* was 120 µg/mL in the four compounds, for *Micrococcus luteos* was respectively 120 µg/mL , 120 µg/mL, 120 µg/mL, 60 µg/mL, for *Pseudomonas aeruginosa* was respectively 120 µg/mL, 120 µg/mL, 250 µg/mL, 120 µg/mL and *Stahylococcus aureus* oxacillin resistant was respectively 250 µg/mL, 250 µg/mL, 120 µg/mL, 250 µg/mL. Results for JR-13, JR-15, JR-17 and JR-18 for *Acinetobacter baaumannil* were respectively 250 µg/mL, 500 µg/mL, 120 µg/mL and 500 µg/mL for *Bacilus subtilis* was respectively 120 µg/mL, 500 µg/mL, 250 µg/m and 250 µg/mL for *Enterobacter aerogines* was respectively 500 µg/mL, 500 µg/mL, 250 µg/mL and 250 µg/mL for *Micrococcus luteos* was respectively 120 µg/mL , 500 µg/mL, 250 µg/mL and 250 µg/mL for *Pseudomonas aeruginosa* was respectively 500 µg/mL, 250 µg/mL, 500 µg/m and 500 µg/mL and *Stahylococcus aureus* oxacillin resistant was 500 µg/mL. The antibacterial activities corroborate the data from Moura (2016), which emphasizes the chemical structure of the substituted N-acylhydrazonic derivatives, showing that the presence of nitrogen in the JR15 molecule, the presence of the bezene ring-bound bromine in the compound JR- 17 and the presence of nitrogenous heteroaromatic compounds fused to the benzene ring in compound JR-18 are favorable to their biological activity. In addition, recent studies have reported the presence of many bioactive compounds with *N*-acylhydrazonic nuclei at various stages in the experimental phase and in clinical trials with antibacterial activities (4, 5). **CONCLUSION:** In conclusion, *N*-acylhydrazone derivatives showed relevant antibacterial activity, which can lead to the discovery of new antibacterial therapies.

Keywords: *N*-acylhydrazonic. Antibacterial activity. MIC. MBC. Bacterial infections.

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ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM COALHO CHEESE AGAINST PATHOGENIC MICROORGANISMS

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ABSTRACT

INTRODUCTION: Lactic Acid Bacteria (LAB) are considered as the most important group of microorganisms used for fermentative purposes. They are of crucial importance due to their physiological characteristics, such as substrate utilization, metabolic capacity and probiotic properties (1). Due to their ancient use as yeast, especially in the food industry, LABs have been extensively studied as bioprotective agents as they may allow the control of fungal growth and other pathogenic and / or spoilage microorganisms, thereby improving shelf life many fermented products and therefore reduce health risks (2). For this reason, LABs are an interesting group in terms of their antimicrobial efficacy, which may be linked to bacteriocin actions - a group of biomolecules that may represent a good alternative in the replacement of antimicrobial agents in foods, such as antibiotics, as well as other substances that may also be considered as antimicrobials, such as diacetyl, hydrogen peroxide and lactic acid. **AIMS:** The aim of this study was to investigate the antimicrobial activity of lactic acid bacteria isolated from handcrafted coalho cheeses in the Paraíba Sertão against pathogenic microorganisms. **METHODS:** The lactic acid bacteria used in this study were isolated from artisanal coalho cheese from the municipality of Catole do Rocha, located in Sertão Paraibano. Were used two species of LAB such as *Enterococcus faecium* KT990030 and *Streptococcus infantarius subsp. infantarius* KT990067 which were reactivated in 10% Reconstituted Skimmed Milk (LDR) and in Man, Rogosa and Sharpe (MRS) broth, respectively, and incubated at 37 ° C between 18-24 hours. Aliquots were collected and centrifuged at 10,000 xg, 4 ° C for 10 minutes, where the supernatant was obtained for antimicrobial activity evaluation by the broth microdilution method (CLSI, 2018) (3). The antimicrobial action of LAB was performed against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), both species being activated in Mueller-Hinton medium and incubated in a bacteriological greenhouse at 37ºC, between 18-24 hours. After this period, they were adjusted to a standard concentration of 10^7 CFU / mL. The LAB-derived supernatant was tested against pathogens in the following ratios: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1: 128, 1:256 and 1:512. The experiments were performed in triplicate, in a 96-well plate, with 24 hour incubation. **RESULTS AND DISCUSSION:** The plates were then read at 595 nm, and thus determined the percentage of growth inhibition of pathogenic bacteria by the following formula: % = (A0-A) / A0-Ai * 100. The LAB showed an effective action

against *S. aureus* and *E. coli*. *Enterococcus faecium*, for example, showed 100% inhibition against the two pathogenic microorganisms at the first two dilutions (1:2 and 1:4), however, at dilutions 1:8 and 1:16 the percentage inhibition against *E. coli* were 97% and 86%, respectively, while inhibitory activity against *S. aureus* at 1:8 dilution was 75%. *Streptococcus infantarius subsp. infantarius* was lethal to both pathogens at the first two dilutions (1:2 and 1:4), inhibiting their growth 100%, however, at the third dilution (1:8) it presented about 70% inhibition against *E. coli* and 97% against *S. aureus*. According to the results, the inhibition of pathogenic microorganisms occurred in a dose dependent manner, that is, the higher the LAB supernatant concentration, the greater the inhibition of pathogen growth. In a study by Souza et al. (2017) (4) identified that the evaluated lactic acid bacteria were capable of 100% inhibition of a *S. aureus* strain at the first dilution (1:2) and about 85% inhibition at the second dilution (1:4), given which corroborate those found in this research. In evaluating the antimicrobial activity of lactic acid bacteria isolated from commercial yogurt belonging to the genus *Lactobacillus* spp. Prabhurajeshwar and Chandrakanth (2018) (5) identified that the MIC of the Y9 isolate cell-free supernatant was 50 µL, 1: 4 dilution, against *S. aureus* and *E. coli*, values similar to the present study. Due to the virulence presented by several pathogenic microorganisms that can affect humans, studies are being conducted to find new compounds with action against these microorganisms. **CONCLUSION**: Thus, it is concluded that the decrease in the percentage of microorganism inhibition is directly related to the decrease of antimicrobial agent concentration. In all cases, supernatants were found to be effective in inhibiting pathogenic bacteria with high inhibition percentage up to 1:8 dilution, in particular *E. faecium* with high inhibitory activity at 1:16 dilution against *E. coli*.

Keywords: Lactic acid bacteria. Antimicrobian activity. Pathogenic microorganisms. Coalho cheese. Inhibition.

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EVALUATION OF THE POTENTIAL MODIFIER OF THE ACTION OF AMINOGLYCOSIDE OF A PRODUCT OBTAINED FROM THE LEAVES OF *Spondias tuberosa* ASSOCIATED WITH LED

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ABSTRACT

INTRODUCTION: Medicinal plants have been used since ancient times. The Anacardiaceae family is widely distributed in Brazil, mainly in coastal areas. Consisting of about 70 genera and approximately 600 distinct species, it has great economic and pharmacological importance due to the enormous variety of nutrients and compounds present in its leaves, flowers and fruits (1). Among the species of the Anacardiaceae family, we highlight *Spondias tuberosa* Arruda (umbuzeiro), widely used in folk medicine to treat gastrointestinal problems, diabetes, uterine cramps and inflammation, mainly through the infusion of its leaves. The umbuzeiro has in its peel and fruits composed as tannins that promote healing, antioxidant activity by the presence of ascorbic acid and phenols, antitumor and antiviral activity. Several studies conducted with plants indicate that, on the surface of the leaves of some trees there is a membrane responsible for the selectivity and permeability of various substances and this membrane is basically composed of fatty acids (2). **AIMS:** This is an experimental study with a quantitative approach to evaluate the modifying potential of *Spondias tuberosa* antibiotic action associated with LED. *Spondias tuberosa* leaves were collected in Timorante, Exu district, Pernambuco, Brazil. **METHODS:** The product was obtained through the hydrodistillation technique of the fresh leaves of umbuzeiro. Multiresistant bacterial strains from clinical isolates *Escherichia coli (E. coli)* 27 and *Staphylococcus aureus (S. aureus)* 358 were used. The broth microdilution technique was performed using 96-well sterile plates with 1: 1 serial dilutions. were used to evaluate the inhibitory effect of the isolated product. Microbial cultures were picked in brain and heart infusion broth (BHI) and incubated at 35 ° C for 24 h. After this period, the inoculum was standardized, which consisted of the preparation of a suspension in BHI, whose turbidity was similar to the McFarland 0.5 tube (1 x 108 CFU / mL). This suspension was diluted 100-fold in BHI medium, which corresponds approximately to a suspension containing 1 x 106 CFU / mL, from which 100 µL was taken and added to each well of the plate. The test solution was prepared using 10 mg of the solubilized product in 1 mL of dimethylsufoxide (DMSO) to an initial concentration of 10 mg / mL. From this concentration, dilutions were made in sterile distilled water to obtain a stock solution of 1024 µg / mL. The final concentrations of the samples were from 512 to 8

μg / mL. After 24h of incubation, the result was read by adding 20μL of sodium resazurin to each test well. For wells that remained blue growth inhibition was considered and for those that changed their color to pink it showed bacterial growth. Antibiotic modulating activity was performed with an aminoglycoside (amikacin) from the use of multidrug resistant strains where the concentration corresponded to 1,024 μg / mL. The antibiotic was diluted in a volume of 100 μL seriously in the wells and the suspension was inoculated with the multiresistant strain with the product at subinhibitory concentration (MIC / 8). Final concentrations were 512 to 0.5 μg / mL of antibiotics in the culture medium and the plates were incubated for 24 hours at 35 ± 2 °C. After incubation, sodium resazurin was used to evidence modulatory activity. The plates were exposed to the LED fixture. They were divided into four groups: the blue LED light with a wavelength of 415nm, red light of 620 nm and another group the yellow light of 590 nm for 20 minutes each plate. Control plates were made that were not exposed to any light. All tests were performed in triplicate. **RESULTS AND DISCUSSION:** The methodology used refers to the extraction of essential oil, however, the characteristics of the product had the aspect of a "wax" when at room temperature. Studies conducted with waxes reveal that they are basically fatty acids and are insoluble in water. Their main function is to form a selective membrane capable of controlling water exchange with the external environment (3). The microdilution of the product revealed that there was no inhibition of bacterial growth. at any of the concentrations tested. When associated with LED lights the results were not significant either. In the modulation tests against *E. coli*, when amikacin was combined with the product, there was no statistically significant difference in MIC. LED lights were able to decrease the minimum inhibitory concentration of aminoglycoside, however the results were statistically not significant. When amikacin modulation was performed with the product associated with the LED, it was possible to observe an increase in MIC, but it was not statistically significant either. In *S. aureus* tests, LED increased the amikacin MIC, being indifferent to the yellow and red lights, but statistically significant with the blue light, which demonstrates antagonism. The product analyzed was not able to modulate the action of the antibiotic. When amikacin modulation was performed with the product associated with the LED, an increase in MIC was observed, but this was statistically significant only in relation to *S. aureus* with yellow light. **CONCLUSION:** The results show that this product is able to create a protective membrane preventing the interaction of the antibiotic used and the LED lights with the bacterial strains tested, so it is important to verify the mechanism by which this happens in order to elucidate this antagonistic mechanism.

Keywords: LED lights. Modulation. *Spondias tuberosa*.

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ANTITUMORAL ACTIVITY OF β-LAPACHONE IN CELL LINE ACUTE PROMIELOCITIC LEUKEMIA (HL-60)

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ABSTRACT

INTRODUCTION: Cancer in the broader sense refers to more than 277 different types of disease, this disease has as characteristic the disordered growth of cells, invading tissues and organs. Leukemia is a type of cancer characterized by the accumulation of diseased cells in the bone marrow, which replace normal cells. The incidence of this disease is 2.8% of new cases in men and 2.4% in women. There are more than 12 types of Leukemia, with acute myelocytic leukemia being one of the four most common (1). Acute promyelocytic leukemia is a subtype of acute myeloid leukemia resulting from reciprocal and balanced translocation involving the α receptor of retinoic acid in chromosome 17 and in the promyelocytic leukemia gene on chromosome 15, generating the PML-RARαoncogenic fusion protein (2). In order to reduce cancer cases, several therapeutic approaches have been elaborated, aiming to eradicate tumor cells with as little interference as possible in the normal functioning of the body. The efficiency of current cancer therapy is mainly limited by toxicity associated with antineoplastic drugs to normal tissues (3). β-lapachone is a poorly soluble, ortho-naphthoquinone with potential antineoplastic and radiosensitizing activity. (4). According to studies of (5), β-lapachone has promising activity against tumor cells with high selectivity, has been used in trials studying the treatment of cancer, carcinoma, advanced solid tumors, head and neck neoplasms, and carcinoma squamous cell. **AIMS:** The purpose of the present study was to evaluate the cell death pathways caused by the β-lapachone naphthoquinonic derivative in the cellular lineage of acute promyelocytic leukemia. **METHODS:** Cells were maintained in RPMI 1640, for MTT test cells were seeded in 96-well plates (100mL of $3x10^6$ cells/mL for HL-60 and 100mL β-lapachone (0.39–25mg/mL) dissolved in DMSO:Medium (1:99 v/v; 100mL) was added to each well and incubated for 72 h or 24 h. DMSO 1% was used as negative control. After 69 h or 21 h of treatment, 25mL of MTT (5 mg/mL) was added to each well and 3 h later, the MTT-formazan product was dissolved in 100mL of DMSO and the absorbance was measured at 595 nm in a plate spectrophotometer. For the cell viability test the cells were incubated with propidium iodide (10μg/mL). In the analysis of cell cycle and DNA content, triton (0.1%)/ propide iodide (10μg/mL) was used, and for mitochondrial depolarization test a 123 rodamine solution (1μg/mL) was used. All analyses were done on the GuavaEasyCyteHT system (Merck-Millipore) equipment using Guava Soft™ version 2.7 software. Five thousand events were evaluated by experiments carried out

in triplicate and two independent experiments. For cytotoxicity assays, the IC50 values and their 95% confidence intervals were obtained by nonlinear regression with the GraphPad Prism Program Demo (Intuitive Software for Science, San Diego, CA, USA). Mean differences were compared by one- way analysis of variance (ANOVA) followed by Tukey's test or Dunnett's test ($p < 0.05$). **RESULTS AND DISCUSSION:** β-lap had cytotoxic activity against all tumor cell lines tested (0,05 and 1,0 μg/mL) in 72 and 24 h. The concentrations of 0.5 and 1.0 μg/mL were chosen for tumor mechanism tests. β-lap reduced cellular viability in the three concentrations tested 0.5 μg/mL (83%), 1.0 μg/mL (75%) and 1.5 μg/mL (70%) compared to negative control (90%). In the analysis of involvement mitochondrial, β-lap also depolarized 0.5 μg/mL (17%), 1.0 μg/mL (20%) and 1.5 μg/mL (28%), compared to negative control (10%). In the cycle analysis, β-lap (0.5μg/mL) caused cycle stop in phase G1 of the interphase, Suggesting a probable inhibition of cyclins. However, the higher concentrations did not cause cycle stop, since with the increase in the number of cells with fragmented DNA caused the cells to die independently of the phase. In the DNA Fragmentation test, β-lap at concentrations of 0.5 μg/mL, 1.0 μg/mL and 1.5 μg/mL showed a percentage of 4%, 6%, 7% fragmentation, respectively, and were not statistically different when compared with negative control (2.5%). β-lapachone undergoes redox cycles that increase intracellular ROS levels, and this reaction is mostly catalyzed by the enzyme NAD (P) H: quinone oxidoreductase 1 (NQO1), a redox enzyme specifically super-expressed in several cells (4). This enzyme uses NAD and NADPH as co-factors, which promotes the reduction of an electron leads to the formation of a semiquinone-free radical, which in the presence of oxygen is oxidized back to quinone, generating reactive oxygen species (ROS). Into can generate two effects: stimulates the production of detoxifying metabolites, which protect the cell from carcinogenesis and mutagenesis; on the other hand, NQO1 can generate unstable hydroquinone that further increase ROS production or the formation of DNA adducts (5). **CONCLUSION:** Our results demonstrate that β-lapachone is a potent agent causing cell death by different means of induction, except for cell fragmentation. Further studies are needed, thus allowing further trials to be carried out for the confirmation of other death routes and pharmacological safety tests.

Keywords: β-lapachone. Leukemia. Naphthoquinone. cell death. Action mechanism.

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ANTIFUNGAL ACTIVITY OF EXTRACT AND ITS LECTIN-RICH FRACTION FROM POMEGRANATE AGAINST *Candida* AND *Cryptococcus*

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ABSTRACT

INTRODUCTION: *Candida* is a fungal genus that causes major health problems, being associated with superficial and systemic infections. One of the species most related to candidemia is *C. albicans*, but other species are also responsible for a high number of infectious cases (1). Cryptococcosis is a highly invasive fungal infection caused by fungi of the *Cryptococcus* genus. This infection has a high mortality rate in immunocompromised patients. The two species mainly involved with this infection are *Cryptococcus neoformans* and *Cryptococcus gattii* (2). *Punica granatum* is a plant whose fruit is popularly known as "pomegranate", belonging to the Lythraceae family. It is used in food and cosmetic industries and as a therapeutic agent. There are several studies that prove that pomegranate has anti-inflammatory, antioxidant, antibacterial and antitumor actions (3). Due to the increasing number of cases of human invasive mycoses, especially in immunocompromised patients, there has been an increment in the search for new antifungal agents. The current existing antifungals have some limitations such as low efficiency and high toxicity, and some fungal strains have acquired resistance to them (4). Lectins are carbohydrate- binding proteins that have been reported as antifungal agents. **AIMS:** This work aimed to evaluate the antifungal activity of a saline extract from pomegranate juice and its lectin-rich fraction against *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Cryptococcus neoformans* and *Cryptococcus gattii*. **METHODS:** Saline extract was obtained by mixing the pomegranate juice with 0.15 M NaCl at a proportion of 9:1 (v/v) for 6 h. The lectin-rich fraction was obtained as described by Silva et al. (5) after treatment of the extract with 30% ammonium sulphate saturation for 4 h. The fraction corresponded to the supernatant obtained after centrifugation and dialysis. Minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations were determined. The isolates were cultivated in Sabouraud Dextrose Broth (SDB) and the colony density was adjusted turbidimetrically at a wavelength of 600 nm to 3×10^6 colony forming unit (CFU) per mL. The MIC was determined by broth microdilution assay and was defined as the lowest concentration capable of promoting a reduction in growth (48 h) of at least 50% when compared to the 100% growth control. For the determination of MFC, the content of wells containing the sample at concentrations equal or higher than the MIC were inoculated in

plates containing Sabouraud Dextrose Agar and incubated for 48 h. The MFC corresponded to the lowest concentration able to reduce the number of CFU by 99.9% compared to the initial inoculum. **RESULTS AND DISCUSSION:** The saline extract of *P. granatum* presented MIC of 9.75, 9.75 and 2.43 mg/mL for *C. glabrata*, *C. neoformans* and *C. gattii*, respectively. MFC was not detected. The protein fraction presented MIC of 7.14 mg/mL for *C. albicans*, *C. glabrata*, *C. neoformans* and *C. gattii*. Like the extract, the MFC of the fraction was not detected. **CONCLUSION:** The saline extract and protein fraction of *P. granatum* showed fungistatic effect on *Candida* and *Cryptococcus* species.

Keywords: Antifungal. Pomegranate. *Candida*. *Cryptococcus*. *Punica granatum*.

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CYTOTOXIC ACTIVITIES OF THE SECONDARY METABOLITES OF *Streptomyces spp* ISOLATED FROM THE AMAZON-BRAZIL REGION

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ABSTRACT

INTRODUCTION: As bacteria of the genus Streptomyces are gram-positive, soil- isolated bacteria, they are a promising source of bioactive products, with applications in industry, agriculture and the medical field, for various bioactive products composed of substances with antitumor potential (1). **AIMS:** Consider a cytotoxic activity of the secondary metabolites of *Streptomyces* sp. included in the Amazon-Brazil. **METHODS:** Bacterial strains *Streptomyces* sp. ACTMS-4T (UFPEDA 3413), *Streptomyces* sp. ACTMS-5M (UFPEDA 3412) and *Streptomyces* sp. ACTMS-12H (UFPEDA 3405) were isolated from *P. cupana*, a native plant from the Amazon-Brazil, known as "guarana". For extraction of *Streptomyces* spp. 150 ml methanol was added to the culture and left for 48 h at 30 ° C. The culture was extracted twice and the extract was concentrated rotary evaporator at 50 ° C. The methanol extract (0.5 g) from *Streptomyces* sp. (ACTMS-12H) was diluted with distilled water and partitioned with n-hexane (FHex-12H), ethyl acetate (FAcOEt-12H) and 2-butanol (FBuOH-12H) in 1: 3 ratios. The extracts obtained were dried on a rotary evaporator under reduced pressure at 50 ° C, while the aqueous phases of each partition were evaporated at 100 ° C. To evaluate cytotoxic activity, the cell lines used were HEp-2 (laryngeal squamous cell carcinoma), HT-29 (colon cancer), MCF-7 (breast cancer), NCI-H292 (lung mucoepidermoid carcinoma), HL-60 (acute promyelocytic leukemia), K-562 (chronic myelocytic leukemia) and MOLT-4 (acute lymphoblastic leukemia) obtained from the Rio de Janeiro Cell Bank. Cytotoxicity was assessed by the MTT method. Cells were plated in 96-well plates (105 cells / well for adhered cells and 0.3x106 cells / well for suspended cells). After 24 h, extracts and phases were added to the wells in a single concentration (50 µg / mL) and submitted to cytotoxicity testing at various concentrations (0.00019 - 50 µg / mL) to obtain the IC50, extracts and / or fractions that inhibited cell growth above 70%. Doxorubicin (0.009 - 5 µg / mL) was used as a positive control. After 72 h incubation was added in 25 µl MTT (5 mg / mL). After 3 h, excess MTT was aspirated and 100 µL DMSO was added to each well for dissolution of formazan crystals. The absorbance was read in a spectrophotometer at a wavelength of 540

nm. Cytotoxicity to peripheral blood mononuclear cells (PBMCs) was also assessed and obtained from 3 mL of blood. It was used as peripheral for healthy volunteers, according to the standards approved by the Ethics Committee on Research with Human Beings of the Federal University of Pernambuco (process No. 61757616.0.0000.508). FicollHistopaque-1077 3 ml blood samples were added to the blood, followed by 30 min centrifugation at 1500 rpm. To the lymphocyte suspension was added PBS to 11 mL volume and centrifuged for 20 min at 1000 rpm. The supernatant was discarded and the lymphocyte pellet resuspended in 2 mL RPMI 1640 medium, 100 U / mL penicillin, 100 µg / mL streptomycin, obtaining final final concentration of 106 cells / mL. Lymphocyte proliferation was induced by the addition of 3% phytohemagglutinin (2). To determine cytotoxicity with PBMCs, extracts and phases were added to the cell cultures in the 96-well plate at serial concentrations (0.39 - 50 µg / mL). After 48 h of incubation at 37 ° C with 5% CO2 and 95% humidity atmosphere, 10 µL alamar blue was added. The microplate was reincubated for 24 h and absorbances measured at a wavelength of 570 nm and 595 nm (3). **RESULTS AND DISCUSSION:** The EMeOH-12H extract at a concentration of 50 µg / mL showed high cytotoxic activity against NCI-H292, HL-60 and MOLT-4 strains. With the exception of the FHex-12H phase, all other phases showed high cytotoxicity against MOLT-4, with FBUOH-12H also showing activity against HEp-2, HT-29 and HL-60. The IC50 was determined for extracts and phases that showed cell growth inhibition above 75% at a single dose of 50 µg / mL. The EMeOH-12H extract was more cytotoxic to HL-60 and MOLT-4 strains, with IC50 of 5.4 µg / mL and 4.1 µg / mL, respectively. Among the phases, FBuOH- 12H stood out for presenting activity against four of the seven strains tested, being more active against HL-60 (IC50 = 1.4 µg / mL) and MOLT-4 (IC50 = 1.1). µg / ml). For evaluation of cytotoxic activity in PBMCs, extracts and phases that presented cytotoxic activity in at least one tumor lineage were selected, so the EMeOH-4T and EMeOH-5M extracts were not evaluated. The FBuOH 12H phase presented IC50 values below the value determined by the National Cancer Institute (NCI). The cytotoxic activity of the FBuOH-12H phase can be attributed to the presence of saponins, which was found only in this phase. In the literature, few studies report the isolation of *Streptomyce*s saponins, as well as their cytotoxic activity. A compound of *S. diastatochromogenes* MK800-62F with saponin-like chemical structure was isolated and exhibited anticancer activity against small human lung carcinoma cells (Ms-1). Cytotoxicity may be related to the presence of this class of compounds. Other studies carried out with several species of the genus *Streptomyces* have been able to isolate their active compounds and have cytotoxic and antiproliferative potential in tumor cells and have not shown toxic effects on PBMC (4,5). **CONCLUSION:** The species *Streptomyces* spp. it is a promising agent in the discovery of new treatments and development of new anticancer drugs, through technologies that can increase the antitumor effect of the compound and decrease its adverse effects.

Keywords: *Streptomyces* spp. Cytotoxicity. Antiproliferative potential.

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IN VITRO ANTITUMORAL ACTIVITY OF LIPOSOMES CONTAINING ACID BARBACTIC FROM *Cladonia sallzmanii* (LICHEN)

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ABSTRACT

INTRODUCTION: The barbático acid (AB), the main secondary metabolite of the lichen Cladonia salzmannii, has biological activities such as antimicrobial and cytotoxic. However, there are limitations to its pharmacological application, mainly due to its low water solubility and toxicity. The use of systems for controlled drug release contributes, among other things, to enhance the biological activity of molecules with physicochemical property unfavorable. The stealth liposomes show up candidates vehicles for antitumor drugs, especially by extending the plasma half-life of these compounds. **AIMS:** This study aimed to evaluate its *in vitro* antitumor activity of the stealth liposomes containing barbatico acid of Cladonia salzmanii. **METHODS:** Conventional Liposomes (AB-LC) and stealth (AB-LF) containing the AB were prepared by hydration of the lipid film and characterized by determining the size, polydispersity index, surface charge, the content and the encapsulation rate. For in vitro antitumoral activity assay, the MTT assay (bromide of 3- (4,5-dimetiltiazol-2-il) -2,5-diphenilthetrazolium) was used to determine cellular viability. Carcinoma ehrlich cells and undifferentiated cells J774 macrophages were grown in Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with bovine fetal serum (10%) and penicillin-streptomycin (1%) 37ºC and 5% CO2. A density of 1x105 cells/mL were placed in plates of 96 wells and after 24 h of incubation, the nanoparticles were free and immobilized for 24 hours, at final concentrations of 40; 20;10;5 and 2,5 µg/mL of the barbatic acid free and encapsulated. After the treatment period, 20 µL of MTT solution (5mg/mL) was added, and the plates were incubated for 3h. After incubation the supernatant was removed and 100 µL of DMSO (Dimethylsulfoxide) were added. Absorbance was measured in Microplate Reader (BioteK Elx808) at length of 630nm. Cytotoxicity was expressed in cellular viability: (Absorbance of the treated cell population X 100 / Absorbance of the untreated cell population). **RESULTS AND DISCUSSION:** The formulations (AB-LC and AB-LF) proved to be homogeneous, with Tyndall effect and remained stable after 90 days when stored at 4 °C. AB-LC and ABLF showed respectively 115.6 ± 5.88nm (0.255) and 109.3 ± 2.82 (0.307). The encapsulation rate was respectively 90.66 ± 4.57% for AB-LC and 95.15 ± 5.34% for AB-LF. IC50 values (µg/ml) of AB, AB-LC and AB-LF was 12.00 ± 5.24 and 11.53 ± 4.83 front TAE , respectively. For J774 (healthy cells) was not determined IC50 dose of 40µg/ml until tested. The positive control, 5-Fluoracil showed little activity in reducing proliferation at the same

concentrations tested. The IC50 values presented by AB, AB-LC and AB-LF for Ehrlich carcinoma cells were slightly higher than the IC50 of a lichen metabolite with proven antitumor activity (uric acid), at which time cell toxicity was evaluated. of human lung carcinoma, and it was observed that in its free form, uric acid showed IC50 = 10.0 µg / mL whereas nanoencapsulated form showed IC50 = 11.5 µg / mL. **CONCLUSION:** These data suggest that the barbatic acid, promoted an effective tumor inhibition *in vitro*. Furthermore, the encapsulation enhanced the antitumor activity of barbático acid. Further cells proliferative assays will be performed against undifferentiated cancer cell lines using the flow cytometer for better confirmation of these results.

Keywords: Cytotoxicity. Nanotechnology. Liposomes. Barbatic acid. Lichen.

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CYTOTOXIC ACTIVITY EVALUATION OF THE HEXANE EXTRACT OF *Miconia Caiuia* IN TUMOR CELLS

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ABSTRACT

INTRODUCTION: The genus *Miconia* is the largest representant of the *Melastomataceae* family, having more than 1.056 species distributed mainly in tropical areas and in various biomes of Brazil, including the Cerrado, Caatinga, Amazon Forest and Atlantic Forest. (1). In the Northeast were identified 74 species of *Miconia*, usually present in the Atlantic Forest and the state of Pernambuco, the second with the largest number of recognized species (2). According study, some *Miconia* species and isolated compounds have shown significant pharmacological potential. (3). **AIMS:** This work aims to evaluate the cytotoxic profile of *Miconia caiuia* hexane extract in tumor cells and analyze its phytochemical profile. **METHODS:** The lineages NCI-H292 (lung carcinoma), HT-29 (colon cancer), HEp-2 (laryngeal carcinoma), MCF-7 (estrogen receptor-positive breast adenocarcinoma) were used, obtained from cell bank of Rio de Janeiro and maintained at the Cell Culture Laboratory of the Department of Antibiotics - UFPE. Cell suspensions of 10^5 cells/mL (adhered cells) were plated and after 24h the *Miconia* extract was added to the culture plates at a concentration of 50 µg/mL. After 72h incubation MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was added and the plates were reincubated. Posteriorly, dimethylsufoxide (DMSO) was added to dissolve the formazan crystals. The optical reading was performed in an automatic microplate reader (560 nm). The concentration capable of reducing cell viability relative to the control (IC_{50}) by 50% was calculated to define the degree of cytotoxicity by nonlinear regression using *Graph pad* prisma version 7.0 demo software. Phytochemical screening was performed to detect the presence of secondary metabolites by Thin Layer Chromatography (TLC). The extract (5 mg/mL) was dissolved in the solvents: Hex, AcOEt e MeOH. As a stationary phase, F_{254} Silica gel plates were used and as mobile phase the solvents in the following proportions: hexane:ethyl acetate (70:30 v/v), hexane:ethyl acetate (50:50 v/v) and hexane:chloroform:ethyl acetate:methanol (2:3:4:1 v/v/v/v). After elution, the plates were dried and sprayed with specific developers and examined under ultraviolet light (254 and 365 nm) (4). **RESULTS AND DISCUSSION:**

The percentage of cell growth inhibition (IC%) of hexane extract was 82.92 ± 4.20 for HEp-2 and HT-29 lineages; 68.27 ± 4.50 for MCF-7 and 98.86 ± 0.22 for NCI-H292 lineages. These results indicate a moderate to high inhibition potential of the extract for the tested lineages except for MCF-7. In the IC_{50} (µg/mL and 95 % IC) determination, the results were 25.58 (22.12 - 37.39) for HEp-2 and 42.95 (33.90 - 54.42) for the NCI-H292 lineage. Studies with isolated triterpenes and *M. ferruginata* showed inhibition percentages of 98.1% and 98.5% for melanoma and colon lineages, respectively (5). According to the literature, the cytotoxic activity of *M. albicans* and *M. stenostachya* species was at a concentration of 30 mg/mL for *M. albicans* and 60 mg/mL for *M. stenostachya* during genotoxicity and mutagenicity assays (6), These experimental data demonstrate that the results obtained in this work contribute significantly to the importance of cytotoxic studies with species of the genus *Miconia*. In the phytochemical analysis, it was possible to detect the presence of triterpenes, steroids and tannins. The triterpenes presence was also verified in crude extracts of other *Miconia* species such as *M. stenostachya*, *M. ligustroides* and *M. sellowiana* with the isolation of three triterpenes for each species (7). **CONCLUSION:** *Miconia caiuia* hexane extract showed cytotoxic activity for the cell lineages used, highlighting its antitumor profile for the Hep-2 cell lineage. The presence of the terpenoid class in the qualitative analysis of the studied extract may justify its efficiency, because these compounds show tumoral action.

Keywords: Melastomataceae. Phytochemistry. Anticancer.

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ANTIFUNGAL ACTIVITY OF THE AQUEOUS EXTRACT OF *Crotalaria breviflora* DC. AGAINST *Candida* sp. STRAINS

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ABSTRACT

INTRODUCTION: The genus *Candida* is composed of a heterogeneous group of organisms that grow as yeasts. Among them, the most common species is *Candida albicans* which can cause infections in humans and other animals. In addition, they are among the main agents causing hospital infections as well as *C. tropicalis* (RODRIGUES et al., 2019). On the other hand, natural products of plant origin have been gaining prominence for being effective, presenting good microbiological activity. In this sense, many plants were evaluated not only for their antifungal activity, but also as a modifier of antibiotic resistance (MORAIS-BRAGA et al., 2016). The species *Crotalaria breviflora*, native to Brazil, belongs to the Fabaceae family, which despite its recognized toxic effect, has some species used in popular medicine to treat several diseases. **AIMS:** In this context, this study aimed to evaluate the antifungal activity and combinatorial action of aqueous extract from the leafs of *C. breviflora* against *C. albicans* e *C. tropicalis* strains. **METHODS:** Plant material was collected in March 2018 in a rural area in the Monte Alverne district, Crato-CE, Brazil. Subsequently, an infusion was performed and allowed to stand for 72 h, then frozen and subjected to a lyophilizer for release of the aqueous solvent. Two yeasts of standard strains were used: *Candida albicans* (CA 77) and *C. tropicalis* (CT 23). Fluconazole was used as the reference antifungal drug. Minimum Fungicidal Concentration (MFC) was determined by subculture in Sabourad Dextrose Agar. The effect of the combination extract/fluconazole was verified by microdilution, with the extract in subinhibitory concentrations (MFC/16). Statistical analysis was performed using GraphPad Prism, version 6.0, by one-way ANOVA followed by Bonferroni post hoc test to evaluate the difference between the experimental groups. **RESULTS AND DISCUSSION:** In analysis of the results, it was possible to observe that the aqueous extract of the leaves had no effects on the species of *C. tropicalis* and *C. albicans* tested, that is, there was no reduction in the growth of fungal strains. Similar data were observed in combination with fluconazole, which had no effect on the activity of the drug used as a control. It is important to note that extracts are complex mixtures, where there is interaction of compounds present in the sample, in constant modifications depending on the environmental conditions in which the plant is found, and this can enhance or delay the action of certain compounds (GALVÃO et al.,

2017). To date there are no literary reports when the microbiological activity of this species, however, other species of the genus present significant results with different microbiological species (GOMEZ-CHANG, et al., 2018). **CONCLUSION:** The aqueous extract of the leaves of *C. breviflora* showed no antifungal effect for *Candida* species, i.e., it did not potentiate the effect of fluconazole in the combinatorial assay, nor in growth inhibition - MIC. Further tests shall be carried out to elucidate these results.

Keywords: Fabaceae, Candida, MFC, microdilution.

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ANTIMICROBIAL EVALUATION OF *Streptomyces* sp. BIOMASS EXTRACTS

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ABSTRACT

INTRODUCTION: In the last decades, several natural products have been discovered and characterized, many of them with therapeutic actions and isolated from microorganisms. Actinobacteria are important producers of bioactive compounds, which stand out for presenting different biological activities such as antiviral, antifungal, insecticide, cytotoxic, and antimicrobial – being evidenced, therefore, as a group of great biological and economic importance (OLANO et al, 2011). Metabolites are produced to inhibit the proliferation of other microorganisms, increasing the chances of spore germination and their survival. Among actinobacteria, the genus *Streptomyces* stands out as an important producer of the main classes of antimicrobials, such as: aminoglycosides, glycopeptides, β-lactams, and macrolides. In Brazil, *Streptomyces* spp. (Sp-1 and Sp-2) whose secondary metabolites showed antifungal activity against *Aspergilus niger, A. fumigatus, Trichophyton rubrum*, and *Trichosporon inkin* (OLIVEIRA et al, 2010). Rosly et al. (2014) demonstrated the *in vitro* inhibition against *Helicobacter pylori* ATCC bacterium of the acetonic extract from the fermentation of *Streptomyces* sp. H7372. **AIMS:** To evaluate the antimicrobial activity of the biomass extracts from *Streptomyces* sp UFPEDA 3407 (20G). **METHODS:** The bacterial strain *Streptomyces* sp. UFPEDA 3407 (20G), isolated in the region of Maués - Amazon State - Brazil, was provided by the UFPEDA Microorganism Collection of the Department of Antibiotics of the Federal University of Pernambuco. To evaluate actinobacterial antimicrobial activity, the primary assay - agar diffusion test was performed. The strain was reactivated in liquid ISP-2 medium for 48 h and a plate with the same medium was inoculated for 5 days at 37 °C. After growth, 6 mm diameter circular blocks were removed and inoculated in Müeller Hinton (37 °C) or Sabouraud (30 °C) medium, where other microorganisms had been previously inoculated for antimicrobial activity evaluation (*Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Bacillus subtillis*, *Candida krusei* and *Candida albicans*). The test was performed in triplicate. After the cultivation period, the diameter of the halos was measured. For the secondary assay, a fermentation in submerged culture was performed where 6 blocks of agar (10 mm) were inoculated in ISP-2 medium and cultured for 13 days at 37 °C and immediately after this process, 10% of the pre-inoculum was added to a container containing MPE medium is added followed by stirring at 180 rpm for 96 h. Antimicrobial activity and pH were monitored every 24 h. After fermentation, the cell mass was separated by centrifugation (10,000 rpm for 5 min). The extraction of the active principle from biomass was performed with water miscible solvents (ethanol, methanol, and acetone). Then, the agar diffusion test was performed against the same microorganisms previously tested (LYRA et al, 1964). The analyzes followed with the susceptibility tests for the determination of the Minimum Inhibitory Concentration (MIC),

using the microdilution technique (1000 - 0.48 µg/mL). The plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for yeast. After the culture period, the bacterial microplates were developed with 25 µL of 0.01% resazurin and incubated for 1-3 h. Similar procedure with yeast microplates was done, using 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) salt as the developer and incubation for 1-3 h (CLSI, 2010). **RESULTS AND DISCUSSION:** The results obtained from the agar diffusion test showed that actinobacteria have a good action spectrum with activity for bacteria (Gram-positive) and fungi (yeast), with inhibition halos ranging from 19 to 26 mm. In the secondary test, the best time was 96 h for four of the five microorganisms that were sensitive (halos > 15 mm), determining the best fermentation time. After determining the best fermentation time (96 h), actinobacteria was submitted to the fermentation process and its bioactive compound was extracted from the biomass by acetone, ethanol, and methanol solvents (yields of 1.29%, 2.22% and 0,32%, respectively). Whose results showed a good action spectrum with activity for bacteria (Gram-positive) and fungi (yeast), presenting average inhibition halos between 14 and 16.5 mm. In the MIC test, the biomass methanolic extract showed antimicrobial activity against Gram-positive bacteria with MIC values equal to or below 31.2 µg/mL, while for yeast 31.2 µg/mL (*C. albicans*) and 125 µg/mL (*C. krusei*). The acetonic biomass extract showed similar MIC values to the crude methanolic extract for Gram-positive bacteria but was less effective for yeasts with values ranging from 500 to 1000 µg/mL. The ethanolic biomass extract showed MIC values equal to or below 31.2 µg/mL when tested against bacteria. None of the three extracts showed antimicrobial activity for the *P. aeruginosa* Gram-negative microorganism, the lack of sensitivity of these bacteria can be related to the structural difference of Gram-negative bacteria in relation to the Gram-positive. **CONCLUSION:** Therefore, the strain *Streptomyces* sp UFPEDA 3407 shows to be an actinobacterium with high potential due to the production of metabolites with antimicrobial activity. It is important to highlight that the MIC results of the crude extracts obtained in this study are expressed in µg/mL.

Keywords: Actinobacteria; actinomycetes; antimicrobial; anti-bacterial; antifungal.

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NANOENCAPSULATION SNEDDS-TYPE AND PHYSICOCHEMICAL CHARACTERIZATIONS OF THE NATURAL ANTIMUTAGENIC TRANS-DEHYDROCROTONIN FOR FURTHER ANTICANCER ASSESSMENT

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ABSTRACT

INTRODUCTION: The use of extracts from Brazilian medicinal plants in the treatment of human disease is a common practice; however, for many species little information is available about the risks that these products may pose to health. This is not the case of *Croton cajucara* Benth, an Amazonian medicinal plant, that has been largely studied including its assessment of the possible mutagenic potential. Many tumors result from alterations in the homeostatic control of cell differentiation and apoptosis. So, studies have demonstrated that some anticancer agents can induce apoptosis in human leukemic cells, although these agents show serious cytotoxicity not only to malignant cells but also to normal tissues, including myelocytes and cells of the immune system. The HL60 (human leukemic cells) for example, can be induced to undergo terminal differentiation and apoptosis by a variety of chemical and biological agents. In this sense, *Croton cajucara* extracts and its isolated compound trans-dehydrocrotonin (t-DCTN) have been searched for their therapeutic effectiveness on the discovery of anticancer agents. It was evidenced that *Croton cajucara* is a powerful antitumor source with antigenotoxicity and cytotoxic effects against Ehrlich carcinoma and human K562 leukemia and HL60 cells. Indeed, t-DCTN showed anticancer, antimutagenic, and antigenotoxicity properties (AGNER et al, 2001). The protective effect of t-DCTN was evidenced against induction of micronuclei and apoptosis by different mutagenic agents in vitro and was cytotoxic to HL60 cells. Aiming at to improve the biochemical profiling of the lead compound t-DCTN, its encapsulation into liposomes was carried out specifically for enhancement of its antitumor activity (LAPENDA et al, 2013). Other systems such as PLGA microspheres and hydroxypropyl-β-cyclodextrin into microparticles and a mixed monolayer system of t-DCTN and phospholipids were also assessed. **AIMS:** The aims of the present study were i) optimize t-DCTN loading into a liquid nanosystem to improve its oral availability by using a stable colloidal system, such as SNEDDS (self-nanoemulsion drug delivery system); ii) perform physicochemical and release characteristics of the t-DCTN-SNEDDS formulation; iii) improve the bioavailability of the t-DCTN for further anticancer pharmacological purposes. **METHODS:** The SNEDDS ternary phase diagram was constructed using the surfactant mass titration methodology into the aqueous and oily phases in order to obtain a polar o/w nanoemulsion region. The surfactant (Tween´s mixed composition) was mixed with a specific oil phase, on the following weight ratios 9:1 of surfactant and oil phase (respectively), 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. The mixtures were diluted dropwise with distilled water and the nanoemulsion region was produced by

using the mechanical stirring Vortex (IKA, Germany), at ambient temperature. The pH was measured by using a pre-calibrated pH meter PG-2000 (Gehaka, Brazil), at 25 ± 2 °C. The refractive index was determined using Abbe's refractometer (Bellingham plus Stanley Limited, England), at 25 ± 2 °C. The average droplet size and Zeta potential was measured by Zeta Potential Analyzer (ZetaPlus, Brookhaven Instruments Corporation, USA), with a detector angle of 90 °. Rheological property was determined by using oscillatory Haake Mars rheometer (Thermo Fisher Scientific, Germany), at 25.0 ± 0.1 °C using a thermostatic bath. Analysis were carried out by applying a shear rate sweep from 0 to 103 s^{-1}. Surface tension was carried out using a SensaDyne tensiometer (model QC-6000, USA) employing the maximum pressure bubble technique, using nitrogen as gas phase. The result of the surface tension was analyzed with the SensaDyne tensiometer software, version 1.21. **RESULTS AND DISCUSSION:** The phase diagram of the SNEDDS formulation was based on the proposed model of colloidal system designed by Winsor and afforded the Winsor IV region based on the determination of the maximum solubility of the active matter (surfactant) in the aqueous and oils phases, by means of mass titrations. According to this methodology, the mixture of several compounds with different physicochemical properties can generate various colloidal systems including spontaneous monophasic systems. The obtained single-phase o/w-type nanoemulsion (c.m.c. 8.492 x 10^{-3} g/mL) remained isotopically stable after water dilution (upon thirty dilutions) ensuring no phase change and characterize as self-colloidal drug delivery system (SNEDDS) which was applied to load t-DCTN (5 mg/mL to 10mg/mL) by using a solubilization method. The t-DCTN-load into the SNEDDS formulation was isotropic when observed under polarized light microscopy consistently with usual optical properties. The measurements of pH (4.78), refractive index, droplet size (10.94 nm), viscosity (3.4 x 10^{-3} N·s/m².), surface tensions (4.877 x 10^{-2} N/m) are consistent with colloidal systems. Zeta potential detected the total charge of t-DCTN-SNEDDS formulation, allowing prediction of its physic stability. The release characteristic of the t-DCTN-load into the SNEDDS formulation was in agreement with others t-DCTN encapsulation systems. **CONCLUSION:** The colloidal SNEDDS carrier was obtained in order to improve t-DCTN solubility and its bioavailability aiming at to improve t-DCTN anticancer assessment in a modern biotechnologic health therapies. The applied SNEDDS system was composed by a surfactant (Tween composition) on a preferential range 10% to 15%, in the presence of bidistilled water (89% to 82%) and a vegetable oil phase (a conventional food oil) on a preferential range 1% to 3%. After physicochemical characterization the obtained formulation was classified as SNEDDS-type nanoemulsion system. This target formulation become available for several t-DCTN bionanotechnological investigations.

Keywords: *Croton cajucara Benth*, trans-dehydrocrotonin, self-nanoemulsion drug delivery system, physicochemical characterization, bioavailability for anticancer therapeutic assessments.

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ANNALS OF THE
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CYTOTOXIC POTENTIAL OF NEW 2-SUBSTITUTED FURAN-DERIVATIVES IN CANCER CELLS

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ABSTRACT

INTRODUCTION: Cancer is still a second disease with the highest mortality rates in the world, having about 100 distinct types, as the main common characteristics such as disordered cell growth and metastatic capacity. Although there are some anticancer drugs used in chemotherapy, most of them have low specificity, also attacking normal cells, causing adverse effects (INCA, 2019). Molecules containing furan ring have been found to be biologically active in several organisms. But not all furan-containing compounds are toxic. In the literature, a significant number of publications describes furan derivatives as with therapeutic properties such as antibacterial, antiviral, antifungicide, anti-inflammatory and mainly in antiproliferative against cancer cells (LISA, 2013; ZHANG et al, 2015). **AIMS:** In this work, 2-substituted furanyl compounds (PIRES et al, 2016) were evaluated for antiproliferative activity against different human and normal cancer cell lines. **METHODS:** In cell culture, NCI-H292 (human lung carcinoma), Hep-2 (human laryngeal carcinoma), MCF-7 (human breast adenocarcinoma), HL-60 (human acute promyelocytic leukemia), K562 (human chronic myelocytic leukemia) and human peripheral blood mononuclear cells (PBMC) were used cytotoxicity evaluation. The derivative (**2a**) was dissolved in dimethyl sulfoxide and added to a 96-wells plate at a final concentration of 25 μg/mL. The doxorubicin drug (5 μg/mL) was used as a standard. The cell lines presented inhibition above 70 % were incubated in concentrations ranging from 0.2 to 25 μg/mL by 72 h, to determine the concentration that inhibits 50% of growth in relation to the negative control (IC_{50}). Doxorubicin (0.034 - 5 μg/mL) was used as a standard. Human red blood cells (RBC) were used to evaluate hemolytic activity. The molecule **2a** was incubated at concentrations ranging from 1.95 to 250 μg/mL and Triton X-100 1% was used as a positive control. Experiments with human blood were performed after approval by the UFPE Research Ethics Committee (CAAE 60366816.8.0000.5208). **RESULTS AND DISCUSSION:** The molecule **2a** showed inhibition > 70% against HL-60, NCI-H292, and K562 cell lines and IC_{50} values ranging from 10.45 to 27.10 μg/mL. In the PBMC test, presented 73.23% inhibition. However, it did not cause erythrocyte hemolysis even at the highest concentration used. **CONCLUSION:** Our results showed the antiproliferative potential of **2a** since it was cytotoxic against three cancer cell lines and did not caused hemolysis in human RBC. However, more tests are necessary to discover the mechanisms of action of this compound and how the chemical structure changes during this process. The details of this work will be presented in the poster.

Keywords: Cytotoxicity, MTT Assay, Hemolytic Activity.

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FROM QSAR TO ARTIFICIAL INTELLIGENCE: PERSPECTIVES TO NEGLECTED TROPICAL DISEASES

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ABSTRACT

INTRODUCTION: QSAR is one of the methods most successfully employed during the drug discovery and lead optimization stages, due to the low cost and the efficacity to evaluate the compound activities in a short period. Outlined in 1870, when Crum-Brown and Fraser proposed that the biological activity (BA) was a mathematical function of the molecular structure: BA=f (molecular-structure). However, this process was only developed in1964 when Hansch and Fujita proposed an advance in the QSAR modelrelating the biological activity with physico-chemical parameters (HANSCH; FUJITA, 1964). Many statistical techniques are employed to build QSAR modelssuch as Multiple Linear Regression (MLR), Neural Networks (NN), Partial Least Square (PLS) and Complex Networks (CN), combined with different molecular descriptors (physical-chemical properties, topological indices, and 3D descriptors) (PIRHADI et al, 2015). In the last decades, several QSAR studies have been performed in order to identify new organic compounds with many different biological activities, like cancer and neurological disorders, but also in the field of the Neglected Tropical Diseases (NTD). **STATE OF ART:** Lima et al. (2018) reported the discovery of new inhibitors of deoxy-uridinetriphosphatase of *Plasmodium falciparum* (PfdUTPase) with *in vitro* potency against *P. falciparum* multidrug-resistant using a combi-QSAR approach followed by virtualscreening and in vitro experimental evaluation. They used a dataset containing known nucleosides reported in the literature asPfdUTPase inhibitors to build a 2D-QSAR using Hologram-QSAR(HQSAR) with different combinations of fragment distinction as atoms, bonds, connectivity, hydrogen atoms, chirality and hydrogen bond donor/acceptor, whereas Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA) were used to build 3D-QSAR models. Hit2Lead library of ChemBridge database was used in the virtual screening allowing the identification of five new potencial PfdUTPase inhibitors. The virtual hits were evaluated in several properties including logP and logS, toxicity, carcinogenicity, and hERG affinity, showing promising results in all the tests. In the end of the study they developed robust and externally predictive consensus QSAR models, merging 2D (HQSAR) and 3D-QSAR (CoMFA and CoMSIA) models to predict the activity and selectivity of new nucleosides PfdUTPase-inhibitors. In 2019, da Silva et al. realized a 2D and 3D-QSAR study with all Praziquantel (PZQ) derivatives, the first line drug for the treatment of human schistosomiasis, found in the

literature, using Generalized Linear Model (GLM), and 3D-QSAR, through a pharmacophore construction using the software Phase. The 3D-QSAR model failed due to the high similarity of the molecules, however two 2D-QSAR models were created, the first elucidated the difference between active and inactive PZQ derivatives, and the second model elucidated the difference between molecules less and more active than the PZQ. In the end of the work, they proposed and virtually screened 28 new PZQ derivates aiming the development of a second generation of PZQ (RIBEIRO DA SILVA et al, 2019). In a recently published work, Zhavoronkov et al. (2019) developed a generative tensorial reinforcement learning (GENTRL), a machine learning (ML) approachto accelerate the drug design. It provided a fast (21 days) identification of new potent inhibitors of discoidin domain receptor 1 (DDR1), a kinase target implicated in many diseases, for example fibrosis.In less than 2 months, 46 days, they identified, after the synthesis andbiological evaluation, four active compounds in the biochemical assays, two of them also actives in the cell-based assay, and one lead candidate with a promisingparmacokinetics profiles. Their work proved the ability of the Artificial Intelligence (A.I.) techniques to reduce the time and cost of drug design, especially when A.I. design is combined with QSAR techniques. **CONCLUSION & PERSPECTIVES:** The construction of QSAR models is today one of the most powerful tools to accelerate the drug discovery, hit-to-lead, and lead optimization process in many difference fields of the medicinal chemistry. Especially when combined with innovative techniques able to automate the design and virtual generation of new molecules as the Artificial Intelligence, it allows to predict the activity and to elaborate strategies to synthetize only the most biologically interesting molecules. In the NTD field, where investment from the pharmaceutical industry is lacking, it helps to reduce the costs of the project. The Drugs for Neglected Diseases Initiative (DNDi), an international non-profit research and development organization working in the field of drug design and development to NTDs, is already working in collaboration with the French start-up Iktos-Artificial Intelligence for New Drug Design, combining techniques like QSAR and A.I. to accelerate the process of drug discovery. The main perspective of the combination of QSAR and A.I. techniques is the decrease of time and cost of the drug discovery process. DNDi in collaboration with Iktos hope to discovery, in the next years, new treatments against the NTD.

Keywords: Neglected Tropical Diseases, QSAR, Deep Learning Artificial Intelligence.

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IN VITRO SENSITIZER EFFECTS OF β-LAPACHONE AND 3-METHYL-1,4-NAPHTHOQUINONE IN HL-60 ACUTE PROMIELOCITIC LEUKEMIA CELLS

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ABSTRACT

INTRODUCTION: β-lapachone is a natural product with several pharmacological uses, found in abundance in South America and has cytotoxic potential in several types of tumors in vitro and in vivo (PARK et al, 2014). Is a natural compound that is obtained from bark of the lapacho tree, and it has been reported to be a natural product that activates cell death in several cancer cell lines. The cell-specific death-inducing effects of β-lapachone are known to be directly correlated with the enzymatic activity of NAD(P)H: quinine oxidoreductase 1 (NQO1) (ZHANG et all, 2015). Synergistic activities are already described in literature as a pathway of antitumor treatment, where diseases that have an sensitizing potential can be used before effective treatment in order to promote better pharmacological action, thus such as decreasing therapeutic doses, which helps patients who have adverse effects on the high doses of convectional drugs. Studies have already demonstrated the sensitizing potential of β-lapachone for antitumor, antibacterial chemotherapy and radiotherapy (LAMBERTI et al, 2018). **AIMS:** The purpose of the present study was to evaluate the sensitizing and synergistic effect of β-lapachone with a naphthoquinic-derived compound in promyelocytic leukemia cells. **METHODS:** HL-60 cells were plated at concentration (0.3x106/mL), after β-lapachone (β-lap) at three concentrations (0.5, 1.0 and 1.5 μg/mL), was added to the plate 30 minutes and 1 hour before incubation with derivatives naphthoquinonic. Subsequently the 3-methyl-1.4 naphthoquinone (MNTq) was added at the concentration of 1.0 μg/mL. The choice of concentration was based on previous tests performed through the MTT test. For the cell viability test the cells were incubated with propidium iodide (10μg/mL). In the analysis bof cell cycle and DNA content, triton (0.1%)/propide iodide (10μg/mL) was used, and for mitochondrial depolarization test a 123 rodamine solution (1μg/mL) was used. All analyses were done on the GuavaEasyCyteHT system (Merck-Millipore) equipment using Guava Soft™ version 2.7 software. Five thousand events were evaluated by experiments carried out in triplicate and two independent experiments. For cytotoxicity assays, the IC50 values and their 95% confidence intervals were obtained by nonlinear regression with the GraphPad Prism Program Demo (Intuitive Software for Science, San Diego, CA, USA). Mean differences were compared by one-way analysis of variance (ANOVA) followed by Tukey's test or Dunnett's test ($p < 0.05$). **RESULTS AND DISCUSSION:** Morphological analysis presented dead cells with apoptotic and autophagic induction pattern, where cells showed formation of autophagic vacuoles, chromatin condensation and formation of apoptotic bodies. In the cellular viability test, associative treatment with 30 minutes of sensitivity of β-

lapachone at concentrations of 0.5 and 1.0 μg/mL sensitized the substance MNTq in the time 30 min of sensitization (0.5 μg/mL) was more sensitized than at the time of 1 however, at the concentration of 1.0 μg/mL of β-lap, sensitization was more effective in the time of 30 min. In the mitochondrial depolarization test, 1-hour time sensitization increased depolarization at all concentrations in the two tested substances. Regarding cell cycle analysis, the substance MNTq sensitized with 0.5 μg/mL of β-lap for 30 min caused cycle stop in phase G2/M, compared with negative control. However, at the time of 1 hour, cycle stop occurred in phase G1 caused by sensitization of 1.5 μg/mL of β-lap. Substances with the naphthoquinone ring are known to cause oxidative stress in the cell, which increases levels of free radicals, causing the cell to stop at cycle phases involving cyclin scan, which occurs between phases G1/ S and G2/M, which corroborates our results. In the DNA fragmentation assay, at all sensitization concentrations tested, an increase in DNA (ARAÚJO et al, 2012). Fragmentation was observed, which reiterates cell death demonstrated in the viability test. β-lap was shown in our study as a potent sensitizer, similar results were found in the study of (SUZUKI et al, 2006) that tested the sensitizing effect of β-lap the ionizing radiation on lung cancer cells. The tested compound causes increased cell death in sublethal doses when sensitized with β-lapachone, however the compound 1.4 naphthoquinone presented the most promising results in all trials, where the concentration of 0.5 μg/mL (1/2 of the value of IC_{50}) sensitized with 0.5 μg/mL of β-lapachone caused cell death and cycle stop, showing that it is possible to decrease doses, with the maintenance of therapeutic activity. **CONCLUSION:** Our results have shown that β-lapachone at low concentrations is a potent sensitizer in leukemia cells, reducing its side effects and increasing the effectiveness of other substances. MNTq caused increased cell death in sublethal doses when sensitized with β-lapachone, showing that it is possible to decrease doses, with the maintenance of therapeutic activity. These results are unheard of for these molecules together.

Keywords: Synergism, Naphthoquinone, β-lapachone , Leukemia, Cell Death.

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