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Metabolites from *Streptomyces* spp. isolated of Amazonian rivers against *Plasmodium falciparum* (FCR-3 strain)

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ABSTRACT. *Plasmodium falciparum* is an important parasite that causes malaria, which affects people in tropical and subtropical regions where it is an important public health problem. The genus *Streptomyces* is an important producer of antibiotics, in this context, the present work evaluates the antiplasmodial activity of *Streptomyces* isolated from sediments of the Madeira and Purus rivers. This activity was determined by using flow cytometry against the *P. falciparum* strain (FCR3), and by cytotoxicity tests on MRC5 and Vero cells. *Streptomyces* were able to inhibit *P. falciparum* FCR3 growth, with the best inhibition results obtained by the APUR32.5 and MPUR40.3 isolates. In fractionation obtained with ethyl acetate at a concentration of 3.12 µg/mL, the APUR32.5F isolate showed 84.5% inhibition, and the MPUR40.3F isolate showed 86.6% inhibition, in both cases superior to quinine (81.5%), with toxicity less than or equal to doxorubicin for both fractions. *Streptomyces* isolated from the Purus and Madeira rivers which are tributaries of the Amazon River, proved to be an important source of new compounds with antiplasmodial activity.
Key words: *Streptomyces;* Malaria; Antimalarial; Antiplasmodial; *Plasmodium falciparum.*

INTRODUCTION

Malaria is a serious infectious disease caused by the parasite *Plasmodium falciparum* and transmitted by the bite of infected female mosquitoes of the genus *Anopheles*. The disease is endemic in several regions of the world, mainly in tropical and subtropical areas, where it is an important public health problem. Globally, there were about 241 million cases of malaria and approximately 627 thousand deaths in 2020 (WHO, 2021). The ability of *Streptomyces* species to produce a wide variety of bioactive compounds is extremely valuable and has been intensively studied. The use of these compounds in the pharmaceutical industry has proven to be extremely beneficial, since they have therapeutic properties and are used as raw materials for the manufacture of medicines (Procópio et al., 2012; Al-Shaibani et al, 2021). Several works have shown that *Streptomyces* produce metabolites with antimalarial properties (Ahmad et al., 2021; Happi et al., 2023). Endophytic strains such as *Streptomyces* SUK10, obtained from the bark of the *Shorea ovalis* tree, produced diketopiperazine gancidin, which inhibited *Plasmodium berghei* PZZ1/100 (Zin et al., 2017). In the search for bioactive compounds from microbial extracts, a species of *Streptomyces* (S.4) from a marine sponge collected in the Florida Keys exhibited antiplasmodial activity against the *P. falciparum* strain Dd2, producing a dipeptide called xestostreptin (Harinantenaina Rakotondraibe et al., 2015). *S. asterosporus* DSM 41452 produces molecules related to the non-ribosomal cyclodepsipeptide WS9326A and the polyketide animicin B, which exhibited inhibitory activity against *P. falciparum* (Zhang et al., 2018). Bioassay-guided fractionation of the fermentation broth extract of *S. spectabilis* BCC 4785 led to the isolation of three main antimalarial agents, metacycloprodigiosin, bafilomycin A1 and spectinabilin. Metacycloprodigiosin exhibited potent *in vitro* activity against *P. falciparum* K1 (Isaka et al., 2002), whereas the PR3 strain of *Streptomyces* produced cyclic ethers with antiplasmodial activity in combination with valinomycin, synergistically improving the antiplasmodial activity of valinomycin and reducing its cytotoxicity (Watson et al., 2021). *S. hygroscopicus* produced a compound derived from isoquinoline that could be formulated as an antimalarial drug candidate (Nugraha et al., 2021).

P. falciparum, the main causative agent of malaria, has high adaptability and has become resistant to almost all antimalarial drugs (Wellems, 2002), so new classes of bioactive compounds are needed to combat *P. falciparum* and reduce the resistance risk. Therefore, the objective of this work was to evaluate extracts and fractions of *Streptomyces* isolated from the sediments of rivers in the Amazon against *P. falciparum* FCR3.

MATERIALS AND METHODS

Preparation of extracts and fractionation

For the tests, we used seven strains of *Streptomyces* isolated from sediments of the Madeira (MAD42) and Purus rivers (APUR39.1, APUR36.3, MPUR21.2, MPUR36.1, APUR32.5, MPUR40.3), belonging to the collection of the Embrapa Western Amazon research unit, isolated by Silva, 2021. For the production of the pre-inoculum, the strains were reactivated on a plate containing IPS2 agar medium (10 g of starch; 4 g of yeast extract, 10 g of malt extract, 4 g of dextrose and 1000 mL of distilled water, with the pH adjusted to 7.2. Subsequently, the isolates were grown in liquid ISP2 under stirring at 150 rpm for 7 days at 28 °C. After the growth of *Streptomyces,* the culture was centrifuged and the aqueous part was lyophilized to obtain the extract used in the tests. For fractionation, isolates APUR 32.5 and MPUR 40.3 were selected. They were grown in

ISP2 liquid medium for 10 days at 28 °C under orbital agitation at 150 rpm, after which fractionation was performed with ethyl acetate (EtOAc) 1:1 (v:v). The organic phase was separated from the aqueous phase in a decantation funnel and the organic phase was dried in a rotary evaporator. The fractionation and subsequent testing against growth of *Streptomyces* were carried out at the Embrapa laboratory.

Antiplasmodial activity

The chloroquine-resistant strain of *P. falciparum* (FCR3) was cultured in RPMI medium with 10% AB+ human plasma and normal human erythrocytes in a low-oxygen atmosphere, following the protocol of Trager and Jenson (1978). The infected erythrocyte suspension was incubated at 37 °C in a controlled system (with 5% CO2, 5% O2, and 90% N2 gas mixture). The antimalarial tests were carried out on the Biotechnological Bioassays Platform of the IPCCB-FMT (RPT11H) and on the Flow Cytometry of the ILM/FIOCRUZ - Amazonas (RPT08J). Samples with concentration of 10 mg/mL were serially diluted in complete RPMI medium to six concentrations (3.12µg/mL, 6.25µg/mL, 12.5µg/mL, 25µg/mL, 50µg/mL and 100µg/mL) in plates of 96 flat bottom wells. Erythrocytes containing asynchronous *P. falciparum* FCR3 were cultured together with the diluted samples, with final parasitemia, 2% hematocrit and 80% young trophozoites (rings). After 72 hours of incubation, the samples were stained with ethidium bromide and washed with 1X PBS buffer. At the end, the samples were resuspended in 200 µl of 1X PBS for analysis in a BD FACSCanto II flow cytometer (BD Biosciences, San Jose, USA) in the FL-1 channel with software Getting Started with BD FACSDiva e FlowJo 1.0. Parasite-free erythrocytes were used as negative control, and infected red blood cells as positive control. Quinine was used as the reference control drug and tested at the same concentrations.

In vitro cytotoxicity

The extracts and fractions were solubilized in 100 µL of DMSO at a concentration of 10 mg/mL and subsequently evaluated at the six concentrations mentioned previously: 3.12 µg/mL, 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL and 100 µg/ml. The strains were grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco), supplemented with 10% inactivated fetal bovine serum (Gibco) and penicillin (50 µg/mL). All assays were performed in triplicate. The MRC5 and Vero cell lines were used under the culture conditions adapted by the Laboratory of RPT11H-Bioassays of Biotechnological Compounds of the Leônidas and Maria Deane Institute (ILMD). Assays were carried out by the Alamar Blue™ method according to Ahmed et al. (Ahmed et al., 1994). Cells were plated at a concentration of 1.0 x 10^4 cells/well in a 96-well plate and treated with samples at the six concentrations. After 24 hours of incubation and adherence of the cells in a 5% CO_2 oven at 37 °C, they were treated with the peptides, after which the plates were kept in a CO_2 incubator for 72 hours with 5% CO_2 at 37 °C. After this period, 10 µL of 0.4% resazurin (diluted 1:20) was added to each well and the Alamar Blue (Sigma-Aldrich, Brazil) metabolization time of 2 hours was used. Fluorescence was monitored with a microplate reader (GloMax® Explorer), with emission wavelength 580-640 nm and excitation of 520nm. Cell growth was used as positive control, and 0.1% DMSO was used as negative control. As drug control, doxorubicin (Sigma-Aldrich) was used, tested at the same concentrations as the samples. The percentage of cell viability was calculated according to the formula: % Viability = Ft x 100/Fb, where Ft= (fluorescence of cells + medium + substance + resazurin) and Fb= (fluorescence of cells + medium + resazurin).

Molecular identification of *Streptomyces* strains

Streptomyces were cultured in 5.0 mL of ISP2 liquid medium stirred at 180 rpm at 28 °C for 72 hours. Then the cells were centrifuged and the supernatant was discarded. Silica was added, and 700 µL of 2% CTAB was shaken in a TissueLyser for 20 minutes, then incubated at 65 °C for 30 minutes with agitation every 10 minutes to homogenize the suspension. After incubation, 600 µL of CIA (chloroform and isoamyl alcohol 1:24) was added and the solution was homogenized and centrifuged at 10,000 rpm for 5 minutes. Next, 400 µL of isopropanol (-20 °C) was added to the supernatant, homogenized and centrifuged at 4,000 rpm for 10 minutes to precipitate the DNA. The pellet containing the DNA was washed with 70% ethanol and allowed to dry, then resuspended in 100 µL of TE buffer containing RNAse and incubated in a water bath at 37 °C for 30 minutes. The DNA was quantified in a NanoDrop spectrophotometer and the integrity of the material was observed in 0.8% agarose gel.

For amplification of the 16S rRNA gene, PCR reactions were performed in a final volume of 25 µL, containing 17.25 µL of autoclaved ultrapure water, 2.5 µl of 1X buffer, 1 µl of 10 mM dNTPs. 1 µL of 50 mM MgSO4, 1 µl of each of the primers 235F (3' CCGTACTCCCCCAGGCGGG 5') and 878R (5' CGCGGCCTATCAGCTTGTTG 3') at 5 µM, 1 µL of DNA at a concentration of 50 ng/µL, and 0.25 µL of Taq DNA Polymerase enzyme 5U/µ (EasyTaq® DNA Polymerase, Transgen Biotech). The parameters used in the amplification were initial denaturation of 5 min at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at 65 °C and 1 min at 72 °C, and a final extension of 7 min at 72 °C. PCR fragments were visualized by electrophoresis using 1.5% agarose gel.

For sequencing reactions, PCR obtained were treated with 0.5 µL of Exo-Sap and incubated at 37 °C for 15 min and 80 °C for 15 min for enzymatic activation and inactivation, and then were sequenced using the Brilliant Dye v3 kit. Reactions were analyzed with a Genetic Analyzer 3500 sequencer (Thermo Fisher). The sequences obtained were compared with those deposited in the GenBank (National Center for Biotechnology Information-NCBI) using the BLASTn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic distribution of the 16S rRNA gene was based on a dataset constructed from sequences deposited in the GenBank. The 16S rRNA gene alignments were performed using the Clustal-W software and the trees were constructed based on the neighbor joining (NJ) method with 500 bootstrap repetitions, and the distance matrix was generated using the Kimura two-parameter model with the MEGA 11 program (Molecular Evolutionary Genetics Analysis).

RESULTS

Activity against *P. falciparum* FCR3 and cytotoxicity

In the tests carried out with the extracts of the seven strains of *Streptomyces* at a concentration of 3.12 µg/mL, only the isolate APUR39.1 MPUR40.3 showed activity against *P. falciparum* FCR3. However, against all strains at a concentration of 100 µg/mL it showed activity, ranging from 40.6 to 82.5% inhibition (Table 1). At the lowest concentration (3.12 µg/mL), the extract of APUR39.1 strain caused inhibition of 77.6% while MPUR40.3 caused inhibition of 85.7%. These were the most efficient in relation to the standard drug (quinine), which caused 81% inhibition.

The isolates APUR32.5, MAD42, APUR36.3, MPUR21.2 and MPUR36.1, with negative values at the concentration of 3.12 µg/mL, indicated that the extracts were favorable to *P falciparum*

Table 1. Percentage of inhibition of *P. falciparum* FCR3 by the extracts and fractions (APUR32.5F and MPUR40.3F) of the different *Streptomyces* tested.

Concentration	Quinine	MAD42	APUR39.1	APUR36.3	MPUR21.2	MPUR36.1	APUR32.5	MPUR40.3	*APUR32.5F*	*MPUR40.3F*
3.12 µg/mL	***81.5±1.4***	-28.7±6.0	77.6±4.9	-1.1±28.1	-12.5±8.5	-22.7±2.2	-4.9±9.6	85.7±1.9	*84.5±1.4*	*86.4±1.7*
6.25 µg/mL	***85.9±0.2***	-6.0±1.3	58.3±0.6	-3.2±22.1	-13.8±7.4	-19.2±4.6	9.6±6.7	85.8±0.4	*85.5±0.8*	*88.6±0.7*
12.5 µg/mL	***84.3±2.7***	6.7±3.4	68.0±0.6	5.8±17.3	-12.0±4.7	-17.0±3.6	17.6±5.8	85.3±1.6	*82.6±1.2*	*88.3±0.9*
25 µg/mL	***82.0±1.5***	18.7±2.9	82.4±2.2	18.4±19.1	-11.8±9.6	-2.0±9.7	34.3±6.1	85.1±0.9	*83.0±2.4*	*86.8±2.9*
50 µg/mL	***81.7±1.3***	60.1±21.4	76.8±1.9	33.1±19.9	14.5±2.5	-1.0±2.8	72.3±14.9	85.4±1.5	*82.8±2.2*	*87.1±0.7*
100 µg/mL	***73.9±1.6***	75.6±5.4	78. ±1.3	60.8±8.0	63.1±5.0	40.6±4.9	68.2±8.7	82.5±1.9	*78.2±3.4*	*79.9±2.6*
Hemolysis	***N***	25 µg/mL	50 µg/mL	N	N	N	25 µg/mL	25 µg/mL	*N*	*N*

N = There was no hemolysis.

Table 2. Viability of the MRC5 cells against the extracts and fractions (APUR32.5F and MPUR40.3F) of the different *Streptomyces* tested.

Concentration	Doxorubicin	MAD42	APUR39.1	APUR36.3	MPUR21.2	MPUR36.1	APUR32.5	MPUR40.3	*APUR32.5F*	*MPUR40.3F*
3.12 µg/mL	***78.1±1.0***	134.3±4.2	94.6±4.9	94.1±1.6	88.5±1.1	87.4±0.4	142.0±7.1	79.6±0.8	*72.7±1.1*	*62.0±1.7*
6.25 µg/mL	***66.9±1.3***	133.6±0.4	105.2±5.0	98.7±1.2	93.5±0.9	92.9±0.3	132.1±1.9	81.8±0.8	*73.7±0.4*	*68.5±5.1*
12.5 µg/mL	***49.0±2.3***	110.4±0.4	99.1±4.1	101.0±1.9	98.3±0.6	97.5±0.2	109.7±12.2	85.7±0.3	*77.2±0.5*	*70.8±0.1*
25 µg/mL	***38.3±1.8***	92.4±3.0	83.1±10.0	105.7±5.9	99.5±1.0	99.2±0.6	95.9±4.8	81.0±0.8	*73.8±0.5*	*68.1±6.6*
50 µg/mL	***37.7±0.3***	77.2±6.3	86.5±3.4	89.9±0.3	94.3±4.2	91.3±1.6	82.0±1.3	76.9±1.0	*66.5±0.9*	*59.0±0.6*
100 µg/mL	***36.3±0.1***	72.9±0.6	89.7±8.1	85.0±4.9	91.4±2.1	90.3±0.6	75.6±1.1	73.9±0.5	*63.6±0.4*	*56.6±4.0*

Values above 100%, there was a stimulation of cell growth.

Table 3. Viability of the Vero cell against the extracts and fractions (APUR32.5F and MPUR40.3F) of the different *Streptomyces* tested.

Concentration	Doxorubicin	MAD42	APUR39.1	APUR36.3	MPUR21.2	MPUR36.1	APUR32.5	MPUR40.3	*APUR32.5F*	*MPUR40.3F*
3.12 µg/mL	***43.4 ±0.7***	100.0±0.4	99.4±0.9	99.6±0.6	136.1±0.8	108.4±1.1	104.5±4.2	65.6±0.4	*44.3±2.0*	*44.4±0.3*
6.25 µg/mL	***38.2±0.8***	99.7±0.1	99.0±0.5	103.4±1.2	133.5±0.2	111.1±0.3	99.8±1.1	56.6±1.9	*46.0±0.1*	*42.1±0.1*
12.5 µg/mL	***37.1±0.6***	90.2±0.1	100.2±0.5	100.5±0.6	123.5±0.4	116.6±0.5	99.3±0.3	52.2±0.5	*44.9±0.3*	*40.5±0.1*
25 µg/mL	***35.6±0.1***	88.7±0.5	94.6±0.1	99.6±0.1	119.0±0.5	111.8±0.1	98.1±0.7	44.4±0.9	*42.2±0.4*	*39.3±0.1*
50 µg/mL	***35.5±0.3***	87.7±0.2	91.2±0.5	99.7±0.1	116.21.1	123.9±0.5	96.4±1.0	40.4±0.2	*41.8±0.2*	*33.8±0.6*
100 µg/mL	***33.5±0.1***	86.9±0.3	89.9±0.2	89.3±0.3	116.0±0.8	122.1±0.7	96.6±0.9	36.2±0.1	*36.3±0.5*	*25.9±0.6*

FCR3 multiplication. The extracts of isolates APUR36.3, MPUR21.2 and MPUR36.1 did not show hemolysis, while APUR32.5, MAD42 and MPUR40.3 showed hemolysis from 25 µg/mL and APUR39.1 from 50 µg/mL. Two isolates (APUR32.5 and MPUR40.3) were selected for fractionation, after which the APUR32.5F isolate had improved inhibition results and there was no hemolysis. MPUR40.3F produced the same inhibition results and there was also no hemolysis, showing that the fractionation was able to separate what causes hemolysis from what inhibits growth of *P. falciparum* FCR3 (Table 1). After fractionation, the results of APUR32.5F and MPUR40.3F showed greater inhibition than that of quinine, mainly at a concentration of 3.12 µg/mL (Figure 1).

In the cytotoxicity tests, some extracts promoted cell proliferation. For example, APUR32.5 and MAD42 induced MCR5 cell growth at concentrations of up to 12.5 µg/mL (Table 2). For the Vero cells, the isolates MPUR21.2 and MPUR36.1 induced cell proliferation at all concentrations (Table 3). MPUR40.3 showed the greatest toxicity against both MRC5 and Vero cells (Tables 2 and

3). There was lower toxicity of the extracts versus the MRC5 cells, with the value of the fractions being less toxic than that of doxorubicin (Figure 2). Among the fractions, only MPUR40.3F at concentrations of 50 and 100 µg/mL presented higher toxicity values than doxorubicin for the Vero cells (Figure 3).

The fractions produced better results than the extracts. The best *P. falciparum* FCR3 inhibition result at different concentrations was MPUR4.3F at a concentration of 6.25 µg/mL. There was an increase in inhibition at concentrations from 3.12 µg/mL to 6.25 µg/mL, and a tendency towards a reduction in activity at concentrations from 12.5 µg/mL to 100 µg/mL. When compared to quinine, almost all fractions had similar or superior results to quinine at almost all concentrations (Figure 1).

In the cytotoxicity tests, only the fractions at concentration of 3.12 µg/mL prevented the growth of the MCR5 cells more than doxorubicin, with the other fractions of APUR32.5F and MPUR40.3F being less toxic to the MCR5 cells than doxorubicin, as can be seen in Figure 2.

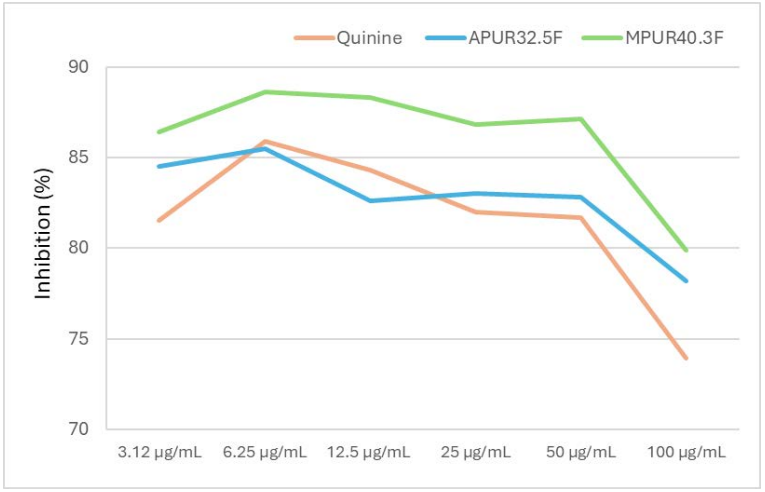

Figure 1. Graph of the percentage of *P. falciparum* FCR3 inhibition, against the MPUR40.3F and MPUR32.5F fractions at different concentrations.

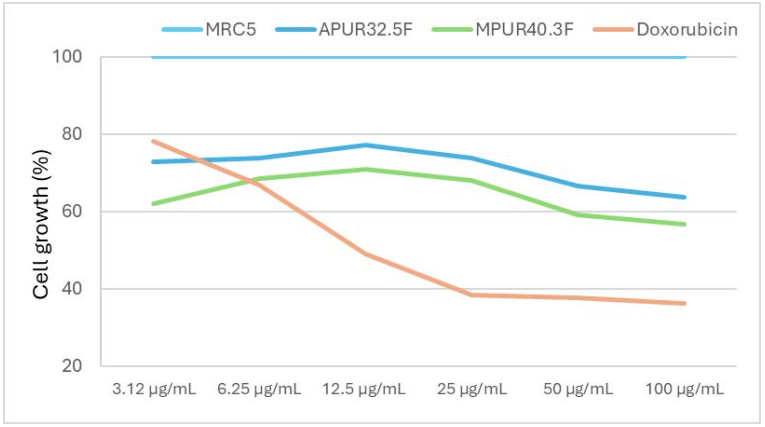

Figure 2. MCR5 cell growth graph against doxorubicin and MPUR40.3 and APUR32.5 fractions at different concentrations.

The Vero cells were more sensitive than the MRC5 cells, presenting smaller growth compared to the tested fractions, with doxorubicin being more toxic than MPUR40.3F and APUR32.5F up to a concentration of 25 µg/mL, and APUR32.5F being less toxic than MPUR40.3F, as can be seen in Figure 3, indicating that MPUR40.3F was more toxic than doxorubicin at concentrations of 50 µg/mL and 100 µg/mL.

Phylogeny of *Streptomyces* MPUR40.3 and APUR32.5.

Phylogeny based on the 16S rRNA gene of isolates APUR32.5 and MPUR40.3, showed that these *Streptomyces* are phylogenetically close to *S. rochei* and *S. plicatus*. The 16S rRNA gene of *Streptomyces* is highly conserved, as can be seen in the bar of Figure 4, and with values of 100% in the bootstrap analysis, allowing grouping only at the genus level. As can be seen in the phylogeny, in which the two species of *Streptomyces*, *S. rochei* and *S. plicatus*, presented 100% similarity between the sequences of the 16S rRNA gene, the *Streptomyces* used in the phylogeny were strains with the genome described in the GenBank (standard lineages and with the correct identification).

DISCUSSION

The discovery of new antiplasmodial drugs with lower cytotoxicity is fundamental for more effective treatment of malaria. Bacteria of the genus *Streptomyces* are a natural source of compounds with wide application. In this work, we observed that the fractions of the strains of *Streptomyces* sp. (MPUR32.5 and APUR40.3) isolated from the sediment of rivers in the Amazon region had excellent activity against *P. falciparum* FCR3, as can be seen in figure 1. *Streptomyces* strains are potential sources of novel bioactive molecules. Na et al. (2008) reported that the guided antimalarial fractionation of the culture of *Streptomyces* sp. marine strain H668 led to the isolation of a new polyether metabolite, which showed *in vitro* antimalarial activity against *P. falciparum* clones susceptible (D6) and resistant (W2) to chloroquine, without cytotoxicity to normal Vero cells (Na

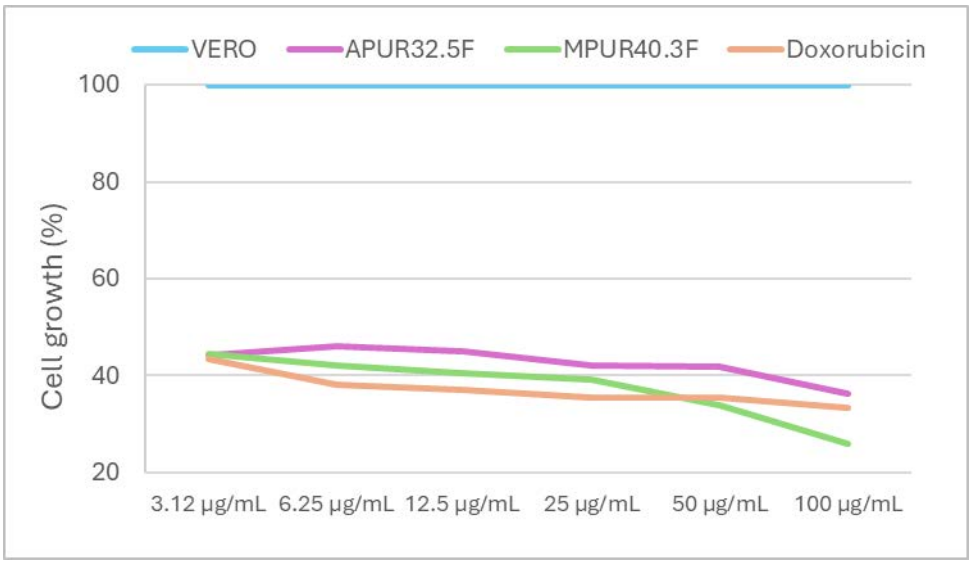

Figure 3. Graph of Vero cell growth against doxorubicin and MPUR40.3 and APUR32.5 fractions at different concentrations.

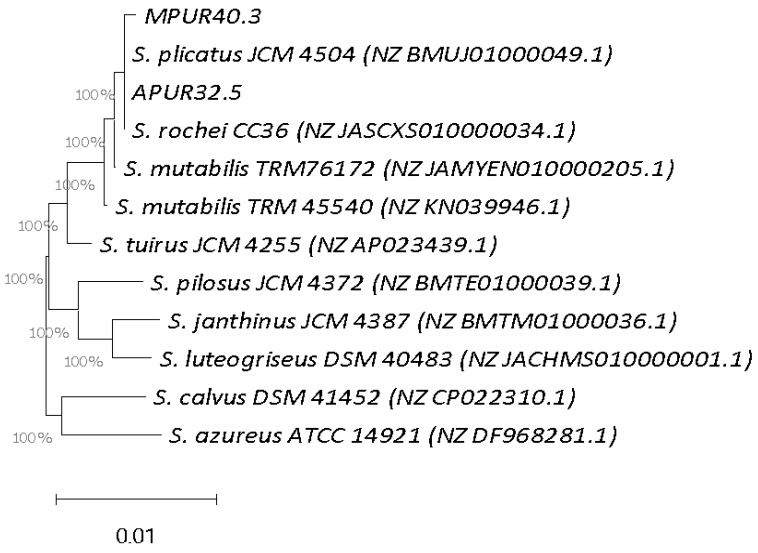

Figure 4. Phylogenetic tree of *Streptomyces* MPUR32.5 and APUR40.3, based on the sequence of the 16S rRNA gene, compared with sequences deposited in the GenBank.

et al., 2008). Isaka et al. (2002) observed that bioassay-guided fractionation of an extract from the fermentation broth of *Streptomyces spectabilis* BCC 4785 led to the isolation of three principle antimalarial agents, metacycloprodigiosin, bafilomycin A_1, and spectinabilin. Metacycloprodigiosin exhibited potent *in vitro* activity against *Plasmodium falciparum* K1, while its cytotoxicity was much weaker (Isaka et al., 2002). According to Zin et al. (2017a), diketopiperazine gancidin W (GW) from *Streptomyces* SUK10, obtained from the bark of the *Shorea ovalis* tree, was isolated and tested *in vivo* against *P. berghei* PZZ1/100. GW exhibited a *P. berghei* inhibition rate of nearly 80% in mice, and had low toxicity, making it a good candidate for potential use as an antimalarial agent (Zin et al., 2017a). Happi et al. (2023) found that *Streptomyces* are important sources of bioactive metabolites that can be considered interesting candidates for the discovery of new antiplasmodial drugs (Happi et al., 2023). They observed that many compounds derived from *Streptomyces* demonstrated strong activities, and were in some cases more effective than the reference drugs. Zin et al. (2017b) fractionated the crude extract of *Streptomyces endophytic* SUK 08 in different solvents, where hexane was used as nonpolar solvent, ethyl acetate and dichloromethane as semipolar solvents and ethanol as polar solvent (Zin et al., 2017b). Of these four solvents, ethyl acetate was the best for extraction, exhibiting the highest antimalarial activity against *P. berghei*, without any significant cytotoxic effect on cells. They further stated that such observations were likely due to the semipolar nature of ethyl acetate, thus attracting polar and nonpolar SUK 08 compounds in the crude extract. Fractionation using ethyl acetate was important because it did not cause hemolysis and increased antiplasmodial activity of the APUR32.5 isolate. As for the toxicity of the fractions, the authors observed that both fractions (MPUR32.5 and APUR40.3) presented lower toxicity in comparison with doxorubicin, which is a standard drug in cytotoxicity tests, and is used for the treatment of cancer (Nicoletto and Ofner, 2022). One of the most important factors in a new drug is its low toxicity. Hence, a balance is necessary between clinical dose, efficacy and toxicity, so that the ideal action is on a specific target at low concentration (Sun et al., 2022).

The phylogenetic tree using the 16S rRNA gene sequence from the APUR32.5 and MPUR40.3 isolates with the corresponding sequences in the GenBank showed that these strains form a cluster with *S. rochei* and *S. plicatus*, with 100% similarity, allowing identification only at the genus level. The genus *Streptomyces* includes hundreds of species that produce many bioactive metabolites of clinical importance. Most members of this group share highly similar phenotypes. While 16S rRNA sequencing is more appropriate for the discrimination of more dissimilar *Streptomyces*, it is not efficient for separating phylogenetically closely related *Streptomyces* species (Guo et al., 2008). Bacteria of the genus *Streptomyces* isolated from the Amazon region are an important source of specialized metabolites with high structural diversity, which can be used in for wide range of biological purposes.

CONCLUSION

Strains of *Streptomyces* isolated from the sediment of the Madeira and Purus rivers were able to inhibit the growth of *P. falciparum* FCR3, with the best inhibition results obtained by the APUR32.5 and MPUR40.3 isolates. In the fraction obtained with ethyl acetate at a concentration of 3.12 µg/mL, the APUR32.5 isolate produced 84.5% inhibition and the MPUR40.3 isolate showed 86.6% inhibition, both superior to quinine (81.5%). The toxicity of both fractions were less than or equal to that of doxorubicin. However, further studies are needed targeting a *Streptomyces* compounds with antimalarial activity.

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