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Preliminary Study on the Chemical and Biological Properties of Propolis Extract from Stingless Bees from the Northern Region of Brazil

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Abstract: Natural products are historically regarded as the main sources of bioactive compounds to fight the most diverse diseases; among them, propolis deserves to be highlighted due to several biological activities, such as antioxidant, anti-inflammatory, healing, antibacterial and antileishmanial activity. In this study, the antioxidant and antipromastigote activities and the cytotoxicity of propolis of the *Scaptotrigona* aff. *postica* stingless bee were evaluated. Propolis extract was analyzed by infrared spectroscopy, thermogravimetry and spectrophotometry for total polyphenols and flavonoid contents. Antioxidant activity was assessed spectrophotometrically by the DPPH, ABTS and FRAP methods, while antipromastigote activity and cytotoxicity were assessed by the MTT assay. The infrared spectra showed the characteristic bands of both phenols and alcohols. The thermogravimetry study revealed two events and thermal stability around 370 °C, while differential scanning calorimetry showed two endothermic events and an exothermic event. Total polyphenol and flavonoid contents were 21.29 ± 0.003 mg/g and 8.17 ± 0.00 mg/g, respectively, and the antioxidant activities identified by the DPPH, ABTS and FRAP methods were 910.09 ± 0.0264 μM Trolox (IC_{50} 18.9 ± 0.01 μg/mL), 859.97 ± 0.0854 μM Trolox (IC_{50} 692 ± 0.01 μg/mL) and 1613.67 ± 0.2610 μM Trolox (IC_{50} 80 ± 0.1 μg/mL), respectively. The satisfactory antioxidant activity of propolis can be related to the high content of phenolic compounds, which adds value to this product and can contribute to the development of meliponiculture in the Brazilian state of Pará. The propolis extract caused a toxic effect on promastigote forms of *Leishmania amazonensis* at all concentrations tested, with an IC_{50} value of 1.50 μg/mL and a statistically significant difference compared to the negative control ($p < 0.001$). These results show that the propolis extract from *S. postica* bees may be a promising alternative for treatment against promastigote forms of *L. amazonensis*.

Keywords: propolis; total phenols; cytotoxicity; *Scaptotrigona*; antioxidant activity; antipromastigote activity

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1. Introduction

Stingless bees (meliponines), which are part of the Meliponinae subfamily (Apidae family), have different characteristics from stinging bees, such as colony size, indication biology, brood selection, production of queen bees, method of bee storage and bee recruitment instrument [1]. Meliponine females do not have a stinger or have it in an atrophied

form, as they have created different ways of protection, such as a strong bite [2,3]. It is reported that around 600 varieties have been identified and are widespread throughout tropical and subtropical areas [3], including South and Central America, southern North America, Africa, Southeast Asia and northern Oceania [1]. Around 200 of them, belonging to 29 genera, are distributed throughout Brazil, with 89 endemic species, which represents around 20% of neotropical stingless bees [2,3].

The genera *Plebeia*, *Trigona*, *Melipona*, *Scaptotrigona* and *Trigonisca* contain a wide variety of popular species [3]. The genus *Scaptotrigona*, composed of 24 species commonly known as canudo bees, mandaguari, tubi and tubiba, is widespread throughout the Neotropical region and includes species that build their nests in pre-determined locations [4]. The species *Scaptotrigona affinis postica* Latreille (*S. postica*), popular as canudo and tucanaíra, is geographically located in Bolivia, Brazil and Peru [4].

S. postica is a stingless bee commonly found in the Amazon, which has broad potential for making honey and is used to pollinate native crop species [5], due to the visit to a large number of botanical species, tolerance to management, and ability to reproduce on a large scale [6–8]. Stingless bees produce geopropolis by adding plant resin to wax and soil [9,10]; however, *S. postica* does not use soil to produce propolis [11].

Stingless bee propolis is a substance made up of oils and resins collected by bees in different parts of plants, from exudates from cuts or buds and flower buds, which are mixed with wax they produce in their oral apparatus [12,13]. This substance is used in nest structures (inlet tubes, casing), in gap seals, and in small portions to protect against intruders [7]. Propolis is an apicultural product largely applied in traditional medicine since ancient times to carry out asepsis and to heal wounds [5,14,15]. More recently, thanks to its biological activities related to its complex chemical composition [16], it has been considered one of the most promising products in the chemoprevention and treatment of various diseases, especially cancer [17].

Some studies have demonstrated that the presence of compounds such as flavonoids, aromatic acids and their esters, terpenoids and carotenoids, among others [18–20], is related to biological properties such as the antioxidant, antimicrobial and antileishmanial activities found in propolis [21–24]. In this sense, American Cutaneous Leishmaniasis (ACL) is a polymorphic, spectral disease of the skin and mucous membranes, which is divided into several clinical forms, such as the cutaneous, mucosal cutaneous and diffuse cutaneous ones [24]. Its therapy still faces challenges due to low patient adherence to the first-choice medications used for its treatment, which are responsible for numerous adverse reactions [24]. As a way to minimize this problem, natural products can be used, including propolis.

Propolis is traditionally used in wound healing and as an antiseptic [17]. It is important to highlight that, despite the biological properties of propolis, contact with it can cause anaphylaxis and local irritations, due to the allergic effect that occurs not because of the intrinsic nature of propolis but rather because of the vulnerability of the individual [14]. A study carried out on the hydroethanolic extract of *S. postica* propolis indicated that it may be important in controlling tumor growth in models of experimental solid tumors [17]. The effects of propolis from *Scaptotrigona* sp. were studied in human brain tumor (glioblastoma) cells for cell proliferation, clonogenic capacity and cell death, and the extract was observed to inhibit proliferation in glioblastoma and fibroblast cell lines [25].

Studies have shown the potential of propolis to combat electron transfer mediating molecules in the skin, which makes its use safe in the treatment of burns. This natural product acts speeding up the tissue repair of burned skin and leads to the remodeling of damaged tissue due to the ability of flavonoids to reduce lipid peroxidation and prevent cell death [26,27]. Polyphenols act as antioxidants through a complex mechanism, preventing the formation of reactive oxygen species (ROS), chelating metal ions involved in both the production and elimination of ROS, affecting the reactions that induce lipid peroxidation and collaborating synergistically with other antiradical compounds [28].

Research has revealed that stingless bee propolis has great potential in preventing harmful changes in cellular metabolism due to its antiradical action and inflammatory processes, in addition to having immunomodulatory potential [18,29]. Stingless bee propolis has an antioxidant effect and acts by directly scavenging free radicals and inhibiting lipid peroxidation [30–32]. Lipid peroxidation, due to the attack of ROS on cell lipids, promotes the generation of carcinogenic compounds in the body and causes insertions, removals and substitutions of nucleotides [33].

Therefore, studies on quality assurance of extracts from natural sources by physicochemical characterization techniques become necessary to ensure product effectiveness, quality and safety. There are several methods used for this purpose, such as thermogravimetric analysis (TG/DTG), differential scanning calorimetry (DSC), Fourier-transform infrared (FT-IR) spectroscopy, and ultraviolet–visible (UV-Vis) spectrophotometry.

In search of new bioproducts focused on the cosmetic and pharmaceutical sectors, studies are required on extracts from bee products such as propolis, which has a complex chemical composition and several biological properties. Most research to date has focused on propolis produced by *Apis mellifera* and *Melipona scutellaris*, while only a few studies were carried out on that produced by the stingless bee *S. postica*. So, research efforts to assess its physicochemical profile by TG/DTG and DSC as well as its antioxidant potential and biological activities are expected to expand the possibility of obtaining new bioproducts to be exploited in the most diverse pharmaceutical areas.

From this perspective, in this study, the propolis extract of *S. postica* was characterized physicochemically and evaluated for its antioxidant potential and in vitro antipromastigote activity, seeking its possible use as a leishmanicidal agent.

2. Materials and Methods

2.1. Propolis Samples

The *S. postica* propolis was provided by Embrapa Eastern Amazon. The material was collected in Igarapé Açu, a city of the Brazilian Pará state, in an irrigated area of açaí (*Euterpe oleracea*) monoculture (1°01′46″ S 47°35′03″ W). The samples (two samples), taken in a commercial apiary, were divided into small portions, packed in plastic bags, and kept frozen (−18 °C) for 24 h before the extract preparation.

2.2. Reagents and Standards

L-ascorbic acid, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6)-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazoline bromide (MTT) and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma-Aldrich (St. Louis, MO, USA), while potassium persulfate, Folin–Ciocalteu reagent, sodium carbonate, aluminum chloride, potassium bromide, sodium acetate trihydrate and ferric chloride hexahydrate were acquired from LabSynth (São Paulo, Brazil).

2.3. Extract Preparation and Physicochemical Characterization

2.3.1. Propolis Extract Preparation

Extraction was carried out using the maceration method using 100 g of crushed propolis and 1 L of 70% ethanolic solution in a closed amber bottle placed in the dark at room temperature (25 °C) for 7 days. The obtained tincture was vacuum-filtered and placed in a rotary evaporator (Buchi R-210, Geneva, Switzerland) at 40 °C. The vacuum was controlled for a period of 3 h until ethanol was completely removed, thus obtaining the concentrated extract, which was kept in an amber bottle under refrigeration. Then, the extract was dried by lyophilization for later analyses [34].

2.3.2. Fourier-Transform Infrared Spectroscopy

The Fourier-Transform infrared (FTIR) spectroscopy spectra of the extract were obtained in a spectrometerIR Prestige-21 (Shimadzu®, Kyoto, Japan). For this purpose, 1 mg

of the lyophilized extract was mixed in a smooth agate mortar with 0.99 mg of dry and sprayed potassium bromide (KBr). The mixture was then pressed in special molds, under pressure of around 70,000–100,000 kPa, until the formation of a transparent tablet. The spectra were analyzed in the wavenumber range from 4000 to 400 cm^{-1}, with 32 scans and a resolution of 4 cm^{-1} [35,36].

2.3.3. Thermogravimetric Behavior

Thermogravimetric curves (TGs) of extract samples (3 mg) were recorded in a TGA-50 thermal analyzer (Shimadzu®) using an aluminum crucible under a nitrogen atmosphere (flowrate of 50 mL/min), temperature of 600 °C and heating rate of 10 °C/min. The TA-50W software, version 2.21 (Shimadzu®) was used to investigate the results of thermogravimetric analysis [37].

2.3.4. Thermal Behavior by Differential Scanning Calorimetry

Propolis extract differential scanning calorimetry (DSC) curve was recorded using DSC-60 plus equipment (Shimadzu®). Samples (3 mg) were deposited in an aluminum crucible and analyzed under a nitrogen atmosphere (flowrate of 50 mL/min) at a heating rate of 10 °C/min and temperature of 350 °C [37].

2.3.5. Total Polyphenols

The content of total polyphenols was quantified in a UV 1800 spectrophotometer (Shimadzu®) using a standard curve of gallic acid at different concentrations. The extract was prepared by weighing 20 mg of the lyophilized in a beaker and solubilizing it in 10 mL of distilled water contained in a volumetric balloon, in order to obtain the final concentration of 2 mg/mL. The extract was then placed in an ultrasonic bath (Cleaner Kondentech® CD-4820, São Paulo, Brazil) for 1 min to complete homogenization. Quantification was carried out on a mixture of 100 µL of extract, 500 µL of Folin–Ciocalteu reagent, 6 mL of distilled water and 2 mL of 20% (*w*/*v*) sodium carbonate solution. After 2 h of reaction, readings were obtained in the spectrophotometer at 760 nm. Gallic acid standard curve was obtained under the same conditions using 100 µL of solutions at different concentrations (5 to 75 mg/mL) [38]. The concentration of total polyphenols was calculated based on the straight-line equation $y = 0.0106\,x + 0.094$ ($R^2 = 0.9927$) and expressed in mg/mL.

2.3.6. Total Flavonoids

Total flavonoid determination was performed in the same spectrophotometer as above at a wavelength of 425 nm, using a standard curve of quercetin at different concentrations. Flavonoids were quantified on a mixture of 800 µL of the extract and 1 mL of 2.5% (*w*/*v*) aluminum chloride solution. After 30 min of reaction, readings were made in the spectrophotometer at 425 nm. Quercetin standard curve was obtained under the same conditions using 800 µL of solutions at different concentrations (5 to 30 mg/mL) [38]. The concentration of total flavonoids was calculated based on the straight-line equation $y = 0.0258\,x + 0.0838$ ($R^2 = 0.9905$) and expressed in mg/mL.

2.4. Antioxidant Activity

2.4.1. ABTS Radical Cation Scavenging Assay

Antioxidant activity by capture of $ABTS^{\bullet+}$ was performed in the same spectrophotometer. After 16 h from the preparation of the radical cation solution from the 7 mM ABTS stock solution and the 140 mM potassium persulfate solution, an aliquot of 30 µL of the diluted extract and 3000 µL of the $ABTS^{\bullet+}$ solution was vortexed in a dark environment for 6 min, and readings were then taken at a wavelength of 734 nm. Antioxidant capacity was measured using a Trolox standard curve (100–2000 µM) described by the straight-line equation $y = -0.0003\,x + 0.7131$ ($R^2 = 0.9988$). The percentage of $ABTS^{\bullet+}$ inhibition was calculated according to Equation (1) [39], using the initial absorbance of the radical cation

solution as a control. The half maximal inhibitory concentration (IC_{50}) was calculated after stabilization of the absorbance read every 6 min [39].

$$\%\ \text{inhibition} = \frac{(\text{Absorbanceofthecontrol} - \text{Absorbanceofthesample})}{\text{Absorbanceofthecontrol}} \times 100 \quad (1)$$

2.4.2. DPPH Radical Scavenging Assay

Antioxidant activity by capture of the DPPH• radical was performed in the same spectrophotometer. After preparing the DPPH• solution, a 150 µL aliquot of the diluted extract was mixed with 5850 µL of the DPPH• solution, and the mixture was vortexed. After 30 min of reaction, readings were then taken at a wavelength of 515 nm. Antioxidant capacity was measured using a Trolox standard curve (50–1000 µM) described by the straight line equation $y = -0.0005\ x + 0.6516$ ($R^2 = 0.9984$). The inhibition percentage was calculated according to Equation (1) [40], using the initial absorbance of the radical solution as a control. IC_{50} was calculated after stabilization of the absorbance read every 30 min.

2.4.3. FRAP Assay

Antioxidant activity by the FRAP assay was determined in the same spectrophotometer at a wavelength of 595 nm. After preparing the FRAP reagent, aliquots of 90 µL of the diluted extract were mixed with 270 µL of distilled water and 2700 µL of the FRAP reagent, vortexed for 1 min and kept in a water bath at 37 °C for 30 min. After the necessary time, readings were carried out at a wavelength of 595 nm, using the FRAP reagent to calibrate the spectrophotometer. Antioxidant capacity was measured using a standard Trolox curve (160–1600 µM) described by the straight-line equation $y = 0.0009\ x - 0.1088$ ($R^2 = 0.9948$), using the initial absorbance of the FRAP reagent solution as a control. The IC_{50} value was also calculated similarly to the previous two sections [41].

2.5. In Vitro Cytotoxicity Assay

2.5.1. Cell Culture

The kidney epithelial fibroblast cell line from the African green monkey (Vero) was kindly provided by the Structural Biology and Parasitology Laboratory of the Federal University of Pará (UFPA). Initially, the cells were placed in a humidified atmosphere with CO_2 (5%) in an oven at a temperature of 37 °C in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). After replacing the media until the formation of a cell monolayer, cells were washed with trypsinized phosphate-buffered saline (PBS), centrifuged at 1200 rpm for 10 min and counted in a Neubauer chamber. Then, in order to stabilize them, cells were divided into 96-well plates at a concentration of 1×10^4 cells/mL of DMEM and incubated in an atmosphere of CO_2 (5%) and air (95%) for 24 h [42].

2.5.2. Analysis of Cell Viability

Cell viability analysis was performed using the MTT method. To this end, Vero cells at a concentration of 1×10^4 cells/mL were cultivated in microplates and treated with propolis extract at different concentrations (2.5, 5, 10, 20, 50, 100, 250 and 500 µg/mL), in an incubator at 37 °C under 5% CO_2 atmosphere for 24 h. After this period, the supernatant was removed, PBS was used to wash the microplate, MTT diluted in PBS (0.5 mg/mL) was added, and cells were incubated for 3 h in an oven at 37 °C under 5% CO_2 atmosphere. The supernatant was then removed, and the microplate washed again with PBS. The solution was placed on another microplate, and readings were performed at a wavelength of 570 nm on a microplate reader (model 450, Bio-Rad Laboratories, Richmond, CA, USA). A suspension of dead cells in 15% formaldehyde was used as a control [42].

2.6. In Vitro Antipromastigote Activity Assay

2.6.1. Obtaining and Cultivation of the Parasite

The promastigotes of *Leishmania amazonensis* (strain MHOM/BR/26361), kindly provided in Novy–Nicolle–McNeal (NNN) medium by the Evandro Chagas Institute Leishmaniasis Program, Belém/Pará, were cultivated at 24 °C in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with FBS (10%) within a Biological Oxygen Demand incubator. To perform the experiments, the strain was maintained by six weekly transfers, and promastigotes in growth stage (4th day) were used. After centrifugation of promastigote forms for 10 min at 2500 rpm, the cell precipitate was suspended in 1 mL of RPMI medium. After counting in a Neubauer chamber, 1×10^6 parasites/mL were collected and placed in the wells of 24-well culture plates for 72 h incubation at different extract concentrations [43].

2.6.2. Viability According to the MTT Method

The MTT technique was also used to evaluate the mitochondrial viability of promastigote forms of *L. amazonensis* treated with the extract. For this purpose, parasite promastigotes in the exponential phase were incubated at 25 °C for 24 h together with the extract at different concentrations of (2.5, 5, 10, 20, 50, 100, 250 and 500 µg/mL). Aliquots (200 µL) of the protozoan suspensions were then transferred to the microplate, treated with aliquots (20 µL) of MTT (2 mg/mL) and then incubated for 4 h. After incubation, 20 µL of DMSO per well was added to solubilize the crystals of formazan, and the plate was stirred for 30 min. The color intensity of the final solution was quantified using the microplate reader mentioned above under the same conditions. Cultures of *L. amazonensis* promastigotes not treated with extract were used as the experimental control of cell viability, while a 0.5 µg/mL amphotericin B solution was used as the experimental positive control [43]. After experiments, the data were analyzed to determine IC_{50}.

2.7. Statistical Analysis

The analysis of variance (ANOVA) and Tukey's post hoc test were used to verify the level of significance ($p < 0.05$) of the data obtained in the assessment of cell viability and antipromastigote activity using the Graphpad 5.0 software package.

3. Results and Discussion

3.1. Physicochemical Characterization of Propolis

3.1.1. FTIR Spectrum

The FTIR spectra of propolis extract are shown in Figure 1. The propolis extract showed an absorption band at 3296 cm^{-1} referring to the symmetrical axial deformation vibration of hydrogen bonding in primary amines, an absorption signal at 2927 cm^{-1} due to the C-H axial deformation oscillation in alkanes, the symmetrical angular strain in the N-H plane in amines close to 1600 cm^{-1}, a band at 1416 cm^{-1} ascribable to symmetrical angular deformation in the plane of the terminal methylene group characteristic of the vinyl group absorption, the axial deformation of C-H in alkanes at 1372 cm^{-1}, C-N axial strain vibrations at 1293 cm^{-1} ascribable to secondary aromatic amines, the absorption signal at 1022 cm^{-1} due to C-O-C symmetric axial deformation in ethers (alkyl–aryl–ethers), the out-of-plane angular deformation of C-H in vinyl at 889 cm^{-1} and the C-H angular deformation at 771 cm^{-1} [44].

In short, the spectra of propolis extract highlighted a set of absorption bands referring to groups of important classes such as phenols, esters, aldehydes, ketones and carboxylic acids present in flavonoids, isoflavones and waxes, among others [35,44].

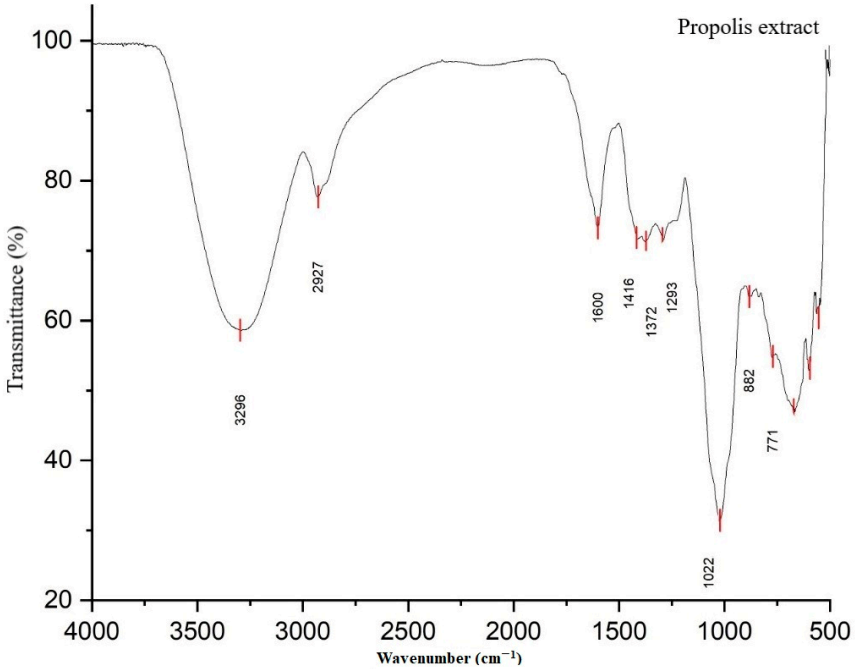

Figure 1. FTIR spectra of propolis extract in the wavenumber range from 4000 to 600 cm^{-1}, with 32 scans and 4 cm^{-1} resolution.

3.1.2. Thermal Behavior by Thermogravimetry

The TG/DTG curves up to 600 °C of propolis extract are shown in Figure 2. A single mass loss of 30.77% was observed in the temperature range of 143–500 °C due to successive reactions of degradation of organic compounds [45–47] including sugars [48].

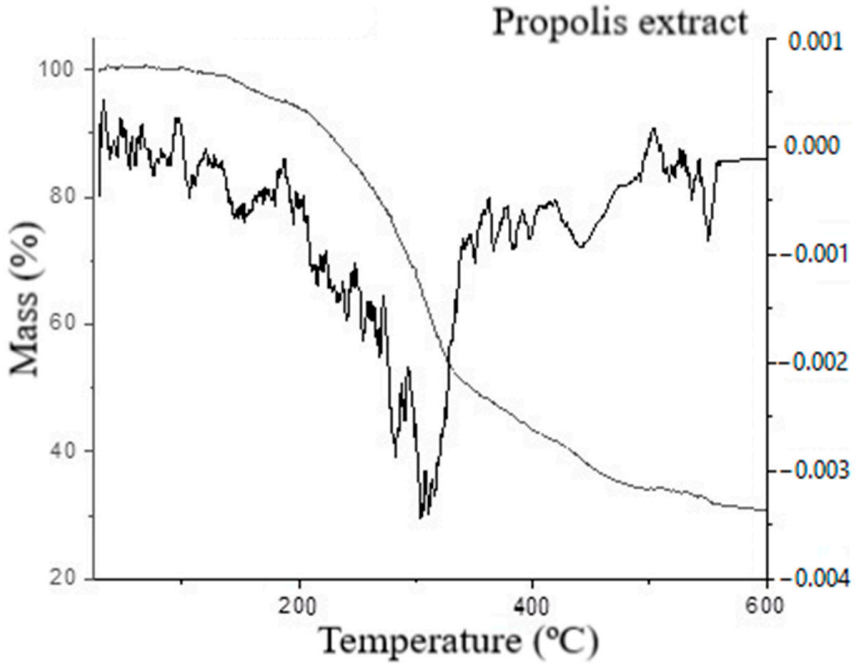

Figure 2. TG/DTG curves of propolis extract. Conditions: nitrogen atmosphere, flowrate of 50 mL/min, heating rate of 10 °C/min, temperature of 600 °C.

The study of the thermal stability of the natural product used as raw material is necessary to guarantee the safety and effectiveness of the herbal medicine obtained.

Thermogravimetry is not a selective technique, so this event may have resulted from the simultaneous decomposition of various substances present in the propolis extract. In the DTG curve, it is possible to identify the existence of various simultaneous and overlapping mass loss events, without a clear definition of their beginning and end, whose complexity is typical of plant extracts. However, it can be assumed that low-molecular-weight substances can evaporate at low temperatures, while more complex substances can evaporate or even decompose at higher temperatures; for example, aromatic phenolic compounds may decompose slowly due to their rigid chemical structures [49,50]. Although the TG/DTG curve of the propolis extract did not give clear insights, the events observed in this work occurred in a temperature range close to the one reported for red propolis (115–620 °C) [49–51].

3.1.3. Thermal Behavior Shown by Differential Scanning Calorimetry

The DSC curves of propolis extract are shown in Figure 3. The extract showed two endothermic events: one in the temperature range 31.67–116.91 °C (ΔH = 249.19 J/g) and the other in the range 160.53–170.59 °C (ΔH = 3.58 J/g), both due to thermal decomposition of constituents [35], and an exothermic one between 293.13 and 297.29 °C (ΔH = 1.51 J/g) likely due to material degradation [37]. The peak temperature of the extract was between 295.7 and 300.0 °C.

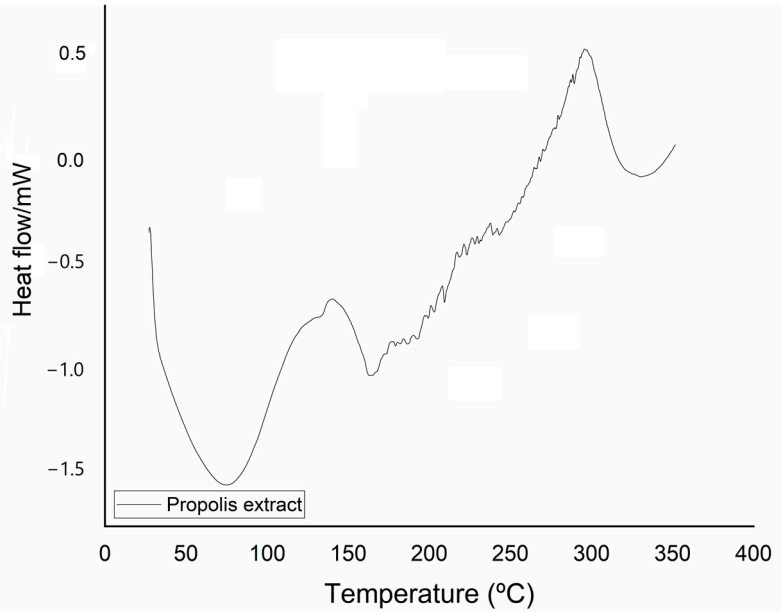

Figure 3. DSC curve of propolis extract. Conditions: nitrogen atmosphere, flowrate of 50 mL/min, heating rate of 10 °C/min, temperature of 350 °C.

DSC study showed extract stability around 300 °C, consistent with the results of thermogravimetry.

3.2. Total Polyphenols and Total Flavonoids

Total phenolic compounds in propolis can be quantified using a simple and quick tool such as UV-Vis spectroscopy [52,53]. Phenolics are compounds found in a wide range of plant species, which are considered the main group with potential to combat free radicals [54] and to exert a large number of therapeutic properties [54,55]. Research has revealed that flavonoids are bioactive components abundant in the propolis of meliponines living in temperate and tropical areas [20,29]. The contents of polyphenols and flavonoids

found in propolis extract are within the limits established in current legislation for these compounds in propolis and its derivatives (Table 1).

Table 1. Contents of phenolic compounds found in *S. postica* propolis extract in this study and results reported in the literature for propolis from other species.

Species	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)
Scaptotrigona postica	21.29 ± 0.003	8.17 ± 0.01
Tetrigona apicalis [56]	28.57	____
Heterotrigona itama [56]	34.17	____
Geniotrigona thoracica [56]	55.16	____
Melipona quadrifasciata [57]	84.4–189.5	0.74–0.79
Tetragonisca angustula [57]	34.9–42.0	0.82–0.93
Melipona quadrifasciata quadrifasciata [58]	3.87	0.14
Tetragonisca angustula [58]	1.26	0.15

Results of tests performed in triplicate are reported as mean and standard deviation. GAE = gallic acid equivalent, QE = quercetin equivalent.

It is hard to compare these values with those reported in the literature not only due to the paucity of studies on *S. postica* propolis but also because the chemical constituents of propolis vary in concentration depending on the botanical origin of the resin; the phytogeography; the region climate [56–61]; the geographic location, that is, the visits of bees to different vegetation; and even the type of soil collected for propolis production [62,63]. Another important factor is the range of bee action. For instance, *S. postica* bumblebees [64] have a flight radius of just over 600 m, being the only report for male meliponines [65], while Araújo et al. [66] estimated a maximum flight distance for medium-sized individuals of the same species to be in the 1159–1710 m range.

Nonetheless, there are only a few reports on the chemical characterization of *S. postica* propolis. Isidorov et al. [67] identified, using GC-MS in an Argentinian propolis extract of this species, diterpenes, abietic and dehydroabietic phenolic acids and pentacyclic triterpenes. Some studies have revealed the substances contained in propolis from several other species by different methods (Table 2).

Table 2. Chemical components reported in the literature for propolis from different meliponines.

Species	Chemical Components
Scaptotrigona mexicana [68]	Phenolic lipids, anacardic and phenolic acids, terpenes, lignans
Melipona orbignyi [69]	Benzoic acids, phenolic acid, dihydrocinnamic acids and Cinnamic acids
Tetragonula sapiens [70]	Mangiferonic and ambolic acids, cycloartenol
Chilean propolis [71]	Galangin, quercetin, apigenin, caffeic acid phenethyl ester
Lithuanian propolis [72]	*p*-Cumaric, ferulic, caffeic and vanillic acids, apigenin, galangin, vanillin
Tetrigona melanoleuca [73]	3-O-acetylursolic acid, dipterocarpol, ocotillones I and II, oleanolic aldehydes
Tetragonula laeviceps [73]	α-Mangostin, 8-desoxigartanin, gartanin, garcinone B, methylpinoresinol

In this study, it was only possible to determine the phenolic compounds of the *S. postica* propolis extract by UV–Vis spectrophotometry. However, a more detailed study of its chemical composition is in progress, and the complete data will be presented in a new publication.

In general, the content of phenolic compounds found in the propolis extract investigated in this study is consistent with both the FTIR and thermogravimetry profiles discussed in the previous two sections and may be relevant in view of the antioxidant and antipromastigote activities demonstrated in this study.

3.3. Antioxidant Activity

The values of antioxidant activities of the propolis extract determined by the DPPH, ABTS and FRAP methods are listed in Table 3 and compared with those reported in the literature for both ascorbic acid taken as a standard and other propolis samples. The antioxidant activity was expressed either as concentration (μM Trolox equivalent/g) or as percentage of radical inhibition (%) by the DPPH and ABTS methods, while only the concentration (μM Trolox equivalent/g) was used for the FRAP method.

Table 3. Antioxidant activities of *S. postica* propolis extract and ascorbic acid (standard) assessed in this study, as well as of different propolis extracts reported in the literature.

	DPPH•		ABTS•+		FRAP
	μM TE/g	**Inhibition %**	**μM TE/g**	**Inhibition %**	**μM TE/g**
Scaptotrigona postica (Brazil, this study)	910.09 ± 0.0264	63.95	859.97 ± 0.0854	34.43	1613.67 ± 0.2610
Ascorbic acid	1132.68 ± 0.005	85.54	2380.04 ± 0.0000	91.31	2408.57 ± 0.058
Canadian propolis [73]	_____	64.0–93.7	_____		_____
Tetragonisca angustula (Brazil) [74]	_____	19.6–30.5	_____		_____
Scaptotrigona mexicana [68]	_____	1.8–17.7	_____		_____
Scaptotrigona depilis (Brazil) [32]	_____	14.9	_____	73.4	_____
Melipona quadrifasciata anthidioides (Brazil) [32]	_____	97.5	_____	99.3	_____
Melipona orbignyi (Brazil) [75]	_____	96	_____		_____
Tetragonisca fiebrigi (Brazil) [31]	_____		_____	86.5	_____

Results of tests performed in triplicate are expressed as mean and standard deviation. TE = Trolox equivalent.

It can be seen that the results referring to the extract and the ascorbic acid obtained using the DPPH and FRAP assays are not very different, which confirms the antioxidant potential of the extract. On the other hand, the wide variation in the antioxidant activity of propolis from different origins can be attributed, similarly to what was observed for the contents of bioactive compounds, to several factors related to seasonality [76].

The food industry uses synthetic antioxidants for food preservation, but their use raises concerns about safe doses and toxicity; therefore, the interest of researchers in the search for natural substitutes has increased [74]. Secondary metabolites, including flavonoids, are the compounds mainly responsible for the antiradical power of plant extracts and bee products [74]. Antioxidants are compounds that, in small quantity compared to the oxidizable substrate, delay the start or prevent the onset or propagation of the oxidation reaction chain, in addition to inhibiting lipid peroxidation as well as the oxidation of other macromolecules and DNA [52]. Therefore, they prevent the gap between the formation and the deactivation of ROS, which triggers several changes in the body [28].

In this study, the IC_{50} of propolis extract and ascorbic acid standard were also evaluated using the three assays selected to determine the antioxidant activity (Table 4). As expected, the IC_{50} value of the extract was higher than that of the standard. It is noteworthy that, usually, the lower the IC_{50}, the greater the antioxidant potential [60]. The IC_{50} value identified by the FRAP assay is worth highlighting, being close to that of the selected antioxidant standard. This suggests that the antioxidant substances present in the extract have a greater capacity to reduce iron. The IC_{50} values of the extract by the DPPH and ABTS assays were more than twice that observed for ascorbic acid. Based on the IC_{50} values, the highest antioxidant activity was detected using the FRAP method, which corroborates the result of the evaluation of concentration (μM Trolox equivalent/g). Therefore, the IC_{50}

values together with those of the concentration confirm the antioxidant potential of the propolis extract assessed by the three different methods.

Table 4. Values of IC_{50} of the *S. postica* propolis extract and ascorbic acid (standard) assessed in this study, as well as of different propolis extracts reported in the literature.

	IC_{50} (µg/mL)		
	DPPH•	**ABTS•+**	**FRAP**
Scaptotrigona postica (Brazil, this study)	18.9 ± 0.01	692 ± 0.01	80 ± 0.1
Ascorbic acid	6.80 ± 0.02	310 ± 0.02	60 ± 0.2
Mandaçaia [77]	11.05 ± 0.6	_____	_____
Melipona quadrifasciata [57]	117.5	_____	
Heterotrigona itama [78]	630.31 ± 0.76	321.58 ± 3.67	_____
Tunisian propolis extract [79]	20.1–43	244–616	375–780
Melipona fasciculata (Viana, Brazil) [9]	76.16 ± 1.05	13.28 ± 0.11	_____
Melipona fasciculata (Pinheiros, Brazil) [9]	265.91 ± 0.29	58.94 ± 0.09	_____

Resuming, the propolis extract showed antioxidant potential and IC_{50} values that confirm its antiradical power with the three methods evaluated, as the likely result of its high phenolic compound content. They are within the range reported in the literature for other propolis extracts and, according to Al Naggar et al. [73], may be exploited to stop the emergence of diseases related to oxidative stress. In addition, these promising results suggest its use in the wound-healing process due to the benefits offered by antioxidants in fighting free radicals.

3.4. Cell Viability Test

The preliminary test to assess the toxicity of any material intended to be used for the manufacture of biomedical devices is in vitro cytotoxicity. Once its non-toxicity is proven, the study of its biocompatibility follows [50].

Propolis extract cytotoxicity was evaluated in fibroblast cells from monkey (Vero) by the MTT method. This cell line was chosen because it proved successful in previous cytotoxicity studies [80,81]. Figure 4 shows the results in terms of cell viability obtained using eight different concentrations of the propolis extract.

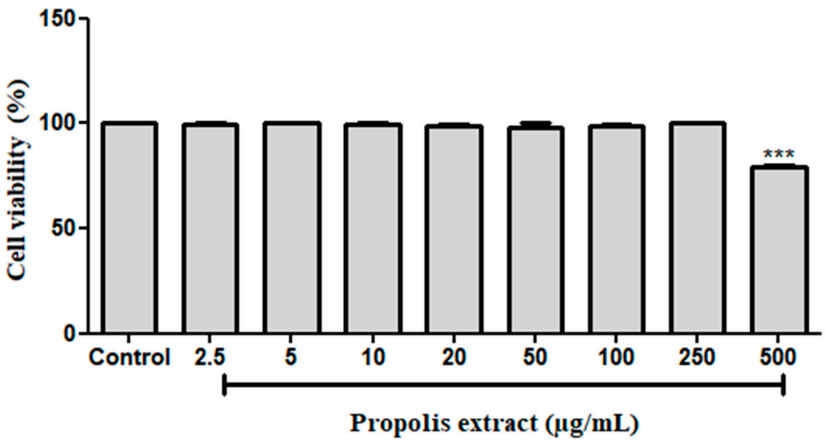

Figure 4. Cytotoxicity of propolis extract against fibroblast cells treated for 24 h in an oven at a temperature of 37 °C (Control = control without treatment). The results were compared using the analysis of variance followed by Tukey's post hoc test, *** $p < 0.01$.

The extract, which proved to be moderately cytotoxic at the maximum tested concentration (500 µg/mL), did not lead to any decrease in cell viability up to the concentration of 250 µg/mL, where it may be safely used without causing cell damage. These results allowed us to estimate a concentration that caused cytotoxicity by 50% (CC50) of 321.4 µg/mL. The groups treated with the extract showed no statistically significant difference compared to the control group after 24 h of the cell viability test, which indicates that the propolis extract did not induce cell death in fibroblasts at concentrations lower than the maximum tested one.

3.5. In Vitro Antipromastigote Assay

Leishmaniasis is a disease with high global impact responsible for a public health problem whose treatment is still limited, mainly due to drug toxicity [82]. In the search for alternative treatments, propolis has shown promising leishmanicidal activity against different species of *Leishmania* [83]. In particular, in vitro experiments showed propolis' ability to reduce the diameter of injuries, cause morphological changes in promastigotes or amastigotes or even improve immune response to *Leishmania* spp.-activating macrophages [22].

Based on these studies, the antileishmanicidal effect of propolis extract was investigated in in vitro tests carried out at different concentrations (2.5–500 µg/mL) against promastigotes of *L. amazonensis*, whose results are illustrated in Figure 5. Propolis extract caused a toxic effect to promastigotes across the entire concentration range tested and had a IC_{50} value of only 1.50 µg/mL. The groups treated with propolis extract showed statistically significant differences compared to both the negative control ($p < 0.001$) and the group treated with amphotericin B ($p < 0.001$, $IC_{50} = 0.50$ µg/mL).

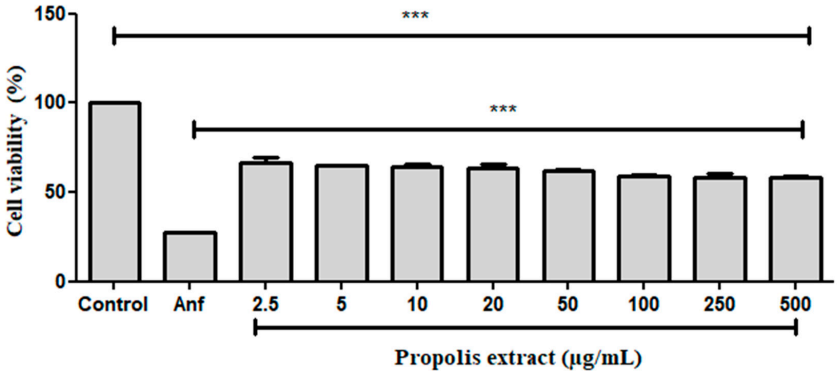

Figure 5. Cell viability of promastigotes of *L. amazonensis* treated with propolis extract at different concentrations for 24 h in an oven at temperature of 25 °C. Control = control without treatment; Anf = treatment with amphotericin B. The results were compared using the analysis of variance followed by Tukey's post hoc test, *** $p < 0.001$.

Over 75 years, the therapeutic window for treating leishmaniasis has been quite limited due to resistance and toxicity, which has led to the search for new drug options [84]. The results of the present study demonstrated that a natural product such as propolis, used since ancient times to treat other illnesses, non-toxic to humans and easy to acquire, is capable of exerting a toxic effect on promastigote forms of *L. amazonensis*. This may have a positive impact on the search for new drugs for the treatment of leishmaniasis. In particular, propolis extract can be used as an alternative ingredient not only to obtain new drugs for oral use, but also to prepare topical formulations, such as gels and hydrogels, which act directly at the site of lesions caused by leishmaniasis. This extract may also be used in association with drugs already used to treat leishmaniasis as a way of enhancing their antileishmanial effect.

Cavalcante et al. [83] observed comparable activity against *L. amazonensis* amastigotes ($IC_{50} = 1.3 \pm 0.1$ µg/mL) of the hexane fraction of propolis collected at the Bahian semiarid region (Brazil). Ayres et al. [85] observed that different propolis ethanolic extracts were able

to reduce the parasitic load of *L. amazonensis* promastigotes and amastigotes in parasite-infected macrophages. The extract of Adana propolis was able to inhibit the growth of *Leishmania tropica* [86], and Machado et al. [24] reported the leishmanicidal potential of extracts of propolis collected in Brazil and Bulgaria against four different species belonging to the *Leishmania* genus, namely *L. amazonensis*, *L. braziliensis*, *L. chagasi* and *L. major*. Finally, the green propolis exhibited antileishmanial potential against *L. braziliensis* [54].

The ways in which propolis exerts its potential to cause death in amastigote and promastigote forms of *Leishmania* species requires elucidation. However, it has been suggested that it may be the result of the activation of macrophages, cells that participate in various activities, including the removal of pathogens and cell debris, generation of metabolites and inflammation mediators, among others [22].

Despite the excellent results of parasite growth inhibition against promastigote forms of *L. amazonesis*, it is necessary to study the extract's effect against amastigote forms to confirm its leishimanicidal potential, and in vivo studies with amastigotes and promastigote forms are needed to prove its potential in the healing of skin lesions caused by *L. amazonesis*.

4. Conclusions

Quality control parameters of propolis from *Scaptotrigona postica* from the northeast of the Brazilian Pará state showed values consistent with those obtained in previous studies for propolis produced in other geographic areas, within the standards established by the Brazilian legislation and suitable for obtaining pharmacognostic specifications. The extract showed total polyphenol content of 21.29 ± 0.003 mg/g and total flavonoid content of 8.17 ± 0.00 mg/g, thermal stability around 370 °C and antioxidant activities identified by the DPPH, ABTS and FRAP assays of 910.09 ± 0.0264 µM Trolox (IC_{50} 18.9 ± 0.01 µg/mL), 859.97 ± 0.0854 µM (IC_{50} 692 ± 0.01 µg/mL) and 1613.67 ± 0.2610 µM Trolox (IC_{50} 80 ± 0.1 µg/mL), respectively. Finally, cell viability and antipromastigote activity tests allowed us to determine CC_{50} and IC_{50} values of 321.4 µg/mL and 1.50 µg/mL, respectively. These results provide the basis for research aimed at identifying and quantifying the major substances that are part of the chemical composition of *S. postica* propolis, as well as verifying other biological activities for its possible incorporation in innovative formulations for the pharmaceutical and cosmetic industries. The propolis extract, with its excellent antipromastigote activity, proved to have promising potential to be used in the development of new herbal drugs to treat leishmaniasis, a neglected disease that requires a constant search for new drugs due to the particularities of existing treatments. In addition, they can add value to propolis and contribute to the development of meliponiculture in the state of Pará.

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