Antioxidant, antimicrobial and cytotoxic activities of secondary metabolites from *Streptomyces* sp. isolated of the Amazon - Brazil region

Atividades antioxidante, antimicrobiana e citotóxica de metabólitos secundários de *Streptomyces* sp. isolado da região Amazônica – Brasil

Actividades antioxidante, antimicrobiana y citotóxica de los metabolitos secundarios de *Streptomyces* sp. aislado de la región Amazónica - Brasileña

Sandrine Maria de Arruda Lima
ORCID: https://orcid.org/0000-0002-2898-3534
Universidade Federal de Pernambuco, Brazil
E-mail: sandrinearruda@gmail.com
Pedro Silvino Pereira
ORCID: https://orcid.org/0000-0002-0001-7238
Universidade Federal de Pernambuco, Brazil
E-mail: pedro.sillvino@gmail.com
Bruno Iraquitan Miranda da Silva
ORCID: https://orcid.org/0000-0002-0633-5261
Universidade Federal de Pernambuco, Brazil
E-mail: brunoiraquitan@ufpe.br
Natalie Emanuelle Ribeiro
ORCID: https://orcid.org/0000-0003-2235-729X
Universidade de Pernambuco, Brazil
E-mail: natalierrodrigues@gmail.com
Elizabeth Fernanda de Oliveira Borba
ORCID: https://orcid.org/0000-0003-1460-027X
Universidade Federal de Pernambuco, Brazil
E-mail: elizabethfernanda_7@hotmail.com
Cícera Datiane de Morais Oliveira-Tintino
ORCID: https://orcid.org/0000-0003-1316-7064
Universidade Regional do Cariri, Brazil
E-mail: datianemorais@gmail.com
Karina Perrelli Randau
ORCID: https://orcid.org/0000-0002-4486-4420
Universidade Federal de Pernambuco, Brazil
E-mail: karina.prandau@ufpe.br
Henrique Douglas Melo Coutinho
ORCID: https://orcid.org/0000-0002-6634-4207
Universidade Regional do Cariri, Brazil
E-mail: hdmcoutinho@gmail.com
Gláucia Manoella de Souza Lima-Gomes
ORCID: https://orcid.org/0000-0002-1941-8912
Universidade Federal de Pernambuco, Brazil
E-mail: glaucia.mslima@ufpe.br
Mónica Lizeth Chávez-González
ORCID: https://orcid.org/0000-0001-6535-2558
Universidad Autonoma de Coahuila, México
E-mail: monicachavez@uadec.edu.mx
Gloria Alicia Martinez-Medina
ORCID: https://orcid.org/0000-0003-2179-4623
Universidad Autonoma de Coahuila, México
E-mail: alicia.martinez@uadec.edu.mx
Maria das Graças Carneiro-da-Cunha
ORCID: https://orcid.org/0000-0002-7219-5527
Universidade Federal de Pernambuco, Brazil
Email: mgcc@ufpe.br
Teresinha Gonçalves-Silva
ORCID: https://orcid.org/0000-0002-5971-0029
Universidade Federal de Pernambuco, Brazil
E-mail: teresinha.goncalves@ufpe.br

Abstract

Bacteria of *Streptomyces* genus are a promising source of biologically active products, with applications in medicine, industry and agriculture. Therefore, the objective of this work was to evaluate the cytotoxic, antioxidant and antimicrobial activities of fermented rice extract and their semipurified fractions from *Streptomyces* spp. isolated of the rhizosphere of *Paullinia cupana*, Amazon-Brazil. For this, a bioguided study was carried out by the cytotoxic activity with methanolic extract of *Streptomyces* sp. ACTMS-12H UFPEDA 3405 (EMeOH-12H) partitioned with n-hexane, ethyl acetate and 2-butanol. The antioxidant activity was analyzed using the DPPH, ABTS and phosphomolybdenum methods, while the antimicrobial activity was investigated by microdilution method to determine the minimum inhibitory concentration (MIC) against species of bacteria and yeast. In the cytotoxicity test, the butanolic phase (FbuOH-12H) presented IC_{50} of 1.1 µg/mL against MOLT-4, with cell death probably by apoptosis, but did not cause cytotoxicity on peripheral blood mononuclear cell (PBMC) or human erythrocytes. Chemical prospecting detected the presence of saponins and reducing sugars on 2-butanol fraction (FBuOH-12H), which can be related to cytotoxicity. On the antioxidant activity by ABTS, the partition with ethyl acetate (FAcOEt-12H) showed antioxidant capacity of 1161.7 ± 0.04 µM of Trolox/g of extract, indicating an expressive reactivity of the phase with this radical. The aqueous phases (from hexane, ethyl acetate and methanol extracts) were active in all tested microorganisms, except *E. faecalis*.

Keywords: Actinobacteria; Anticancer; Phenolic compounds; Citotoxicity.

Resumo

As bactérias de gênero *Streptomyces* são fontes promissoras de produtos biologicamente ativos, com aplicações na medicina, indústria e agricultura. Portanto, o objetivo deste trabalho foi avaliar as atividades citotóxica, antioxidante e antimicrobiana do extrato fermentado em arroz e suas frações semipurificadas de *Streptomyces* spp. isolado da rizosfera de *Paullinia cupana*, Amazônia, Brasil. O estudo bioguiado foi realizado através da atividade citotóxica com o extrato metanólico de *Streptomyces* sp. ACTMS-12H UFPEDA 3405 (EMeOH-12H) particionado com n-hexano, acetato de etila e 2-butanol. A atividade antioxidante foi analisada pelos métodos do DPPH, ABTS e fosfomilibidênio, enquanto que a atividade antimicrobiana foi feita pelo método da microdiluição para determinar a concentração inibitória minima (MIC) frente a espécies de bactérias e levedura. No teste de citotoxicidade, a fase butanólica (FbuOH-12H) apresentou IC_{50} de 1,1 µg/mL frente à linhagem MOLT-4, com morte celular provavelmente por apoptose, mas não causou citotoxicidade em células mononucleares do sangue periférico (PBMC) nem em eritrócitos humanos. A prospecção fitoquímica detectou a presença de saponinas e açúcares redutores na fração 2-butanol (FBuOH-12H), e estes compostos podem estar relacionados à citotoxicidade. Na atividade antioxidante pelo ABTS, a partição com acetato de etila (FAcOEt-12H) apresentou capacidade antioxidante de 1161,7 ± 0,04 µM de Trolox/g do extrato, indicando uma expressiva reatividade da fase com este radical. A fase aquosa (dos extratos hexano, acetato de etila e metanol) foi ativa para todos os microrganismos testados, exceto *E. faecalis*.

Palavras-chave: Actinobactéria; Anticâncer; Compostos fenólicos; Citotoxicidade.

Resumen

Las bacterias del género *Streptomyces* son una fuente prometedora de productos biológicamente activos, con aplicaciones en medicina, industria y agricultura. Por lo tanto, el objetivo de este trabajo fue evaluar las actividades citotóxicas, antioxidantes y antimicrobianas de los extractos y fracciones semipurificadas de *Streptomyces* spp. aislada de la rizósfera de *Paullinia cupana*, Amazonas-Brasil. Para ello, se realizó un estudio bioguiado de la actividad citotóxica con extracto metanólico de *Streptomyces* sp. El extracto ACTMS-12H UFPEDA 3405 (EMeOH-12H) fue fraccionado con n-hexano, acetato de etilo y 2-butanol. La actividad antioxidante fue analizada a través de los métodos de DPPH, ABTS y fosfomolibdeno, mientras que la actividad antimicrobiana fue investigada por el método de microdilución para determinar la concentración minima inhibitoria (MIC) contra especies de bacterias y levaduras. En la prueba de citotoxicidad la fase butanólica (FbuOH-12H) presentó una IC_{50} de 1.1 µg/mL contra MOLT-4, con muerte celular probablemente por apoptosis, pero no causó citotoxicidad en las células mononucleares de sangre periférica (PBMC) o en eritrocitos humanos, La prospección química detectó la presencia de saponinas y azúcares reductores en la fracción de 2-butanol (FBuOH-12H), que pueden estar relacionados con la citotoxicidad. Sobre la actividad antioxidante por ABTS, la fracción de acetato de etilo (FAcOEt-12H) mostró una capacidad antioxidante de 1161.7 ± 0.04 µM de Trolox/g de extracto, indicando una expresiva reactividad de la fase con este radical. Las fases acuosas (de los extractos de hexano, acetato de etilo y metanol) fueron activas en todos los microorganismos probados, excepto E. faecalis.

Palabras clave: Actinobacteria; Anticancer; Compuestos fenólicos; Citotoxicidad.

1. Introduction

Bacteria of the genus *Streptomyces* are known to produce a wide variety of antibiotics and other bioactive compounds, being this genus the major producer in number and diversity of new antibiotics when compared to other bacterial genera

(Rashad et al., 2015). The main classes of antibiotics produced by *Streptomyces* include aminoglycosides, anthracyclines, glycopeptides, β-lactams, macrolides, nucleosides, peptides, polyenes, polyesters and tetracyclines (Kekuda, Shobha, Onkarappa, 2010; Jakubiec-Krzesniak et al., 2018).

Streptomyces can be found in a diversity of habitats, particularly in the soil and in aquatic or endophytic environments. Besides the antibiotic activity, secondary metabolites produced by *Streptomyces* are described in the literature as antifungal (Sharma, Manhas, 2020), antiviral (Jakubiec-Krzesniak et al., 2018), anticancer (Tan et al., 2015; Nguyen et l., 2020), antioxidants (Tan et al., 2018) and cytotoxic agents (Lima et al., 2017).

In the market, there is a range of antibiotic drugs of various chemical classes; however, it is still necessary to investigate new molecules due to the indiscriminate use of these drugs and the increasingly frequent emergence of multiresistant bacterial strains. Many of these antibiotics are also used in the fight against cancer. According to the American Cancer Society, cancer is the second-largest cause of death in the world, behind only cardiovascular diseases (Tan et al., 2015). Compared to microorganisms, cancer cells can develop resistance for existing drugs by two mechanisms: increased expression of efflux pumps and overexpression of anti-apoptotic molecules (Creixell, Peppas, 2012). Therefore, is important to find new molecules with anticancer activities. However, the introduction of a new drug on the market is a lengthy process, laborious and often flawed (Khanna, 2012). Thus, pre-clinical tests in animal models and *in vitro* tests on cells, tissues or biochemical functions are fundamental to guarantee the safety and continuity of the prospection of new drugs in humans (Scott, Peters, Dragan, 2013).

Therefore, the objective of this work was to investigate the antimicrobial, antioxidant and cytotoxic activities of *Streptomyces* spp. secondary metabolites isolated of the rhizosphere of *Paullinia cupana* from Amazon-Brazil region. It is justified by the potential that the *Streptomyces* genus presents for producing useful metabolites for the pharmaceutical industry, besides to contribute to the knowledge of the biotechnological potential of microorganisms in this region.

2. Methodology

This study is an experimental research (Pereira et al.,2018) characterized by directly manipulating variables related to the object of study and aims to test the hypothesis that the chemical constituents present in fermented from *Streptomyces* sp. have promising biological activities.

2.1 Isolation of Streptomyces spp.

The bacterial strains *Streptomyces* sp. ACTMS-12H (UFPEDA 3405) was isolated from the rhizosphere soil of *P. cupana* (Kunth) var. sorbilis (Mart.) Ducke, a plant native to the Amazon-Brazil, known as "guaraná". The isolation of *Streptomyces* spp. was performed using the scattering technique (Ellaiah et al., 2004), in which 10 g of soil were suspended in 90 mL of sterile saline phosphate buffer solution. The sample was shaken (180 rpm, 15 minutes), incubated at 50 °C for 10 minutes and serially diluted until 10-5. Aliquots of 100 µL of 10-3 to 10-5 were dispersed in yeast extract medium - solid Arginine (ALA) (0.03 L-arginine; 0.1 Glucose; 0.1 Glycerol; 0.03 K_2HPO_4; 0.02 $MgSO_4 \cdot 7H_2O$; 0.03 NaCl; 0.1 yeast extract; 0.1 mL of trace solution (1 $FeSO_4 \cdot 7H_2O$; 0,1 $MnSO_4 \cdot 7H_2O$; 0,1 $CuSO_4 \cdot 5H_2O$; 0,1 $ZnSO_4 \cdot 7H_2O$) supplemented with benlate 100 µg/mL and penicillin 3.5 µg/mL. The plate was incubated at 30 °C for 21 days. The strain was deposited in the Collection of Microorganisms of the Department of Antibiotics (UFPEDA), Federal University of Pernambuco (Brazil), under immersion in mineral oil and lyophilized.

2.2 Fermentation, extraction and partitioning of extracts

The pre-inoculum of *Streptomyces* sp. ACTMS-12H (UFPEDA 3405) occurred in Erlenmeyer flask of 500 mL

containing 100 mL of medium ISP-2 liquid. The culture was inoculated with agarose blocks in the lineage ISP-2, cultured for 14 days at 30 °C and subjected to agitation for 48 h (200 rpm 30 °C). About 10 mL of this pre-inoculum was added in 500 mL Erlenmeyer flask with 90 g of cooked rice incubated for 21 days at 30 °C. To the culture, 150 mL of methanol was added and left for 48 h a 30 °C. The culture was extracted twice and the extract was dried on a rotary evaporator at 50 °C. The methanol extract (0.5 g) from *Streptomyces* sp. ACTMS-12H (UFPEDA 3405) was diluted in distilled water and partitioned with n-hexane (FHex-12H), ethyl acetate (FAcOEt-12H) and 2-buthanol (FBuOH-12H) in the proportions of 1:3. The extracts obtained were dried in a rotary evaporator under reduced pressure at 50 °C, while the aqueous phases from each partition were evaporated at 100 °C.

2.3 Chemical prospecting and total phenolic content

Aliquots of 10 μL of the extracts and phases were analysed by thin layer chromatography (TLC) on silica gel aluminium chromate sheets 60 F254 (Merck) to identify the classes of secondary metabolites: alkaloids, reducing sugars, phenolic compounds, flavonoids, tannins, triterpenes - steroids and saponins. Several mobile phases and specific developers were employed (Harborne, 1998). The total phenolic content was determined according to the Folin-Ciocalteu method (Ainsworth, Gillespie, 2007) using gallic acid as a standard phenolic compound. The extract and phases were solubilized in ethanol at a final concentration of 1 mg/mL. An aliquot of 30 μL of each sample was transferred to the 96-well plate, followed by the addition of water, 15% m/v sodium carbonate and Folin-Ciocalteu reagent at the final concentration of 100 μg/mL. After 2 h, the absorbance of the samples was measured using a spectrophotometer at 760 nm. The analyses were performed in triplicate and the total phenol content was determined by interpolating the absorbance of the samples against a calibration curve constructed with solutions of the gallic acid standard and expressed as milligrams of gallic acid per gram of extract (mg EAG/g).

2.4 Cytotoxicity

2.4.1 Tumoral Cells

The cell lines used were HEp-2 (laryngeal squamous cell carcinoma), HT-29 (human colon cancer), MCF-7 (human breast cancer), NCI-H292 (human mucoepidermoid carcinoma of lung), HL-60 (promyelocytic leukemia acute), K-562 (chronic myelocytic leukemia) and MOLT-4 (acute lymphoblastic leukemia) obtained from Rio de Janeiro Cell Bank, Brazil. The cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 nM L-glutamine at 37 °C with 5% CO_2. The cells were plated in 96-well plates (10^5 cells/well for adhered cells and 0.3×10^6 cells/well for cells in suspension). After 24 h, the extracts and phases were added to the wells in a single concentration (50 μg/mL). The extracts and/or fractions that inhibited cell growth above 70% in more than three strains using a concentration of 50 μg/mL, were subjected to cytotoxicity testing in various concentrations (0.39 - 50 μg/mL) to obtain the IC_{50}. Doxorubicin (0.39 - 5 μg/mL) was used as a positive control. After 72 h of incubation with the products, 25 μL of MTT (5 mg/mL) was added to each well. After 3 h, the culture medium with the excess of MTT was aspirated and 100 μL of DMSO was added to each well for the dissolution of formazan crystals (Alley et al., 1988). The absorbance was read on a spectrophotometer at a wavelength of 540 nm.

2.4.2 Peripheral blood mononuclear cell (PBMCs) cytotoxicity

PBMC cells (peripheral blood mononuclear cells - lymphocytes and monocytes) were obtained from peripheral blood from healthy volunteers (3 mL), that was added to 5 mL of phosphate-buffered saline. The experiment was carried out in accordance with the rules approved by the Ethics Committee on Human Research at the Federal University of Pernambuco (n°

61757616.0.0000.508). The steps to isolation included the addition of 3 mL of Ficoll Histopaque-1077, followed by 30 min of centrifugation at 1500 rpm and aspiration of the mononuclear cells present on the intermediate region between the red cells and the serum. The lymphocyte suspension was transferred to another tube and PBS was added to a volume of 11 mL and centrifuged for 20 min at 1000 rpm. The supernatant was discarded and the lymphocyte pellet was resuspended in 2 mL of RPMI 1640 medium supplemented with 20% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin to a final concentration of 10^6 cells/mL. Lymphocyte proliferation was induced by the addition of 3% phytohemagglutinin, a mitogen that acts on T lymphocytes (Liu et al., 2004).

To determine cytotoxicity with PBMCs, extracts and phases were added into cell cultures in the 96-well plate on serial concentrations (0.39 - 50 µg/mL). After 48 h of incubation of the products at 37 °C with an atmosphere of 5% CO_2 and 95% humidity, 10 µL of the stock solution (0.312 mg/mL) of alamar blue was added. The microplate was reincubated for 24 h and the absorbances were measured on a spectrophotometer at a wavelength of 570 nm (reduced) and 595 nm (oxidized) (Ahmed, Gogal, Walsh, 1994).

2.5 Hemolytic activity

The hemolytic assay was performed with peripheral blood from healthy volunteers in accordance with the standards approved by the Ethics Committee for Research with Human Beings of the Federal University of Pernambuco, Brazil (nº 61757616.0.0000.508). The erythrocytes were washed with saline solution (0.85% NaCl + 10 mM $CaCl_2$) by centrifugation (3000 rpm/5min), the supernatant was discarded and the precipitate resuspended in saline to obtain a 2% erythrocyte suspension (ES).

This experiment was carried out in 96-well plates, distributed as follows: 100 µL of saline solution (negative control); 50 µL of saline solution and 50 µL of the vehicle (white); 80 µL of saline solution + 20 µL of Triton X - 100 to 1% (positive control); 100 µL of saline solution + 100 µL of the extracts and phases that showed cytotoxicity on tumor cells (0.0152 - 2,000 µg/mL) were plated. Then, 100 µL of the erythrocyte solution was plated in all wells. After 1 h incubation, under constant agitation at room temperature, the plate was allowed to stand for 1 h, where the supernatant was transferred to another plate and the absorbance was measured in a microplate reader at a wavelength of 540 nm. Samples with EC_{50} values < 200 µg/mL are considered hemolytic (Costa-Lotufo et al., 2002).

2.6 Morphological analysis

MOLT-4 line cells were distributed in 24-well plates at a concentration of 10^6 cells/mL and incubated with the butanol phase (FBuOH-12H) at concentrations equal to IC_{50}, 1/2 of IC_{50} and 2x IC_{50} determined at 24 h. Doxorubicin was used as a positive control and the negative control was treated with the vehicle (DMSO; 0.1%). After incubating the cells with the samples, aliquots of the cell suspension (50 µL) were placed in the slide centrifuge for slide preparation (cytospin). After the cells adhered to the slide, fixation was done with methanol for 1 min, followed by staining by May-Grunwald for 10 seconds, Giemsa for another 10 seconds and washed under running water. The slides were stained and analyzed under an optical microscope to assess their morphological characteristics. The recording of cellular changes was made by photography.

2.7 Antioxidant activity
2.7.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activities of the extracts were determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as free radicals according to (Brand-Williams et al., 1995), with modifications described by (Ma et al., 2011). Through preliminary screening, appropriate amounts of the extracts and phases prepared in concentrations ranging from 0.1 to 5 mg/mL

were transferred to 96-well plates and the DPPH solution was added (23.6 µg/mL in EtOH). Ascorbic acid was used as a positive control. After 30 min under agitation and protection from light, absorbance was read on a spectrophotometer at 517 nm. The scavenging activity (SA%) was expressed as a percentage according to the equation: SA% = 100 x [(Abs control – Abs test)/Abs control]. The EC_{50} value is the concentration of sample necessary to sequester 50% of the free radical DPPH.

2.7.2 Antioxidant activity by 2,2–azino bis (3–ethyl benzothiazoline–6–sulphonic acid) Radical (ABTS) assay

The antioxidant activity of the extract was performed by ABTS assay was performed according to the methodology of Re et al. (2002) adapted by De Almeida et al. (2017). The cationic radical ABTS+ was formed by the reaction between 2.45 mM of potassium persulfate and 7 mM of 2,2–azino bis (3–ethyl benzothiazoline–6–sulphonic acid) radical (ABTS), stored in the dark at room temperature for 16 h. Once formed, the radical ABTS+ it was diluted in ethanol until the absorbance of 0.70 ± 0.05 was obtained at a wavelength of 754 nm. Then, 20 µL of the extracts and phases (1 mg/mL) was added to 980 µL of the solution containing the radical, and the solution was stirred for a few seconds. After 6 min of the reaction in the dark, absorbance was measured. Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) was used as a standard at concentrations of 0; 500; 1000; 1500; 2,000 and 2,500 mM in ethanol. ABTS antioxidant capacity was express in µM of TEAC (Trolox equivalent antioxidant capacity) per gram of the extract and in the percentage of oxidative inhibition.

2.7.3 Phosphomolybdenum method

This assay is based on the spectrophotometric determination of the reduction of Mo^{+6} to Mo^{+5}, with subsequent formation of a phosphate-Mo^{+5} complex (Prietro, Pineda, Aguilar, 1999). Aliquots of 0.1 mL of the extracts and phases (1 mg/mL) were dissolved in distilled water and added with 1 mL of the reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were kept in closed tubes and incubated at 95 °C for 90 min. After cooling to room temperature, absorbance was determined at 695 nm. The total antioxidant activity was expressed in relation to ascorbic acid and calculated using the formula: TAA% = (Abs samples - Abs white) x 100/(Ascorbic acid abs - White abs).

2.8 Antimicrobial activity

2.8.1 Microorganisms

The microorganisms used in the antimicrobial activity test were: *Staphylococcus aureus* (UFPEDA 02), *Staphylococcus aureus* (UFPEDA 700), *Staphylococcus aureus* (UFPEDA 705), *Staphylococcus aureus* (UFPEDA 707), *Staphylococcus aureus* (UFPEDA 729), *Bacillus subtilis* (UFPEDA 86), *Micrococcus luteus* (UFPEDA 100), *Enterococcus faecalis* (UFPEDA 138), *Escherichia coli* (UFPEDA 224), *Serratia marcescens* (UFPEDA 352), *Psedomonas aeruginosa* (UFPEDA 416), *Pseudomonas aeruginosa* (UFPEDA 261), *Pseudomonas aeruginosa* (UFPEDA 262), *Pseudomonas aeruginosa* (UFPEDA 64); an acid-resistant alcohol bacteria: *Mycobacterium smegmatis* (UFPEDA 71) and a yeast *Candida albicans* (UFPEDA 1007). All microorganisms were obtained from the Microbial Collection of the Department of Antibiotics of the Federal University of Pernambuco- Brazil (UFPEDA).

2.8.2 Minimum Inhibitory Concentration (MIC), Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

The determination of the minimum inhibitory concentration (MIC) was performed using the microdilution technique, in 96-well plates, according to the Clinical and Laboratory Standards Institute (CLSI, 2008; CLSI, 2017). Minimum bactericidal concentration and minimum fungicidal concentration (MBC and MFC) were determined by the subculture of the MIC wells in a Petri dish containing the solid medium BHI (brain heart infusion), MH (Mueller Hinton) or SAB (Sabouraud), incubated at 37 °C for 18-24 h for bacteria and 30 °C for 48 h for yeast.

2.9 Statistical analysis

Antimicrobial activity was performed in triplicate and the diameter of the halos was expressed by the mean ± standard deviation. The EC_{50} values of antioxidant activity were calculated from non-linear regression in the GraphPad Prism 5.0 program, where each sample was tested in triplicate. The IC_{50} and EC_{50} values of cytotoxicity and hemolytic activity, respectively, with their 95% confidence intervals, were calculated from non-linear regression, using the software GraphPad Prism.

3. Results

3.1 Isolation of Streptomyces spp.

Based on cultural, micromorphological, biochemical characterizations and antibiotic resistance, strain isolated from the rhizosphere soil of *P. cupana* (Kunth) var. sorbilis (Mart.) Ducke, Amazonas-Brasil, belong to the genus *Streptomyces*. This genus belongs to the family Streptomycetaceae, suborder Streptomycineae, order Actinomycetales, subclass Actinobacteridae, class Actinobacteria, phylum Actinobacteria, domain Bacteria and has the largest number of described species.

The strain of *Streptomyces* spp. showed good growth in the middle of the rice. From 90 g of parboiled rice, was found the yield 18,74% to the EMeOH-12H.

The EMeOH-12H (0.5 g) was partitioned with n-hexane, producing a hexane phase with a yield of 0.48% (0.0024 g); when EMeOH-12H was partitioned with ethyl acetate, the yield of the fraction was 3.56% (0.018 g), and when partitioned with 2-butanol, showed an n-butanol fraction with a yield of 56.88% (0.28 g) (Scheme 1).

Scheme 1. Flowchart of liquid-liquid partition of the methanolic extract of the fermented *Streptomyces* sp. ACTMS-12H UFPEDA 3405 (EMeOH-12H).
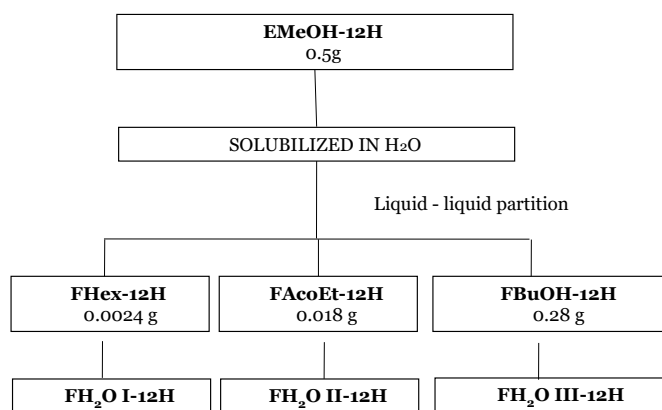
FHex-12H: Hexane phase; FAcOEt-12H: Acetoethyl phase; FBuOH-12H: Butanolic phase; FH2O I-12H: Aqueous phase I 12H; FH2O II-12H: aqueous phase II; FH2O III-12H: Aqueous phase III.
Source: Authors.

3.2 Chemical prospecting and quantification of the total phenolic content

The results of chemical prospecting of the extract EMeOH-12H indicated the presence of flavonoids, monoterpenes/sesquiterpenes, triterpenes/steroids, saponins and reducing sugars. On FHex-12H were found monoterpenes/sesquiterpenes and triterpenes/steroids; on FAcOEt-12H were found flavonoids and minor quantity of triterpenes and steroids; and in FBuOH were found flavonoids, saponin and reducing sugars (Table 1).

Table 1. Chemical prospecting by thin layer chromatography (TLC) of secondary metabolites produced by *Streptomyces* sp. UFPEDA 3405.

Metabolites	*Streptomyces* sp. UFPEDA 3405			
	EMeOH-12H	FHex-12H	FAcOEt-12H	FBuOH-12H
Polyphenols				
Flavonoids	+++	-	+++	+++
Cinnamic derivatives	-	-	-	-
Cumarins	-	-	-	-
Hydrolysable tannins	-	-	-	-
Condensed tannins	-	-	-	-
Anthraquinones	-	-	-	-
Alcaloids	-	-	-	-
Monoterpenes and sesquiterpenes	++	++	-	-
Triterpenes and steroids	++	+	+	+
Saponins	+	-	-	+
Reducing sugars	+	-	-	+

EMeOH-12H: Methanolic extract of the secondary metabolites from *Streptomyces* sp. ACTMS-12H UFPEDA 3405; FHex-12H: Hexane phase; FAcOEt-12H: Acetoethyl phase; FBuOH-12H: Butanolic phase; FH2O I-12H: Aqueous phase I 12H; FH2O II-12H: Aqueous phase II; FH2O III-12H: Aqueous phase III. Presence of metabolites: (+) up to two bands; (++) from two to five bands; (+++) from five to eight bands; (-) Absence.
Source: Authors.

The content of total phenolics found in the extracts and phases ranged from 2.3 to 5.8 mg EAG/g, with no total phenolic compounds being detected in the FHex-12H and FH_2O III-12H fractions, confirming the result of chemical prospecting (Table 2).

Table 2. Total phenolic content of extract and fractions from *Streptomyces* sp. UFPEDA 3405.

Extracts/ Partitions	Total phenolic content (mg EAG/g)
EMeOH-12H	5.8 ± 0.5
FHex-12H	n.d.
FAcOEt-12H	2.3 ± 0.2
FBuOH-12H	5.5 ± 0.4
FH_2O I-12H	5.3 ± 0.5
FH_2O II-12H	2.9 ± 0.2
FH_2O III-12H	n.d.

EMeOH-12H: Methanolic extract of *Streptomyces* sp. ACTMS-12H UFPEDA 3405; FHex-12H: Hexane phase; FAcOEt-12H: Acetoethyl phase; FBuOH-12H: Butanolic phase; FH2O I-12H: Aqueous phase I; 12H; FH2O II-12H: aqueous phase II; FH2O III-12H: aqueous phase III; n.d.: not detected.
Sorce: Authors.

3.3 Cytotoxic activity on tumor cells

EMeOH-12H extract, at a concentration of 50 µg/mL, showed high cytotoxic activity against the strains NCI-H292, MOLT-4 and HL-60. With the exception of the FHex-12H phase, all other phases showed high cytotoxicity against MOLT-4,

with emphasis for FBuOH-12H, which also exhibited activity against HEp-2, HT-29 and HL-60 (Table 3).

Table 3. Percentage of cell growth inhibition (IC%) of extract and partitions of the *Streptomyces* sp. UFPEDA 3405 on tumor line cells at a concentration of 50 µg/mL by the MTT method, after 72 h of incubation.

Extract/Partitions	Lines cells (% inhibition of cell growth)						
	HEp-2	HT-29	MCF-7	NCI-H292	HL-60	K-562	MOLT-4
EMeOH-12H	50.7 ± 3.3	54.2 ± 1.8	52.9 ± 1.1	97.5 ± 0.6	83.2 ± 5.2	56.5 ± 0.8	95.9 ± 1.4
FHex-12H	57.2 ± 5.1	45.3 ± 2.8	50.3 ± 4.1	68.0 ± 0.2	84.2 ± 0.5	61.9 ± 0.8	54.2 ± 4.6
FAcOEt-12H	73.4 ± 3.4	44.7 ± 3.4	42.4 ± 2.8	58.0 ± 0.7	61.5 ± 4.2	29.6 ± 2.4	86.2 ± 1.6
FBuOH-12H	77.6 ± 2.4	89.9 ± 0.7	57.6 ± 1.4	64.8 ± 0.9	81.5 ± 0.2	71.5 ± 1.9	91.8 ± 1.7
FH$_2$O I-12H	45.6 ± 4.0	64.2 ± 1.7	30.4 ± 0.8	50.0 ± 1.2	16.4 ± 0.3	50.2 ± 2.9	92.2 ± 1.5
FH$_2$O II-12H	78.3 ± 1.8	61.9 ± 2.9	51.3 ± 2.8	43.8 ± 1.4	69.9 ± 0.5	$72.4 \pm 1,6$	90.0 ± 1.5
FH$_2$O III-12H	71.5 ± 1.9	52.9 ± 1.8	38.6 ± 1.2	58.9 ± 1.3	33.3 ± 0.7	33.2 ± 2.0	91.6 ± 2.7
Doxorubicin	79.4 ± 2.6	75.3 ± 1.3	74.8 ± 2.1	94.2 ± 1.9	92.9 ± 0.6	79.0 ± 0.1	96.6 ± 0.9

EMeOH-12H: Methanolic extract of *Streptomyces* sp. ACTMS-12H UFPEDA 3405; FHex-12H: Hexane phase; FAcOEt-12H: Acetoethyl phase; FBuOH-12H: Butanolic phase; FH$_2$O I-12H: Aqueous phase I 12H; FH$_2$O II-12H: aqueous phase II; FH$_2$O III-12H: Aqueous phase III. Low activity: $IC_{50} \leq 50\%$; moderate activity: IC_{50} between 51 - 75%; high activity: $IC_{50} > 75\%$ (Mahmoud et al., 2011). The percentage of inhibition x log concentration calculated from non-linear regression, using the GraphPad Prism 5.0 program.
Source: Authors.

The IC_{50} was determined only for extracts and phases that showed inhibition of cell growth above 75% when tested a single dose of 50 µg/mL. The EMeOH-12H extract was more cytotoxic for the HL-60 and MOLT-4 strains, with IC_{50} 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 the HL-60 (IC_{50} = 1.4 µg/mL) and MOLT-4 (IC_{50} = 1.1 µg/mL) (Table 4).

Table 4. Inhibitory concentration (IC_{50}) and effective concentration (EC_{50}) of the extracts and partitions from *Streptomyces* sp. UFPEDA 3405.

Extracts/ Partitions	IC$_{50}$ /IC 95%						CE$_{50}$ /IC 95%
	HEp-2	HT-29	NCI-H292	HL-60	MOLT-4	PBMC	Erythrocytes
EMeOH-12H	n.t.	n.t.	15.0	5.4	4.1	> 50	> 200
			12.9 - 17.6	4.2 - 6.8	3.6 - 4.6		
FHex-12H	n.t.	n.t.	n.t.	7.6	n.t.	> 50	> 200
				6.4 - 9.1			
FAcOEt-12H	n.t.	n.t.	n.t.	n.t.	13.4	> 50	> 200
					10.7 - 16.7		
FBuOH 12H	10.6	6.2	n.t.	1.4	1.1	> 50	> 200
	8.2 - 13.7	5.2 - 7.4		1.2 - 1.5	0.9 - 1.4		
FH$_2$O I-12H	n.t.	n.t.	n.t.	n.t.	7.3	> 50	> 200
					6.2 - 8.6		
FH$_2$O II-12H	26,5	n.t.	n.t.	n.t.	4.3	> 50	> 200
	17,9 - 39,3				3.3 - 5.6		
FH$_2$O III-12H	n.t.	n.t.	n.t.	n.t.	14.5	> 50	> 200
					10.6 - 19.9		
Doxorubicin	0.7	0.4	0.01	0.06	0.04	n.t	n.t.
	0.3 - 1.4	0.3 - 0.5	0.004 - 0.3	0.05 - 0.08	0.04 - 0.05		

EMeOH-12H: Methanolic extract of *Streptomyces* sp. ACTMS-12H UFPEDA 3405; FHex-12H: Hexane phase; FAcOEt-12H: Acetoethyl phase; FBuOH-12H: Butanolic phase; FH2O I-12H: Aqueous phase I 12H; FH2O II-12H: aqueous phase II; FH$_2$O III-12H: aqueous phase III; n.t.: not tested; IC_{50} and EC_{50} with their 95% confidence intervals (IC 95%) were calculated from non-linear regression, using the GraphPad Prism 5.0 program. Each sample was tested in triplicate in two independent experiments.
Source: Authors.

3.4 Cytotoxic activity on peripheral blood mononuclear cells (PBMC) and erythrocytes

The purpose of this test is to assess the selectivity of the compound. The EMeOH-12H and its phases had $IC_{50} > 50$ µg/mL (Table 4). To assess hemolytic activity in human erythrocytes, extract and phases that presented cytotoxic activity in at least one tumor line were selected. EMeOH-12H and its phases showed $EC_{50} > 200$ µg/mL (Table 4), being considered non-hemolytic.

3.5 Morphological analysis

The morphology of MOLT-4 cells was evaluated by the May-Grunwald-Giemsa staining, after treatment of 24 h with FBuOH-12H at concentrations of 9, 18 or 36 µg/mL, values corresponding to ½ IC_{50}, IC_{50} and 2x IC_{50}, respectively (Table 5). The FBuOH-12H phase was selected for a brief study concerning its possible mechanism of action, as it presents cytotoxic activity in tumor lines and does not exhibit cytotoxicity in PBMC or hemolytic activity in human's erythrocytes.

The analysis by optical microscopy revealed several morphological changes induced by FBuOH-12H. The cells of the negative control (untreated) exhibited typical morphology of non-adherent cells, such as: intact membrane, pleomorphic cells and clear visualization of the plasma and nuclear membranes. In the groups treated with 9, 18 or 36 µg/mL of FBuOH-12H, the cells showed a morphology consistent with cells undergoing apoptosis, including reduced cell volume and chromatin condensation. At the highest concentration, loss of membrane integrity was observed, a characteristic of necrosis (Figure 1).

Table 5. Values of the concentration of the butanol partition that inhibits 50% of cell growth (IC_{50}) and the 95% confidence interval (95% CI) in MOLT-4 after 24 and 48 h of incubation.

	MOLT-4	
Samples	**24 h**	**48 h**
FBuOH-12H	17.56	5.56
	14.33-21.52	4.53 – 6.81
Doxorubicin	3.28	0.09
	2.35 – 4.57	0.08 – 0.11

IC_{50} with its 95% confidence intervals were calculated from non-linear regression, using the GraphPad Prism 5.0 program. Each sample was tested in triplicate in two independent experiments.
Source: Authors.

Figure 1. Morphological analysis by the May-Grunwald-Giemsa stain after 24 h of incubation with the FBuOH-12H partition.
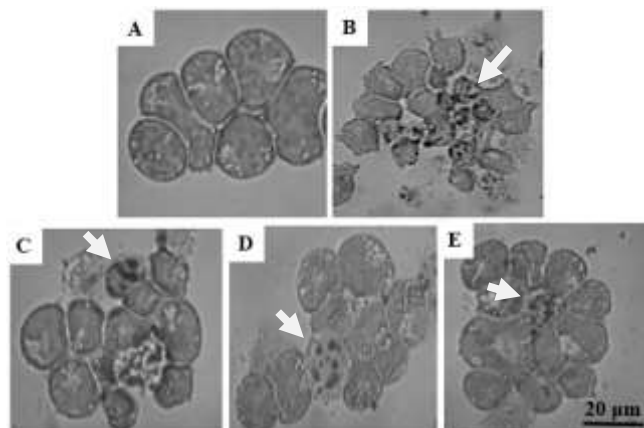
A: Negative control (DMSO); B: Positive control (Doxorubicin 3 µg/mL); C: FBuOH-12H (9 µg/mL), D: FBuOH (18 µg/mL); E: FBuOH (36 µg/mL). Visualized under an optical microscope at 1,000X magnification. Arrow: cells with features of cell death by apoptosis. Source: Authors.

3.6 Antioxidant activity

The antioxidant activity of the extracts and phases by the DPPH method showed EC_{50} values > 500 µg/mL (concentration of the sample necessary to sequester 50% of the free radical of DPPH), while the ascorbic acid at EC_{50} was 4.82 ± 0.06 µg/mL. On the ABTS method, the lowest CAET value was for EMeOH-4T (40.0 ± 0.01 µM of Trolox/g of the extract) with a 10% oxidative inhibition percentage at a concentration of 20 µg/mL, and the highest value for FAcOEt-12H (1161.7 ± 0.04 µM of Trolox/g of the extract) with a percentage of 50% at the same concentration.

No activity was detected on FBuOH-12H using ABTS method. On the phosphomolybdenum method, the best activity was found for EMeOH-12H extract, which presented 5.0 ± 0.3% of the total antioxidant capacity. The FAcOET-12H presented no activity on phosphomolybdenum method (Table 6).

Table 6. Antioxidant activity of extract and fractions obtained of strains of *Streptomyces* sp. UFPEDA 3405.

Extract/fractions	DPPH (CE$_{50}$ µg/mL ± SD)	ABTS CAET (µM/g) / (% ± SD)	Phosphomolibidenium (% ± SD)
EMeOH-12H	> 500	333.3 ± 0.02 / 22.5 ± 0.2	5.0 ± 0.3
FHex-12H	> 500	480.0 ± 0.02 / 23.3 ± 0.1	1.7 ± 0.1
FAcOEt-12H	> 500	1161.7 ± 0.05 / 52.8 ± 0.5	n.d.
FBuOH12H	> 500	n.d.	1.2 ± 0.0
FH$_2$O I-12H	> 500	601.7 ± 0.02 / 31.7 ± 1.0	1.5 ± 0,1
FH$_2$O II-12H	> 500	573.3 ± 0.01 / 32.4 ± 0.9	1.7 ± 0.0
FH$_2$O III-12H	> 500	508.3 ± 0.02 / 26.7 ± 0.8	1.4 ± 0.0
Ascorbic acid	4.8 ± 0.06	-	-

EMeOH 12H: Methanolic extract of *Streptomyces* sp. ACTMS-12H UFPEDA 3405; FHex 12H: Hexane phase; FAcOEt 12H: Acetoethyl phase; FBuOH 12H: Butanolic phase; FH$_2$O I 12H: Aqueous phase I 12H; FH$_2$O II 12H: Aqueous phase II; FH$_2$O III 12H: Aqueous phase III; n.d.: not detected.
Source: Authors.

3.7 Antimicrobial activity

All aqueous phases from *Streptomyces* sp. ACTMS-12H UFPEDA 3405 showed activity against Gram-positive,

Gram-negative, acid- alcohol resistant bacteria and yeast. The FH_2O III-12H was active against *E. coli, S. marcences, P. aeruginosa, M. smegmatis* and *C. albicans* with MIC values of 620, 620, 310, 620, 310 µg/mL, respectively (Table 7). The FAcOEt-12H and FBuOH-12H were active only against *P. aeruginosa* UFPEDA 416 with MIC of 310, 310 µg/mL, respectively.

Table 7. Antimicrobial activity of *Streptomyces* sp. ACTMS-12H UFPEDA 3405 (µg/mL).

Microorganismos	FAcOEt-12H		FBuOH-12H		FH$_2$O I-12H		FH$_2$O II-12H		FH$_2$O III-12H	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus UFPEDA 02	>1000	>1000	>1000	>1000	620	>1000	620	>1000	310	>1000
B. subitilis UFPEDA 86	>1000	>1000	>1000	>1000	620	>1000	310	>1000	310	>1000
M. luteus UFPEDA 100	>1000	>1000	>1000	>1000	620	>1000	620	>1000	>1000	>1000
E. faecalis UFPEDA 138	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
E. coli UFPEDA 224	>1000	>1000	>1000	>1000	620	>1000	620	>1000	620	625
S. marcencens UFPEDA 352	>1000	>1000	>1000	>1000	620	>1000	620	>1000	620	625
P. aeruginosa UFPEDA 416	310	>1000	310	>1000	310	>1000	310	>1000	310	>1000
M. smegmatis UFPEDA 71	>1000	>1000	>1000	>1000	620	>1000	310	>1000	620	>1000
		MFC		MFC		MFC		MFC		MFC
C. albicans UFPEDA 1007	>1000	>1000	>1000	>1000	310	>1000	310	>1000	310	>1000

FHex-12H: Hexane phase; FAcOEt-12H: Acetoethyl phase; FBuOH-12H: Butanolic phase; FH$_2$O I-12H: Aqueous phase I 12H; FH$_2$O II-12H: aqueous phase II; FH$_2$O III-12H: Aqueous phase III. Each sample was tested in triplicate in two independent experiments.
Source: Authors.

4. Discussion

Although the Amazon region has an immense biodiversity, a large part of its species and their phylogenetic relationships are unknown, especially with regard to microbiological diversity and its interactions with other organisms (Genuário et al., 2019; Nascimento et al., 2014), justifying the interest of the present study in the microorganisms of this region.

In this work, the strain of *Streptomyces* sp. ACTMS-12H UFPEDA 3405 was isolated from *Paullinia cupana* rhizosphere and characterized. This strain showed good growth in the middle of rice. The fermentative process in a solid medium has been shown to be an efficient method to obtain enzymes, antibiotics and other bioactive products, with the advantage of use less water and energy when compared to fermentation in submerged culture (Niladevi, Sukumaran, Prema, 2007). Compared to the results obtained with previous study by Lima et al. (2017) using parboiled rice on fermentation in a solid medium, obtained a yield of 6.6%, EMeOH-12H extract showed good productive efficiency, since it presented a percentage of yield above 10%.

On the chemical prospecting of extracts and phases from *Streptomyces sp.*, the results found were similar to previous studies that identified the presence of flavonoids in the extract of *Streptomyces sp.* (ERINLG-4) (Balachandran et al, 2014). Saponins have also been detected in fermented *S. diastatochromogenes* MK800-62F1 (Yoshimoto et al., 2000), and in *Streptomyces sp.* L74 were found reducing sugars and triterpenes (Luo et al., 2013), corroborating with the results found in our study.

Concerning cytotoxicity in tumor cells, the National Cancer Institute (NCI-USA) considers it promising for purification, a crude extract that has an IC$_{50}$ below 30 µg/mL (Suffnes, Pezzuto, 1990). The FBuOH-12H phase presented IC$_{50}$ value of between 1.1 - 10.6 µg/mL, for different cell lines, values considered of high cytotoxicity.

The cytotoxic activity of the FBuOH-12H phase can be attributed, at least in part, to the presence of saponins, which were the metabolites found only in this phase. Cytotoxicity and the chemopreventive role of saponins was discussed in a

several of review papers (Bachran et al., 2008; Fuchs et al., 2009; Podolak, Galanty, Sobolewska, 2010). Triterpenoid saponins isolated from *Aesculus pavia* were tested against a panel of 59 cell lines from nine different human cancers such as leukemia, non-small cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate and breast (Zhang, Li, 2007). These authors observed that compounds with two-acyl groups at C-21 and C-22 had cytotoxic activity for all cell lines tested with GI_{50} 0.175–8.71 µM. Few studies report the isolation of saponins of the *Streptomyces* genus in the literature, as well as their cytotoxic activity. Yoshimoto et al. (2000) described a compound with a chemical structure similar to saponin isolated from *S. diastatochromogenes* MK800-62F, that exhibited anti-cancer activity against small cells of human lung carcinoma (Ms-1).

The cytotoxicity test on human lymphocytes is essential in the discovery of new drugs, since most antineoplastic agents are nonspecific and cause myelosuppression or immunosuppression, one of the most common adverse effects in cancer patients (Vasievich, Huang, 2011). The extract and phases tested in this work showed low cytotoxicity in peripheral blood mononuclear cells, being in agreement with study carried out by Anibou et al. (2008), that demonstrated that two compounds isolated from the aqueous extracts of *Streptomyces* sp. T5 and *Streptomyces* sp. AS8, showed cytotoxic effects on tumor cells, without toxicity in PBMC.

A substance is considered hemolytic when it has an $EC_{50} < 200$ µg/mL (Costa-Lotufo et al., 2002). The evaluation of hemolytic activity is performed to determine the potential that a substance has in causing damage to the plasma membrane of erythrocytes, a dynamic structure that responds directly to interactions with drugs (Saurav, Kannabiran, 2012). Hemolysis then becomes one of the criteria for determining the toxicity in normal cells of anticancer, antimicrobial and antioxidant agents. As EMeOH-12H and its phases have no presented membrane damage, its cytotoxicity in tumor lines seems to be related to other death mechanisms than necrosis. Haque et al. (2016) showed that the acetoethyl extract of *Streptomyces* sp. AIAH-10 did not present hemolytic activity, corroborating the present study.

The inhibition of cell proliferation and drug-induced cell death is facilitated by the induction of apoptotic processes, since apoptosis is a controlled cell deletion without the occurrence of inflammation. In the present study, MOLT-4 cells treated with FBuOH had their cell viability decreased, presenting cell morphology typical of the apoptosis process, with reduced cell volume and chromatin condensation. This kind of cell death has been found in cancer cells treated with metabolites of *Streptomyces* spp. A study by Naine et al. (2015) showed a higher percentage of HepG2 cells that suffered apoptosis when treated with acetoethyl extract from *S. parvulus* VITJS11. More than 400 studies have reported the ability of saponins to induce apoptosis in cancer cells (Weng et al., 2011). Therefore, we can suggest that cell death due to apoptosis may be related to the presence of saponin in the extracts, however, more specific studies are needed to prove it.

Several methods have been employed to determine the antioxidant activity *in vitro* of extracts and isolated substances. One of the most used consists of evaluating the sequestering activity of the free radical 2,2-diphenyl-1-picryl-hydrazil (DPPH). While greater is the DPPH consumption or sequesting by the sample, the EC_{50} value is lower and therefore greater its antioxidant activity. This method has been widely used in the analysis of the reaction mechanism of polyphenolic compounds with free radicals (Pisoschi, Cimpeanu, Predoi, 2015). The extract and all fractions obtained from strains of *Streptomyces* sp. evaluated presented low antioxidant activity, probably due to the low content of phenolic compounds present. These results are in agreement with the studies performed by Lertcanawanichakul et al. (2015) and Naine et al. (2015) that found EC_{50} of 3,400 and 500 µg/mL for the acetoethyl extract of *S. lydicus* A2 and *S. parvulus* VITJS11, respectively.

Since the extracts and phases have low reactivity with DPPH, the importance of investigating the antioxidant activity by several methodologies is justified. The ABTS radical is more reactive than DPPH radical. The idea of the method is to monitor the decay of the ABTS radical produced by the oxidation of $ABTS^+$, generated by the addition of a water-soluble or fat-soluble sample containing antioxidants. The advantage of this method is its relative simplicity, allowing its application in any laboratory routine (Gupta, 2015; Re at al., 1999). The FAcOEt-12H fraction exhibited a 50% oxidative inhibition

percentage at a concentration of 20 µg/mL, with 1161.7 ± 0.04 µM of Trolox/g of the extract, indicating an expressive reactivity with the radical ABTS. Lee et al. (2014) evaluate the antioxidant activity of acetoethyl extract of *Streptomyces sp.* MJM 10778 at a concentration of 135 µg/mL, and found 50% oxidative inhibition.

The phosphomolybdenum method is a simple and inexpensive way to assess the total antioxidant capacity of a complex mixture of compounds, such as extracts obtained from microorganisms. This method has the advantage of evaluating the antioxidant capacity of water-soluble or fat-soluble samples, as well as the ABTS radical (Prietro et al., 1999). The extract and phases showed low antioxidant activity by this method. It should be noted that they also had a low content of total phenolics. According to Leouifoudi et al. (2015), there is a direct relationship between the antioxidant potential and the phenolic content, where the lower the amount of phenolics, the less intense the antioxidant potential, suggesting that this correlation explains the data obtained.

Regarding the antimicrobial activity, FacOEt-12H and FBuOH-12H presented relevant activity with MIC of 310 µg/mL against *P. aeruginosa*, being in these phases detected the presence of flavonoids, triterpenes and steroids. It is also described in the literature that the metabolites produced by *Streptomyces* spp. showed a broad spectrum of antimicrobial activity, regardless of the fact that Gram-negative bacteria have a complex nature of the cell wall, being more resistant to the action of antibiotics due to the lipid barrier of their outer layer (Higginbotham, Murphy, 2010).

Pseudomonas aeruginosa is a pathogen with a resistance rate to carbapenem antibiotics (imipenem and meropenem) of 15 to 20.4% among 152 strains of this bacterium (Balachandran et al., 2016; Savaş et al., 2005), therefore, finding new antibiotics against this Gram-negative bacterium is extremely valuable. In this study, several fractions showed a clinically relevant MIC against *P. aeruginosa* of 310 µg/mL. Choi et al. (2012) obtained for the acetate extract of *Streptomyces* sp. BCNU 1001, a MIC of 250 µg/mL against *P. aeruginosa*. Sosovele et al. (2012) obtained acetoethyl extracts of *Streptomyces* spp with MIC of 500 µg/mL against *P. aeruginosa*. All these studies corroborate our findings.

5. Conclusion

In conclusion, *Streptomyces* sp. ACTMS-12H produces secondary metabolites with antioxidant, antimicrobial and cytotoxic actions against tumor cells. On antimicrobial activity, the action *Streptomyces* sp. ACTMS-12H UFPEDA 3405 against *P. aeruginosa* can be highlighted, because it is a Gram-negative bacterium, being usually resistant to several extracts. The butanol phase FBuOH-12H was able to induce morphological changes in MOLT-4 cells, probably due to the presence of saponins in this fraction. Efforts to isolate the compounds responsible for these biological activities and to elucidate their mechanisms of action are underway.

Acknowledgments

References

Ahmed, S. A., Gogal, R. M., Jr, & Walsh, J. E. (1994). A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay. *Journal of immunological methods, 170*(2), 211–224. https://doi.org/10.1016/0022-1759(94)90396-4.

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature protocols, 2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102.

Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., Abbott, B. J., Mayo, J. G., Shoemaker, R. H., & Boyd, M. R. (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer research, 48*(3), 589–601.

Anibou, M., Chait, A., Zyad, A., Taourirt, M., Ouhdouch, Y., & Benherref, A. (2008). Actinomycetes from Moroccan habitats: isolation and screening for cytotoxic activities. *World Journal of Microbiology and Biotechnology, 24:*2019–2025. https://doi.org/10.1007/s11274-008-9705-7.

Bachran, C., Bachran, S., Sutherland, M., Bachran, D., & Fuchs, H. (2008). Saponins in tumor therapy. *Mini reviews in medicinal chemistry, 8*(6), 575–584. https://doi.org/10.2174/138955708784534445.

Balachandran, C., Sangeetha, B., Duraipandiyan, V., Raj, M. K., Ignacimuthu, S., Al-Dhabi, N. A., Balakrishna, K., Parthasarathy, K., Arulmozhi, N. M., & Arasu, M. V. (2014). A flavonoid isolated from *Streptomyces* sp. (ERINLG-4) induces apoptosis in human lung cancer A549 cells through p53 and cytochrome c release caspase dependant pathway. *Chemico-biological interactions, 224*:24–35. https://doi.org/10.1016/j.cbi.2014.09.019.

Balachandran, C., Duraipandiyan.V., Arun, Y., Sangeetha, B., Emi, N., Al-Dhabi, N. A., et al. (2016). Isolation and characterization of 2-hydroxy-9,10-anthraquinone from *Streptomyces olivochromogenes* (ERINLG-261) with antimicrobial and antiproliferative properties. *Revista Brasileira de Farmacognosia, 26*:285–95. https://doi.org/10.1016/j.bjp.2015.12.003.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Choi, H. J., Kim, D.W., Choi, Y. W., Lee, Y.G., Lee, Y-I., Jeong, Y. K., & Woo, H. J. (2012). Broad-spectrum *in vitro* antimicrobial activities of *Streptomyces* sp. strain BCNU 1001. *Biotechnology and Bioprocess Engineering, 17*:576–83. https://doi.org/10.1007/s12257-011-0151-2.

Clinical and Laboratory Standards Institute - CLSI. (2008). M38-A2 *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*; Approved Standard. 2nd ed. USA.

Clinical and Laboratory Standards Institute - CLSI. (2017). *M100 Performance Standards for Antimicrobial Susceptibility Testing*. 27th ed. USA.

Costa-Lotufo, L. V., Cunha, G. M., Farias, P. A., Viana, G. S., Cunha, K. M., Pessoa, C., Moraes, M. O., Silveira, E. R., Gramosa, N. V., & Rao, V. S. (2002). The cytotoxic and embryotoxic effects of kaurenoic acid, a diterpene isolated from Copaifera langsdorffii oleo-resin. *Toxicon: official journal of the International Society on Toxinology, 40*(8), 1231–1234. https://doi.org/10.1016/s0041-0101(02)00128-9.

Creixell, M., & Peppas, N. A. (2012). Co-delivery of siRNA and therapeutic agents using nanocarriers to overcome cancer resistance. *Nano today, 7*(4), 367–379. https://doi.org/10.1016/j.nantod.2012.06.013.

de Almeida, C., Brito, S. A., de Santana, T. I., Costa, H., de Carvalho Júnior, C., da Silva, M. V., de Almeida, L. L., Rolim, L. A., Dos Santos, V. L., Wanderley, A. G., & da Silva, T. G. (2017). *Spondias purpurea* L. (Anacardiaceae): Antioxidant and Antiulcer Activities of the Leaf Hexane Extract. Oxidative medicine and cellular longevity, 2017, 6593073. https://doi.org/10.1155/2017/6593073

Ellaiah, P, Ramana, T., Raju, K., Sujatha, P., & Sankar, A. U. (2004). Investigations on marine actinomycetes from Bay of Bengal near Kakinada coast of Andhra Pradesh. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 6:*53–6.

Fuchs, H., Bachran, D., Panjideh, H., Schellmann, N., Weng, A., Melzig, M. F., Sutherland, M., & Bachran, C. (2009). Saponins as tool for improved targeted tumor therapies. *Current drug targets, 10*(2), 140–151. https://doi.org/10.2174/138945009787354584.

Genuário, D. B., Vaz, M. G. M. V., Santos, S. N., Kavamura, V. N., & Melo, I. S. (2019). Cyanobacteria From Brazilian Extreme Environments. *Microbial Diversity in the Genomic Era*, 265–284. https://doi.org/10.1016/b978-0-12-814849-5.00016-2.

Gupta D. (2015). Methods for determination of antioxidant capacity: A review. *International Journal of Pharmaceutical Sciences and Research, 6*:546–66. https://doi.org/10.13040/IJPSR.0975-8232.6(2).546-66.

Haque, M. A., Sarker, A. K., Rahman, M. A., Chouduri, M. A. U., & Islam, M. A. U. (2016). Evaluation of antifungal, hemolytic and cytotoxic potential of ethyl acetate extract of a new marine *Streptomyces* sp. AIAH-10. *Bangladesh Pharmaceutical Journal, 16*:19:37–43. https://doi.org/10.3329/bpj.v19i1.29235.

Harborne J. B. 1998. *Phenolic compounds in phytochemical methods – a guide to modern techniques of plant analysis*. Third edition. Chapman & Hall, New York, pp. 66-74.

Higginbotham, S. J., & Murphy, C. D. (2010). Identification and characterisation of a *Streptomyces* sp. isolate exhibiting activity against methicillin-resistant *Staphylococcus aureus*. *Microbiological research, 165*(1), 82–86. https://doi.org/10.1016/j.micres.2008.12.004.

Jakubiec-Krzesniak, K., Rajnisz-Mateusiak, A., Guspiel, A., Ziemska, J., & Solecka, J. (2018). Secondary Metabolites of Actinomycetes and their Antibacterial, Antifungal and Antiviral Properties. *Polish Journal of Microbiology, 67*(3), 259–272. https://doi.org/10.21307/pjm-2018-048.

Kekuda, T. R. P., Shobha, K. S., & Onkarappa, R. (2010). Fascinating diversity and potent biological activities of actinomycete metabolites. *Journal of Pharmacy Research, 3*(2):250-256.

Khanna I. (2012). Drug discovery in pharmaceutical industry: productivity challenges and trends. *Drug discovery today, 17*(19-20), 1088–1102. https://doi.org/10.1016/j.drudis.2012.05.007.

Lee, D. R., Lee, S. K., Choi, B. K., Cheng, J., Lee, Y. S., Yang, S. H., & Suh, J. W. (2014). Antioxidant activity and free radical scavenging activities of Streptomyces sp. strain MJM 10778. *Asian Pacific journal of tropical medicine, 7*(12), 962–967. https://doi.org/10.1016/S1995-7645(14)60170-X.

Leouifoudi, I., Harnafi, H., & Zyad, A. (2015). Olive Mill Waste Extracts: Polyphenols Content, Antioxidant, and Antimicrobial Activities. *Advances in pharmacological sciences, 2015*, 714138. https://doi.org/10.1155/2015/714138.

Lertcanawanichakul, M., Pondet, K., & Kwantep, J. (2015). In vitro antimicrobial and antioxidant activities of bioactive compounds (secondary metabolites) extracted from *Streptomyces lydicus* A2. *Journal of Applied Pharmaceutical Science, 5*(2): 017–021. https://doi.org/10.7324/JAPS.2015.50204.

Lima, S. M., Melo, J. G., Militão, G. C., Lima, G. M., Do Carmo A. Lima, M., Aguiar, J. S., Araújo, R. M., Braz-Filho, R., Marchand, P., Araújo, J. M., & Silva, T. G. (2017). Characterization of the biochemical, physiological, and medicinal properties of *Streptomyces hygroscopicus* ACTMS-9H isolated from the Amazon (Brazil). *Applied microbiology and biotechnology, 101*(2), 711–723. https://doi.org/10.1007/s00253-016-7886-9.

Liu, C. P., Tsai, W. J., Lin, Y. L., Liao, J. F., Chen, C. F., & Kuo, Y. C. (2004). The extracts from Nelumbo nucifera suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells. *Life Sciences, 75*(6), 699–716. https://doi.org/10.1016/j.lfs.2004.01.019.

Luo, J., Wang, Y., Tang, S., Liang, J., Lin, W., & Luo, L. (2013). Isolation and identification of algicidal compound from Streptomyces and algicidal mechanism to Microcystis aeruginosa. *PloS One, 8*(10), e76444. https://doi.org/10.1371/journal.pone.0076444.

Ma, X., Wu, H., Liu, L., Yao, Q., Wang, S., Zhan, R., Xing, S., & Zhou, Y. (2011). Polyphenolic compounds and antioxidant pro perties in mango fruits. *Scientia Horticulturae*, 129(1), 102–107. https://doi.org/10.1016/j.scienta.2011.03.015.

Mahmoud, T. S., Marques, M. R., Pessoa, C. O., Lotufo, L. V. C., Magalhães, H. I. F., Moraes, M. O., Lima, D. P., Tininis, A. G., & Oliveira, J. E. (2011). In vitro cytotoxic activity of brazilian middle west plant extracts. *Brazilian Journal of Pharmacognosy*, 21(3):456–464. https://doi.org/10.1590/S0102-695X2011005000061.

Naine, S. J., Devi, C. S, & Mohanasrinivasan, V. B. V. (2015). Antimicrobial, antioxidant and cytotoxic activity of marine *Streptomyces parvulus* VITJS11 crude extract. *Brazilian Archives of Biology and Technology, 58*:198–207. https://doi.org/10.1590/S1516-8913201400173.

Nascimento, T. C., Jos, E. G. G., Nascimento, P. L. A., Da Silva, A. C., Medeiros, E. V., Falcão, R. E., Teixeira, M. F. S., Souza Lima, G. M., Porto, A. L. F., & Moreiara, K. A. (2014). Partial biochemical characterization of a thermostable chitinase produced by *Streptomyces owasiensis* isolated from lichens of the Amazonian region. *African Journal of Microbiology Research, 8*:2830–4. https://doi.org/10.5897/AJMR2014.6822.

Nguyen, H. T., Pokhrel, A. R., Nguyen, C. T., Pham, V., Dhakal, D., Lim, H. N., Jung, H. J., Kim, T. S., Yamaguchi, T., & Sohng, J. K. (2020). *Streptomyces* sp. VN1, a producer of diverse metabolites including non-natural furan-type anticancer compound. *Scientific reports*, *10*(1), 1756. https://doi.org/10.1038/s41598-020-58623-1.

Niladevi, K. N., Sukumaran, R. K., & Prema, P. (2007). Utilization of rice straw for laccase production by *Streptomyces psammoticus* in solid-state fermentation. *Journal of industrial microbiology & biotechnology, 34*(10), 665–674. https://doi.org/10.1007/s10295-007-0239-z.

Pereira, A. S. et al. (2018). Metodologia da pesquisa científica. UFSM. https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf?sequence=1

Podolak, I., Galanty, A., & Sobolewska, D. (2010). Saponins as cytotoxic agents: a review. *Phytochemistry reviews: proceedings of the Phytochemical Society of Europe, 9*(3), 425–474. https://doi.org/10.1007/s11101-010-9183-z.

Pisoschi, A. M., Cimpeanu, C., & Predoi, G. (2015). electrochemical methods for total antioxidant capacity and its main contributors determination: A review. *Open Chemistry, 13*:824-856. https://doi.org/10.1515/chem-2015-0099.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry, 269*(2), 337–341. https://doi.org/10.1006/abio.1999.4019.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology & medicine, 26*(9-10), 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3.

Saurav, K., & Kannabiran, K. (2012). Cytotoxicity and antioxidant activity of 5-(2,4-dimethylbenzyl)pyrrolidin-2-one extracted from marine Streptomyces VITSVK5 spp. *Saudi Journal of Biological Sciences*, *19*(1), 81–86. https://doi.org/10.1016/j.sjbs.2011.07.003.

Savaş, L., Duran, N., Savaş, N., Önlen, Y., & Ocak, S. (2005). The prevalence and resistance patterns of Pseudomonas aeruginosa in intensive care units in a university hospital. *Turkish Journal of Medical Sciences, 35*:317–22.

Scott, C. W., Peters, M. F., & Dragan, Y. P. (2013). Human induced pluripotent stem cells and their use in drug discovery for toxicity testing. *Toxicology letters, 219*(1), 49–58. https://doi.org/10.1016/j.toxlet.2013.02.020.

Sharma, M., & Manhas, R. K. (2020). Purification and characterization of salvianolic acid B from Streptomyces sp. M4 possessing antifungal activity against fungal phytopathogens. *Microbiological research*, *237*, 126478. https://doi.org/10.1016/j.micres.2020.126478.

Sosovele, M. E., Hosea, K. M., & Lyimo, T. J. (2012). *In vitro* antimicrobial activity of extracts from marine *Streptomyces* isolated from mangrove sediments of Tanzania. *Journal of Biochemical Technology, 3*:431–435.

Suffnes, M., & Pezzuto, J. (1990). *Assays related to cancer drug discovery*. In: Hostettmann K (ed.), *Methods in Plant Biochemistry*. Assays Bioactivity, London: Academic Press; p. 71–133.

Rashad, F. M., Fathy, H. M., El-Zayat, A. S., & Elghonaimy, A. M. (2015). Isolation and characterization of multifunctional Streptomyces species with antimicrobial, nematicidal and phytohormone activities from marine environments in Egypt. *Microbiological research*, 175, 34–47. https://doi.org/10.1016/j.micres.2015.03.002.

Tan, L. T., Ser, H. L., Yin, W. F., Chan, K. G., Lee, L. H., & Goh, B. H. (2015). Investigation of Antioxidative and Anticancer Potentials of *Streptomyces* sp. MUM256 Isolated from Malaysia Mangrove Soil. *Frontiers in microbiology, 6*, 1316. https://doi.org/10.3389/fmicb.2015.01316.

Tan, L. T., Chan, K. G., Chan, C. K., Khan, T. M., Lee, L. H., & Goh, B. H. (2018). Antioxidative Potential of a *Streptomyces* sp. MUM292 Isolated from Mangrove Soil. *Biomed Research International*, 2018, 4823126. https://doi.org/10.1155/2018/4823126.

Vasievich, E. A., & Huang, L. (2011). The suppressive tumor microenvironment: a challenge in cancer immunotherapy. *Molecular pharmaceutics, 8*(3), 635–641. https://doi.org/10.1021/mp1004228.

Weng, A., Thakur, Melzig, & Fuchs. (2011). Chemistry and pharmacology of saponins: special focus on cytotoxic properties. *Botanics Targets and Therapy, 2011*(1):19-29. https://doi.org/10.2147/BTAT.S17261.

Yoshimoto, Y., Sawa, T., Kinoshita, N., Homma, Y., Hamada, M., Takeuchi, T., & Imoto, M. (2000). MK800-62F1, a new inhibitor of apoptotic cell death, from *Streptomyces diastatochromogenes* MK800-62F1. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activity. *The Journal of antibiotics, 53*(6), 569–574. https://doi.org/10.7164/antibiotics.53.569.

Zhang, Z., & Li, S. (2007). Cytotoxic triterpenoid saponins from the fruits of *Aesculus pavia* L. *Phytochemistry, 68*(15), 2075–2086. https://doi.org/10.1016/j.phytochem.2007.05.020.