Experimental Parasitology 270 (2025) 108899

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

In vitro and *in vivo* leishmanicidal and trypanocidal activities of isoflavans from *Tabebuia chrysantha* (Jacq.) G. Nicholson timber by-products

Edwin Correa [a], Sara M. Robledo [b,*], Fernando Echeverri [a], Wiston Quiñones [a], Natalia Arbeláez [c], Javier Murillo [c], Tatiana Pineda [c], Fernando Torres [a,**]

[a] Grupo de Química Orgánica de Productos Naturales, Instituto de Química, Universidad de Antioquia-UdeA. Calle 70 # 52-21, Medellín, Colombia
[b] PECET- Facultad de Medicina, Universidad de Antioquia-UdeA. Calle 70 # 52-21, Medellín, Colombia
[c] Grupo Estudios Preclínicos, Corporación de Innovación para El desarrollo de productos – CIDEPRO. Carrera 51A #62–42, P2, T7. Medellín, Colombia

ARTICLE INFO

Keywords:
Sawdust
Leishmania braziliensis
Trypanosoma cruzi
Cutaneous leishmaniasis
Chagas disease
Sativan
Vestitol
Bio-guided fractionation

ABSTRACT

Cutaneous Leishmaniasis and Chagas disease are neglected tropical diseases that affect millions worldwide. Despite the high morbidity associated with these infections, current treatments are often highly toxic and are showing diminishing efficacy. Thus, new therapeutic options are urgently needed. In this study, bio-guided assays were conducted on the sawdust of *Tabebuia chrysantha* ("guayacán") to identify promising bioactive compounds. The ethanolic crude extract, five chromatography fractions, pure isoflavans sativan and vestitol, and a mixture were evaluated in vitro against *Leishmania braziliensis* and *Trypanosoma cruzi*. High leishmanicidal and trypanocidal activities were observed in the crude extract, fraction F2 (rich in sativan and vestitol), and the two pure isoflavans. Given the abundance and ease of obtaining the isoflavan mixture, its therapeutic potential was further evaluated *in vivo* in hamsters infected with *L. braziliensis* and mice infected with *T. cruzi*. Remarkably, topical and intraperitoneal administration of the chromatography fraction achieved a 67% clinical cure in hamsters with *L. braziliensis* infection and a 75% reduction in parasitemia in *T. cruzi*-infected mice. While the antiparasitic effects of certain flavonoids have been documented, this study is the first to demonstrate the efficacy of isoflavans in animal models for both diseases. The potential efficacy observed against *T. cruzi* and *L. braziliensis*, two pathogens with limited treatment options and a significant drawback of the available treatments, highlights the therapeutic potential of this combination of sativan and vestitol, which can be derived from timber industry waste, presenting an abundant and accessible source for further development.

1. Introduction

Leishmaniasis and Chagas disease are classified as neglected tropical diseases (NTDs), which, along with 19 other parasitic, bacterial, and viral infections, contribute significantly to global morbidity and mortality, impacting the world's most impoverished populations. These diseases have devastating health, social, and economic consequences, particularly in resource-poor settings (Hotez et al., 2020; WHO, 2012). The World Health Organization estimates that NTDs affect one billion people annually, with over 70% of affected countries classified as low- and lower-middle-income economies. Because of their association with poverty and lack of profitability, NTDs are often overlooked by drug manufacturers, exacerbating the social and economic burdens on afflicted communities (Hotez et al., 2020; WHO, 2020).

Cutaneous leishmaniasis (CL) and Chagas disease are vector-borne infections caused by the protozoan parasites *Leishmania* species and *Trypanosoma cruzi*, respectively. These diseases affect millions globally, predominantly in developing and underdeveloped regions. Current treatments, although available, are often highly toxic and are becoming increasingly less effective. Conventional antileishmanial drugs, such as pentavalent antimonials, amphotericin B, and miltefosine, are associated with significant side effects and toxicity. For instance, pentavalent antimonials can cause cardiotoxicity and pancreatitis, while amphotericin B (AMB) is known for its nephrotoxicity. Miltefosine, although orally administered, has been linked to gastrointestinal disturbances and teratogenic effects (Brindha et al., 2021). The primary drugs used to treat Chagas disease are benznidazole (BNZ) and nifurtimox. Both are associated with significant adverse effects, including anorexia, weight

* Corresponding author.
** Corresponding author.
E-mail addresses: sara.robledo@udea.edu.co (S.M. Robledo), luis.torres@udea.edu.co (F. Torres).

https://doi.org/10.1016/j.exppara.2025.108899
Received 30 October 2024; Received in revised form 27 December 2024; Accepted 9 January 2025
Available online 23 January 2025

loss, excitability, sleepiness, and digestive disturbances (Castro et al., 2006). These adverse effects can lead to treatment discontinuation, compromising therapeutic outcomes.

The efficacy of leishmanicidal and trypanocidal drugs varies based on multiple factors, including parasite species, geographic region, stage of infection, and the emergence of drug resistance (Falk et al., 2022; Tunalı et al., 2022; Roatt et al., 2020).

The phenomenon of drug resistance in *Leishmania* spp. is well-documented, with several mechanisms identified, such as decreased drug uptake and overexpression of ATP-binding cassette (ABC) transporters, which results in enhanced drug efflux, reducing their intracellular concentrations and efficacy (Moncada-Diaz et al., 2024). Notably, detoxification mechanisms play a significant role in the neutralization of drug-induced oxidative stress, thereby aiding in parasite survival (Croft et al., 2006) and reducing their intracellular concentrations and efficacy. Although less extensively documented than in *Leishmania*, the identified resistance mechanisms in *Trypanosoma cruzi* are of significant importance; these include the upregulation of efflux pumps that can extrude drugs from the parasite, decreasing their intracellular concentrations and effectiveness (Buckner et al., 1998) and mutations in specific enzymes targeted by therapeutic agents that can reduce drug binding affinity, leading to diminished efficacy (De Rycker et al., 2023). All of these drawbacks complicate CL and Chagas disease management, underscoring an urgent need for new therapeutic alternatives.

Natural products derived from plants, fungi, and other natural sources have shown tremendous potential as a source of bioactive compounds with diverse therapeutic effects and generally lower toxicity than synthetic drugs (Atanasov et al., 2021; Cheuka et al., 2016). In many endemic regions, traditional remedies derived from local plants have been used by communities to treat diseases like leishmaniasis and Chagas (Rizvi et al., 2022). Natural products offer sustainability and accessibility, especially in resource-limited settings. However, bioactive compounds in natural sources are often found in small quantities, necessitating large amounts of raw material to meet the demands of chemical synthesis and preclinical and clinical evaluations (Rathore et al., 2018).

Wood industry by-products such as bark, sawdust, and foliage present a valuable and sustainable source of raw materials, often produced in substantial quantities. These by-products could yield enough bioactive molecules for comprehensive preclinical and clinical testing (Torres-León et al., 2018). Despite their potential, utilization of these wood residues is minimal; only about 60% of a timber tree is used, with the remaining 40% often left as waste. For instance, from a tree approximately 20 m tall and weighing 400 kg, around 160 kg could be utilized as by-products, yielding an estimated 16–24 kg of chemical extracts (da Silva and Rodrigues, 2014).

This study presents the first report of the in vitro and vivo leishmanicidal and trypanocidal effects of isoflavans isolated from ethanolic sawdust extracts of Tabebuia chrysantha (Jacq.) G. Nicholson (Bignoniaceae), commonly known as the golden trumpet tree, is a widely exploited timber tree valued for its ornamental beauty and durability (Gentry, 1992), leading to overexploitation and habitat loss. We emphasize the necessity of sustainable sourcing practices, such as utilizing by-products from the timber industry or exploring alternative methods to obtain therapeutic compounds without further endangering the species.

2. Materials and methods

2.1. Plant material

Sawdust from *T. chrysantha* was obtained in Expomaderas (Medellín, Colombia), and their identification was made by Hernán Darío Cañola at the Colegio Mayor de Antioquia, Laboratorio de Maderas; a voucher was deposited under No. M2. SIU 21012021.

^{1}H and ^{13}C NMR: 300 and 75 MHz, respectively (Bruker Biospin GmbH at 300 MHz, Rheinstetten, Germany). Mass spectrometry: UHR ESI–QqTOF, positive ion mode (Bruker Daltonik GmbH, Bremen Germany. HPLC (Chromaster, 5430 DAD, 5160 pump, Hitachi).

2.2. Extraction and compound isolation

Sawdust of *T. chrysantha* (5 kg) was extracted using ethanol through a percolation method, yielding 60 g of crude extract after solvent evaporation. A total of 4.2 g of the extract was then fractionated by exclusion chromatography on Sephadex LH-20, employing 100% methanol as the eluent, resulting in the collection of five fractions. These chromatography fractions were subsequently evaluated in vitro for their antiparasitic activity and cytotoxicity.

Fractions 1 (F1) and 2 (F2), totaling 2 g, were pooled based on thin-layer chromatography using a petroleum ether acetate solvent system (2:1 v/v). This pooled fraction was subjected to column chromatography on silica gel 60, using a gradient of petroleum ether acetate with the following elution volumes: 250 mL (4:1), 400 mL (3:1), 300 mL (2:1), and 200 mL (1:1). Eleven subfractions were collected, from which subfraction 1 (700 mg) and subfraction 2 (700 mg) were evaluated for in vitro antiparasitic activity.

Further purification of F2 via successive chromatography on silica gel yielded 1 g each of the two primary pure compounds, sativan and vestitol, used for subsequent in vitro and *in vivo* biological activity assays. The structures of compounds were confirmed using 1D and 2D NMR spectroscopy (Figs. S1 and S2, Supplementary Material) and by comparison with previously published literature (Takashima et al., 2010; Luniwal and Erhardt, 2011; Hasan et al., 2012).

2.3. Quantification of sativan and vestitol

A crude extract at 1 mg/mL in methanol was used to develop the HPLC method. A C8 reverse-phase column (150 mm, 5 μm, 4.6 mm ID Supelcosil™, Supelco) was maintained at 25 °C, providing excellent separation of the sample. The gradient mixture consisted of Water-Methanol at 80:20 (for 10 min), transitioning from 80:20 to 70:30 (over 5 min), then holding at 70:30 (for 5 min), followed by a transition from 70:30 to 50:50 (over 10 min), maintaining at 50:50 (for 10 min), then 0:100 (for 10 min), transitioning back to 80:20 (over 5 min), and holding at 80:20 (for 5 min) at a flow rate of 1 mL/min. A 10 μL sample was injected, and DAD detection of the compounds indicated a maximum absorption wavelength of 270 nm.

A stock solution of each pure compound, sativan and vestitol, was prepared at 1.0 mg/mL in methanol. Five working solutions were then prepared at the following concentrations: for sativan, 12.5, 37.5, 62.5, 87.5, and 112.5 μg/mL; and for vestitol, 20, 40, 60, 80, and 100 μg/mL. Subsequently, 10 μL of each working solution was injected to construct a calibration curve.

2.4. Parasites and culture

The L. *braziliensis* strain (MHOM/CO/88/UA301-EGFP) used in this work was isolated from a patient with CL and subsequently transfected with green fluorescent protein (GFP) (Pulido et al., 2012). The *T. cruzi* strain (Tulahuen DTU VI transfected with the beta-galactosidase gene) was donated by FS Buckner (1996). Both L. *braziliensis* and *T. cruzi* were maintained as promastigotes and epimastigotes, respectively, in culture at 26 °C in Novy-MacNeal-Nicholle (NNN) medium and phosphate-buffered saline (PBS) supplemented with glucose, pH 6.9 (WHO, 2010).

For trypomastigotes obtention, U-937 cells were infected with epimastigotes of T. cruzi (12–15 days of growth) at a parasite-to-cell ratio of 5:1 in RPMI with 10% FBS, and plates were incubated at 34 °C in a 5% CO2 atmosphere. After 24 h of infection, non-internalized parasites were removed by two washes with PBS. Then, fresh enriched RPMI-1640 medium was added, and the plates were incubated for 10 days at

37 °C in a 5% CO_2 atmosphere. At day 12–15 post-infection, extracellular trypomastigotes found in the culture medium were recovered in 50 ml falcon tubes. They were then centrifuged at 1200 rpm for 5 min to remove the existing cellular debris. The supernatant containing the trypomastigotes was recovered and again centrifuged at 4000 rpm for 20 min. After this, the supernatant containing the trypomastigotes was recovered and again centrifuged at 4000 rpm. After centrifugation, the supernatant was removed, and the trypomastigote pellet was resuspended in 1 ml of medium to count parasites in the Neubauer chamber (De Arias and Ferro, 1988). The parasites obtained were used to infect donor mice, a crucial step that paves the way for the *in vivo* evaluation of therapeutic response.

2.5. Cells and culture

Human U-937 promonocytes (ATCC® CRL1593.2™) were cultured in RPMI 1640 medium (Sigma) enriched with 10% Fetal Bovine Serum (FBS) (Invitrogen) and 1% ready-to-use antibiotic mix of penicillin (10,000 units/mL) and streptomycin (10,000 μg/mL) under standard conditions at 37 °C, 5% CO_2, with medium changes every three days until use. Human red blood cells (huRBC) were collected with informed consent from healthy donors (n = 3), with an average total blood volume of 10 mL per donor. The RBC fraction was obtained by centrifuging heparinized blood at 1100–1300 g for 10 min.

2.6. Animals in experimentation

Golden hamsters (*Mesocricetus auratus*), both male and female, six weeks of age, were used as the animal model for CL. In contrast, BALB/c mice (*Mus musculus*), also both male and female, nine weeks of age, were used as the experimental model for *T. cruzi* infection. Both hamsters and mice were housed under the following macroenvironmental conditions: 22 °C temperature, 60% relative humidity, 16–20 air changes/hour regulated by the ventilation system, and 12-h light/dark cycles using artificial white lighting, controlled by a timer.

The microenvironmental conditions included groups of three animals of the same sex housed in transparent polypropylene or acrylic boxes (19 cm high x 20 cm wide x 30 cm long), filled two-thirds with autoclaved shavings. Mice and hamsters had continuous access to sterilized water and food (LabDiet®) *ad libitum*.

2.7. In vitro toxicity evaluation

The cytotoxicity of the crude extract, fractions, and metabolites was evaluated in the human promonocytic cell line U-937 (ATCC® CRL-1593.2™) (Gaithersburg, MD, USA), as described by Upegui et al. (2020). Briefly, cells were cultured under standard conditions in RPMI-1640 medium supplemented with 10% inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. Cells (100,000 cells/well) in the log growth phase (3 days old) were dispensed into each well of a 96-well culture plate and exposed to 100 μL of each of the five evaluated extract, fraction, or metabolite concentrations: 200, 50, 12.5, 3.125, and 0.78 μg/mL. The plates were incubated at 37 °C in a 5% CO_2 atmosphere. After 72 h of incubation, 10 μL/well of MTT solution (0.5 mg/mL) was added, and the plates were incubated at 37 °C for 3 h. Then, 100 μL/well of dimethyl sulfoxide (DMSO) was added to each well. The plates were read at 570 nm in a spectrophotometer (Varioskan™ Flash Multimode Reader, Thermo Waltham, MA, USA), and the absorbance was recorded as optical density (OD). Non-exposed cells served as the negative control (no toxicity), while doxorubicin was used as the positive control (cytotoxicity). The RPMI-1640 medium plus 0.2% DMSO was used as a blank solution to correct for nonspecific absorbance. Three assays were performed, with each concentration in triplicate.

The cytotoxicity of each extract, fraction, or metabolite was determined based on the percentage of mortality, calculated using Eq. (1). These percentages were then used to calculate the median cytotoxic concentration (CC_{50}) using the Probit linear regression model in Prism GraphPad 8.0.

$$\%\text{Mortality} = 100 - \left[\frac{\text{OD Exposed cells}}{\text{OD Control cells}} \times 100\right] \quad (1)$$

The potential toxicity of derivatives of *T. chrysanta* sawdust was complemented with the quantification of hemolytic activity. Briefly, 500 mL of huRBC at a 2% hematocrit in RPMI-1640 medium enriched with 3% FBS were exposed to serial dilutions of each crude extract, fractions, or metabolites (200, 50, 12.5, 3.125, and 0.78 μg/mL). After 6 h of incubation at 37 °C, the free hemoglobin concentration was determined based on the optical density (OD) measured at 550 nm in a spectrophotometer (Varioskan™). HuRBC in ultrapure water served as the positive control (hemolysis), while huRBC in phosphate-buffered saline (PBS) served as the negative control (no hemolysis) (Insuasty et al., 2017). Three assays were conducted for each concentration in triplicate.

The hemolytic activity was calculated based on the percentage of hemolysis using Eq. (2). These percentages were then used to determine the median hemolytic concentration (HC50) through the Probit linear regression model in Prism GraphPad 8.0.

$$\%\text{Hemolysis} = 100 - \left[\frac{\text{OD Exposed cells}}{\text{OD Control cells}} \times 100\right] \quad (2)$$

2.8. In vitro leishmanicidal activity

U-937 macrophages (300,000 cells/mL) were cultured in enriched RPMI-1640 medium supplemented with 0.1 μg/mL of phorbol 12-myristate 13-acetate (PMA) in 24-well tissue culture plates. The cells were incubated at 37 °C in a 5% CO2 atmosphere. After 72 h of incubation, the cells were infected with 5-day-old promastigotes of *L. braziliensis* at a parasite-to-cell ratio of 15:1 and incubated at 34 °C with 5% CO_2 for 3 h. The cells were then washed twice with phosphate-buffered saline (PBS), and 1 mL of fresh enriched RPMI-1640 was added to each well. The plates were incubated again at 34 °C and 5% CO_2.

After 24 h of incubation, the culture medium was replaced with fresh enriched RPMI-1640 medium containing each extract, fraction, or metabolite at four serial dilutions (100, 25, 6.25, and 1.56 mg/mL). The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 72 h.

Cells were harvested using 100 μL of a trypsin/EDTA solution and centrifuged at 1100 rpm for 10 min at 4 °C. The supernatant was discarded, and the cells were washed twice with PBS. The cells were then suspended in 500 μL of PBS and analyzed using a flow cytometer (Cytomics F.C. 500 MP L, Brea, CA, USA) with an argon laser, measuring at 488 nm (excitation) and 525 nm (emission) while counting 10,000 events. The number of amastigotes was determined based on the median fluorescence intensity (MFI) of the green fluorescence events (Pulido et al., 2012). Infected cells served as a negative control, while infected cells treated with amphotericin B (AMB) were used as a positive control for leishmanicidal activity. Nonspecific fluorescence was corrected by subtracting the fluorescence of unstained cells. Three assays were conducted for each concentration in triplicate.

Leishmanicidal activity was calculated based on the percentage decrease in amastigotes using Eq. (3). These percentages were then used to determine the median effective concentration (EC_{50}) through the Probit linear regression model in Prism GraphPad 8.0.

$$\%\ \textit{reduction of amastigotes} = 100 - \left[\frac{(\textit{MFI infected treated cells})}{(\textit{MFI infected non} - \textit{treated cells})}\ x\ 100\right] \quad (3)$$

2.9. In vitro trypanocide activity

The anti-trypanosomal activity of compounds was tested in intracellular amastigotes of *T. cruzi*. Briefly, the U-937 macrophages (25,000

cells in 100 mL of enriched RPMI 1640 medium with 0.1 μg/mL of PMA) were placed into each well of the 96-well cell culture plates. The cells were then infected with epimastigotes of *T. cruzi* (10 days of growth) at a parasite-to-cell ratio of 5:1 in RPMI with 10% FBS, and plates were incubated at 34 °C, 5% CO_2 atmosphere. After 24 h of infection, non-internalized parasites were removed by two washes with PBS. Then, fresh enriched RPMI 1640 medium was added containing each of the four serial diluted concentrations of each extract, fraction, or pure metabolite (the same as those used in the leishmanicidal assay), and the plates were incubated for 48 h at 37 °C in a 5% CO_2 atmosphere.

Next, 100 mM chlorophenol red-β-D-galactopyranoside (CPRG; Sigma-Aldrich, St. Louis, MO, USA) and 1% Nonidet P-40 were added to each well, and the plates were incubated at room temperature for 3 h. The plates were read at 570 nm using a spectrophotometer (VarioskanTM), and absorbance was recorded as optical density (OD). Infected cells served as a negative assay control, while infected cells exposed to benznidazole (BNZ) were used as a positive assay control (for trypanocidal activity). In contrast, the RPMI-1640 medium was used as a negative control. Nonspecific absorbance was corrected by subtracting the OD of the blank sample. Determinations were performed in triplicate in two independent experiments (Buckner et al., 1996).

Trypanocidal activity was determined based on the percentage decrease of intracellular amastigotes using Eq. (4); these percentages were then used to calculate the median effective concentration (EC_{50}) using the Probit linear regression model in Prism GraphPad 8.0.

$$\%\ reduction\ of\ amastigotes = 100 - \left[\frac{(OD\ \ infected\ treated\ cells)}{(OD\ infected\ non-treated\ cells)-}\ x\ 100\right] \quad (4)$$

2.10. In vivo therapeutic response of sativan and Vestitol in hamsters infected with L. braziliensis

In this study, male and female golden hamsters (*Mesocricetus auratus*), aged six to seven weeks, were used as the animal model. The hamsters were intradermally inoculated with 1×10^8 stationary phase promastigotes of *L. braziliensis* (MHOM/CO/88/UA301-EGFP) in the dorsum of the skin, following established procedures (Robledo et al., 2012).

Once the hamsters developed a skin ulcer greater than 25 mm^2, they were randomly assigned to four experimental groups (n = 6 per group). Sativan, vestitol, and F2 fraction were solubilized in castor oil to a final concentration of 3%. Three groups of hamsters received topical treatment with 60 μL per lesion per day of either 3% sativan (Group 1), 3% vestitol (Group 2), or 3% F2 (Group 3) for a duration of 30 days. The fourth group (Group 4) received meglumine antimoniate (MA) administered via intralesional injection at a dosage of 100 μL (2 mg/mL) three times per week for four weeks.

Hamsters were monitored for an additional 90 days post-treatment. Every two weeks, starting from the onset of treatment (TD0) to the conclusion of the study (PTD90), the area of the ulcer was measured using an electronic caliper. At the end of the study, outcomes were recorded as cure (complete re-epithelialization), improvement (decrease in lesion area >20%), relapse (reactivation of the lesion after initial improvement or cure), or failure (increase in lesion size).

Toxicity was evaluated according to weight loss, changes in the appearance or behavior of the animals, and visualization of macroscopic alterations of organs and tissues during necropsy and, if any altered organ was present, by histopathological study of the altered tissue. For this, animals were monitored every day documenting appearance, behavior and body condition for each hamster. The body weight was recorded at before administration of treatment and monthly thereafter for three months. Serum levels of ALT, BUN and creatinine were measured before and eight days of treatment (TD8) to detect any alterations caused by the compounds. At the end of the study, hamsters were euthanized for necropsy, and histological analyses were performed only on those organs that exhibited abnormalities during necropsy. Hamsters were inspected daily to record mortality.

2.11. In vivo effectiveness of sativan, vestitol, and the combination of both compounds in the treatment of BALB/c mice infected with T. cruzi

To obtain infective trypomastigotes, donor BALB/c mice were experimentally inoculated with 500 trypomastigotes (obtained after infection of U-937 cells) in 100 μL saline solution. After 21 days post-inoculation, mice were anesthetized intraperitoneally (i.p) with a 9:1 mixture of ketamine (50 mg/mL) and xylazine (20 mg/mL) for intracardiac blood sampling. Thirty microliters were taken in capillaries with EDTA, centrifuged at 1872 g for 5 min to count the blood-plasma interface in the Neubauer chamber (De Arias and Ferro, 1988).

Thirty male and female BALB/c mice were intraperitoneally inoculated with 100 trypomastigotes of *T. cruzi* in 100 μL of blood, and parasitemia was monitored through blood concentration using the microhematocrit method and subsequent counting in a Neubauer chamber (De Arias and Ferro, 1988). Once the establishment of infection was verified (approximately one week after inoculation), the mice were randomly assigned to five experimental groups (n = 6 per group).

Sativan, vestitol, and F2 fraction were solubilized in castor oil to achieve a final concentration of 30 mg/mL. Groups 1 to 4 received oral treatment with 100 μL (100 mg/kg/day, approximately 3 mg/day) of 3% sativan (Group 1), 3% vestitol (Group 2), 3% F2 fraction (Group 3), or benznidazole (BNZ, Group 4) for 25 days. The untreated control group (Group 5) was orally administered 100 μL/day of PBS.

Parasite load and body weight were recorded at the end of the treatment and monthly thereafter for three months. The mice's health status was monitored daily throughout the study. At the end of the study (90 days post-treatment), the mice were euthanized for necropsy, and histological analyses were performed only on those organs that exhibited abnormalities during necropsy.

The *in vivo* trypanocidal effectiveness was evaluated based on the reduction in parasitemia in response to treatment. For this assessment, the parasite load before and after treatment for each mouse was compared. At the end of the study, clinical outcomes were classified as cure (clearance of parasitemia), improvement (reduction in parasite load by at least 10%), or failure (increase in parasite load). The toxicity of compounds.

As described for the evaluation of the therapeutic response in the LC model, toxicity was evaluated in the mice according to alterations in the appearance and behavior of the animals but also in the body weight and serum levels of ALT, BUN and creatinine (. Body weight was recorded before treatment (TD0), at the end of treatment (TD30), and every month during follow-up until the end of the study (PTD90). Blood samples for seric levels of ALT, BUN, and creatinine determination were analyzed in the Animal diagnostic unit of the Faculty of Agricultural Sciences of the University of Antioquia. Mice were inspected daily to record mortality.

2.12. Data analysis

In vitro cytotoxicity was categorized based on CC_{50} values as follows: high (CC_{50} < 100 μg/mL for extracts/fractions or < 100 μM for pure compounds), moderate (CC_{50} between 100 and 200 μg/mL or μM), and low (CC_{50} > 200 μg/mL or μM). Similarly, in vitro leishmanicidal and trypanocidal activities were classified by EC_{50} values: high activity (EC_{50} < 25 μg/mL or μM), moderate (EC_{50} between 25 and 50 μg/mL or μM), and low (EC_{50} > 50 μg/mL or μM). The selectivity index (SI) was calculated as $SI = CC_{50}/EC_{50}$.

No statistical comparisons were performed between lesion sizes or parasitemias across extracts, fractions, pure compounds, and standard drugs (AMB and BNZ). Instead, descriptive comparisons were made for each animal relative to changes over time during the follow-up period.

Statistical analysis was only conducted for body weight comparisons

between changes over time during the follow-up period and treatment groups. Normality was assessed using the Shapiro-Wilk test in Prism v8.0 (GraphPad Software, USA). Statistical significance was determined via two-way ANOVA, with p-values <0.05 considered significant.

2.13. Ethical aspects

All procedures with hamsters and BALB/c mice and hamsters were approved by the Animal Experimentation Ethics Committee of the University of Antioquia (Act 129, Aug 14, 2018) and followed the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (Percie du Sert et al. 2020). On the other hand, the procedure for collecting venous blood from healthy donors was endorsed by the Universidad de Antioquia Bioethical Committee (Act 16-05-727).

3. Results

3.1. Isolation and structure assignments

The ethanolic crude extract of *T. chrysantha* was fractionated using a bioassay-guided approach, resulting in five fractions (F1 to F5) and two pure metabolites: sativan and vestitol (Fig. 1). All pure compounds were isolated from F2 fraction by successive chromatography columns and identified spectroscopically based on 1D and 2D NMR data, as well as comparisons with literature references (Fig. 1) (Takashima et al., 2010; Luniwal et al., 2011; Hasan et al., 2012).

The first compound, sativan, an isoflavan, demonstrated high activity against *Leishmania braziliensis*, with an EC_{50} of 5.5 μg/mL (19.2 μM) and a SI of 1.3. It also showed effectiveness against *T. cruzi*, with an EC_{50} of 6.6 μg/mL (23.1 μM) and an SI of 1.7. The second molecule, vestitol, another isoflavan, exhibited significant activity against *L. braziliensis* (EC_{50} = 5.3 μg/mL - 19.5 μM, SI = 1.9) and *T. cruzi* (EC_{50} = 7.1 μg/mL −26.1 μM, SI = 1.4) (Table 1).

Given the lengthy and complex separation process for sativan and vestitol, assays were conducted using the F2 fraction in animal models of *L. braziliensis* and *T. cruzi*, as it contains a mixture of these compounds as the primary components. To determine the quantitative composition of the bioactive components in both the crude extract and the F2 fraction, we constructed an HPLC calibration curve using pure sativan and vestitol. The overlapped chromatograms of the standard compounds and the crude extract exhibited good resolution of the compound peaks, with retention times of 31.3 min for sativan and 25.1 min for vestitol. The calibration curves yielded the following equations.

- For sativan: Y = 9.7731x−20.0903Y = 9.7731x - 20.0903Y = 9.7731x−20.0903 (r = 0.9988)
- For vestitol: Y = 7007.69x−30.8830Y = 7007.69x - 0.8830Y = 7007.69x−30.8830 (r = 0.9981)

Accordingly, the crude extract contained 13.5% sativan and 10.2% vestitol. In the F2 fraction, these percentages increased to 36.7% and 31.8%, respectively. This significant enrichment explains the observed increase in in vitro activity.

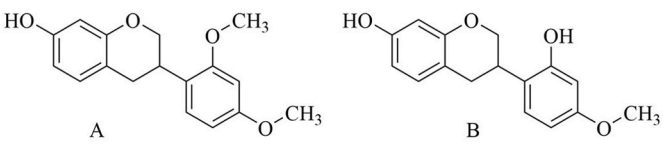

Fig. 1. Structure of antiparasitic compounds isolated from *Tabebuia chrysantha* sawdust. A. Sativan; B. Vestitol.

3.2. Antiparasite activity, cytotoxicity in U-937 macrophages and hemolytic activity

The ethanolic crude extract and sub-fractions of *T. chrysantha* sawdust demonstrated significant antiparasitic activity against all tested parasites, with EC_{50} values of 17.7 μg/mL for *L. braziliensis* and 21.3 μg/mL for *T. cruzi* (Table 1). However, this extract also exhibited high cytotoxicity toward U-937 cells, which are used as hosts for both *L. braziliensis* and *T. cruzi*, with a CC_{50} value of 60.8 μg/mL. When evaluating the relationship between antiparasitic and cytotoxic activities, SI values were calculated to be 3.4 for *L. braziliensis* and 2.9 for *T. cruzi*. The hemolytic activity of the crude extract was >500 μg/mL.

The extract was further analyzed through chromatographic separation using Sephadex LH-20, yielding five distinct fractions. All fractions exhibited notable activity against *L. braziliensis*, with EC_{50} values ranging from 3.3 μg/mL to 23.3 μg/mL. Notably, fractions F2 and F4 displayed a broad activity profile, with F2 exhibiting antiparasitic effects against both *L. braziliensis* and *T. cruzi*, with EC_{50} values of 11.1 μg/mL and 10.3 μg/mL, respectively. Fractions F1, F3 and F4 showed cytotoxic activity for U-937 macrophages with CC_{50} of 13.2, 7.7 and 7.9 μg/mL, respectively. The F2 was non-cytotoxic to U-937 macrophages, with a CC_{50} of 210 μg/mL while F5 showed moderate cytotoxicity with CC_{50} values of 128.9 μg/mL (Table 1). The hemolytic activity of fractions and pure metabolites sativan and vestitol was >500 μg/mL.

3.3. Therapeutic response to treatment with sativan, Vestitol, and the F2 fraction in golden hamsters infected with L. braziliensis

Topical treatment with a 3% sativan cream over 30 days resulted in clinical cure (complete re-epithelialization) in 2 out of 6 hamsters (30%). Additionally, lesion size reduction was observed in 3 out of 6 hamsters (50%) during a 90-day post-treatment follow-up period (Table 2). For the 3% vestitol treatment, the clinical cure was achieved in 4 out of 6 hamsters (67%), with a reduction in lesion size noted in 2 out of 6 hamsters (33%).

In contrast, treatment with the 3% F2 fraction, which contains a combination of sativan and vestitol as the main components, along with intralesional MA, led to complete healing of the ulcers in 4 out of 6 hamsters (67%) by the end of the study. In this group, one hamster (17%) did not respond to the treatment, while another showed improvement in lesion size.

In comparison, the group treated with MA alone exhibited a decrease in lesion size of 47% and 84% in 2 out of 6 hamsters (Table 2 and Supplementary Material Figs. S3–S6).

Before treatment, there was observed a statistically significant difference in the body weight between F2, vestitol and sativan groups of hamsters vs. MA, with p-values of 0.0401 (F2 *vs.* MA), 0.0155 (V *vs.* MA) and 0.0045 (S *vs.* MA) (Fig. 2). Nonetheless, no significant weight loss was noted in any of the treated animals throughout the study. Additionally, there were no statistically significant differences in body weight among the treatment groups over time with p-values >0.05 as calculated by two-way ANOVA (Fig. 2).

No alterations in liver enzyme ALT and renal function impairment (creatinine and BUN) were detected, being all metabolites evaluated in the normal ranges, as follows:

45–80 U/L for ALT, 15 y 25 mg/dL for BUN and 0.2–0.8 mg/dL for creatinine (Washington and Van Hoosier, 2012) (Table 3).

3.4. Therapeutic response to the treatment with a sativan, vestitol, and F2 fraction in BALB/c mice infected with T. cruzi

Oral treatment with sativan (100 mg/kg/day for 25 days) resulted in a parasitological cure (clearance of parasitemia) in 2 out of 6 mice (33%), while parasitemia was reduced in another two out of six mice (33%) after a 90-day post-treatment follow-up period. The remaining two out of six mice did not respond to the treatment (Table 4). In the

Table 1
In vitro hemolytic, cytotoxic, and antiparasitic activities of *Tabebuia chrysantha* crude extract, fractions and pure compounds against Leishmania braziliensis and Trypanosoma cruzi.

Product	Hemolytic activity	Cytotoxicity U937 macrophages	Antiparasite activity			
	huRBC		*Leishmania braziliensis*		*Trypanosoma cruzi*	
	CC_{50}, μg/mL[a]	CC_{50}, μg/mL[b]	EC_{50} (μg/mL) [c]	SI [d]	EC_{50} (μg/mL) [c]	IS [d]
Crude extract	>500	60.8 ± 5.2	**17.7 ± 1.4**	3.4	**21.3 ± 1.9**	2.9
F1	>500	13.2 ± 1.7	**7.9 ± 0.3**	1.7	58.9 ± 8.3	0.2
F2	>500	210.0 ± 22.3	**11.1 ± 1.4**	18.9	**10.3 ± 0**	20.4
F3	>500	7.7 ± 0.3	**6.7 ± 1.7**	1.2	47.7 ± 12.4	0.2
F4	>500	7.9 ± 0.3	**3.3 ± 0.3**	2.4	32.8 ± 5.8	0.2
F5	>500	128.9 ± 28.2	**23.3 ± 4.2**	<2.57	36.7 ± 4.2	3.8
Sativan	>500	7.3 ± 0.7	**5.5 ± 0.5**	1.3	**6.6 ± 0.8**	1.7
Vestitol	>500	10.0 ± 2.4	**5.3 ± 0.4**	1.9	**7.1 ± 0.5** **7.1 ± 0.5**	1.4
AMB[d]	18.0 ± 3.0	36.6 ± 3.0	**0.3 ± 0.1**	122.0	NA[e]	NA
BNZ[f]	250 ± 17.5	>200.0	NA	NA	**15.8 ± 2.6**	>12.7
DOX[g]	27.2 ± 1.7	1.0 ± 0.2	NA	NA	NA	NA

Data represent the mean value ± standard deviation. Bold values are active compounds (EC_{50} < 25 μg/mL). [a]HC_{50}: Median hemolytic concentration in huRBC; [b]CC_{50}: Median cytotoxic concentration in U-937 cells. [c]EC_{50}: Effective concentration on intracellular amastigotes of *L. braziliensis* or *T. cruzi*. [d]IS: Selectivity index for *L. braziliensis* and *T. cruzi* calculated by CC_{50}/EC_{50}. [e]AMB: Amphotericin B (used as internal assay control for leishmanicidal activity); [f]NA: Not applicable. [g]BNZ: Benznidazole (used as internal assay control for trypanocidal activity). [h]DOX; doxorubicin (used as internal assay control for cytotoxicity).

Table 2
Clinical outcome in hamsters with cutaneous leishmaniasis following treatment with sativan, vestitol or fraction F2.

Product	Code[a] (sex)	Lesion size (mm^2)[b]					↓ LS PTD90 (%)[h]	Final outcome
		TD0[c]	TD30[d]	PTD30[e]	PTD60[f]	PTD90[g]		
3% S[i]	561-OD (♂)	13.0	22.9	12.9	0	0	100	Cure
	562-AO (♂)	20.4	63.4	21.8	17.5	13.0	36.2	Improvement
	563-SM (♀)	92.1	49.0	66.7	0	0	100	Cure
	563-OI (♂)	34.9	25.5	27.6	31.3	23.0	34.0	Improvement
	564-AO (♀)	30.7	20.	20.1	28.9	18.6	39.3	Improvement
	564-SM (♀)	14.7	12.1	15.4	23.5	25.2	−72.2[e]	Failure
3% V[j]	556-OI (♂)	35.9	24,7	6.4	0	0	100	Cure
	562-SM (♀)	36.1	0	0	0	0	100	Cure
	562-OD (♂)	21.4	53.6	0	21.7	14.9	30.5	Improvement
	563-AO (♀)	19.8	5.7	7.2	12.8	0	100	Cure
	564-OD (♂)	43.4	27.0	48.0	29.6	36.3	16.4	Improvement
	563-ODx2 (♀)	16.3	0	0	0	0	100	Cure
3% F2[k]	530-OD (♂)	66.2	19.4	0	0	0	100	Cure
	530-OI (♂)	28.1	0	0	0	0	100	Cure
	531-OI (♀)	47.8	0	0	18.2	27.3	43.0	Relapse
	532-OD (♀)	50.0	0	0	29.3	53.1	−6.2[h]	Failure
	532-OI (♀)	20.0	21,3	12,6	3.9	0	100	Cure
	534-AO (♂)	87.0	5,9	0	0	0	100	Cure
MA[l]	796-MPI (♂)	18.6	48.7	13.0	19.2	23.1	84.1	Improvement
	797-MAD ♀)	35.4	34.2	0	0	0	100	Cure
	797-MAI (♀)	26.1	36.1	0	0	0	100	Cure
	816-MAI (♂)	27.7	34.9	14.4	12.6	14.6	47.4	Improvement
	816-MPD (♂)	23.2	7.8	0	0	0	100	Cure
	823-MAD (♀)	20.8	2.4	0	0	0	100	Cure

The data presented correspond to the clinical evolution of cutaneous leishmaniasis following treatment. [a]The abbreviations OD, AO, SM, OI, ODx2, MPI, MAD, MAI, and MPD refer to internal codes used to label each hamster within their respective groups. [b]Lesion area in mm^2; [c]TD0: lesion size before treatment; [d]TD30: lesion size at last day of treatment; [e]PTD30: lesion size at day 30 post-treatment; [f]PTD60: lesion size at day 60 post-treatment; [g]PTD90: lesion size at day 90 post-treatment; [h]Percentage of decrease in the lesion size at day 90 posttreatment. A negative value corresponds to an increase in the size instead a decrease; [i]S: sativan; [j]V: vestitol; [k]F2: fraction 2, which corresponds to the combination of vestitol and sativan as the major components in this fraction; [l]MA: meglumine antimoniate.

case of vestitol treatment, a parasitological cure was observed in two out of six mice (33%), while a reduction in parasitemia level (improvement) was noted in four out of six mice (67%). Treatment with the F2 fraction produced a parasitological cure in two out of 6 mice (33%) at the end of the study, with parasitemia reduction ranging from 61% to 94% in the other four out of six mice. As expected, treatment of infected mice with BNZ at 100 mg/kg/day for 25 days resulted in a parasitological cure in all mice in this group (Table 4). There was no mortality at the infection rate of 100 parasites/ml during the study. However, two mice in the sativan-treated group and one mouse in the vestitol-treated group reached the endpoint between days 85 and 88 post-treatment due to a deterioration in their clinical condition, necessitating euthanasia before the completion of the study.

The body weight was monitored during treatment in mice infected with *T. cruzi* to assess weight loss and adjust compound volumes accordingly. No significant weight loss that could compromise the health of mice was recorded. Only the treatment with vestitol resulted in a slight weight loss during the treatment period; however, once the treatment concluded, the mice exhibited expected weight gain (Fig. 3). According to a two-way ANOVA, there were no statistically significant differences in body weight among the treatments ($p > 0.05$).

At the end of the study corresponding to day 90 post-treatment, all mice were euthanized and necropsied. No alterations were observed in any of the target organs (heart, intestine, lung, kidney, liver or spleen) so

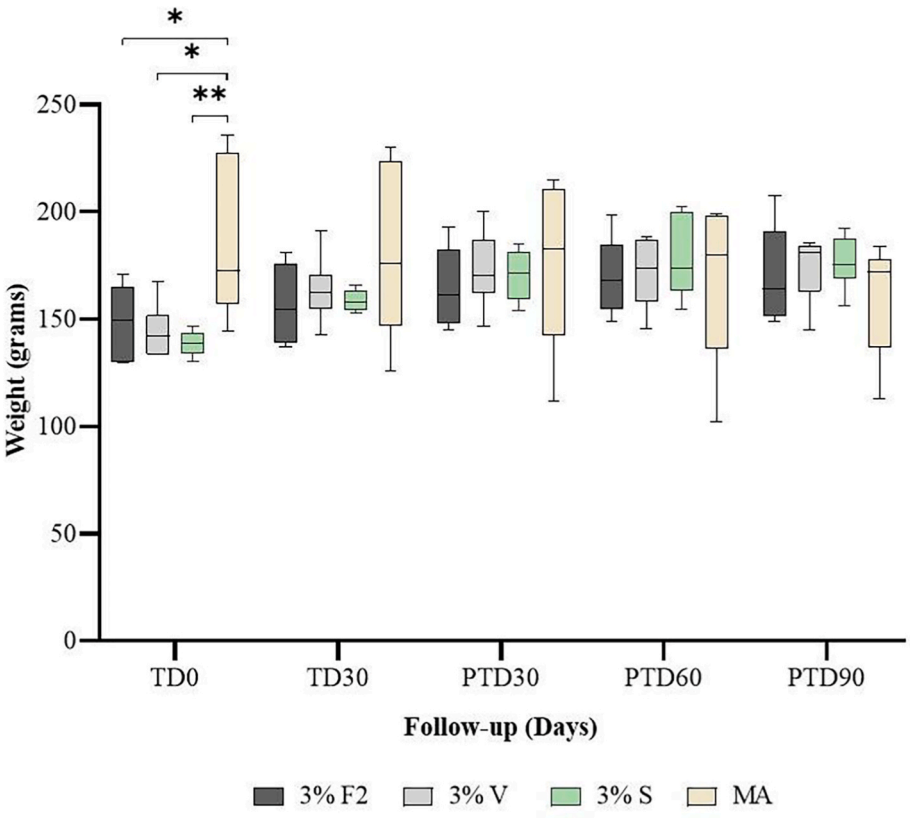

Fig. 2. Changes in body weight of hamsters with cutaneous leishmaniasis caused by *L. braziliensis* during and after treatment. Body weight (g) is shown before and after treatment with a 3% topical formulation of sativan (S), vestitol (V), and F2 fraction, compared with intralesional meglumine antimoniate (MA). The red dashed line represents the mean body weight in each treatment group. Time points: TD0, before treatment; TD30, last day of treatment; PTD15, day 15 post-treatment; PTD30, day 30 post-treatment; PTD45, day 45 post-treatment; PTD60, day 60 post-treatment; PTD90, day 90 post-treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Serum levels of ALT, BUN and creatinine in hamsters treated with sativan, vestitol and Fraction 2.

Compound	ALT (U/L)		BUN (mg/dL)		Creatinine (mg/dL)	
	TD0	TD8	TD0	TD8	TD0	TD8
S	53.0 ± 7.0	57.5 ± 8.8	17.1 ± 3.2	19.5 ± 3.7	0.38 ± 0.03	0.41 ± 0.06
V	46.5 ± 9.5	57.7 ± 4.1	16.8 ± 2.9	21.7 ± 3.9	0.43 ± 0.06	0.45 ± 0.04
F2	46.0 ± 7.5	54.0 ± 4.7	19.3 ± 3.7	18.0 ± 4.7	0.46 ± 0.04	0.48 ± 0.04
MA	53.0 ± 9.1	62.8 ± 6.6	22.5 ± 3.0	22.3 ± 5.4	0.48 ± 0.08	0.51 ± 0.03
Reference values*	45.0 ± 9.4	52.7 ± 2.6	23.9 ± 3.5	21.8 ± 8.3	0.36 ± 0.08	0.37 ± 0.08

Data represent the mean ± standard deviation of serum ALT, BUN, and creatinine levels measured before treatment (TD0) and on day eight of treatment (TD8). *Values are from healthy, uninfected, and untreated 8-week-old male and female hamsters.

no histopathological studies were performed.

Similar to that observed in hamsters, no alterations in ALT, BUN and creatinine were detected. All metabolites were in the normal ranges, as described: 20–90 U/L for ALT, 15–30 mg/dL for BUN and 0.2–0.8 mg/dL for creatinine (Washington and Van Hoosier, 2012) (Table 5).

4. Discussion

For more than 80 years, pentavalent antimony compounds have been considered the first-line treatment for leishmaniasis, despite their numerous disadvantages. These include the need for parenteral administration and side effects such as nausea, vomiting, muscle and abdominal pain, cardiac issues, increased hepatic aminotransferase levels, and chemical pancreatitis. Moreover, treatment adherence is negatively affected by the prolonged duration (often several weeks) and restricted availability due to access limitations. The other option, miltefosine (hexadecylphosphocholine), has potential teratogenicity that makes it contraindicated during pregnancy. Side effects associated with miltefosine include abdominal pain, facial and limb swelling, bloody or tarry stools, chills, fever, dizziness, itching or rash, and pinpointing red spots on the skin (PAHO, 2022).

Currently, only BNZ and nifurtimox are available for treating *T. cruzi* infections, and they have been in use for over 50 years. While effective for acute and recent infections and in preventing mother-to-child transmission, their efficacy diminishes in chronically infected individuals, particularly those over 18 years old. Additionally, both BNZ and nifurtimox are linked to frequent adverse events and high rates of treatment discontinuation, especially among adults (Pérez-Molina et al., 2021). Given this context, there is an urgent need for new treatment options for patients and healthcare providers.

In contrast, *T. chrysantha* is a source of bioactive molecules with various properties, including anti-inflammatory, antioxidant, antimicrobial, anti-diabetic, and antitumoral activities, as well as wound healing and immunomodulatory effects (Pérez et al., 2007, 2009;

Table 4
Clinical outcome in mice infected with *T. cruzi* after treatment with sativan, vestitol or fraction F2.

Product	Code[a] (sex)	Parasite load[b] (parasites/mL)					↓ parasite load PTD90 (%)[h]	Final outcome
		TD0[c]	TD25[d]	PTD30[e]	PTD60[f]	PTD90[g]		
S[i]	C1-OIx2 (♀)	1500	417	333	667	250	83.3	Improvement
	C1-AO (♀)	1750	0	0	167	0	100	Cure
	C2-AO (♀)	1583	250	833	0	0	100	Cure
	C2-SM (♀)	1500	333	750	500	417	73.7	Improvement
	C4-SM (♂)	1750	333	583	167	*[j]	*[j]	Failure
	C4-OD (♂)	1750	500	1000	917	*[j]	*[j]	Failure
V[k]	C2-OD (♀)	833	1000	1000	0	667	19.9	Improvement
	C2-OI (♀)	1750	250	1250	0	0	100	Cure
	C2-ODx2 (♀)	1500	667	667	0	0	100	Cure
	C2-OIx2 (♀)	1583	167	333	333	500	68.4	Improvement
	C5-OI (♂)	1750	667	167	0	167	90.5	Improvement
	C5-AO (♂)	1750	167	667	750	917	47.6	Improvement
F2[l]	19-OI (♀)	1500	1250	1417	667	83	94.5	Improvement
	19-AO (♀)	1583	1250	833	0	0	100	Cure
	19-SM- (♀)	2917	2417	250	250	0	100	Cure
	19- OIx2 (♂)	1750	1583	1083	2083	667	61.9	Improvement
	20-OD (♂)	2667	1667	1417	500	250	90.6	Improvement
	20-OI (♂)	2083	1250	1417	667	200	90.4	Improvement
BNZ[m]	C1-OIx2 (♀)	1000	0	0	0	0	100	Cure
	C1-AO (♀)	2000	0	0	0	0	100	Cure
	C1-OI (♀)	1750	0	0	0	0	100	Cure
	C3-ODx2 (♂)	1833	0	0	0	0	100	Cure
	C3-OI (♂)	1917	0	0	0	0	100	Cure
	C3-OIx2 (♂)	1667	0	0	0	0	100	Cure
C-[n]	1-♀	1000	2333	3167	SM	1000	1000	NA
	2-♀	917	2583	1917	1000	1250	917	NA[o]
	3-♀	1167	1500	1667	1667	833	1167	NA
	1-♂	1667	2083	2917	750	417	1667	NA
	2-♂	1333	1667	3333	833	667	1333	NA
	3-♂	1167	2500	3167	833	833	1167	NA

Data correspond to the clinical evolution of the infection by *T. cruzi* in BALB/c mice after treatment. [a]OIx2, AO, SM, OD, OI and ODx2, correspond to internal codes to label each mouse in each group; [b]amount of parasite in blood; [c]TD0: parasitemia before treatment; [d]TD25: parasitemia at last day of treatment; [e]PTD30: parasitemia at day 30 post-treatment; [f]PTD60: parasitemia at day 60 post-treatment; [g]PTD90: parasitemia at day 90 posttreatment; [h]percentage of decrease in the parasitemia at day 90 posttreatment respect to before treatment; [i]S: sativan; j*: end point of the study and sacrifice; [k]V: vestitol; [l]F2: fraction 2, which corresponds to the combination of vestitol and sativan as the major components in this fraction; [m]BNZ: benznidazol; [n]C-: negative control (infected and untreated mice). [o]NA: no applicable.

Garzón et al., 2008; Franco et al., 2013; Panda et al., 2019, 2020). However, its anti-parasitic activity has not been explored until now.

In this study, we report for the first time the presence of the isoflavonoids sativan and vestitol in the crude extract of *T. chrysantha* sawdust, along with their activity against *L. braziliensis* and *T. cruzi* through in vitro and *in vivo* studies.

The cytotoxicity evaluation of F2 and its isolated compounds (sativan and vestitol) was performed in U-937 macrophages and huRBC as a preliminary assessment. These cell models were chosen due to their relevance to the biological context of the study: U-937 macrophages as host cells for intracellular *parasites L. braziliensis* and *T. cruzi* and huRBC to evaluate potential hemolytic toxicity. While these results suggest a favorable toxicity profile in the tested systems, we recognize the need for broader evaluations to confirm safety. Future studies should include assessments in diverse cell lines (e.g., hepatocytes, fibroblasts, and epithelial cells) and additional toxicity evaluations in animal models to provide an understanding of the safety profile of F2 and its components. Furthermore, *in vivo* toxicity studies would provide critical insights into pharmacokinetics, biodistribution, and potential adverse effects, essential for advancing these compounds toward preclinical development.

While parasitemia often becomes undetectable between 15 and 45 days post-infection in specific models, this duration varies significantly depending on the specific *T. cruzi* strain and host factors. Our study utilized a strain with demonstrated virulence that ensures persistent infection in mice, maintaining detectable parasitemia up to 240 days post-infection (data not shown). This strain exhibits a pronounced tropism for intestinal tissue, notably sparing organs like the heart.

Confirming a parasitological cure involves demonstrating complete parasite elimination by day 90 post-treatment and ensuring no relapse under immunosuppressive conditions. We recognize that a stringent criterion beyond a mere 10% reduction in parasitemia is also essential for accurately assessing therapeutic efficacy. Nonetheless, it is important to recognize that our current study represents an initial phase in the drug development process for sativan and vestitol compounds.

Because the scope of our study was to identify the leishmanicidal and trypanocidal properties of Sativan and Vestitol, our findings offer initial insights into their antiparasitic potential. So, further research with rigorous evaluation criteria is essential to confirm their efficacy and safety as therapeutic agents.

Sativan has been isolated from *Spatholobus suberectus,* also inhibition of tumor development in triple negative breast cancer has been reported (Peng Fu et al., 2020), antituberculosis activity is reported for Sativan and Isovestitol from *Sesbania grandiflora* (Hasan N. et al., 2012). This research focuses on identifying new bioactive molecules and exploring known compounds' activities, justifying further scale-up, formulation, and eventual development of clinical trials for rapid drug development or phytotherapeutics.

We hypothesize that the leishmanicidal and trypanocidal activities of satan and vestitol could be related to the activation of M2-like macrophages, which play a crucial role in anti-inflammatory activity (Arabpour et al., 2021) and wound healing and tissue repair (Martinez and Gordon, 2014). These are two properties that are fundamental in the healing process of CL, in addition to the specific antiparasitic activity.

Sativan, vestitol, and other isoflavonoids are known to modulate macrophage activity by influencing the production of inflammatory mediators such as nitric oxide and cytokines, which play critical roles in the control of intracellular pathogens (Faruk et al., 2022; Bueno-Silva et al., 2020, 2022). Isoflavonoids can also modulate cellular processes

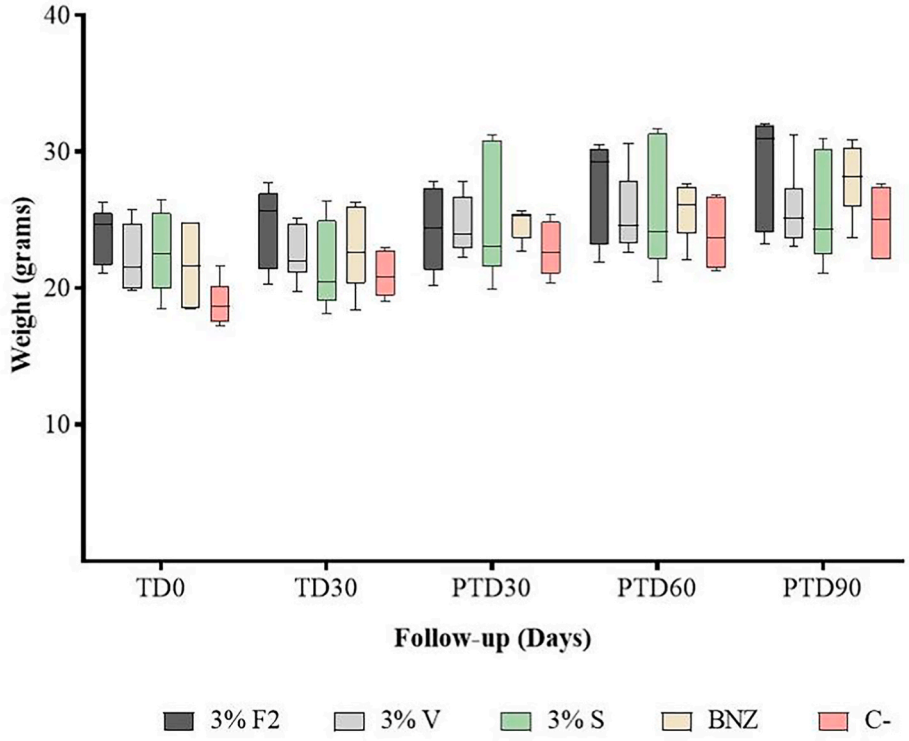

Fig. 3. Evolution of body weight in BALB/c mice infected with *T. cruzi* before and after treatment. Body weight (g) is shown for mice treated orally with 100 mg/kg/day of sativan (S), vestitol (V), and F2 fraction over 25 days, compared with oral benznidazole (BNZ). The red dashed line represents the mean body weight in each treatment group. Time points: TD0, before treatment; TD30, last day of treatment; PTD15, day 15 post-treatment; PTD30, day 30 post-treatment; PTD45, day 45 post-treatment; PTD60, day 60 post-treatment; PTD90, day 90 post-treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 5
Serum levels of ALT, BUN and creatinine in BALB/c mice treated with sativan, vestitol and Fraction 2.

Compound	ALT (U/L)		BUN (mg/dL)		Creatinine (mg/dL)	
	TD0	TD8	TD0	TD8	TD0	TD8
S	40.8 ± 6.9	44.7 ± 1.5	21.5 ± 4.5	27.3 ± 2.6	0.50 ± 0.09	0.50 ± 0.08
V	42.7 ± 6.8	47.2 ± 4.5	24.0 ± 2.3	27.8 ± 3.2	0.32 ± 0.09	0.43 ± 0.07
F2	47.0 ± 4.2	58.7 ± 8.7	21.7 ± 2.9	25.5 ± 2.9	0.43 ± 0.05	0.44 ± 0.07
MA	41.2 ± 2.3	51.2 ± 5.2	22.6 ± 1.5	30.1 ± 3.8	0.44 ± 0.02	0.46 ± 0.05
Reference values*	39.2 ± 1.6	42.8 ± 5.7	19.2 ± 1.9	23.7 ± 2.3	0.42 ± 0.06	0.39 ± 0.02

Data represent the mean ± standard deviation of serum ALT, BUN, and creatinine levels measured before treatment (TD0) and on day eight of treatment (TD8). *Values are from healthy, uninfected, and untreated 8-week-old male and female BALB/c mice.

such as collagen synthesis, keratinocyte proliferation, and fibroblast migration, which are crucial for wound repair (Zulkefli et al., 2023).

While these findings suggest a potential link between the anti-inflammatory and antiparasitic activities of sativan and vestitol, our study does not directly assess their impact on macrophage function or innate immune responses. Future studies are required to determine whether these compounds exert their effects through immunomodulatory mechanisms or direct antiparasitic activity.

The in vitro trypanocidal activity observed for sativan and vestitol highlights their potential as starting points for drug discovery against *T. cruzi*. However, the *in vivo* results did not show the same level of efficacy, which could be attributed to several factors, including bioavailability, pharmacokinetics, or the inability of the compounds to reach therapeutic concentrations at the target sites. It is also possible that the inflammatory and immune-modulating effects of the compounds, which are beneficial in the context of CL, may not directly translate to efficacy in treating *T. cruzi* infections.

These discrepancies underscore the need for further research to optimize the pharmacological properties of these compounds. Future studies should focus on evaluating the pharmacokinetics and biodistribution of sativan and vestitol in animal models and exploring combination therapies with existing trypanocidal drugs to enhance efficacy. Despite the modest *in vivo* results against *T. cruzi*, the findings are valuable as they provide a basis for refining and optimizing the activity of these compounds. This iterative process is critical in early-stage drug discovery and highlights the need for preclinical studies to bridge the gap between in vitro efficacy and *in vivo* application.

Modifying the therapeutic regimen, such as increasing the dose or extending the treatment duration, may enhance cure rates in future studies. Additionally, formulating the compounds with excipients may improve skin penetration (for CL) or oral absorption (for *T. cruzi* infection).

The activity of F2, which comprises various bioactive components including sativan and vestitol, as the majority constituents, suggests the potential for combined or complementary effects that may not be fully replicated by the individual compounds alone. Although the concentrations of sativan and vestitol in F2 differ from those used in the assays with the isolated compounds, the overall efficacy of F2 underscores the

value of exploring natural fractions as multi-target therapeutic scaffolds. These findings warrant further preclinical investigation to determine whether synergistic, additive, or independent mechanisms underlie the observed bioactivity. Such studies would also provide critical insights into dose optimization and formulation strategies, paving the way for eventual scale-up and translational development. Future studies could be directed to investigate potential synergistic or antagonistic interactions between sativan and vestitol in combination and explore pharmacokinetics and bioavailability differences between F2 and isolated compounds.

The comparable efficacy observed in the F2 and isolated metabolites, supports the use of standardized fractions over pure compounds derived from natural sources for therapeutic purposes. The purification process can be expensive, extensive, and yield limited quantities, while a multi-component fraction, such as that containing sativan and vestitol, is cheaper, faster, and produces better yields. Furthermore, the toxicity profile of the F2 fraction (which contains the majority of sativan and vestitol) showed no cytotoxicity in U-937 cells and huRBC compared to the pure molecules.

While our findings highlight the potential of isoflavans derived from *T. chrysantha* wood industry residues as promising candidates for developing new antiparasitic drugs, addressing the ethical and sustainability considerations associated with utilizing materials from threatened species is imperative to ensure that drug development does not exacerbate the depletion of threatened species. Adhering to ethical guidelines for field research on rare plant species (www.mass.gov) and compliance with international agreements like the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) 2016 (www.fs.usda.gov) is necessary to regulate the trade of endangered species and prevent their exploitation but also is essential to minimize harm to native populations. Lastly, to increase pharmaceutical sustainability, sustainable sourcing practices are necessary for responsibly obtaining natural compounds and seeking alternatives to non-renewable resources.

Additionally, using crude extracts or mixtures of these isoflavans instead of pure compounds could be a more accessible and cost-effective alternative for developing new drugs against neglected anti-parasitic diseases, particularly in developing countries.

While the results are promising, further research is necessary to validate these findings in clinical settings and assess these compounds' long-term safety and efficacy. Comprehensive pharmacological studies and clinical trials are essential to establish the therapeutic potential of sativan and vestitol as viable treatments for leishmaniasis and Chagas disease.

5. Conclusions

This study presents the first evidence of the anti-parasitic activity of the isoflavonoids sativan and vestitol, extracted from *T. chrysantha*, demonstrating their potential as novel therapeutic agents against *L. braziliensis* and *T. cruzi*. These findings underscore the importance of exploring natural products in the quest for effective treatments for neglected diseases. The F2 fraction, which contains these compounds, offers a cost-effective and efficient alternative to pure compounds, thereby supporting the use of standardized plant extracts in therapeutic applications.

Moreover, utilizing waste from the *T. chrysantha* wood industry contributes to sustainable practices by minimizing waste and creating new commercial opportunities. This approach aligns with the principles of the circular economy, transforming by-products into valuable bioactive compounds and promoting environmental sustainability while addressing public health challenges. The development of crude extracts or mixtures containing isoflavonoids provides an accessible and economically viable option for treating neglected anti-parasitic diseases, particularly in low-resource settings.

CRediT authorship contribution statement

Edwin Correa: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Sara M. Robledo:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Fernando Echeverri:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Wiston Quiñones:** Writing – review & editing, Funding acquisition. **Natalia Arbeláez:** Writing – original draft, Methodology, Data curation. **Javier Murillo:** Writing – original draft, Methodology, Data curation. **Tatiana Pineda:** Writing – original draft, Methodology, Data curation. **Fernando Torres:** Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Data availability statement

The data supporting this study are available from the corresponding authors upon reasonable request.

Funding

This work was supported by the Colombian Ministery of Science, Technology, and Innovation [Grant number 111571249866], the Universidad de Antioquia [grant numbers ES84230004 and ES84190049], and Corporación de Innovación para el Desarrollo de Productos – CIDEPRO.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

To technicians responsible for supervision and care of the hamsters and mice used in therapeutic response assays.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exppara.2025.108899.

Data availability

Data will be made available on request.

References

Arabpour, M., Saghazadeh, A., Rezaei, N., 2021. Anti-inflammatory and M2 macrophage polarization-promoting effect of mesenchymal stem cell-derived exosomes. Int. Immunopharm. 97, 1567. https://doi.org/10.1016/j.intimp.2021.107823.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., the International Natural Product Sciences Taskforce, Supuran, C.T., 2021. Natural products in drug discovery: advances and opportunities. Nat. Rev. Drug Discov. 20, 200–216. https://doi.org/10.1038/s41573-020-00114-z.

Brindha, J., Balamurali, M.M., Chanda, K., 2021. An overview on the therapeutics of neglected infectious diseases-leishmaniasis and Chagas diseases. Front. Chem. 9, 622286. https://doi.org/10.3389/fchem.2021.622286.

Buckner, F.S., Verlinde, C.L., La Flamme, A.C., Van Voorhis, W.C., 1996. An efficient technique for screening drugs for activity against *Trypanosoma cruzi* using parasites expressing beta-galactosidase. Antimicrob. Agents Chemother. 40, 2592–2597. https://doi.org/10.1128/AAC.40.11.2592.

Buckner, F.S., Wilson, A.J., White, T.C., Van Voorhis, W.C., 1998. Induction of resistance to azole drugs in *Trypanosoma cruzi*. Antimicrob. Agents Chemother. 42, 3245. https://doi.org/10.1128/AAC.42.12.3245.

Bueno-Silva, B., Rosalen, P.L., Alencar, S.M., Mayer, M.P.A., 2020. Vestitol drives LPS-activated macrophages into M2 phenotype through modulation of NF-κB pathway. Int. Immunopharm. 82, 106329. https://doi.org/10.1016/j.intimp.2020.106329.

Bueno-Silva, B., Bueno, M.R., Kawamoto, D., Casarin, R.C., Pingueiro, J.M.S., Alencar, S. M., Rosalen, P.L., Mayer, M.P.A., 2022. Anti-inflammatory effects of (3S)-Vestitol on peritoneal macrophages. Pharmaceuticals 15, 553. https://doi.org/10.3390/ph15050553.

Castro, J.A., deMecca, M.M., Bartel, L.C., 2006. Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis). Hum. Exp. Toxicol. 25, 471. https://doi.org/10.1191/0960327106het653oa.

Cheuka, P.M., Mayoka, G., Mutai, P., Chibale, K., 2016. The role of natural products in drug discovery and development against neglected tropical diseases. Molecules 22, 58. https://doi.org/10.3390/molecules22010058.

Croft, S.L., Sundar, S., Fairlamb, A.H., 2006. Drug resistance in leishmaniasis. Clin. Microbiol. Rev. 19, 111. https://doi.org/10.1128/CMR.19.1.111-126.2006.

da Silva, V.C., Rodrigues, C.M., 2014. Natural products: an extraordinary source of value-added compounds from diverse biomasses in Brazil. Chem Biol Technol Agric 1, 14. https://doi.org/10.1186/s40538-014-0014-0.

De Arias, A.R., Ferro, E.A., 1988. Quantification of *Trypanosoma cruzi* parasitaemia by direct micromethod. Trans. R. Soc. Trop. Med. Hyg. 82, 248.

De Rycker, M., Wyllie, S., Horn, D., Read, K.D., Gilbert, I.H., 2023. Anti-trypanosomatid drug discovery: progress and challenges. Nat. Rev. Microbiol. 21, 35. https://doi.org/10.1038/s41579-022-00777-y.

Falk, N., Berenstein, A.J., Moscatelli, G., Moroni, S., González, N., Ballering, G., Freilij, H., Altcheh, J., 2022. Effectiveness of nifurtimox in the treatment of Chagas disease: a long-term retrospective cohort study in children and adults. Antimicrob. Agents Chemother. 66, e02021–e02022.

Faruk, E.M., Fouad, H., Hasan, R.A.A., Taha, N.M., El-Shazly, A.M., 2022. Inhibition of gene expression and production of iNOS and TNF-α in experimental model of neurodegenerative disorders stimulated microglia by Soy nano-isoflavone/stem cell-exosomes. Tissue Cell 76, 101758. https://doi.org/10.1016/j.tice.2022.101758.

Franco Ospina, L.A., Castro Guerrero, J.P., Ocampo Buendía, Y.C., Pájaro Bolívar, I.B., Díaz Castillo, F., 2013. Anti-inflammatory, antioxidant and antibacterial activity of two species of *Tabebuia* genus. Rev. Cubana Plantas Med. 18, 34–46.

Gentry, A.H., 1992. A synopsis of bignoniaceae ethnobotany and economic botany. Ann. Mo. Bot. Gard. 79, 53–64. https://doi.org/10.2307/2399809.

Hasan, N., Osman, H., Mohamad, S., Chong, W.K., Awang, K., Zahariluddin, A.S.M., 2012. The chemical components of *Sesbania grandiflora* root and their antituberculosis activity. Pharmaceuticals 5, 882–889. https://doi.org/10.3390/ph5080882.

Hotez, P.J., Aksoy, S., Brindley, P.J., Kamhawi, S., 2020. What constitutes a neglected tropical disease? PLoS Neglected Trop. Dis. 14, e0008001. https://doi.org/10.1371/journal.pntd.0008001.

Insuasty, D., Robledo, S.M., Vélez, I.D., Cuervo, P., Insuasty, B., Quiroga, J., Nogueras, M., Cobo, J., Abonia, R., 2017. A Schmidt rearrangement-mediated synthesis of novel tetrahydro-benzo[1,4]diazepin-5-ones as potential anticancer and antiprotozoal agents. Eur. J. Med. Chem. 141, 567–583. https://doi.org/10.1016/j.ejmech.2017.10.024.

Luniwal, A., Erhardt, P., 2011. Total syntheses of (±)-Vestitol and bolusanthin III using a wittig strategy. Synlett 11, 1605–1607. https://doi.org/10.1055/s-0030-1260783.

Martinez, F.O., Gordon, S., 2014. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 6, 13. https://doi.org/10.12703/P6-13.

Moncada-Diaz, M.J., Rodríguez-Almonacid, C.C., Quiceno-Giraldo, E., Khuong, F.T.H., Muskus, C., Karamysheva, Z.N., 2024. Molecular mechanisms of drug resistance in *Leishmania* spp. Pathogens 13, 835. https://doi.org/10.3390/pathogens13100835.

Panda, S.P., Panigrahy, U.P., Panda, S., Jena, B.R., 2019. Stem extract of *Tabebuia chrysantha* induces apoptosis by targeting sEGFR in Ehrlich Ascites Carcinoma. J. Ethnopharmacol. 235, 219–226. https://doi.org/10.1016/j.jep.2019.02.023.

Panda, S.P., Panigrahy, U.P., Prasanth, D., Gorla, U.S., Guntupalli, C., Panda, D.P., Jena, B.R., 2020. A trimethoxy flavonoid isolated from stem extract of *Tabebuia chrysantha* suppresses angiogenesis in angiosarcoma. J. Pharm. Pharmacol. 72, 990–999. https://doi.org/10.1111/jphp.13272.

Percie du Sert, N., Hurst, V., Ahluwalia, A., et al., 2020. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. BMC Vet. Res. 16, 242–249. https://doi.org/10.1186/s12917-020-02451-y.

Pérez-Molina, J.A., Crespillo-Andújar, C., Bosch-Nicolau, P., Molina, I., 2021. Trypanocidal treatment of Chagas disease. Enferm. Infecc. Microbiol. Clín. 39, 458–470. https://doi.org/10.1016/j.eimce.2020.04.012.

Pérez, C.J.E., Isaza, M.G., Acosta, S.M., 2007. Actividad antibacteriana de extractos de *Phenax rugosus* y *Tabebuia chrysantha*. Bios 6, 59–68.

Pérez, C., Sepúlveda-Arias, J.C., Acosta Agudelo, S.M., Isaza, Mejía G., 2009. Effect of aqueous and methanolic extracts of Phenax rugosus (Poir.) Wedd. and Tabebuia chrysantha G. Nicholson, on the production of IgG2b and IgM antibodies in rats, a preliminary study. Bios 8, 37–46.

Pulido, S.A., Munoz, D.L., Restrepo, A.M., Mesa, C.V., Alzate, J.F., Vélez, I.D., Robledo, S. M., 2012. Improvement of the green fluorescent protein reporter system in Leishmania spp. for the in vitro and in vivo screening of antileishmanial drugs. Acta Trop. 122, 36–45. https://doi.org/10.1016/j.actatropica.2011.11.015.

Rathore, A.S., Kumar, D., Kateja, N., 2018. Role of raw materials in biopharmaceutical manufacturing: risk analysis and fingerprinting. Curr. Opin. Biotechnol. 53, 99–105. https://doi.org/10.1016/j.copbio.2017.12.022.

Rizvi, S.A.A., Einstein, G.P., Tulp, O.L., Sainvil, F., Branly, R., 2022. Introduction to traditional medicine and their role in prevention and treatment of emerging and Re-emerging diseases. Biomolecules 12, 1442. https://doi.org/10.3390/biom12101442.

Roat, B.M., de Oliveira Cardoso, J.M., De Brito, R.C.F., Coura-Vital, W., de Oliveira Aguiar-Soares, R.D., Reis, A.B., 2020. Recent advances and new strategies on leishmaniasis treatment. Appl. Microbiol. Biotechnol. 104, 8965–8977. https://doi.org/10.1007/s00253-020-10856-w.

Robledo, S.M., Carrillo, L.M., Daza, A., Restrepo, A.M., Muñoz, D.L., Tobón, J., Murillo, J.D., López, A., Ríos, C., Mesa, C.V., Upegui, Y.A., Valencia-Tobón, A., Mondragón-Shem, K., Rodríguez, B., Vélez, I.D., 2012. Cutaneous leishmaniasis in the dorsal skin of hamsters: a useful model for the screening of antileishmanial drugs. J. Vis. Exp. (62), 3533. https://doi.org/10.3791/3533.

Takashima, Y., Kaneko, Y., Kobayashi, Y., 2010. Synthetic access to optically active isoflavans by using allylic substitution. Tetrahedron 66, 97–207. https://doi.org/10.1016/j.tet.2009.10.116.

Torres-León, C., Ramírez-Guzman, N., Londoño-Hernandez, L., Martinez-Medina, G.A., Díaz-Herrera, R., Navarro-Macias, V., Alvarez-Pérez, O.B., Picazo, B., Villarreal-Vázquez, M., Ascacio-Valdes, J., Aguilar, C.N., 2018. Food waste and by-products: an opportunity to minimize malnutrition and hunger in developing countries. Front. Sustain. Food Syst. 2, 52. https://doi.org/10.3389/fsufs.2018.00052.

Upegui, Y.A., Echeverri, F., Quiñones, W., Torres, F., Nacher, M., Rivas, L.I., Dos Santos Meira, C., Gedamu, L., Escobar, G., Archbold, R., Vélez, I.D., Robledo, S.M., 2020. Mode of action of a formulation containing hydrazones and saponins against Leishmania spp. Role in mitochondria, proteases, and reinfection process. Int. J. Parasitol.: Drugs Drug Resist. 13, 94–106. https://doi.org/10.1016/j.ijpddr.2020.06.004.

Washington, I.M., Van Hoosier, G., 2012. Clinical biochemistry and hematology. The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents, pp. 57–116. https://doi.org/10.1016/B978-0-12-380920-9.00003-1.

World Health Organisation, 2010. Control of the Leishmaniasis: Report of a Meeting of the WHO Expert Committee on the Control of Leishmaniases, 949, pp. 22–26. http://apps.who.int/iris/bitstream/10665/44412/1/WHO_TRS_949_eng.pdf. (Accessed 28 January 2024).

World Health Organization, 2012. Global report for research on infectious diseases of poverty. https://www.who.int/tdr/capacity/global_report/en/. (Accessed 28 January 2024).

World Health Organization, 2020. Control of Neglected Tropical Diseases. WHO, Geneva. http://www.who.int/neglected_diseases/diseases/en. (Accessed 28 January 2024).

Zulkefli, N., Che Zahari, C.N.M., Sayuti, N.H., Kamarudin, A.A., Saad, N., Hamezah, H.S., Bunawan, H., Baharum, S.N., Mediani, A., Ahmed, Q.U., Ismail, A.F.H., Sarian, M.N., 2023. Flavonoids as potential wound-healing molecules: emphasis on pathways perspective. Int. J. Mol. Sci. 24, 4607. https://doi.org/10.3390/ijms24054607.