JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
2022, VOL. 40, NO. 23, 13001–13016
https://doi.org/10.1080/07391102.2021.1978320

Leishmanicidal and cytotoxic activity of essential oil from the fruit peel of *Myrciaria floribunda* (H. West ex Willd.) O. Berg: Molecular docking and molecular dynamics simulations of its major constituent onto *Leishmania* enzyme targets

Deyzi Caroline da Silva Barbosa[a], Vanderlan Nogueira Holanda[a,b], Arabinda Ghosh[c], Rafael Trindade Maia[d], Welson Vicente da Silva[a], Vera Lúcia de Menezes Lima[b], Márcia Vanusa da Silva[b], Maria Tereza dos Santos Correia[b]* and Regina Celia Bressan Queiroz de Figueiredo[a]*

[a]Departamento de Microbiologia, Instituto Aggeu Magalhães, Recife, Pernambuco, Brazil; [b]Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil; [c]Microbiology Division, Department of Botany, Gauhati University, Guwahati, Assam, India; [d]Centro de Desenvolvimento Sustentável do Semiárido, Universidade Federal de Campina Grande, Sumé, Paraíba, Brazil

Communicated by Ramaswamy H. Sarma

ABSTRACT

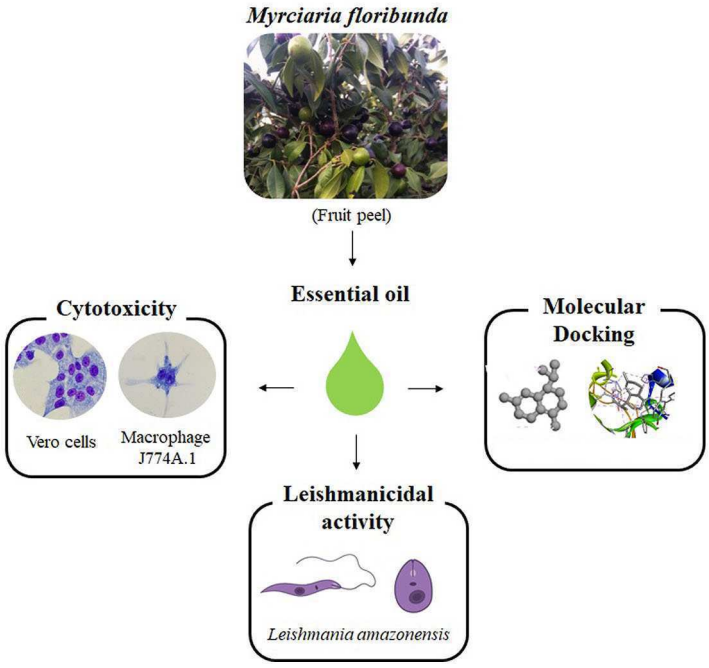
Cutaneous Leishmaniasis (CL) is a neglected disease characterized by highest morbidity rates worldwide. The available treatment for CL has several limitations including serious side effects and resistance to the treatment. Herein we aimed to evaluate the activity of essential oil from the peel of *Myrciaria floribunda* fruits (MfEO) on *Leishmania amazonensis*. The cytotoxic potential of MfEO on host mammalian cells was evaluated by MTT. The *in vitro* leishmanicidal effects of MfEO were investigated on the promastigote and intracellular amastigote forms. The ultrastructural changes induced by MfEO were evaluated by Scanning Electron Microscopy (SEM). The molecular docking of the major compounds δ-Cadinene, γ-Cadinene, γ-Muurolene, α-Selinene, α-Muurolene and (E)–Caryophyllene onto the enzymes trypanothione reductase (TreR) and sterol 14-alpha demethylase (C14DM) were performed. Our results showed that MfEO presented moderate cytotoxicity for Vero cells and macrophages. The MfEO inhibited the growth of promastigote and the survival of intracellular amastigotes, in a dose- and time- dependent way. The MfEO presented high selectivity towards amastigote forms, being 44.1 times more toxic for this form than to macrophages. Molecular docking analysis showed that the major compounds of MfEO interact with *Leishmania* enzymes and that δ-Cadinene (δ-CAD) presented favorable affinity energy values over TreR and C14DM enzymes, when compared with the other major constituents. Molecular dynamics (MD) simulation studies revealed a stable binding of δ-CAD with lowest binding free energy values in MMGBSA assay. Our results suggested that δ-CAD may be a potent inhibitor of TreR and C14DM enzymes.

ARTICLE HISTORY
Received 29 January 2021
Accepted 3 September 2021

KEYWORDS
Myrciaria floribunda; molecular docking; δ-cadinene; cutaneous leishmaniasis; MD simulation

CONTACT Regina Celia B. Q. Figueiredo regina.bressan@fiocruz.br Laboratório de Biologia Celular de Patógenos, Departamento de Microbiologia, Instituto Aggeu Magalhães – IAM/FIOCRUZ-PE, Avenida Professor Moraes Rego s/n, Recife, Pernambuco 50670-465, Brazil
*Both authors contributed equally to this work.

Supplemental data for this article can be accessed online at http://dx.doi.org/10.1080/07391102.2021.1978320



Abbreviations: ADMET: Absorption, distribution, metabolism, excretion, and toxicity; Ama: Amastigote; AmB: Amphotericin B; CL: Cutaneous Leishmaniasis; $CaCl_2$: Calcium chloride; CC_{50}: Concentration required to reduce cell viability by 50%; CO_2: Carbon dioxide; C14DM: Sterol 14-alpha demethylase; DHO: Dihydroorotate; DHODH: Dihydroorotate dehydrogenase; DMSO: Dimethyl sulfoxide; EOs: Essential oils; GC/MS: Gas Chromatography Mass Spectrometry; FAD: flavin adenine dinucleotide; IC_{50}: Inhibitory concentration for 50% of parasites; MM-GBSA: Molecular mechanics energies combined with the Poisson–Boltzmann or generalized Born and surface area continuum solvation; MfEO: Essential oil form fruit the peel of *M. floribunda*; MD: Molecular dynamics; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; n.d: Not determined; NTDs: Neglected tropical diseases; ORO: Orotate; PDB: Protein Data Bank; Pro: Promastigote; PTR: Pteridine reductase; Rg: Radius of gyration; RMSD: Root Mean Square Deviation; RMSF: Root Mean Square Fluctuation; SEM: Scanning Electron Microscopy; FBS: Fetal Bovine Serum; SeI: Selectivity index; TreR: Trypanothione reductase

1. Introduction

Essential oils (EOs) are natural products extracted from plants and have important biological properties in the context of neglected diseases. Furthermore, EOs are easy to obtain, have low cost, and are effective in the treatment of parasitic infections (Badirzadeh et al., 2020). EOs from the species of Myrtaceae family have attracted attention due to their proven biological activities, such as antimicrobial (Correa et al., 2019), anti-inflammatory (Costa et al., 2020), antioxidant (Hennia et al., 2019) and leishmanicidal (Durazzini et al., 2019). These potential medicinal applications are related to the ability of EOs constituents, mainly terpenes and terpenoids molecules, to interact with target molecules (Kim et al., 2014).

Myrciaria floribunda (H. West ex Willd.) O. Berg, a medicinal plant belonging to the Myrtaceae family and popularly known as Cambuí, has been reported as having insecticidal (Tietbohl et al., 2020), antibacterial and antioxidant activities (de Azevedo et al., 2019). The EOs from the leaves and flowers of this plant also demonstrated an inhibitory potential on the acetylcholinesterase for the treatment of neurodegenerative disorders (Tietbohl et al., 2012). However, at our best knowledge, the investigation on the biological potential of the essential oil from fruit peel of *M. floribunda* (MfEO) against parasites of medical and veterinary importance as *Leishmania* spp, is still lacking. Furthermore, the fruit peels are usually discarded as useless material despite being a source of metabolites with higher concentration of bioactive components compared to the pulp (Saidani et al., 2017; Czech et al., 2020). In a previous work we have described the chemical composition of MfEO and the ADMET profile of their major compounds, suggesting that this EO may be promising for pharmacological prospecting and treatment of diseases (da Silva Barbosa et al., 2020).

Leishmaniasis are neglected tropical diseases (NTDs) caused by protozoa of *Leishmania* genus, responsible for the higher rates of morbidity. These diseases have a worldwide distribution being found in about 89 countries (World Heald Organization, 2020; Valero & Uriarte, 2020). Among the clinical forms of these diseases, Cutaneous Leishmaniasis (CL) stands out for its higher capacity to cause physical deformities (Holanda et al., 2020). *Leishmania amazonensis* is considered as the most relevant epidemic species, due to its wide geographical distribution, and the ability to cause different

forms of CL (Christensen et al., 2019). Although many ways to combat the leishmaniasis have been proposed, such as the adoption of sanitary measures (Okwor & Uzonna, 2016), research on the vaccine development and for the discovery of effective drugs against the etiological agents (Sousa et al., 2019), these diseases are still a health concern, mainly in the development countries.

Currently, there are no vaccines for leishmaniasis and for more than seventy years, the treatment for these illnesses has been based on the use of parenteral administration of pentavalent antimonials (Menezes et al., 2015). The first-line drugs Meglumine antimoniate and sodium stibogluconate have several disadvantages, such as a long period of therapeutic regimen, variations in the effectiveness, cases of resistance (Sundar et al., 2019) and serious side effects (Marques et al., 2019). The main adverse effects observed for these drugs are the cardiotoxicity, hepatotoxicity, and nephrotoxicity (Taheri et al., 2019), which, in some cases, can result in the patient death (Costa et al., 2003; Lima et al., 2007). In this regard in the present work, we investigated for the first time, the effects of MfEO on the mammalian cells and its inhibitory activity on the promastigote and amastigote forms of *Leishmania amazonensis*. We also used *in silico* approaches to predict the potential of MfEO major constituents as inhibitors of relevant enzyme targets of *Leishmania*.

2. Material and methods

2.1. M. Floribunda fruit peel essential oil (MfEO)

The fruits of *Myrciaria floribunda* were collected in the municipality of Exu, in the state of Pernambuco, Brazil (voucher 92722) and the MfEO was extracted using the hydrodistillation method (Clevenger apparatus). For this, 200 g of the fruit peel was mixed with 2.5 L of distilled water and subjected to hydrodistillation for 3 h. After extraction, the oil was stored at 5 °C. The chemical characterization of MfEO was performed through GC/MS analysis, as described by da Silva Barbosa et al. (2020).

2.2. In vitro evaluation of the effect of MfEO on mammalian cells

The cytotoxic potential of MfEO was evaluated according to Holanda et al. (2020) with minor modifications. Briefly, Vero and macrophage J774A.1 cells (5×10^5 cells/well) were seeded in 96-well plates containing 100 µL of RPMI medium, supplemented with 10% inactivated FBS and incubated for 3 h at 37° C in 5% CO_2 atmosphere. After this period, non-adherent cells were removed, and the remaining adhered Vero cells and macrophages were cultured for 48 h in RPMI in the absence or presence of different concentrations of MfEO (31.25 to 500 µg/mL) diluted in 0.1% DMSO. Subsequently, treated, and untreated cells were washed and incubated in 100 µL of fresh RPMI medium containing 5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma- Aldrich, St. Louis, MO, USA), for 3 h at 37 °C. Then, the precipitate of formazan crystals derived from the reduction of MTT was solubilized in isopropanol solution (100 µL/well) and the absorbance was read in a spectrophotometer at 540 nm. The cytotoxic concentration of MfEO (CC_{50}) was determined by regression analysis using the SPSS 8.0 software.

2.3. Leishmanicidal activity assay

2.3.1. Effect of MfEO on promastigotes forms

To evaluate the effects of MfEO in the promastigote forms of *Leishmania amazonensis* (LTB0016), 10^6 parasites/mL were cultured in Schneider's medium supplemented with 10% FBS, in the absence or presence of different concentrations of MfEO (25 to 200 µg/mL diluted in 0.1% DMSO) and incubated at 26 °C for 48 h. The parasite viability was evaluated using the CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega, USA) at 24 and 48 h, and the concentration of MfEO capable of inhibiting 50% of the parasite viability (IC_{50}) was determined by regression analysis using the SPSS 8.0 software.

2.3.2. Effect of MfEO on intracellular amastigotes forms

To verify the action of MfEO on the intracellular amastigote forms of *L. amazonensis*, macrophages J774A.1 (10^6 cells/mL) were plated in 24-well culture plates containing RPMI medium supplemented with 10% FBS. Macrophages were allowed to adhere for 3 h at 37 °C in 5% CO_2 atmosphere and subsequently infected with *L. amazonensis* promastigotes at 20:1 parasite/cell ratio at 37 °C for 14 h. The non-internalized parasites were removed by washing and the adhered cells were incubated for 24 h at the same cultivation conditions with 1/8, 1/4, 1/2 and 1x of the IC_{50} value of MfEO for promastigote forms. The untreated cells or treated with the reference drugs (Meglumine Antimoniate and Amphotericin B) were used as negative and positive controls, respectively. After the incubation time, the cells were washed in PBS, fixed in methanol and stained with panoptic staining. The total number of amastigote/infected cells was determined by counting 150 randomly chosen macrophages in duplicate. The survival index of intracellular amastigote was determined by multiplying the percentage of infected macrophages by the average number of parasites per infected cell. The IC_{50} of MfEO for amastigote forms was determined by regression analysis as already described. The selectivity index (SeI) of MfEO for promastigotes and amastigotes was determined by the ratio of CC_{50} for macrophages and the IC_{50} for both developmental forms of *L. amazonensis*.

2.4. Ultrastructural assay

To analyze the possible changes in the ultrastructure of *L. amazonensis* promastigotes in response to the MfEO treatment, the parasites were treated with IC_{50} or 2x the IC_{50} value, for 48 h. Untreated cells were used as a control. Subsequently, the cells were fixed for 2 h at room temperature in a solution containing 2.5% glutaraldehyde/4% paraformaldehyde in 0.1 M phosphate buffer pH 7.2. After washing in the same buffer, the cells were post-fixed for 1 h with 1% osmium tetroxide/0.8% potassium ferrocyanide/5 mM $CaCl_2$ in 0.1 M cacodylate buffer, pH 7.2. After washing

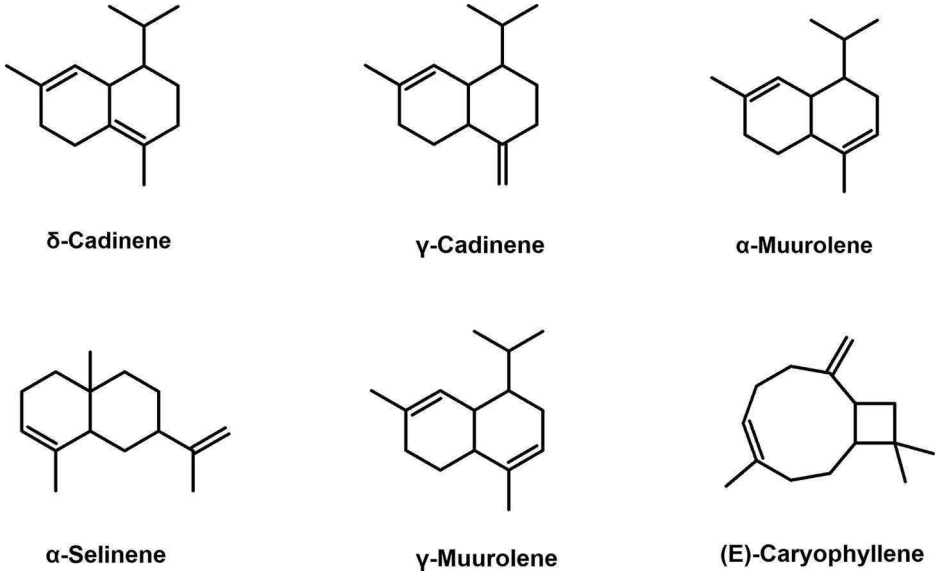


Figure 1. Chemical structures of the major compounds of MfEO.

with cacodylate buffer, the cells were adhered to the coverslips coated with poly-L-lysine. The samples were dehydrated in ethanol, dried at a critical point HCP-2 (Hitachi, Tokyo, Japan), coated with a 20 nm thick gold layer, and observed in a JEOL T-200 Scanning Electron Microscope.

2.5. Molecular docking of major compounds of MfEO on Leishmania target proteins

The molecular docking of the main MfEO constituents onto *Leishmania* target proteins was carried out using AutoDock 4.2 software (Morris et al., 2009). The target proteins were selected according to both: the stereochemical parameters provided by Ramachandran analysis, using the PROCHECK server (Laskowski et al., 2006) and its metabolic role on the *Leishmania* survival and proliferation, as described by Herrera-Acevedo et al. (2021). The Molecular docking was performed based on the confrontation of the optimized ligand structures with the selected protein targets from *Leishmania*. The crystal structures of target proteins were obtained from Protein Data Bank (PDB) under the codes: dihydroorotate dehydrogenase (3C61), sterol 14-alpha demethylase (3L4D), trypanothione reductase (2YAU) and pteridine reductase (1W0C). The validation of target protein-ligand complex structures was performed using the co-crystallized standard ligand of target proteins to ensure the virtual screening process. For this, sterol 14-alpha demethylase was redocked with its co-crystalized ligand fluconazole. For each ligand-protein complex 10-docking poses were generated using Lamarckian Genetic Algorithm (López-Camacho et al., 2015). The pose with the lowest affinity energy value was selected as the final docking result. The interactions of the target-ligand complex were analyzed and rendered using Discovery Studio v.20.1.0.19295.

2.6. Molecular dynamics (MD) simulations

MD simulations studies of the δ-Cadinene (δ-CAD) interaction with the proteins trypanothione reductase (TreR) and sterol 14-alpha demethylase (C14DM) were performed for 50 ns using the Desmond 2018-4 from Schrödinger, LLC. The OPLS-2005 force field (Bowers et al., 2006; Chow et al., 2008; Shivakumar et al., 2010) and explicit solvent model with the SPC water molecules were used in this system (Jorgensen et al., 1996). Na^+ ions and 0.15 M, NaCl solution were added to the system to neutralize the charge and simulate the physiological environment, respectively. The NPT ensemble was set up by using the Nose-Hoover chain-coupling scheme (Martyna et al., 1992). The temperature was maintained at 300 K with a relaxation time of 1.0 ps and pressure 1 bar in all the simulations. A time step of 2 fs was used. The Martyna-Tuckerman–Klein chain coupling scheme (Martyna et al., 1994) barostat method was used for pressure control with a relaxation time of 2 ps. The particle mesh Ewald method (Toukmaji & Board, 1996) was used for calculating long-range electrostatic interactions and the radius for the Coulomb interactions were fixed at 9 Å. RESPA integrator was used to calculate the non-bonded forces. The root-mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), protein ligand interactions were monitored to observe the stability of the complex in MD simulations.

2.7. MM-GBSA free energy calculations

The binding free energy calculation of the protein-ligand docking complexes was estimated by using the MM/GBSA by using OPLS-2005 force field (Genheden & Ryde, 2015). The MM-GBSA method calculates the binding free energy as follows:

$$dG_{bind} = G_{complex} - (G_{protein} + G_{ligand}) \quad (1)$$

Where, dG_{bind} = binding free energy, $G_{complex}$, $G_{protein}$, and G_{ligand} are the free energies of the protein-ligand complex, protein and ligand, respectively. The other contributing energies along with dG_{bind} obtained are presented as the mean ± standard deviation (SD).

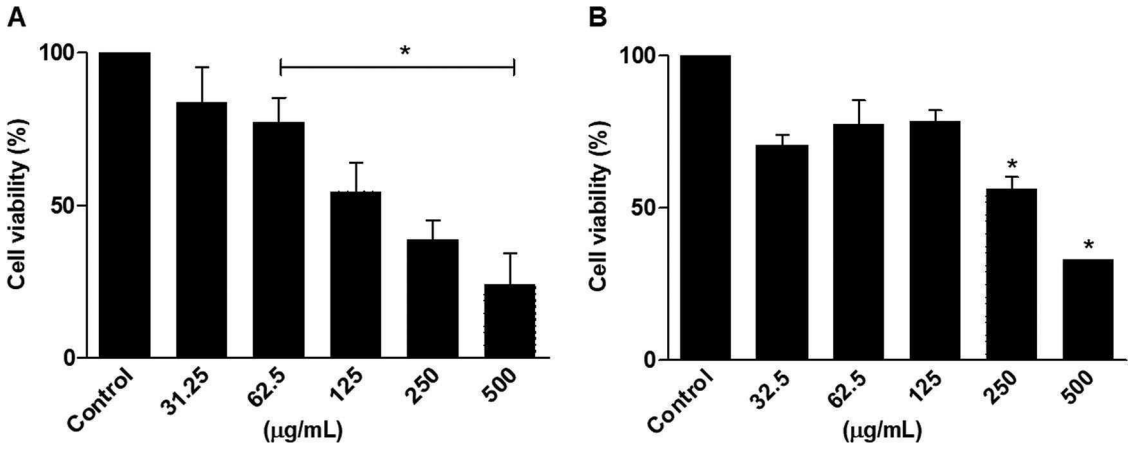


Figure 2. Effects of MfEO on mammalian cells after 48 h of treatment. (A) Vero cells, (B) Macrophages. The bars represent the mean ± SD of three independent experiments in triplicate. * Significant difference compared to the control group ($p < 0.05$).

Table 1. Leishmanicidal and cytotoxic effects of essential oil of *M. floribunda*.

	MfEO (μg/mL)				
		IC_{50}		Sel	
Cell Type	CC_{50}	Pro	Ama	Pro	Ama
Vero	260.9 ± 6.3	–	–	4.67	n.d.
J774	332.9 ± 13.1	–	–	5.96	44.15
L. amazonensis	–	55.84 ± 1.1	7.54 ± 0.6		

MfEO: Essential oil from the *Myrciaria floribunda* peel fruit; CC_{50}: Concentration required to reduce cell viability by 50%; IC_{50}: Inhibitory concentration for 50% of parasites; Sel: Selectivity Index; Pro: Promastigote; Ama: Amastigote; n.d: not determined. The Data represent the mean ± SD of three independent experiments performed in triplicate.

2.8. Statistical analysis

Linear regression was performed using SPSS 8.0 software (IBM Co., New York, USA). Nonparametric data were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni tests. Statistical analysis was performed using the Prism 5.0 software (GraphPad, San Diego, CA, USA). All statistical analyzes were performed at the level of significance $p < 0.05$ for *in vitro* assays.

3. Results and discussion

3.1. Effect of MfEO on the mammalian cells

Essential oils are a mixture of compounds rich in metabolites that present several biological activities, being a promising starting point for the development of new drugs against infectious diseases (Oliveira et al., 2020). In this regard, the EOs from the Myrtaceae family have been reported as a source of secondary metabolites with pharmacological properties against various diseases (Durazzini et al., 2019; Mancianti & Ebani, 2020). The chemical composition of MfEO revealed that this EO is rich in terpene compounds having as their main constituents the δ-Cadinene (26.8%), γ-Cadinene (15.69%), γ-Muurolene (6.21%), α-Selinene (6.11%), α-Muurolene (6.11%) and (E)-Caryophyllene (5.54%) (Figure 1)

An important criterion to be observed in the search for new bioactive compounds is its cytotoxic potential to mammalian cells (Bazana et al., 2019). In this regard, this parameter was evaluated in the Vero cells and J774A.1 macrophages treated with MfEO, using the MTT method after 48 h of incubation with the EO (Figure 2). The MfEO presented a CC_{50} of 260.9 μg/mL and 332.9 μg/mL for Vero cells and macrophages, respectively (Table 1). Accordingly, to a classification made by Ríos et al. (2008), EOs having a CC_{50} between 100 and 500 μg/mL are considered as moderately toxic. Our results showed that the macrophages were more resistant to MfEO when compared to the Vero cells. This observation is important, since the macrophage is the main target cell of *Leishmania* spp. The resistance of J774A.1 macrophages to MfEO treatment may be related to the intense metabolic activity of these cells and its ability to neutralize potentially toxic chemical constituents (Chowdhary et al., 2016). The moderate cytotoxicity of MfEO prompted us to investigate the effects of MfEO on the promastigote forms of *L. amazonensis*.

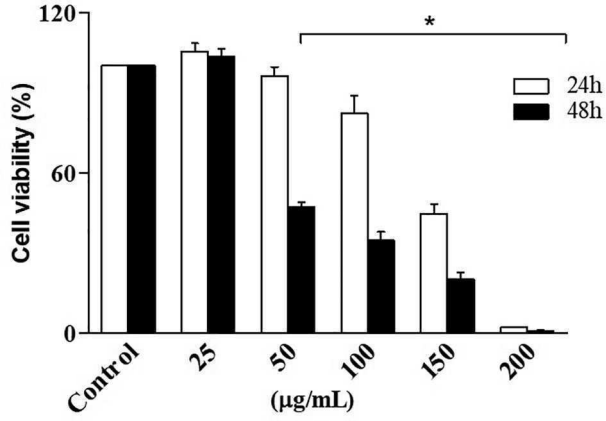


Figure 3. The effects of MfEO on promastigote forms of *L. amazonensis* after 24 and 48 h of treatment. The bars represented the mean ± SE of three independent experiments in triplicate. * Significant difference when compared to the control group ($p < 0.05$).

3.2. Leishmanicidal effect of MfEO on promastigotes and intracellular amastigotes of Leishmania amazonensis

The inhibitory effects of EOs on pathogenic Trypanosomatids has been widely investigated over the years, with emphasis to the EOs from the Myrtaceae family (Souza et al., 2017; Durazzini et al., 2019). In this study we investigated the

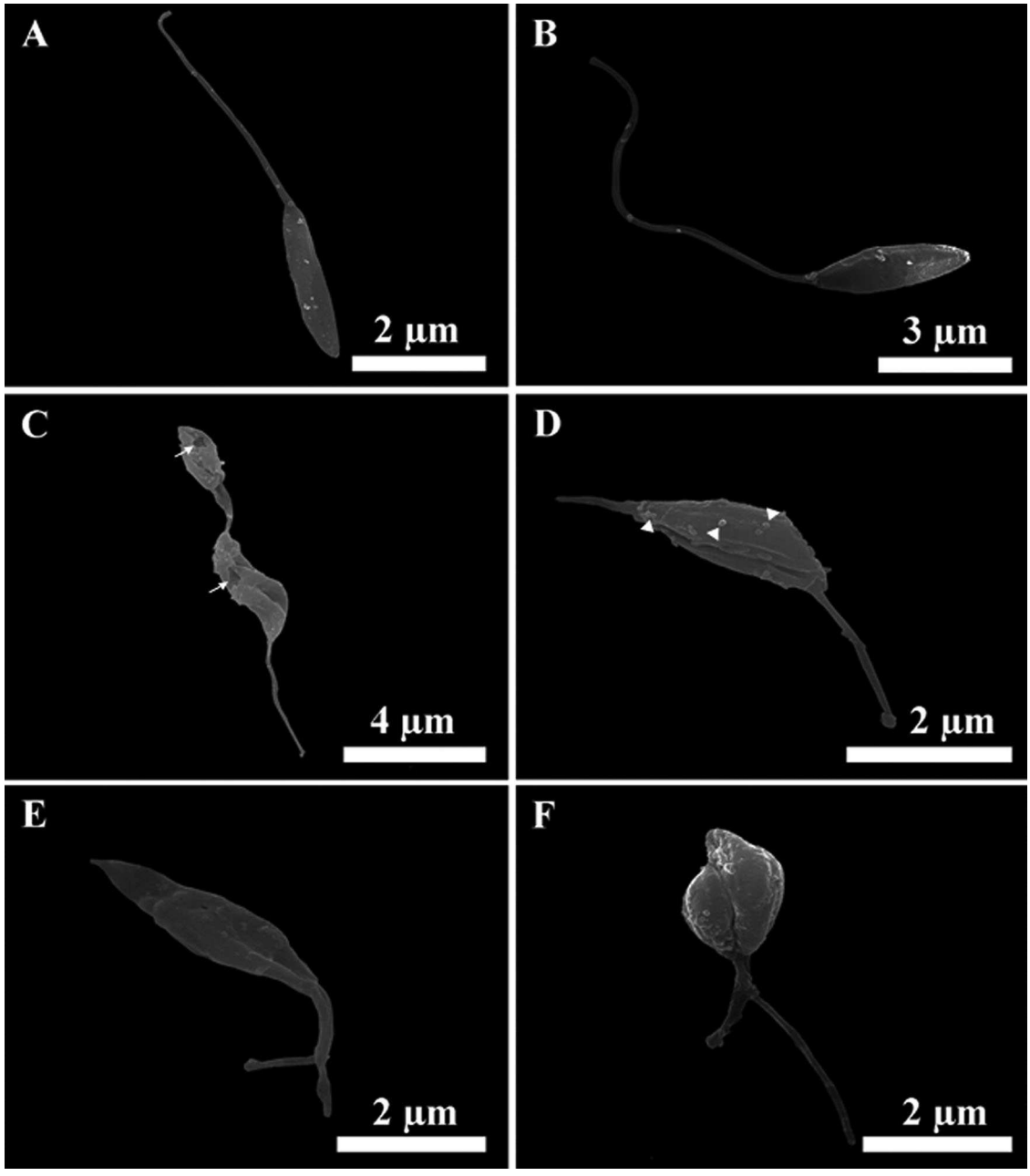


Figure 4. The effects of MfEO on *L. amazonensis* ultrastructure as observed by scanning electron microscopy. (A-B) Control promastigotes (C-D) Promastigotes treated with 55.84 µg/mL of MfEO. (C) Note the presence of perforations on the plasma membrane (arrow). (D) Detail of exocytic vesicles budding from cell surface (arrowhead). (E-F) Promastigotes treated with 111.68 µg/mL of MfEO.

leishmanicidal activity of MfEO on *L. amazonensis,* the etiological agent of cutaneous leishmaniasis. Our results showed that MfEO was able to significantly inhibit the viability of *L. amazonensis* promastigote after 24 h of cultivation, at concentrations higher than 100 µg/mL. At 48 h, the inhibitory effect of MfEO on the parasite is earlier observed, for concentrations superior to 50 µg/mL (Figure 3). The concentration that inhibited the viability of promastigotes by 50% (IC_{50}) was 55.84 µg/mL (Table 1). The major constituents of the MfEO, the δ-Cadinene (Andrade et al., 2016; Ferreira et al., 2020), α-Muurolene (Macêdo et al., 2020) and (E)-Caryophyllene (da Silva et al., 2018; Moreira et al., 2019) have also been described as chemical constituents of other EOs with inhibitory activity on *L. amazonensis.*

It is interesting to note that at the concentration of 200 µg/mL of MfEO the viability of promastigotes was inhibited to almost 100%. Although there is no statistic difference compared to the control cells, at 25 µg/mL the viability of treated cells, mainly at 24 h of cultivation, was slightly higher than the untreated cells. This effect could be due to a shift on cellular metabolism to circumvent the stress induced by treatment with EO (Monzote et al., 2018). When the cytotoxicity of MfEO for mammalian cells was compared with the leishmanicidal effect on promastigote we found that this essential oil was about 6 and 5 times more toxic for the parasite than to the macrophages and Vero cells, respectively (Table 1).

In the next step of our work, we analyzed the effects of MfEO treatment on the ultrastructure of *L. amazonensis*. For

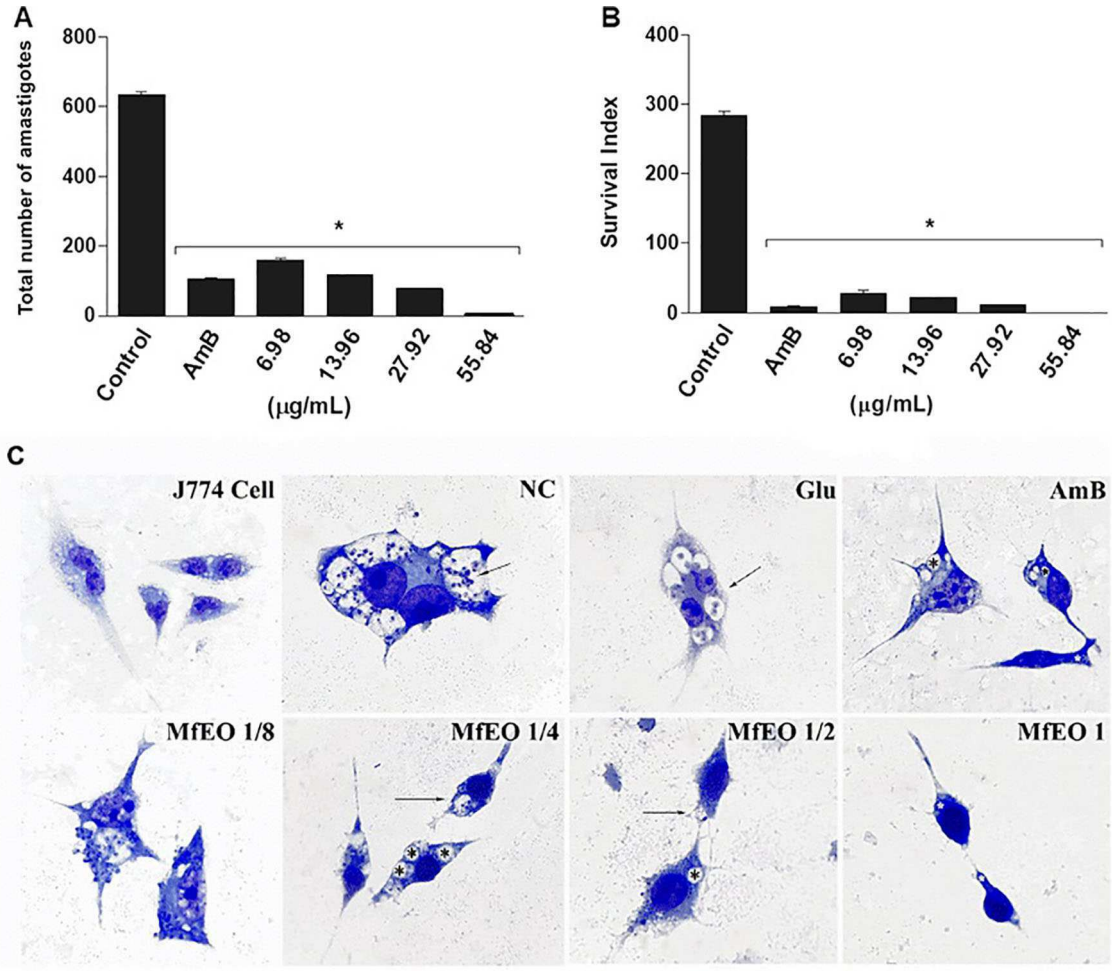


Figure 5. Effects of the MfEO on intracellular amastigotes forms of *L. amazonensis*. (A) Total number of intracellular amastigotes; (B) Survival index of amastigotes inside macrophages; (C) Light microscopy of J774A.1 infected-macrophage cultures treated or not with different concentrations of MfEO. Amphotericin B (AmB) and Glucantine (Glu) were used as reference drugs. Arrows point to the amastigote forms inside large parasitophorous vacuoles. Each bar represents the mean ± SD of three independent experiments performed in duplicate. (*) Significant difference compared to the control group ($p < 0.05$).

this, promastigote forms were treated with 1x and 2x the IC_{50} value for 48 h and analyzed by Scanning Electron Microscopy (Figure 4). The control cells (Figure 4A-B) showed usual morphology, with spindle-shaped cell body and preserved elongated flagellum. The treated cells showed drastic ultrastructural changes such as the appearance of aberrant dividing cells, the wrinkling and rupture of the plasma membrane (Figure 4C and D), the presence of exocytic vesicles (Figure 4D) and a reduction of the cell body (Figure F). These changes are compatible with the loss of cell viability in *Leishmania* cells (Tiuman et al., 2005; da Silva et al., 2018).

The effects of MfEO on the promastigote ultrastructure may be related to the lipophilic nature of this essential oil. The partition coefficient logarithm between octanol and water (cLogP) of the major component of MfEO were ≥ 4 (lipophilicity), which facilitates the passage of such compound through the biological membranes facilitating the interaction with intracellular targets (da Silva Barbosa et al., 2020). Pinto et al. (2014) evaluating a series of molecules found that the nonpolar character of a given compound was directly related to its effectiveness against protozoa. Previous study showed that the treatment of *L. chagasi* with *Cymbopogon citratus* essential oil leads to the appearance of aberrant-shaped cells, suggesting an impairment of cell division at the end of telophase (Oliveira et al., 2009).

In view of our promising results in the promastigotes, we evaluated the action of MfEO on the intracellular amastigotes. The investigation of the leishmanicidal activity in this developmental stage of the parasite is an effective way to correlate the *in vitro* and *in vivo* anti-*leishmania* activity of tested substances candidates to formulations and drugs (Russell & Talamas-Rohana, 1989). In this regard, infected macrophages were treated or not with MfEO, at concentrations corresponding to 1/8, 1/4, 1/2 and 1x the IC_{50} values previously obtained for the promastigote form.

The treatment of the infected-macrophages with both MfEO and Amb significantly reduced the number of amastigotes inside these cells, in a dose dependent way, when compared to untreated-infected ones. At the concentrations of 27.92 μg/mL and 55.84 μg/mL, the effectiveness of MfEO against intracellular amastigotes, were comparably to the reference drug amphotericin B (reference drug) at the concentration of 9.75 μg/mL (Figure 5A). At the highest concentration (55.84 μg/mL), the survival rate of amastigotes was close to zero (Figure 5B). The MfEO was more effective against intracellular amastigote than promastigote forms,

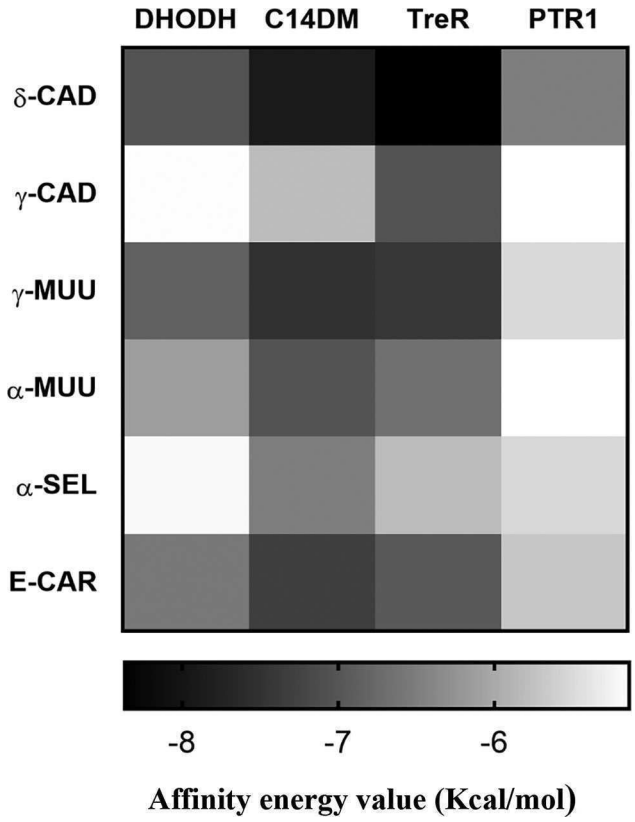


Figure 6. Analysis of the affinity energy value of δ-CAD, γ-CAD, γ-MUU, α-MUU, α-SEL and E-CAR on *Leishmania* proteins. The affinity energy values are represented as black colour range scale. As closer to black more favourable is the affinity energy value.

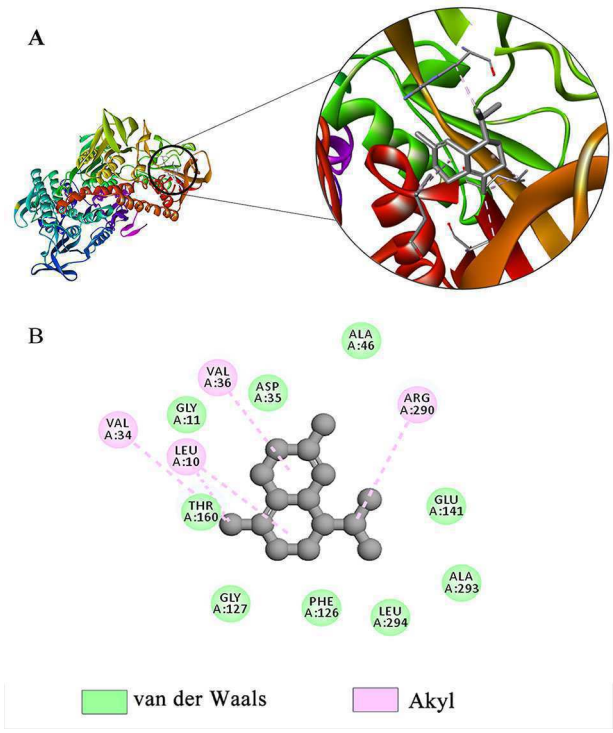


Figure 7. The molecular docking of δ-CAD onto TreR. (A) Representative diagram of docking result of δ-CAD onto TreR; (B) Binding interactions of δ-CAD with TreR.

with an estimated IC_{50} value of 7.54 μg/mL, about 7.4 times lower than those required for promastigote forms.

The effects of MfEO on macrophage infection by *L. amazonensis* could be better visualized by light microscopy (Figure 5C). The analysis of untreated-infected cells showed a high infection rate with numerous internalized amastigotes inside large parasitophorous vacuoles. As expected, the treatment of infected cells with both MfEO and the reference drugs induced a significant decrease in the number of amastigote/parasitophorous vacuole and consequently, in the rate of infection (Figure 5C). The uninfected and untreated macrophage controls showed usual morphology with polygonal cells, membrane projections and strong adhesion to the substrate are visualized at different stages of cell division (Figure 5C). In the infected macrophages treated with MfEO or AmB it is possible to observe, mainly at higher concentrations, the presence of empty parasitophorous vacuoles (Figure 5C, asterisks).

One of the desirable characteristics of a promising leishmanicidal drug is its selective toxicity to the parasite without causes damage to the host cells. Our results showed that MfEO was 44.1 times more selective for the amastigotes than to the macrophages. This value was considerably higher than those reported for the reference drugs which are very toxic to mammalian cells (Marques et al., 2019). The effects of MfEO on macrophage infection could be related to the direct action of essential oil components on the amastigote. However, we cannot rule out the possibility of MfEO is also acting on macrophages, making them more competent to fight the parasite (Ueda-Nakamura et al., 2006; Jihene et al., 2020). Previous studies showed that the lipophilic constituents of EOs, mainly the terpenes, can interact with liposaccharides, fatty acids, and membrane phospholipids of *Leishmania*, impairing key metabolic activities, leading to the cell death by necrosis or apoptosis (Díaz et al., 2018, Herrera-Acevedo et al., 2021).

3.3. The molecular docking of major constituents of MfEO on Leishmania target proteins

Based on our *in vitro* results, we hypothesized that the major constituents of MfEO could be acting on key enzymes or other molecules that are essential for survival and proliferation of *L. amazonensis*. In the last decades, the identification and validation of biochemical pathways present in trypanosomatids but absent or divergent from their host has been provide promissory targets for the rational design of novel drugs against *Leishmania* spp. (Romero & López, 2017).

The advance of analytical and computational techniques, especially molecular docking and molecular dynamic simulations have been used to predict the behavior of the major components of essential oils on validated enzyme targets of the *Leishmania spp*. (Oliveira et al., 2020; Herrera-Acevedo et al., 2021). The molecular docking is one of most useful *in silico* tools to investigate the mechanisms of action of organic molecules on biological systems. In this regard, it is possible to predict the interaction of compounds with molecular targets providing important information for the design of similar compounds with enhanced biological

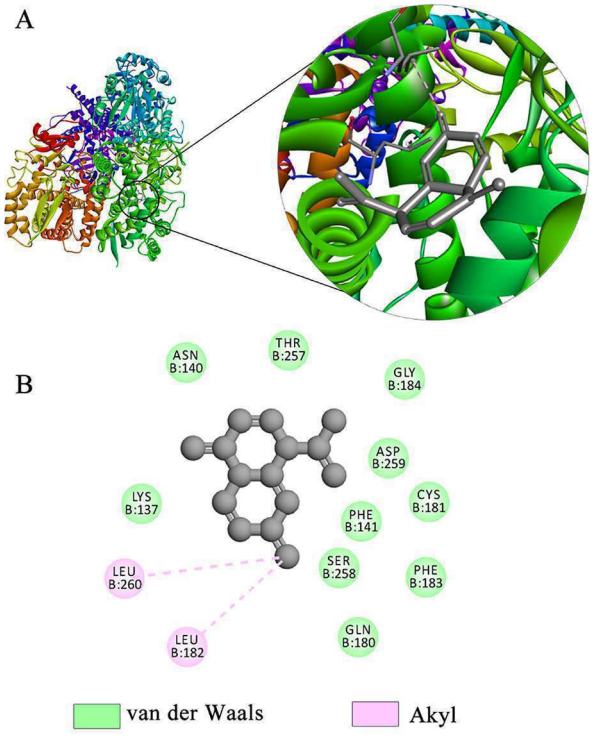


Figure 8. The molecular docking of δ-CAD with C14DM. (A) Representative diagram of docking result of δ-CAD with C14DM; (B) Binding interaction of δ-CAD with C14DM.

activity (Pagadala et al., 2017). Herrera-Acevedo et al. (2021), for example, have used these *in silico* approaches, to select, from an in-house database containing 360 natural-derived kaurene diterpenoids, structures with potential activity against pteridine reductase 1 (PTR1).

Enzymes that play a role in the metabolic pathways of parasites are considered potential targets for the development of new antiparasitic compounds (Jain & Jain, 2018). Dihydroorotate dehydrogenase (DHODH) is a flavoenzyme that catalyzes the oxidation of dihydroorotate (DHO) to orotate (ORO) in *de novo* pyrimidine biosynthesis, which is vital for DNA synthesis (Boschi et al., 2019). Another key enzyme of *Leishmania spp.* is the sterol 14-alpha demethylase (C14DM), which catalyzes the removal of the 14-α-methyl group from precursors during ergosterol biosynthesis (Macedo-Silva et al., 2015). The enzyme Trypanothione reductase (TreR) participates in the polyamine-dependent redox metabolism and performs antioxidant functions to protect the parasite against oxidative damage (Ilari et al., 2012). Pteridine reductase (PTR) is a short-chain reductase that allows trypanosomatids to reduce and use pterins acquired or recycled from the host, necessary for protein and nucleic acid synthesis (Gourley et al., 2001). In addition, this enzyme has shown an important role in the parasite resistance to oxidative stress. The inhibition of this enzyme leads to an increased sensitivity of trypanosomatids to oxidizing agents (Corona et al., 2012).

In this study we investigate the potential of the major compounds δ-CAD, γ-CAD, γ-MUU, α-MUU, α-SEL and E-CAR as putative inhibitors of target enzymes of *Leishmania* spp. Our molecular docking analysis showed that the major components of MfEO presented favorable affinity energy values onto *Leishmania* spp. enzyme targets. The formation of ligand-enzyme complexes was mainly due to the van der Waals or alkyl interactions. For the validation of our molecular anchorage, the fluconazole, a ligand co-crystallized with the enzyme C14DM from *Leishmania infantum*, was redocked (PDB-ID: 3L4D, Figure S1) with a value of the root-mean-square deviation of 1.6 Å. It is important to highlight that an RMSD value lower than 2 Å is considered as successful (Razzaghi-Asl et al., 2020). The affinity energy value for each ligand were calculated for the complexes (Figure 6 and Tables S1–S6). Our results showed that the major compounds of MfEO presented different inhibition potential on the tested enzymes.

Natural plant compounds from the mevalonate pathway, such as terpenoids, have been described as promising leishmanicidal agents by acting as enzyme inhibitors of *Leishmania* spp. (Gervazoni et al., 2020). It is interesting to note that the ligands (with the exception of E-CAR) are isomeric terpenes that have a similar chemical structure to naphthoquinone compound, already reported to possess antileishmanial properties by inhibiting TreR enzyme (Sharma et al., 2012).

Our *in silico* molecular docking analyzes showed that the δ-CAD was the ligand that presented the most favorable affinity energy values for all tested *Leishmania* enzymes. The δ-CAD-TreR and δ-CAD-C14DM complexes showed greater potential for enzymatic inhibition (Figure 6). Therefore, we decided to investigate in more detail the binding profile between δ-CAD and these enzymes. The van de Waals interactions with the amino acids Glu141, Phe126, Gly127, Asp35, Gly11, Val36 and Thr160 (Figure 7) were observed. TreR is a homodimer comprised by A and B chains with two active sites formed by the interaction of these chains (Pandey et al., 2016). Around the active site, composed by the amino acids Cys52 and Cys57 of the B chain, there are a highly conserved Ser464, Leu399, Met400 and Lys61 residues, which play important functional roles (Verma et al., 2012). Because the δ-CAD did not show any interaction with these residues, it is possible that the potential of this compound as TreR inhibitor occurs through the interaction with the same amino acids that bind to the co-crystallized cofactor, flavin adenine dinucleotide (FAD) in the B chain.

Interactions with the amino acids Val34, Leu10, Arg290, Phe126, Glu141 and Gly11 (Figure S2) suggest that δ-CAD could occupy the adenine binding site preventing FAD to work as a cofactor of TreR.

TreR is one of the main oxidoreductases that acts on the thiol metabolism, being responsible to maintain the intracellular redox balance in *Leishmania spp.* (Pandey et al., 2016). This enzyme has three binding domains to FAD, NADPH and trypanothione. It has been observed that *Leishmania* parasites can increase TreR expression as a mechanism of resistance to the drugs used in the treatment of leishmaniasis (Hefnawy et al., 2017; da Silva et al., 2018). The inactivation of TreR may result in a reduced survival of amastigotes inside the macrophage (Dumas et al., 1997). During the parasite infection, TreR reduces the trypanothione, a molecule of the

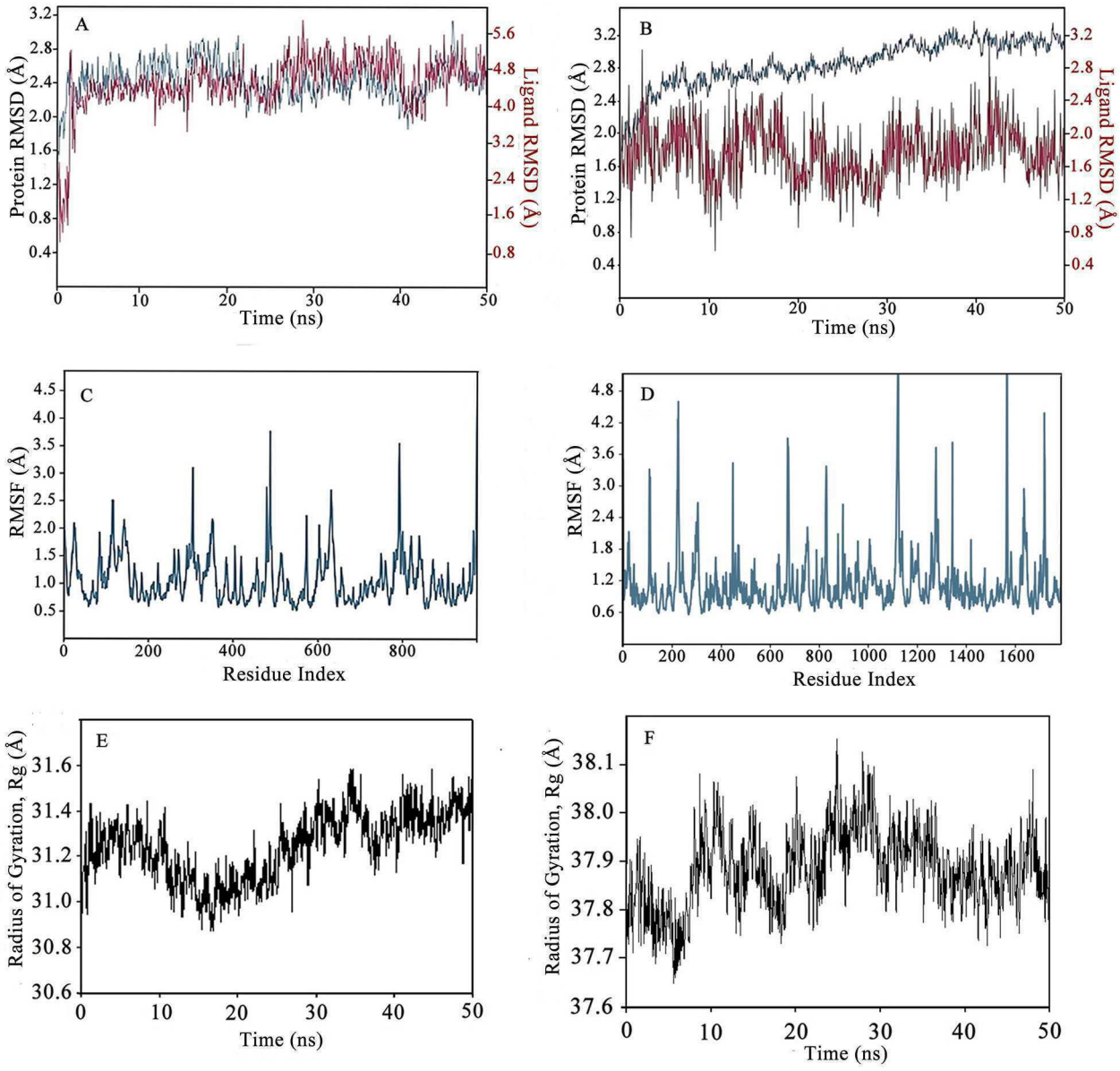


Figure 9. The molecular dynamic (MD) simulations. (A) RMSD plots of TreR ligand δ-CAD and (B) C14DM ligand δ-CAD after 50 ns of simulation. (C) RMSF plots of Cα backbone of TreR and (D) C14DM bound with δ-CAD. (E) Radius of gyration of TreR and (F) C14DM bound with ligand.

tryparedoxin/tryparedoxin peroxidase system, to neutralize the H_2O_2 produced by the macrophages to combat intracellular amastigotes (Turcano et al., 2018).

As observed for TreR, δ-CAD showed an inhibitory potential on C14DM, a key enzyme of lipid metabolism in *Leishmania* spp. The δ-CAD-C14DM complex showed the most favorable affinity energy value among all the other complexes for this target (Figure 6). For this complex, van der Waals and alkyl interactions with the amino acid residues Lys137, Asn140, Thr257, Gly184, Asp259, Cys181, Phe141, Ser258, Phe183, Gln180, Leu182 and Leu280 of the A chain were observed (Figure 8).

Inhibitors of ergosterol biosynthesis have a greater impact on the physiology of *Leishmania* spp. as well as other trypanosomatids (Mukherjee et al., 2020). In this study, our *in silico* analyzes indicated a good potential for chemical interactions between δ-CAD and C14DM. These data can be used as a starting point for the synthesis of cadinene analogs that may act as inhibitor of this enzyme.

Due to the amino acid sequence similarity between the enzymes of *Leishmania* spp and other pathogenic trypanosomatids such as *Trypanosoma cruzi*, C14DM have been reported as an important target for drug therapy against leishmaniasis, Chagas disease and sleeping sickness (Holanda et al., 2020). The inhibition of C14DM can affect the removal of the alpha-methyl group from sterols. These molecules are considered as fundamental constituents of cell membranes as well as regulatory molecules required for parasite growth and survival (Vargas et al., 2019). It has been shown that the inhibition of C14DM severely compromises the mitochondrial physiology of *Leishmania*, leading to an accumulation of reactive oxygen species (Mukherjee et al., 2020), which can result in an irreversible damage. Interestingly, our docking analysis suggested that δ-CAD may simultaneously works as

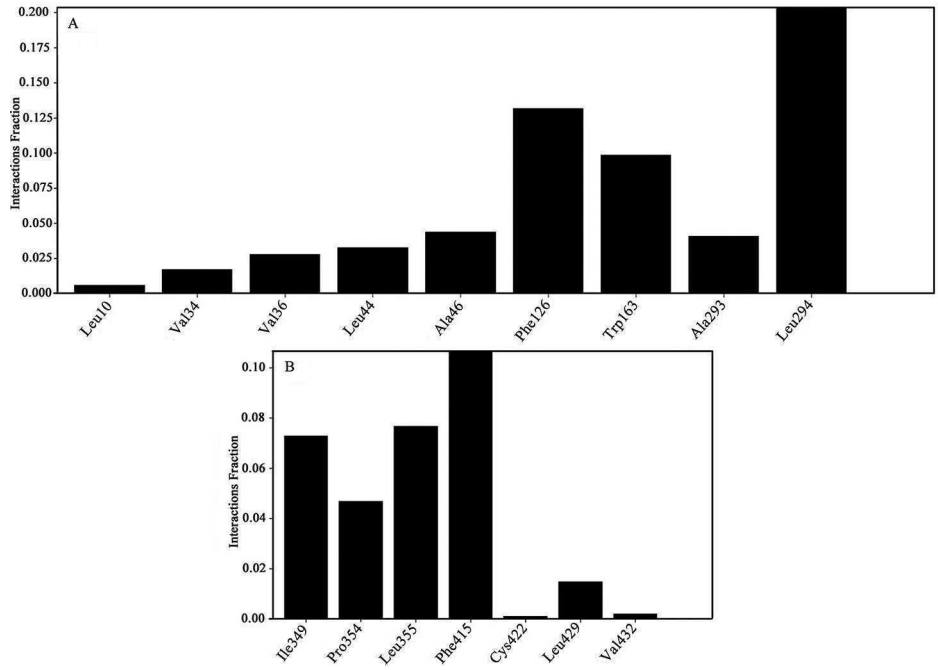


Figure 10. Protein–Ligand contact histogram of the interactions formed between ligand and protein residues during 50 ns MD simulation of δ-CAD in complex with (A) TreR and (B) C14DM.

an inhibitor of both C14DM and TreR, amplifying the deleterious effect on the parasite and lowering the risk of parasite resistance. Because our molecular docking analysis pointed out the δ-CAD-TreR and δ-CAD-C14DM complexes as having the most favorable affinity energy values and predicted inhibition constants, we further performed the molecular dynamics simulation to assess the final convergence and stability for these complexes.

3.4. Molecular dynamics (MD) simulations

The MD simulations were carried out for the best two docked complexes δ-CAD-TreR and δ-CAD-C14DM, which presented lowest affinity energy values in our molecular docking (more -dG), analyzed for 50 ns. Root mean square deviation (RMSD) of δ-CAD-TreR complex displayed small value of deviation with an average of 0.4 Å of the Cα backbone of protein (Figure 9A, blue line), whereas the ligand RMSD also displayed very stable conformation with smaller deviation on aligned to the protein backbone RMSD (Figure 9A, red line). On the hand, δ-CAD bound C14DM complex displayed lower stability as compared to δ-CAD-TreR complex. The RMSD of the Cα backbone of protein displayed an average of 1.8 Å fluctuation, which is the acceptable range during 50 ns of simulation (Figure 9B, blue line). However, the ligand RMSD fluctuated more on aligning to the protein backbone (Figure 9B, red line). This means that the ligand rearranged its position during the entire simulation trajectory. It is very important to understand the individual amino acid residue fluctuations (RMSF) of the protein which determines the stability of the ligand bound complex.

The RMSF of Cα backbone of TreR displayed less fluctuations of amino acid residues in the ligand bound state throughout 50 ns of simulation. The high fluctuations observed among residues 280, 385, 390 and 800 (Figure 9C). On the other hand, the backbone of C14DM displayed much more fluctuating residues on δ-CAD bound state (Figure 9D). Radius of gyration is the assessment of compactness of the protein in ligand bound state. Herein, the Cα backbone of TreR displayed good stable compact structure having significant linearity of the curve at the later part of the simulation 30-50 ns (Figure 9E), whereas C14DM protein displayed much deflection of the Rg curve indicating an initial loose of conformation with later (40-50 ns) packed into a compact structure (Figure 9F). Protein ligand interaction bar plot (Figure 10) displaying the involvement of weak interaction but devoid of H-bonds. The major contribution was due to hydrophobic interaction between TreR with Leu10, Val34, Val36, Leu44, Ala46, Phe126, Trp163, Ala293 and Leu294 at the binding cavity of δ-CAD (Figure 10A). On the other hand, C14DM interacted with IlE349, Pro354, Leu355, Phe415, Cys422, Leu429 and Val432 (Figure 10B). Other contributing interactions were determined by MM-GBSA analysis.

3.5. Molecular mechanics generalized born surface area (MM-GBSA) calculations

Utilizing the MD simulation trajectory, the binding free energy along with other contributing energy in form of MM-GBSA were determined for each δ-CAD ligand complexed with the TreR and C14DM proteins. The results (Table 2) suggested that the maximum contribution to dG_{bind} in the stability of the simulated complexes were due to dG_{bind}Coulomb, dG_{bind}vdW and dG_{bind}Lipo, while dG_{bind}Covalent and dG_{bind}SolvGB contributed to the instability of the corresponding complexes. Although, no hydrogen bonds were formed between the ligands and the protein, the stability of protein-ligand complexes and the binding efficiency observed may be achieved through other non-bound

Table 2. Binding free energy components for the ligand δ-CAD with TreR and C14DM proteins calculated by MM-GBSA (kcal/mol) analysis.

Proteins	dG_{bind}	dG_{bind}Lipo	dG_{bind}vdW	dG_{bind}CoulomB	dG_{bind}SolvGB	dG_{bind}Covalent
TreR	−51.00 ± 2.21	−21.22 ± 1.39	−39.03 ± 1.10	−0.27 ± 0.29	9.06 ± 0.78	0.52 ± 0.78
C14DM	−38.89 ± 1.66	−18.55 ± 0.96	−35.44 ± 6.17	−0.22 ± 0.23	15.05 ± 1.30	0.26 ± 0.25

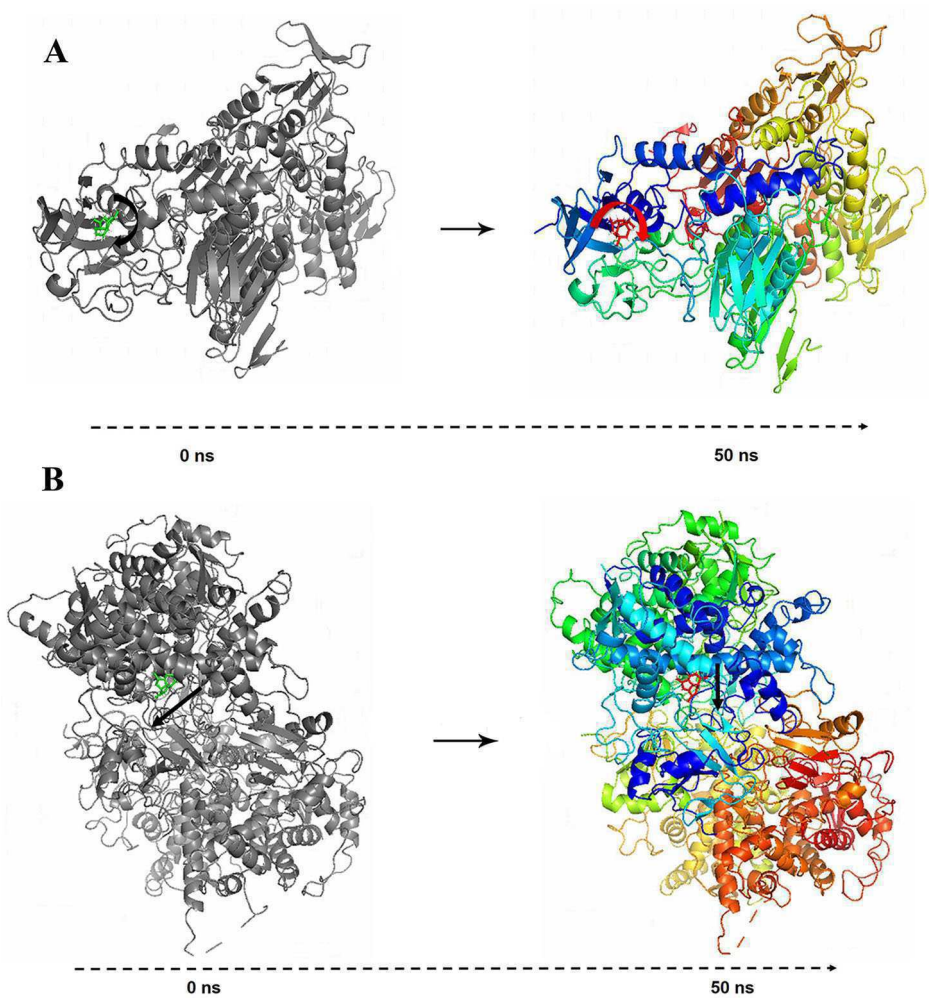


Figure 11. Position and movement of δ-CAD bound to (A) TreR and (B) C14DM at the binding site before simulation (grey, 0 ns) and after simulation (rainbow, 50 ns). Conformational variances between first and last frame of MD simulation trajectories after 50 ns.

interactions. The dG bind was calculated by the equation provided in the methodology section and described elsewhere (Kollman et al., 2000). All other non-bonded energies were calculated for the protein-ligand complex from MMGBSA trajectory as described by Bharadwaj et al. (2021).

The binding free energies dGBind, as an outcome of MMGBSA calculations, were lower than those obtained from molecular docking studies. The more negative binding energies mean very high binding of δ-CAD to TreR and C14DM proteins. Therefore, it can be suggested that δ-CAD has higher affinity to TreR and C14DM, which can impose higher inhibition towards these enzymes, contributing to the potent anti-leishmanial activity found for this compound. Positional movement analysis of δ-CAD was performed from the MD simulation MM-GBSA trajectories in triplicate for better reproducibility of the results. The δ-CAD rotate 90° on the binding cavity of TreR which can be clearly visible form the 0^{th} ns and 50 ns data (Figure 11A, arrow). This positional movement allowed the ligand to establish a stable conformation with the protein. On the other hand, 45° angular rotation was observed in case of C14DM bound complex of δ-CAD (Figure 11B). These angular movements substantially adapted to the best accommodation of the ligand at the binding cavity to attain the best stability during the 50 ns simulation. The prediction of free energy calculation has been widely used for the elucidation of protein-ligand binding due to its accuracy when compared to other computational chemistry techniques (Wang et al., 2019). The dG_{bind} values demonstrated the affinity of δ-CAD to TreR and C14DM complexes and corroborate the predicted score obtained from molecular docking calculations, with the δ-CAD-TreR complex having a favorable binding free energy value as compared with the δ-CAD-C14DM.

4. Conclusions

The research and development of new therapeutic alternatives more effective and with low toxicity are necessary due

to the limitations presented by the available reference drugs. Together, our results demonstrated that MfEO has high potential for the development of a promising leishmanicidal agent against *L. amazonensis*. The MfEO caused drastic changes in the ultrastructure of the promastigotes and showed greater selectivity for the intracellular amastigote form of the parasite, with a significant reduction in the number of infected cells. In addition, the main components of MfEO (δ-CAD) showed binding affinity towards the enzymes TreR and C14DM, which are important validated drug targets in protozoa of the genus *Leishmania*. The MD simulations and MM-GBSA calculations confirmed that the δ-CAD have lowest binding free energy values with TreR and C14DM enzymes. It is possible to notice the presence of similar chemical structures among the major compounds. Finally, this study was a pioneer in describes the leishmanicidal potential of MfEO on *L. amazonensis*, the etiological agent of CL, providing a new perspective for the use of fruit peels in the discovery of bioactive compounds with low cost for pharmacological applications.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo a Ciência e Tecnologia de Pernambuco (FACEPE) and Instituto Aggeu Magalhães for supporting this research.

ORCID

Deyzi Caroline da Silva Barbosa http://orcid.org/0000-0002-9026-6528
Vanderlan Nogueira Holanda http://orcid.org/0000-0001-5309-0036
Arabinda Ghosh http://orcid.org/0000-0002-3891-5949
Rafael Trindade Maia http://orcid.org/0000-0002-5870-1091
Welson Vicente da Silva http://orcid.org/0000-0001-5676-6863
Vera Lúcia de Menezes Lima http://orcid.org/0000-0003-2581-9218
Márcia Vanusa da Silva http://orcid.org/0000-0003-4920-9975
Regina Celia Bressan Queiroz de Figueiredo http://orcid.org/0000-0001-5806-0944

References

Andrade, M. A., dos Santos Azevedo, C., Motta, F. N., dos Santos, M. L., Silva, C. L., de Santana, J. M., & Bastos, I. M. (2016). Essential oils: In vitro activity against Leishmania amazonensis, cytotoxicity and chemical composition. *BMC Complementary and Alternative Medicine*, *16*(1), 444–448. https://doi.org/10.1186/s12906-016-1401-9

Badirzadeh, A., Heidari-Kharaji, M., Fallah-Omrani, V., Dabiri, H., Araghi, A., & Salimi Chirani, A. (2020). Antileishmanial activity of Urtica dioica extract against zoonotic cutaneous leishmaniasis. *PLoS Neglected Tropical Diseases*, *14*(1), e0007843. https://doi.org/10.1371/journal.pntd.0007843

Bazana, M. T., Codevilla, C. F., & Menezes, C. R. (2019). Nanoencapsulation of bioactive compounds: Challenges and perspectives. *Current Opinion in Food Science*, *26*, 47–56. https://doi.org/10.1016/j.cofs.2019.03.005

Bharadwaj, K. K., Sarkar, T., Ghosh, A., Baishya, D., Rabha, B., Panda, M. K., & Pati, S. (2021). Macrolactin A as a novel inhibitory agent for SARS-CoV-2 Mpro: Bioinformatics approach. *Applied Biochemistry and Biotechnology*, *2021*, 1–24. https://doi.org/10.1007/s12010-021-03608-7

Boschi, D., Pippione, A. C., Sainas, S., & Lolli, M. L. (2019). Dihydroorotate dehydrogenase inhibitors in anti-infective drug research. *European Journal of Medicinal Chemistry*, *183*, 111681. https://doi.org/10.1016/j.ejmech.2019.111681

Bowers, K. J., Chow, D. E., Xu, H., Dror, R. O., Eastwood, M. P., Gregersen, B. A., Klepeis, J. L., Kolossvary, I., Moraes, M. A., Sacerdoti, F. D., & Salmon, J. K. (2006). *Scalable algorithms for molecular dynamics simulations oncommodity clusters* [Paper presentation]. SC'06: Proceedings of the2006 ACM/IEEE Conference on Supercomputing, 43. https://doi.org/10.1109/SC.2006.54

Chow, E., Rendleman, C. A., Bowers, K. J., Dror, R. O., Hughes, D. H., Gullingsrud, J., Sacerdoti, F. D., & Shaw, D. E. (2008). Desmond performance on a cluster of multicore processors. *DE Shaw Research Technical Report* DESRES/TR-2008-01. http://deshawresearch.com.

Chowdhary, S. J., Chowdhary, A. M. I. T., & Kashaw, S. U. S. H. I. L. (2016). Macrophage targeting: A strategy for leishmaniasis specific delivery. *International Journal of Pharmacy and Pharmaceutical Sciences*, *8*(11), 16–26.

Christensen, S. M., Belew, A. T., El-Sayed, N. M., Tafuri, W. L., Silveira, F. T., & Mosser, D. M. (2019). Host and parasite responses in human diffuse cutaneous leishmaniasis caused by L. amazonensis. *PLoS Neglected Tropical Diseases*, *13*(3), e0007152. https://doi.org/10.1371/journal.pntd.0007152

Corona, P., Gibellini, F., Cavalli, A., Saxena, P., Carta, A., Loriga, M., Luciani, R., Paglietti, G., Guerrieri, D., Nerini, E., Gupta, S., Hannaert, V., Michels, P. A. M., Ferrari, S., & Costi, P. M. (2012). Structure-based selectivity optimization of piperidine-pteridine derivatives as potent Leishmania pteridine reductase inhibitors. *Journal of Medicinal Chemistry*, *55*(19), 8318–8329. https://doi.org/10.1021/jm300563f

Correa, M. S., Schwambach, J., Mann, M. B., Frazzon, J., & Frazzon, A. P. G. (2019). Antimicrobial and antibiofilm activity of the essential oil from dried leaves of Eucalyptus staigeriana. *Arquivos do Instituto Biológico*, *86*, 1–8 https://doi.org/10.1590/1808-1657000202018

Costa, J. M., Garcia, A. M., Rêbelo, J. M. M., Guimarães, K. M., Guimarães, R. M., & Nunes, P. (2003). [Fatal case during treatment of american tegumentary leishmaniasis with sodium stibogluconate bp 88 (shandong xinhua)]. *Revista da Sociedade Brasileira de Medicina Tropical*, *36*(2), 295–298. https://doi.org/10.1590/s0037-86822003000200013

Costa, W. K., Oliveira, J. R. S. d., Oliveira, A. M. d., Santos, I. B. d S., Cunha, R. X. d., Freitas, A. F. S. d., Silva, J. W. L. M. d., Silva, V. B. G., Aguiar, J. C. R. d O. F. d., Silva, A. G. d., Navarro, D. M. d A. F., Lima, V. L. d M., & Silva, M. V. d. (2020). Essential oil from Eugenia stipitata McVaugh leaves has antinociceptive, anti-inflammatory and antipyretic activities without showing toxicity in mice. *Industrial Crops and Products*, *144*, 112059. https://doi.org/10.1016/j.indcrop.2019.112059

Czech, A., Zarycka, E., Yanovych, D., Zasadna, Z., Grzegorczyk, I., & Kłys, S. (2020). Mineral content of the pulp and peel of various citrus fruit cultivars. *Biological Trace Element Research*, *193*(2), 555–563. https://doi.org/10.1007/s12011-019-01727-1

da Silva Barbosa, D. C., Holanda, V. N., de Assis, C. R. D., de Oliveira Farias de Aguiar, J. C. R., doNascimento, P. H., da Silva, W. V., do Amaral Ferraz Navarro, D. M., Silva, M. V. d., de Menezes Lima, V. L., & dos Santos Correia, M. T. (2020). Chemical composition and acetylcholinesterase inhibitory potential, in silico, of Myrciaria floribunda (H. West ex Willd.) O. Berg fruit peel essential oil. *Industrial Crops and Products*, *151*, 112372. https://doi.org/10.1016/j.indcrop.2020.112372

da Silva, A. D., Dos Santos, J. A., Machado, P. A., Alves, L. A., Laque, L. C., de Souza, V. C., Coimbra, E. S., & Capriles, P. V. S. Z. (2019). Insights about resveratrol analogs against trypanothione reductase of Leishmania braziliensis: Molecular modeling, computational docking and in vitro antileishmanial studies. *Journal of Biomolecular Structure & Dynamics*, *37*(11), 2960–2969. https://doi.org/10.1080/07391102.2018.1502096

da Silva, V. P., Alves, C. C. F., Miranda, M. L. D., Bretanha, L. C., Balleste, M. P., Micke, G. A., Silveira, E. V., Martins, C. H. G., Ambrosio, M. A. L. V., de Souza Silva, T., Tavares, D. C., Magalhães, L. G., Silva, F. G., &

Egea, M. B. (2018). Chemical composition and in vitro leishmanicidal, antibacterial and cytotoxic activities of essential oils of the Myrtaceae family occurring in the Cerrado biome. *Industrial Crops and Products*, *123*, 638–645. https://doi.org/10.1016/j.indcrop.2018.07.033

de Azevedo, M. M. L., Cascaes, M. M., Guilhon, G. M. S. P., Andrade, E. H. A., Zoghbi, M. d G. B., da Silva, J. K. R., Santos, L. S., & da Silva, S. H. M. (2019). Lupane triterpenoids, antioxidant potential and antimicrobial activity of Myrciaria floribunda (H. West ex Willd.) O. Berg. *Natural Product Research*, *33*(4), 506–515. https://doi.org/10.1080/14786419.2017.1402311

Díaz, J. G., Arranz, J. C. E., Batista, D. D. G. J., Fidalgo, L. M., Costa, J. D. L. V., Macedo, M. B., & Cos, P. (2018). Antileishmanial potentialities of Croton linearis leaf essential oil. *Natural Product Communications*, *13*(5), 1934578X1801300. https://doi.org/10.1177/1934578X1801300527

Dumas, C., Ouellette, M., Tovar, J., Cunningham, M. L., Fairlamb, A. H., Tamar, S., Olivier, M., & Papadopoulou, B. (1997). Disruption of the trypanothione reductase gene of Leishmania decreases its ability to survive oxidative stress in macrophages. *The EMBO Journal*, *16*(10), 2590–2598. https://doi.org/10.1093/emboj/16.10.2590

Durazzini, A. M. S., Machado, C. H. M., Fernandes, C. C., Willrich, G. B., Crotti, A. E. M., Candido, A. C. B. B., Magalhães, L. G., Squarisi, I. S., Ribeiro, A. B., Tavares, D. C., Martins, C. H. G., & Miranda, M. L. D. (2019). Eugenia pyriformis Cambess: A species of the Myrtaceae family with bioactive essential oil. *Natural Product Research*, 1–5. https://doi.org/10.1080/14786419.2019.1669031

Ferreira, F. B. P., Herculano Ramos-Milaré, Á. C. F., Gonçalves, J. E., Lazarin-Bidóia, D., Nakamura, C. V., Sugauara, R. R., Fernandez, C. M. M., Gazim, Z. C., Demarchi, I. G., Silveira, T. G. V., & Lonardoni, M. V. C. (2020). Campomanesia xanthocarpa (Mart.) O. Berg essential oil induces antileishmanial activity and remodeling of the cytoplasm organelles. *Natural Product Research*, 1–5. https://doi.org/10.1080/14786419.2020.1827401

Genheden, S., & Ryde, U. (2015). The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. *Expert Opinion on Drug Discovery*, *10*(5), 449–461. https://doi.org/10.1517/17460441.2015.1032936

Gervazoni, L. F., Barcellos, G. B., Ferreira-Paes, T., & Almeida-Amaral, E. E. (2020). Use of natural products in leishmaniasis chemotherapy: An overview. *Frontiers in Chemistry*, *8*, 579891. https://doi.org/10.3389/fchem.2020.579891

Gourley, D. G., Schüttelkopf, A. W., Leonard, G. A., Luba, J., Hardy, L. W., Beverley, S. M., & Hunter, W. N. (2001). Pteridine reductase mechanism correlates pterin metabolism with drug resistance in trypanosomatid parasites. *Nature Structural Biology*, *8*(6), 521–525.

Hefnawy, A., Berg, M., Dujardin, J. C., & De Muylder, G. (2017). Exploiting knowledge on Leishmania drug resistance to support the quest for new drugs. *Trends in Parasitology*, *33*(3), 162–174. https://doi.org/10.1016/j.pt.2016.11.0031.

Hennia, A., Nemmiche, S., Guerreiro, A., Faleiro, M. L., Antunes, M. D., Aazza, S., & Miguel, M. G. (2019). Antioxidant and antiproliferative activities of Myrtus communis L. essential oils from different Algerian regions. *Journal of Essential Oil Bearing Plants*, *22*(6), 1488. https://doi.org/10.1080/0972060X.2019.1687335

Herrera-Acevedo, C., Flores-Gaspar, A., Scotti, L., Mendonça-Junior, F. J. B., Scotti, M. T., & Coy-Barrera, E. (2021). Identification of Kaurane-type diterpenes as inhibitors of Leishmania pteridine reductase I. *Molecules (Basel, Switzerland)*, *26*(11), 3076. https://doi.org/10.3390/molecules26113076

Herrera-Acevedo, C., Perdomo-Madrigal, C., Muratov, E. N., Scotti, L., & Scotti, M. T. (2021). Discovery of alternative chemotherapy options for Leishmaniasis through computational studies of Asteraceae. *ChemMedChem.*, *16*(8), 1234. https://doi.org/10.1002/cmdc.202000862

Holanda, V. N., Silva, W. V. d., Nascimento, P. H. d., Silva, S. R. B., Cabral Filho, P. E., Assis, S. P. d O., Silva, C. A. d., Oliveira, R. N. d., Figueiredo, R. C. B. Q. d., & Lima, V. L. d M. (2020). Antileishmanial activity of 4-phenyl-1-[2-(phthalimido-2-yl)ethyl]-1H-1,2,3-triazole (PT4) derivative on Leishmania amazonensis and Leishmania braziliensis: In silico ADMET, in vitro activity, docking and molecular dynamic simulations. *Bioorganic Chemistry*, *105*, 104437. https://doi.org/10.1016/j.bioorg.2020.104437

Ilari, A., Baiocco, P., Messori, L., Fiorillo, A., Boffi, A., Gramiccia, M., Di Muccio, T., & Colotti, G. (2012). A gold-containing drug against parasitic polyamine metabolism: The X-ray structure of trypanothione reductase from Leishmania infantum in complex with auranofin reveals a dual mechanism of enzyme inhibition. *Amino Acids*, *42*(2-3), 803–811. https://doi.org/10.1007/s00726-011-0997-9

Jain, V., & Jain, K. (2018). Molecular targets and pathways for the treatment of visceral leishmaniasis. *Drug Discovery Today*, *23*(1), 161–170. https://doi.org/10.1016/j.drudis.2017.09.006

Jihene, A., Rym, E., Ines, K. J., Majdi, H., Olfa, T., & Abderrabba, M. (2020). Antileishmanial potential of propolis essential oil and its synergistic combination with Amphotericin B. *Natural Product Communications*, *15*(1), 1934578X1989956–1934578X1989958. https://doi.org/10.1177/1934578X19899566

Jorgensen, W. L., Maxwell, D. S., & Tirado-Rives, J. (1996). Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. *Journal of the American Chemical Society*, *118*(45), 11225–11236. https://doi.org/10.1021/ja9621760

Kim, C., Bu, J. H., Lee, J. S., Hyun, C., & Lee, N. H. (2014). Chemical compositions and anti-inflammatory activities of essential oils from Aster spathulifolius and Vitex rotundifolia maxim. *Journal of Applied Pharmaceutical Science*, *4*(10), 12–15. https://doi.org/10.7324/JAPS.2014.401003

Kollman, P. A., Massova, I., Reyes, C., Kuhn, B., Huo, S., Chong, L., Lee, M., Lee, T., Duan, Y., Wang, W., Donini, O., Cieplak, P., Srinivasan, J., Case, D. A., & Cheatham, T. E. (2000). Calculating structures and free energies of complex molecules: Combining molecular mechanics and continuum models. *Accounts of Chemical Research*, *33*(12), 889–897. https://doi.org/10.1021/ar000033j

Laskowski, R. A., MacArthur, M. W., & Thornton, J. M. (2006). PROCHECK: Validation of protein-structure coordinates. *International Tables for Crystallography*, *25*(2), 722–725.

Lima, M. V. N. D., Oliveira, R. Z. D., Lima, A. P. D., Cerino, D. A., & Silveira, T. G. V. (2007). American cutaneous leishmaniasis with fatal outcome during pentavalent antimoniate treatment. *Anais Brasileiros de Dermatologia*, *82*(3), 269–271. https://doi.org/10.1590/S0365-05962007000300010

López-Camacho, E., Godoy, M. J. G., García-Nieto, J., Nebro, A. J., & Aldana-Montes, J. F. (2015). Solving molecular flexible docking problems with metaheuristics: A comparative study. *Applied Soft Computing*, *28*, 379–393. https://doi.org/10.1016/j.asoc.2014.10.049

Macêdo, C. G., Fonseca, M. Y. N., Caldeira, A. D., Castro, S. P., Pacienza-Lima, W., Borsodi, M. P. G., Sartoratto, A., da Silva, M. N., Salgado, C. G., Rossi-Bergmann, B., & Castro, K. C. F. (2020). Leishmanicidal activity of Piper marginatum Jacq. from Santarém-PA against Leishmania amazonensis. *Experimental Parasitology*, *210*, 107847. https://doi.org/10.1016/j.exppara.2020.107847

Macedo-Silva, S. T., Visbal, G., Urbina, J. A., De Souza, W., & Rodrigues, J. C. F. (2015). Potent in vitro antiproliferative synergism of combinations of ergosterol biosynthesis inhibitors against Leishmania amazonensis. *Antimicrobial Agents and Chemotherapy*, *59*(10), 6402–6418. https://doi.org/10.1128/AAC.01150-15

Mancianti, F., & Ebani, V. V. (2020). Biological activity of essential oils. *Molecules*, *25*(3), 678. https://doi.org/10.3390/molecules25030678

Marques, S. A., Merlotto, M. R., Ramos, P. M., & Marques, M. E. A. (2019). American tegumentary leishmaniasis: Severe side effects of pentavalent antimonial in a patient with chronic renal failure. *Anais brasileiros de dermatologia*, *94*(3), 355–357. https://doi.org/10.1590/abd1806-4841.20198388

Martyna, G. J., Klein, M. L., & Tuckerman, M. (1992). Nose-Hoover chains-the canonical ensemble via continuous dynamics. *The Journal of Chemical Physics*, *97*(4), 2635–2643. https://doi.org/10.1063/1.463940

Martyna, G. J., Tobias, D. J., & Klein, M. L. (1994). Constant pressure molecular dynamics algorithms. *The Journal of Chemical Physics*, *101*(5), 4177–4189. https://doi.org/10.1063/1.467468

Menezes, J. P. B., Guedes, C. E. S., Petersen, A. L. D. O. A., Fraga, D. B. M., & Veras, P. S. T. (2015). Advances in development of new treatment for leishmaniasis. *BioMed Research International*, *2015*, 815023. https://doi.org/10.1155/2015/815023

Monzote, L., Geroldinger, G., Tonner, M., Scull, R., De Sarkar, S., Bergmann, S., Bacher, M., Staniek, K., Chatterjee, M., Rosenau, T., & Gille, L. (2018). Interaction of ascaridole, carvacrol, and caryophyllene oxide from essential oil of Chenopodium ambrosioides L. with mitochondria in Leishmania and other eukaryotes. *Phytotherapy Research: PTR*, *32*(9), 1729–1740. https://doi.org/10.1002/ptr.6097

Moreira, R. R. D., Santos, A. G. d., Carvalho, F. A., Perego, C. H., Crevelin, E. J., Crotti, A. E. M., Cogo, J., Cardoso, M. L. C., & Nakamura, C. V. (2019). Antileishmanial activity of Melampodium divaricatum and Casearia sylvestris essential oils on Leishmania amazonensis. *Revista do Instituto de Medicina Tropical de São Paulo*, *61*, 1-7. http://dx.doi.org/10.1590/s1678-9946201961033

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Mukherjee, S., Moitra, S., Xu, W., Hernandez, V., & Zhang, K. (2020). Sterol 14-α-demethylase is vital for mitochondrial functions and stress tolerance in Leishmania major. *PLoS Pathogens*, *16*(8), e1008810. https://doi.org/10.1371/journal.ppat.1008810

Okwor, I., & Uzonna, J. (2016). Social and economic burden of human leishmaniasis. *The American Journal of Tropical Medicine and Hygiene*, *94*(3), 489–493. https://doi.org/10.4269/ajtmh.15-0408

Oliveira, D. M., Furtado, F. B., Gomes, A. A. S., Belut, B. R., Nascimento, E. A., Morais, S. A. L., Martins, C. H. G., Santos, V. C. O., da Silva, C. V., Teixeira, T. L., Cunha, L. C. S., Oliveira, A. d., & de Aquino, F. J. T. (2020). Chemical constituents and antileishmanial and antibacterial activities of essential oils from Scheelea phalerata. *ACS Omega*, *5*(3), 1363–1370. https://doi.org/10.1021/acsomega.9b01962

Oliveira, V. C., Moura, D. M., Lopes, J. A., de Andrade, P. P., da Silva, N. H., & Figueiredo, R. C. (2009). Effects of essential oils from Cymbopogon citratus (DC) Stapf., Lippia sidoides Cham., and Ocimum gratissimum L. on growth and ultrastructure of Leishmania chagasi promastigotes. *Parasitology Research*, *104*(5), 1053–1059. https://doi.org/10.1007/s00436-008-1288-6

Pagadala, N. S., Syed, K., & Tuszynski, J. (2017). Software for molecular docking: A review. *Biophysical Reviews*, *9*(2), 91–102. https://doi.org/10.1007/s12551-016-0247-1

Pandey, R. K., Verma, P., Sharma, D., Bhatt, T. K., Sundar, S., & Prajapati, V. K. (2016). High-throughput virtual screening and quantum mechanics approach to develop imipramine analogues as leads against trypanothione reductase of leishmania. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *83*, 141–152. https://doi.org/10.1016/j.biopha.2016.06.010

Pinto, E. G., Santos, I. O., Schmidt, T. J., Borborema, S. E., Ferreira, V. F., Rocha, D. R., & Tempone, A. G. (2014). Potential of 2-hydroxy-3-phenylsulfanylmethyl-[1,4]-naphthoquinones against Leishmania (L.) infantum: biological activity and structure-activity relationships . *PloS One*, *9*(8), e105127. https://doi.org/10.1371/journal.pone.0105127

Razzaghi-Asl, N., Ebadi, A., Shahabipour, S., & Gholamin, D. (2020). Identification of a potential SARS-CoV2 inhibitor via molecular dynamics simulations and amino acid decomposition analysis. *Journal of Biomolecular Structure & Dynamics*, 1–16. https://doi.org/10.1080/07391102.2020.1797536

Ríos, Y. K., Otero, A. C., Muñoz, D. L., Echeverry, M., Robledo, S. M., & Yepes, M. A. (2008). Actividad citotóxica y leishmanicida in vitro del aceite esencial de manzanilla (Matricaria chamomilla). *Revista Colombiana de Ciencias Químico-Farmacéuticas*, *37*(2), 200–211.

Romero, A. H., & López, S. E. (2017). In silico molecular docking studies of new potential 4-phthalazinyl-hydrazones on selected Trypanosoma cruzi and Leishmania enzyme targets. *Journal of Molecular Graphics & Modelling*, *76*, 313–329. https://doi.org/10.1016/j.jmgm.2017.07.013

Russell, D. G., & Talamas-Rohana, P. (1989). Leishmania and the macrophage: A marriage of inconvenience. *Immunology Today*, *10*(10), 328–333. https://doi.org/10.1016/0167-5699(89)90188-6

Saidani, F., Giménez, R., Aubert, C., Chalot, G., Betrán, J. A., & Gogorcena, Y. (2017). Phenolic, sugar and acid profiles and the antioxidant composition in the peel and pulp of peach fruits. *Journal of Food Composition and Analysis*, *62*, 126–133. https://doi.org/10.1016/j.jfca.2017.04.015

Sharma, N., Shukla, A. K., Das, M., & Dubey, V. K. (2012). Evaluation of plumbagin and its derivative as potential modulators of redox thiol metabolism of Leishmania parasite. *Parasitology Research*, *110*(1), 341–348. https://doi.org/10.1007/s00436-011-2498-x

Shivakumar, D., Williams, J., Wu, Y., Damm, W., Shelley, J., & Sherman, W. (2010). Prediction of absolute solvation free energies using molecular dynamics free energy perturbation and the OPLS force field. *Journal of Chemical Theory and Computation*, *6*(5), 1509–1519. https://doi.org/10.1021/ct900587b

Sousa, J. K. T., Antinarelli, L. M. R., Mendonça, D. V. C., Lage, D. P., Tavares, G. S. V., Dias, D. S., Ribeiro, P. A. F., Ludolf, F., Coelho, V. T. S., Oliveira-da-Silva, J. A., Perin, L., Oliveira, B. A., Alvarenga, D. F., Chávez-Fumagalli, M. A., Brandão, G. C., Nobre, V., Pereira, G. R., Coimbra, E. S., & Coelho, E. A. F. (2019). A chloroquinoline derivate presents effective in vitro and in vivo antileishmanial activity against Leishmania species that cause tegumentary and visceral leishmaniasis. *Parasitology International*, *73*, 101966. https://doi.org/10.1016/j.parint.2019.101966

Souza, L. I. O., Bezzera-Silva, P. C., Navarro, D. M. D. A. F., Silva, A. G., Santos Correia, M. T., Silva, M. V., & Figueiredo, R. C. B. Q. (2017). The chemical composition and trypanocidal activity of volatile oils from Brazilian Caatinga plants. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *96*, 1055–1064. https://doi.org/10.1016/j.biopha.2017.11.121

Sundar, S., Chakravarty, J., & Meena, L. P. (2019). Leishmaniasis: Treatment, drug resistance and emerging therapies. *Expert Opinion on Orphan Drugs*, *7*(1), 1–10. https://doi.org/10.1080/21678707.2019.1552853

Taheri, A. R., Sabouri Rad, S., & Molkara, S. (2019). Systemic treatments of Leishmaniasis: A narrative review. *Reviews in Clinical Medicine*, *6*(3), 91–97. https://doi.org/10.22038/rcm.2019.42632.1287

Tietbohl, L. A. C., Mello, C. B., Silva, L. R., Dolabella, I. B., Franco, T. C., Enríquez, J. J. S., Santos, M. G., Fernandes, C. P., Machado, F. P., Mexas, R., Azambuja, P., Araújo, H. P., Moura, W., Ratcliffe, N. A., Feder, D., Rocha, L., & Gonzalez, M. S. (2020). Green insecticide against Chagas disease: Effects of essential oil from Myrciaria floribunda (Myrtaceae) on the development of Rhodnius prolixus nymphs. *Journal of Essential Oil Research*, *32*(1), 1–11. https://doi.org/10.1080/10412905.2019.1631894

Tietbohl, L. A., Lima, B. G., Fernandes, C. P., Santos, M. G., Silva, F. E., Denardin, E. L., & Rocha, L. (2012). Comparative study and anticholinesterasic evaluation of essential oils from leaves, stems and flowers of *Myrciaria floribunda* (H. West ex Willd.) O. Berg. *Latin American Journal of Pharmacy*, *31*(4), 637–641.

Tiuman, T. S., Ueda-Nakamura, T., Cortez, D. A. G., Dias Filho, B. P., Morgado-Díaz, J. A., de Souza, W., & Nakamura, C. V. (2005). Antileishmanial activity of parthenolide, a sesquiterpene lactone isolated from Tanacetum parthenium. *Antimicrobial Agents and Chemotherapy*, *49*(1), 176–182. https://doi.org/10.1128/AAC.49.11.176–182.2005.

Toukmaji, A., & Board, J. (1996). Ewald summation techniques in perspective: A survey. *Computer Physics Communications*, *95*(2-3), 73–92. https://doi.org/10.1016/0010-4655(96)00016-1

Turcano, L., Torrente, E., Missineo, A., Andreini, M., Gramiccia, M., Di Muccio, T., Genovese, I., Fiorillo, A., Harper, S., Bresciani, A., Colotti, G., & Ilari, A. (2018). Identification and binding mode of a novel Leishmania Trypanothione reductase inhibitor from high throughput screening. *PLoS Negl Trop Dis*, *12*(11), e0006969. https://doi.org/10.1371/journal.pntd.0006969

Ueda-Nakamura, T., Mendonça-Filho, R. R., Morgado-Díaz, J. A., Maza, P. K., Filho, B. P. D., Cortez, D. A. G., Alviano, D. S., Rosa, M. S. S., Lopes, A. H. C. S., Alviano, C. S., & Nakamura, C. V. (2006). Antileishmanial activity of Eugenol-rich essential oil from Ocimum gratissimum. *Parasitology International*, *55*(2), 99–105. https://doi.org/10.1016/j.parint.2005.10.006

Valero, N. N. H., & Uriarte, M. (2020). Environmental and socioeconomic risk factors associated with visceral and cutaneous leishmaniasis: A

systematic review. *Parasitology Research*, *119*(2), 365–384. https://doi.org/10.1007/s00436-019-06575-5

Vargas, J. A. R., López, A. G., Pérez, Y., Cos, P., & Froeyen, M. (2019). In vitro evaluation of arylsubstituted imidazoles derivatives as antiprotozoal agents and docking studies on sterol 14α-demethylase (CYP51) from Trypanosoma cruzi, Leishmania infantum, and Trypanosoma brucei. *Parasitology Research*, *118*(5), 1533–1548. https://doi.org/10.1007/s00436-019-06206-z

Verma, R. K., Prajapati, V. K., Verma, G. K., Chakraborty, D., Sundar, S., Rai, M., Dubey, V. K., & Singh, M. S. (2012). Molecular docking and in vitro antileishmanial evaluation of chromene-2-thione analogues. *ACS Medicinal Chemistry Letters*, *3*(3), 243–247. https://doi.org/10.1021/ml200280r

Wang, E., Sun, H., Wang, J., Wang, Z., Liu, H., Zhang, J. Z., & Hou, T. (2019). End-point binding free energy calculation with MM/PBSA and MM/GBSA: Strategies and applications in drug design. *Chemical Reviews*, *119*(16), 9478–9508. https://doi.org/10.1021/acs.chemrev.9b00055

World Heald Organization. (2020). Global Health Observatory (GHO) data| Leishmaniasis. www.who.int/gho/neglected_diseases/leishmaniasis/en/.