Antimalarial activity of basic phospholipases A2 isolated from Paraguayan Bothrops diporus venom against Plasmodium falciparum

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ABSTRACT

Malaria is a parasitic infectious disease and was responsible for 400,000 deaths in 2018. Plasmodium falciparum represents the species that causes most human deaths due to severe malaria. In addition, studies prove the resistance of P. falciparum to drugs used to treat malaria, making the search for new drugs with antiplasmodial potential necessary. In this context, the literature describes snake venoms as a rich source of molecules with microbicidal potential, including phospholipases A2 (PLA2s). In this sense, the present study aimed to isolate basic PLA2s from Paraguayan Bothrops diporus venom and evaluate their antiplasmodial potential. Basic PLA2s were obtained using two chromatographic steps. Initially, B. diporus venom was subjected to ion exchange chromatography (IEC). The electrophoretic profile of the fractions from the IEC permitted the selection of 3 basic fractions, which were subjected to reverse phase chromatography, resulting in the isolation of the PLA2s. The toxins were tested for enzymatic activity using a chromogenic substrate and finally, the antiplasmodial, cytotoxic potential and hemolytic activity of the isolated toxins were evaluated. The electrophoretic profile of the fractions from the IEC permitted the selection of 3 basic fractions, which were subjected to reverse phase chromatography, resulting in the isolation of the two enzymatically active PLA2s, BdTX-I and BdTX-II and the PLA2 homologue BdTX-III. The antiplasmodial potential was evaluated and the toxins showed IC50 values of: 2.44, 0.0153 and 0.59 μg/mL, respectively, presenting PLA2 selectivity according to the selectivity index results (SI) calculated against HepG2 cells. The results show that the 3 basic phospholipases isolated in this study have a potent antiparasitic effect against the W2 strain of P. falciparum. In view of the results obtained in this work, further research are necessary to determine the mechanism of action by which these toxins cause cell death in parasites.

1. Introduction

Malaria is an infectious parasitic disease with vector transmission involving a diversity of parasites in different ecological environments. Endemicity occurs in tropical and subtropical regions, mainly in Sub-Saharan Africa, Latin America and Southeast Asia. In 2018, the World Health Organization (WHO) reported 228 million cases of malaria, with an estimated 405,000 deaths; children under the age of 5 represent the most vulnerable group for this endemic disease. Despite decades of efforts, this disease remains a leading cause of death worldwide (WHO, 2020).

The species capable of infection in humans are: Plasmodium falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi (Cox, 2010). Among these, P. falciparum has a higher proportion of cases in the following

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regions: Africa (99.7%), Southeast Asia (50%), Eastern Mediterranean (71%) and Western Pacific Region (65%), while P. vivax appears in 53% of cases in Southeast Asia and the Americas. These are species with the highest epidemiological prevalence (WHO, 2020).

Regarding clinical presentation, the species P. falciparum is responsible for the severest form of malaria and, consequently, causes the majority of deaths. Another factor that makes P. falciparum an important focus of study is the capacity of resistance development by the parasite to antimalarials, especially for the standard scheme treatment. Initially, cases of Chloroquine resistance were recorded in Southwest Asia and then in South America (Nevin and Croft, 2016; Wongsrichanalai et al., 2002). Due this fact, since 2001, combined therapy with artesininin (ATC) has been used in the treatment of malaria (Su and Miller, 2015); however, failed treatments with ATC have been reported in strains of P. falciparum on the Thailand-Cambodia and Myanmar border, reaffirming the need for studies of new molecules with antiplasmodial potential (Ashley et al., 2014; Bhumiratana et al., 2013; Mathenge et al., 2020).

Given this scenario, the application of drugs with different chemical properties, the improvement of available pharmaceuticals and the development of new drugs, present new alternatives that may delay the evolution of parasitic resistance against antimalarials (Okombo and Chibale, 2016).

Regarding the identification of agents with biotechnological potential, biodiversity is very important as a source for the discovery of new molecules (Harvey et al., 2015; Newman and Cragg, 2016). In this sense, molecules of animal origin have stood out in the scientific literature for their potential biotechnological applications and in this context, several authors have reported the antitumor potential (Araya and Lomonte, 2013). Snake Venom PLA2, enzymes dependent on the calcium ion that catalyze hydrolysis in the sn-2 position of phospholipids, triggering the release of fatty acids and lysophospholipids (Dennis et al., 2011; Gutiérrez and Lomonte, 2013). Snake venom PLA2 (svPLA2) are widely studied proteins and are related to several clinical manifestations of snakebite envenoming, such as myotoxicity, neurotoxicity, cardiotoxicity, inhibition of platelet aggregation, and edema, among others (Gutiérrez and Lomonte, 2013; Kini, 2003; Lomonte and Gutiérrez, 2011).

An interesting characteristic of bothropic venoms is the presence of PLA2 isoforms. In addition to enzymatically active PLA2s, which have an aspartic acid residue at position 49 (Asp49-PLA2), the presence of PLA2s with no catalytic properties has been reported, called PLA2-homologues or Lys49-PLA2, due to the presence of a lysine residue at position 49 (Gutiérrez and Lomonte, 2013; Kini, 2003). Within the group of homologous PLA2s, variants have been described such as Ser49-PLA2 (Krizaj et al., 1970), Arg49-PLA2 (Mebes et al., 2006) and Gln49-PLA2 (Sao et al., 2005). It should be noted that, PLA2-homologues are devoid of enzymatic activity, and are responsible for various pharmacological effects (Lomonte et al., 2003).

In recent decades, another aspect of svPLA2 under investigation are their potential biotechnological applications and in this context, several authors have reported the antitumor potential (Araya and Lomonte, 2007), antiviral (Cecilio et al., 2013) and antiparasitic activity of svPLA2 (Grabner et al., 2017), which makes this group of proteins promising targets of study. In view of the above, the present study describes the isolation of basic PLA2s from the venom of Paraguayan Bothrops diporus with the objective of evaluating their antiparasitic potential on intra-erythrocytic forms of Plasmodium falciparum.
(BthTX-II) from B. jararacussu snake venom were used as the negative and positive controls.

2.4. In vitro antimalarial assays against P. falciparum

Chloroquine-resistant and mefloquine-sensitive (W2) P. falciparum clones of the Indochina III/CDC strain were used for the antimalarial activity assays. Parasite cultures were grown in human red blood cells under conditions established by Trager and Jensen (1976), with modifications. Cultures were prepared in TPP bottles with 2% hematocrit, diluted in RPMI 1640 medium culture (supplemented with 25 mM HEPES, 21 mM sodium bicarbonate, 11 mM glucose, 40 μg/mL gentamicin, and 0.5% (v/v) Albumax). The cultures were maintained in desiccators at 37 °C in a standard gas mixture consisting of 5% O2, 5% CO2 and 90% N2. Culture media were changed daily and parasitemia was monitored in blood smears, fixed with methanol, stained with fast panoptic segmentation and visualized under an optical microscope with an immersion objective lens (1000×).

2.4.1. Synchronization of parasites for in vitro tests

Cultures with a predominance of ring forms were obtained through synchronization with sorbitol as described by Lambros and Vanderberg (1979). The culture medium was removed from the bottles and 10 mL of 5% sorbitol and 0.5% glucose were added to the red blood cells containing the parasites. The volume was then transferred to a 15 mL Falcon® tube, incubated at 37 °C for 10 min and centrifuged for 5 min, 70×g at room temperature. The supernatant was removed, and the pellet resuspended in complete RPMI 1640 medium. Hematocrit and parasitemia were adjusted to 2% and 0.5%, respectively.

2.4.2. In vitro assay against P. falciparum

Parasite cultures were distributed in 96-well microplates with 180 μL/well of RPMI 1640 medium containing: 0.5% parasitemia and 2% hematocrit for the SYBR Green I test (Sigma-Aldrich®). Prior to adding the parasite suspension, 20 μL of the samples, previously diluted in PBS 1X, were added to the test plate in serial concentrations from 10 to 0.00488 μg/mL. Artemisinin (0.05 μg/mL) was used as a positive control, infected red cells were used as the negative control, and non-infected red blood cells (blank) as the experimental control. The plates were incubated at 37 °C for 48 h.

After the incubation period, a fluorescence test was performed according to Smilkstein et al. (2004). Briefly, the supernatant was removed and 100 μL of phosphate buffered saline (PBS) 1X was added to each well, and then centrifuged at 700×g for 10 min in order to wash the red blood cells. Afterwards, the supernatant was discarded and 100 μL of lysis buffer (20 mM Tris, pH 7.5; 5 mM EDTA; 0.008%; w/v saponin; 0.08%, Triton X-100 v/v) with SYBR Green I was added and the solution was transferred to 96-well microplates with 100 μL of 1X PBS, after which a reading was performed. After incubation for 30 min at room temperature, fluorescence was measured using a spectrophotometer (Synergy HT-BioTek) with an excitation of 485 nm and an emission of 590 nm.

2.5. In vitro cytotoxicity assays against HepG2 and selectivity index

Cytotoxicity was evaluated against HepG2 cells, in 96-well plates. The samples were tested in serial concentrations from 10 to 0.00488 μg/ml for BdTX-II and 100 to 0.00159 μg/ml for BdTX-I and BdTX-III, with a treatment period of 48 h. Concomitantly, the negative control consisted of untreated cells, while the positive control was cells treated with lysis buffer (20 mM Tris-HCl, 5 mM EDTA, 0.08% Triton X-100, 0.008% saponin in 1X PBS, at pH 7.5) and complete RPMI 1640 medium (control).

Cytotoxicity was determined using the MTT colorimetric method (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide-Sigma-Aldrich). After the treatment period, 20 μL of MTT was added at a concentration of 5 mg/mL in PBS 1x (w/v) to wells and incubated for 4 h at 37 °C with CO2 (Mosmann, 1983); after the incubation period, the supernatant was discarded and 100 μL of DMSO (Sigma-Aldrich) was added to the wells to dissolve the formazan crystals. Optical reading was performed on a plate spectrophotometer (Biochrom model: Expert plus) at wavelengths of 570 nm. Cell viability was determined according to the equation below:

\[ \text{Viability (\%)} = (\text{Test absorbance} - \text{Blank absorbance}) \times 100 \]

(Control absorbance – Blank absorbance)

The selectivity index (SI) was calculated using the ratios between the CC50 and IC50 values of the respective samples. Samples with SI values greater than 10 were considered to be selective/non-toxic, while compounds with SI values less than 10 were considered non-selective/toxic (Nava-Zuazo et al., 2010).

2.6. In vitro hemolytic activity

The hemolysis assay was performed with a suspension of 1% human erythrocytes in 180 μL of incomplete RPMI 1640 medium, distributed in a 96-well microplate with “U” bottom (Kasvi). 20 μL of samples, in serial concentrations from 10 to 0.00488 μg/mL, were deposited in the microplates and then incubated for 30 min at 37 °C, with shaking every 5 min (Wang et al., 2010). The absorbances were read on a spectrophotometer (Biochrom model: Expert plus) at wavelengths of 540 nm. 0.05% saponin (Sigma Aldrich®) was used as the positive control for hemolysis and non-lysed red blood cells cultured in incomplete RPMI 1640 medium were used as the negative control.

2.7. Quality control -Z’-factor

The results of the antimalarial, cytotoxicity and hemolysis tests were subjected to a quality control parameter. The parameter used was the Z'-factor (Zhang et al., 1999), a statistical factor that indicates the reliability of the results based on the degree of difference between positive and negative controls. The Z'-factor values were calculated based on the following formula:

\[ Z' = \frac{1 - 3 \times (\text{dpN} + \text{dpP})}{|\text{MN} - \text{MP}|} \]

where: dpN = negative control standard deviation, dpP = positive control standard deviation, MN = negative control mean, MP = positive control mean.

2.8. Statistical analyses

The analyses were performed with the aid of GraphPad Prism version 6.0 and Origin version 9.1. The results were expressed as mean ± SD of three independent experiments carried out in triplicate. The analysis of growth inhibition of 50% of the parasites (IC50) and cytotoxic concentration for 50% of the cells (CC50) were calculated using dose-response curves as a function of non-linear regression, applying the formula \( y = A_1 + (A_2 - A_1)/(1 + 10^{(\text{LOGx0-x})}) \). ANOVA followed by the Tukey multiple comparison test was used to analyze phospholipase activity and hemolysis assay results. The differences were considered significant when the p value showed a significance level <0.05.

3. Results

3.1. Isolation of PLA2s

The result for IEC was 7 main fractions (Fig. 1A), numbered 1–7, respectively. The initial fractions 1–3, which eluted before applying the gradient, correspond to acidic proteins, while the last four fractions 4–7 correspond to basic proteins, with fraction 4 being obtained with
approximately 30% buffer B and fractions 5, 6 and 7 eluted after approximately 65% buffer B. SDS-PAGE 12.5% was performed in order to determine the apparent molecular masses of putative PLA₂s, as well as of the molecules present in the eluted fractions (Fig. 1B). The electrophoretic profile suggests the presence of a variety of proteins with different molecular masses, which is characteristic of bothropic venoms. Based on the chromatographic and electrophoretic profiles (Fig. 1), the basic fractions F5, F6 and F7 were submitted to RPC. Fig. 2A–C shows the elution profile for the three isolated molecules. The electrophoretic profile allows for the visualization of main bands at

Fig. 1. Chromatographic and electrophoretic profiles of B. diporus venom. (A) This procedure allowed us to obtain seven fractions. The samples were eluted with a 0–100% gradient of buffer B, with a flow rate of 1 mL/min. Elution was monitored at 280 nm. (B) Electrophoretic profile of B. diporus venom (VBd) and seven fractions (F1–F7) eluted in IEC. It can be observed that F5, F6 and F7 fractions present protein bands with molecular mass ≈13 kDa, compatible with that of svPLA₂s.

Fig. 2. Chromatographic profile in RPC of F4-6 IEC fractions from B. diporus venom. Fig. 2A–C shows the RPC elution profile of the three isolated basic PLA₂s: BdTX-I (A), BdTX-II (B) and BdTX-III (C), respectively. The electrophoretic profile (D) demonstrates the presence of main bands with proteins ≈13 kDa, compatible with the molecular weight of svPLA₂s. Lanes: 1- BdTX-I, 2- BdTX-II and 3- BdTX-III.
approximately 13 kDa for each isolated toxins, putative PLA$_{s}$, which were named BdTX-I, BdTX-II and BdTX-III, respectively. It is possible to observe bands with proteins at approximately 25 kDa, corresponding to aggregates of PLA$_{s}$ in dimeric forms.

Phospholipase activity was evaluated using the chromogenic substrate 4N3OBA. In this step, it was demonstrated that BthTX-I showed enzymatic activity; however, the activity of BthTX-II was even more intense (Fig. 3). The enzymatic activity of these toxins was compared with the positive control (CP) BthTX-II (Asp49-PLA$_{s}$). BthTX-III showed no activity on the substrate, comparable to the negative control (CN) (BthTX-I, a Lys49-PLA$_{2}$-homologue). Both controls correspond to toxins isolated from B. jararacussu venom.

The PLA$_{s}$ isolated from fractions 5, 6 and 7 tested in vitro against strains of P. falciparum, showed IC$_{50}$ values of $2.44$ μg/mL, $0.0153$ μg/mL and $0.59$ μg/mL, respectively. Cytotoxicity assays on HepG2 cells indicated that the PLA$_{s}$ were not toxic at the concentrations tested, with CC$_{50}$ ≥ 10 and 100 μg/mL for both. The exact Selectivity Index (SI) cannot be calculated; however, with these results, the fractions were selective and non-toxic, showing SI ≥ 41, ≥653.6 and ≥169.5, respectively (Table 1).

Regarding the hemolytic capacity of these PLA$_{s}$, as a result, proteins did not show hemolytic action at the tested concentrations (10–0.0048 μg/mL). This result demonstrates that the PLA$_{s}$ are selective against the parasite and do not cause damage to erythrocytes.

4. Discussion

The diversity of proteins found in snake venoms explains the motivation of research groups who seek to develop studies aimed at elucidating the physical, chemical, structural and biological characteristics of the molecules that make up these venom and the relationship that these molecules have with the pathophysiology of snake envenoming and biotechnological applications (Kini and Fox, 2013; Simoes-Silva et al., 2018). Thus, the aim of this study was to evaluate the antiparasitic potential of basic isoforms of PLA$_{s}$ isolated from B. diporus venom.

Several authors have reported the purification of svPLA$_{2}$s through the application of different chromatographic techniques (Stabeli et al., 2012). In the present study, we chose to use cation exchange chromatography as the first fractionation step, a procedure from which 7 fractions were eluted (Fig. 1A), which were subjected to 12.5% SDS-PAGE. The electrophoretic profile presents the relative molecular masses of the proteins present in the seven eluted fractions (Fig. 1B).

Both the characteristic elution profile of fractions 5, 6 and 7 corresponding to basic proteins, as well as the relative molecular masses of the proteins observed in the aforementioned fractions, suggest that the molecules eluted in the last three fractions correspond to basic PLA$_{s}$ present in B. diporus venom. Cation exchange chromatography has already been reported by several authors as an efficient step for the fractionation and isolation of the proteins that make up these venoms (Andrião-Escarso et al., 2000; Soares et al., 1998, 2000a, 2000b).

Subsequently, basic fractions compatible with svPLA$_{2}$ molecular weights were subjected to reverse phase chromatography (Fig. 2A–C). The combination of IEC followed by RPC has already been reported by several authors, who mention that these steps contribute considerably to the isolation of basic PLA$_{s}$ from bothropic venoms, guaranteeing the purification of the molecules of interest with a high degree of purity (Alfonso et al., 2019; Grabner et al., 2017; Nunes et al., 2013). The electrophoretic profile of the isolated toxins can be seen in Fig. 2D. The eluted molecules were named BthTX-I, BthTX-II and BthTX-III, and for each of them, a main band of approximately 13 kDa can be observed, compatible with svPLA$_{2}$ molecular masses.

The test performed to evaluate the enzymatic activity of these toxins (Fig. 3) revealed that both BthTX-I and BthTX-II exert enzymatic activity, which indicates that these enzymes belong to the group of Asp49-PLA$_{s}$. On the other hand, considering that BthTX-III did not show catalytic properties, it is suggested that this protein is a homologous PLA$_{s}$ with a lysine residue at position 49 (Maraganore et al., 1984; Owney et al., 1999).

In this study, protein quantification suggests that the isolated proteins correspond to approximately 15% of the B. diporus venom. The literature describes PLA$_{s}$ as representing approximately 24% of the B. diporus venom proteome, with approximately 65% of PLA$_{s}$ corresponding to the Asp49-PLA$_{s}$ group (Gay et al., 2015). Recently, Teixera and coworkers (2018) reported the isolation of a Lys49-PLA$_{2}$ homologue from B. diporus named BthTX-I. The authors describe this toxin as having pharmacological effects such as myotoxicity and edema induction.

Subsequently, from the venom of the same species, Bustillo and coworkers (2019) describe the isolation of PLA$_{s}$-I (Asp49) and PLA$_{s}$-II (Lys49), reporting myonecrotic, cytotoxic action and suggesting that proteins have a synergistic effect that can potentiate their pharmacological effects.

As previously described, svPLA$_{s}$ present in B. diporus venom are responsible for different pharmacological effects. While the literature reports the antiparasitic activity of this group of proteins (Alfonso et al., 2019; Grabner et al., 2017; Nunes et al., 2013; Stabeli et al., 2006), this study aimed to evaluate the cytotoxic capacity of PLA$_{s}$ against the intra-erythrocytic forms of P. falciparum, becoming the first report in scientific literature to perform antiplasmodial assays of toxins isolated from Paraguay B. diporus.

Some authors have demonstrated that antiplasmodial action is more pronounced in enzymatically active PLA$_{s}$ than inactive ones. However, BmajPLA$_{s}$-II (a Lys49-PLA$_{2}$-homologue), isolated by Grabner and coworkers (2017) presents an IC$_{50}$ value comparable to that of the enzymatically active PLA$_{s}$ obtained in this study.

Previously, Zieler and coworkers investigated the antiplasmodial activity of a phospholipase isolated from Crotalus adamanteus; the authors describe that a concentration of 1 μmol.L$^{-1}$ of PLA$_{s}$ is effective to
block the in vitro development of ookinetes, intestinal forms of *P. falciparum* found in the mosquito (Zieler et al., 2001). In this context, Guillaume et al. also report antimalarial effects against intra-erythrocytic forms of *P. falciparum* of seven PLA$_2$s, with IC$_{50}$ values between 512.4 and 0.1 nM, including the snakes *A. kretzoi* and *Naja mossambica mossambica*, *Notechis scutatus scutatus* and *Vipera ammodytes* (Guillaume et al., 2004).

Upon analysis of the activities presented by different bothropic venoms, it is noted that the PLA$_2$s of these venoms are relevant in terms of their antiprotozoal action and antiplasmodial potential as shown in the present study.

The cytotoxicity assessment of the PLA$_2$s of *B. diporus* and *B. xenochlora* was performed against HepG2 cells. This cell line was chosen because hepatocytes are cells that naturally metabolize drugs and where *Plasmodium* replicates during a phase of its cycle in the vertebrate host (Thieleke-Matos et al., 2016). The CC$_{50}$ values observed for the three PLA$_2$s was ≥ 10 and 100 μg/mL; with this result the exact SI cannot be calculated; however, the fractions can be considered potentially safe regarding cytotoxicity parameters, since the SI results were greater than 10 (Nezlin et al., 2003; Katsumo et al., 2015).

The cytotoxic profile obtained in this study agrees with results previously obtained (Castillo et al., 2012). These authors tested an Asp49-PLA$_2$ purified from the venom of *B. asper*, which presented a CC$_{50}$ of 26.98 μg/mL against PBMC and an SI of 19 against *P. falciparum*. In this context, Grabner et al. reported that BmajPLA$_2$ has a CC$_{50}$ of 53.07 μg/mL and an SI of 8.28. It is noted that homologous PLA$_2$s tend to have lower SI than enzymatically active PLA$_2$s; however, in the present study the three protein isoforms presented CC$_{50}$ and SI values characterizing them as selective and non-toxic (Grabner et al., 2017).

In order to observe that the antiprotosomal activity of PLA$_2$s is not due to hemolytic action, an in vitro hemolysis assay was performed, using the same concentrations tested in the antiprotosomal assay. BdTX-I, BdTX-II and BdTX-III did not present hemolysis. The correlation of these results shows that the antiprotosomal activity of these PLA$_2$s may occur directly on the intra-erythrocytic forms of *P. falciparum* and not cause destabilization or lysis of the red blood cell membrane.

5. Conclusion

Natural animal and plant products can provide compounds that have therapeutic biotechnological potential in the face of biological tests; among these include snake venoms, which have been successful in obtaining new drugs. Bothropic venoms are biochemically complex, consisting mostly of protein components, such as metalloproteases, serinoproteases, type C lectins, L-amino acid oxidases (LAAO), myo-toxins, disintegrins and phospholipases A$_2$ (PLA$_2$).

The results presented herein show that PLA$_2$s isolated from *B. diporus* have applicable biotechnological potential regarding their antiplasmodial activity. New studies should be carried out in order to understand the mechanism of death of the parasite in the presence of these proteins.

CRediT authorship contribution statement

Keila A. Vitorino: Conceptualization, Investigation, Methodology.
Jorge J. Alfonso: Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing, Visualization. Ana F. Gómez: Conceptualization, Methodology, Validation, Writing - original draft, Visualization. Ana Paula A. Santos: Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing, Visualization. Ygor R. Antunes: Methodology, Validation. Cleopatra A. da S. Caldeira: Conceptualization, Investigation, Methodology. Celeste V. Gómez: Visualization, Supervision. Carolina B.G. Teles: Conceptualization, Validation, Writing - original draft, Funding acquisition. Andreimar M. Soares: Writing - original draft, Project administration, Funding acquisition. Leonardo A. Calderon: Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| PLA$_2$ | phospholipase |
| A$_2$ | snake venom phospholipase A$_2$ |
| IEC | Ionic Exchange Chromatography |
| SDS-PAGE | Poly-Acrylamide Gel Electrophoresis in the presence of Sodium Dodecyl Sulfate |
| RPC | Reverse Phase Chromatography |
| TFA | trifluoroacetic acid |
| ACN | Acetonitrile |
| 4N3OBA | 4-nitro-3-(octyloxy) benzoic acid |
| RPMI-1640 | Roswell Park Memorial Institute |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyloxazole bromide |
| DMSO | Dimethyl sulfoxide |
| IC$_{50}$ | half maximal inhibitory concentration |
| CC$_{50}$ | half maximal cytotoxic concentration |
| SI | Selectivity Index |

Availability of data and materials

Not applicable.

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Authors’ contributions

KAV, JJA, AFG, YRA, AMS, LAC performed the isolation and biochemical characterization. KAV, APAS, CBGT carried out the plasmocidal assays. KAV, JJA, AFG, YRA, AMS, LAC, CVG, APAS, CBGT analyzed the results. KAV, JJA, AFG, YRA, CASC, CVG, APAS, CBGT, AMS and LAC participated in the discussion of the results, carried out a critical review of the work and assisted in drafting and structuring the manuscript. JJA, AFG, LAC, CBTG, CVG and AMS were responsible for the conception of the study and supervised the experimental work. All the authors read and approved the final manuscript.
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