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Original Article

Antileishmanial activity and chemical composition from Brazilian geopropolis produced by stingless bee *Melipona fasciculata*

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ABSTRACT

Geopropolis is produced by some stingless bee species such as *Melipona fasciculata* and consists of a mixture of plant resins, salivary secretions of the bee, wax, and soil. This study evaluated the antileishmanial activity *in vitro*, cytotoxicity and chemical composition of geopropolis produced by *M. fasciculata* in the savannah region of Maranhão, Brazil. The geopropolis extract was obtained through maceration using in 70% ethanol. The hydroalcoholic extract of geopropolis after liquid–liquid partition yielded the hexane, chloroformic, ethyl acetate and hydroalcoholic fractions. Antileishmanial activity was evaluated against promastigote and intracellular amastigote of *Leishmania amazonensis*. Cytotoxic was realized in BALB/c mice peritoneal macrophages. Chemical analysis was performed by gas chromatography–mass spectrometry and high performance liquid chromatography coupled to an ultraviolet–visible detector. The geopropolis inhibited the *L. amazonensis* promastigotes growth and was effective in reducing the infection of murine macrophages since the number of internalized amastigotes were smaller in cells treated with the geopropolis extract in relation to the untreated group. The ethyl acetate fraction was the most active and showed the highest index of selectivity as antileishmanial product. The geopropolis from *M. fasciculata* had an antileishmanial effect, mainly after the obtention of the ethyl acetate fraction that improved the activity without increasing the cytotoxicity against murine macrophages. Analysis for gas chromatography-mass spectrometer identified as main compounds the gallic and ellagic phenolic acids, either in the extract or in the active fraction. The results obtained by high performance liquid chromatography it was possible to confirm the presence and quantify the concentration gallic and ellagic acid either in the extract or in the active fraction. These results suggest that the antileishmanial activity of geopropolis is related to the presence of derivatives of these phenolic acids, mainly gallic and ellagic acids.

Introduction

Leishmaniasis is an infectious disease that occurs in countries with tropical and temperate climates and it is transmitted to humans by the bite of sand flies infected with protozoa of the genus *Leishmania*. This disease affects approximately 2 million people in the world every year (WHO, 2018).

The severity of disease varies ranging from cutaneous or mucosal to visceral or diffuse cutaneous infection. *Leishmania amazonensis*, a species transmitted mainly in the Amazon region, which is associated with localized cutaneous lesions. In Brazil occur both visceral leishmaniasis (VL) and tegumentary leishmaniasis (TL), endemic disease in Northern and Northeastern Brazil, mainly in the states of Bahia, Ceará, Maranhão, and Piauí (Marzochi et al., 2009).

Currently, the drugs used to treat leishmaniasis include pentavalent antimonial, amphotericin B, and pentamidine, present variable efficacy against different *Leishmania* species, elevated toxicity and can induce resistance of the parasite (Croft and Coombs, 2003; Silva-López, 2010). Therefore, there is an urgent need for new drugs that can be used both for the prophylaxis and for the treatment of leishmaniasis. In this respect, bee products are promising candidates since they are important sources of bioactive compounds (Bankova and Popova, 2007; Lavinas et al., 2019).

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In the State of Maranhão, northeast Brazil, *Melipona fasciculata* Smith, popularly known as "tiúba" or "tiúba-do-maranhão", is the most widely cultivated stingless bee species because of its high productivity of honey and geopropolis. This species is therefore one of the main sources of income for many families in the state (Dutra et al., 2008) and the geopropolis produced is popularly used for the treatment of inflammation, weakness, hemorrhoids, gastritis, cough, and also as a healing agent (Kerr, 1987; Araújo et al., 2016b).

Geopropolis is produced from plant resins that are mixed with pollen, wax, salivary secretions, and soil. In the beehive, this product is used to fill small cracks, to seal entry holes, and as an antimicrobial agent (Araújo et al., 2016b; Sanches et al., 2017).

The chemical composition of geopropolis produced by different species of *Melipona* has shown phenolic acids, flavonoids, coumarins, hydrolysable tannins, prenylated benzophenone, terpenes, steroids, saponins, fatty acids and sugars (Dutra et al., 2008, 2014; Da Silva et al., 2013; Souza et al., 2013, 2014; Araújo et al., 2015; Batista et al., 2016; Cunha et al., 2016).

The following activities have been attributed to this product: antimicrobial (Muli et al., 2008; Liberio et al., 2011; Araújo et al., 2016b; Santos et al., 2017), antioxidant (Da Silva et al., 2013; Souza et al., 2013; Dutra et al., 2014; Batista et al., 2016; Ferreira et al., 2017), anti-inflammatory (Liberio et al., 2011; Franchin et al., 2013), antiproliferative, cytotoxic (Cunha et al., 2013, 2016; Campos et al., 2014), anti-nociceptive (Souza et al., 2014), immunomodulatory (Liberio et al., 2011; Araújo et al., 2015), antitumor (Cinegaglia et al., 2013; Bartolomeu et al., 2016) and gastroprotective (Ribeiro-Junior et al., 2015).

There are studies investigating the antileishmanial activity of propolis produced by *Apis mellifera* bees (Ayres et al., 2007; Machado et al., 2007; Duran et al., 2008, 2011; Pontin et al., 2008). However, there are no reports describing the antileishmanial activity of the *M. fasciculata* geopropolis.

Therefore, the present study investigated the antileishmanial activity, cytotoxicity and chemical composition of geopropolis produced by *M. fasciculata* Smith in order to contribute the bioprospection of new products with activity against *Leishmania* infection.

Material and methods

Geopropolis sample

The geopropolis sample was collected from beehive in meliponary in the municipality of Fernando Falcão, Maranhão, Brazil (2°37′30″S 44°52′30″W), a savannah region from Maranhão State, northeast Brazil in November of 2008. Geopropolis was removed from the hives with a spatula, stored in sterile container, and transported to the laboratory for preparation of the extract and chemical and biological analysis.

Extraction and fractionation of geopropolis extract

The geopropolis sample (500 g) was separately macerated with 1:2 (w/v) in 70% ethanol for 48 h and filtered to separate the inorganic part (soil). The hydroalcoholic extract of geopropolis (HEG) was fractionated by liquid–liquid partition using solvents of increasing polarity, obtaining hexane (HF), chloroform (CF), ethyl acetate (EAF) and hydroalcoholic (HAF) fractions, as described by Dutra et al. (2014).

Parasites

Leishmania amazonensis (MHOM/BR/1987/BA-125) promastigotes were maintained in axenic cultures containing Schneider's medium (Sigma, St. Louis, MO, USA) supplemented with 10% inactivated fetal bovine serum (Gibco, Carlsbad, CA, USA) and gentamicin (50 μg/ml; Sigma), incubated at 24 °C. The growth of the cultures was monitored daily and parasites were counted in a Neubauer chamber. Only *L. amazonensis* promastigote forms in the stationary phase were used in the assays.

Obtention of murine peritoneal macrophages

The cells were obtained by washing the peritoneal cavity from Balb/c mice with cold sterile phosphate-buffered saline (PBS) 5 days after intraperitoneal injection of 1 ml of 3% sterile thioglycolate. The cell suspensions were placed in 96-well flat-bottom microplates and after 3 h of adherence, the nonadherent cells were removed by washing with PBS at 37 °C. The adherent cells in 100 μl of complete medium were used in most of the experiments. The cells were centrifuged (160 × *g*, 10 min, 4 °C) and resuspended in complete medium RPMI and 10% fetal bovine serum (FBS) (Ribeiro-Dias et al., 1999). All experiments involving the use of experimental animals were performed in accordance to the ethical standards of Federal University of Maranhão and were approved by the ethics committee (Protocol: 23115-012975/2008-43).

Evaluation of extract, fractions and phenolic acids against Leishmania amazonensis *promastigotes*

The antileishmanial activity of the extract, fractions and phenolic acids gallic and ellagic (Sigma Aldrich, St Louis, MO, USA) were evaluated against promastigotes of *L. amazonensis* in culture using microplates. The HEG, fractions and phenolic acids were resuspended in PBS and diluted in complete RPMI 1640 medium to final concentrations of 500, 250, 125, 62.5, 31.25, and 15.62 μg/ml. Aliquots of 10 μl of the suspension containing 5×10^6/ml promastigote forms of *L. amazonensis* were added to the wells. Amphotericin B (Sigma Aldrich, St Louis, MO, USA), the reference drug for the treatment of leishmaniasis, was used as positive control in concentrations varying from 0.07 to 10 μg/ml, and compared to the negative control (culture medium). After 24 h of incubation, the total number of live promastigotes was determined by flagella motility in a Neubauer chamber, under a bright-field light microscopy. The concentration that inhibited culture growth by 50% (IC_{50}) was determined by nonlinear regression (Bezerra et al., 2006).

Evaluation of extract and fraction in macrophages infected with Leishmania amazonensis *amastigotes*

Peritoneal macrophages from Balb/c mice were harvested and plated at 2×10^6 cell/ml in a 24-well plate containing a coverslip of 13 mm diameter, allowed to adhere for 2 h at 37 °C in 5% CO_2. After this period the wells were washed three times with unsupplemented RPMI 1640 to remove non-adherent cells. Then the macrophages were infected with promastigotes at ratio of promastigote/macrophage (10:1), and the cells were incubated at 37 °C in 5% CO_2. After 4 h incubation, free promastigotes were removed and counted (Silva et al., 2018). The infected macrophages were treated during 24 h with HEG (47 μg/ml) and EAF (29 μg/ml) according the inhibitory concentration detected at the *in vitro* antipromastigote assay. After incubation, the cells were fixed with methanol, Giemsa stained and examined by light microscopy. The number of amastigotes/100 macrophage cells and the percentage of infected cells were determined. The infection index was calculated by multiplying the percentage of infected macrophages by the mean number of amastigotes per infected cells (Lima Junior et al., 2014; Silva et al., 2018).

Cytotoxicity assay

Peritoneal macrophages were plated at 2×10^5 cells/well on coverslips in 96-well plate for 24 h in the presence or absence of the HEG and fractions in different concentrations (15.62, 31.25, 62.5, 125, 250 and 500 μg/ml). At the end of the period were added 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Aldrich, St Louis, MO, USA) 5 mg/ml and the cells were incubated for 3 h at 37 °C in 5% CO_2. The supernatant was removed and the plates were incubated overnight at room temperature with 100 μl of 10% solution of sodium dodecyl sulfate–HCl to dissolution of formazan crystals. Absorbance was determined in a spectrophotometer (MR 5000, Dynatech Laboratories Inc., Gainesville, VA, USA) at 540 nm. The 50% cytotoxic concentration (CC_{50}) was determined by regression analysis. The selectivity index (SI) was determined as the ratio of CC_{50} for the macrophages to IC_{50} for the promastigote. Each assay was carried out in triplicate in three independent experiments (Ribeiro-Dias et al., 1999).

Sample preparation for gas chromatography–mass spectrometry (GC–MS) analysis

The HEG and fractions of geopropolis (10 mg) were solubilized in pyridine (300 μl) and bis-(trimethylsilyl) trifluoroacetamide (BSTFA, Sigma Aldrich, St Louis, MO, USA) including 1% trimethylchlorosilane (TMCS) (BSTFA/TMCS), 100 μl, and heated at 80 °C for 20 min according to Batista et al. (2016).

Analysis of the extract and fractions by GC/MS

Analysis of the HEG and fractions were performed on gas chromatograph (GC) (Agilent 6890) coupled to a mass spectrometer (MS) (Agilent 5973N MSD), equipped with a HP-5MS fused silica (30 m × 0.25 mm i.d., film thickness 0.25 μm) and operating in the electron impact ionization mode (70 eV), was used for analyses. The temperatures of the injector and detector were maintained at 230 °C and 250 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 ml/min, and the injection volume was 1 μl in split ratio 10:1. Analysis consisted of 5 min of isothermal heating (70 °C), a temperature gradient of 5 °C/min at 310 °C, and a last minute of heating at 310 °C. The interface was maintained at 200 °C and the detector was operated in the scanning mode (*m/z* 50–650) and a scan interval of 2 scan s^{-1}. The identification of geopropolis compounds was based on the percentage of similarity plus comparison of mass spectra (MS) using software NIST AMIDS version 2.0 data library, with the percentage of total ion chromatograms (TIC %).

Analysis of the extract and fractions by HPLC/UV

The identification and quantification of gallic (GA) and ellagic acids (EA) in the HEG and EAF fraction were performed by high performance liquid chromatography coupled to an ultraviolet–visible detector (HPLC–UV). The analysis was proceeded in chromatograph Varian HPLC system equipped with a ProStar Autosampler 410, 2 ProStar 215 pumps, ProStar 325 UV-Vis detector (Varian, Palo Alto, USA) controlled with Galaxie software (Varian v1.9.3.2), using a Phenomenex RP-Gemini C18 column (250 mm × 4.6 mm, 5 μm) at room temperature. The mobile phase was comprised of 0.1% (v/v) formic acid acidified ultrapure water (as solvent A) and acetonitrile (as solvent B). The gradient program was as follows: 90–10% B (5 min), 80–20% B (10 min), 70–30% B (25 min), 65–35% B (30 min). The detection of compounds of interest was performed at a wavelength (λ) of 254 nm, and the volume injected was 20 μl, with the mobile phase flow of 1.0 ml/min.

The concentration of GA and EA in the samples were obtained from the regression-equation resulted by the linear regression of a calibration curve, correlating the chromatographic peak area referred to the gallic acid and ellagic acid purchased from Sigma-Aldrich (St. Louis, MO, USA) and their concentration in the sample (1 mg/ml). Calibration curve (five points) was used to quantify each standard with the range of 5–100 μg/ml for gallic acid, and 2.5–40 μg/ml for ellagic acid.

Table 1
Antileishmanicidal activity of the extract and fractions of geopropolis, gallic acid, and ellagic acid against promastigote forms, cytotoxicity and selectivity index for *Leishmania amazonensis*.

Sample	*L. amazonensis*	Macrophage	
	IC_{50} (μg/ml)[a]	CC_{50} (μg/ml)[a]	SI_{pro}[b]
HEG	47.00 ± 2.70 [a]	292.70 ± 35.49 [a]	6.22
HF	No activity	Nd	Nd
CF	43.21 ± 2.32 [a]	78.34 ± 32.64 [b]	1.81
EAF	29.90 ± 2.93 [b]	244.90 ± 22.60 [c]	8.19
HAF	49.48 ± 1.40 [a]	381.70 ± 9.29 [d]	7.71
Gallic acid	14.48 ± 1.87	Nd	Nd
Ellagic acid	151.7 ± 19.50	Nd	Nd
Amphotericin B	1.0 ± 0.46 [c]	Nd	Nd

[a] Values represent the mean of triplicate measurements ± standard deviation. Different letters in the same column indicate a significant difference (Tukey test, $p < 0.05$). HEG, hydroalcoholic extract of geopropolis; HF, hexane fraction; CF, chloroform fraction; EAF, ethyl acetate fraction; HAF, hydroalcoholic fraction; Nd, not determined.

[b] SI_{pro} (selectivity index) = CC_{50} macrophages/IC_{50} promastigote forms.
IC_{50} = concentration producing 50% inhibition of *L. amazonensis* promastigotes.
CC_{50} = concentration producing 50% inhibition of peritoneal macrophages.

Statistical analysis

All assays were repeated three times and the results expressed are expressed as the mean ± standard deviation and were analyzed. All data were compared by analysis of variance (one-way ANOVA) followed by the Tukey test. Differences were considered significant when $p < 0.05$. All data were analyzed with the GraphPad Prism 7.0 software (San Diego, CA, USA). All analyses were performed in triplicate.

Results

The HEG, CF, EAF, and HAF showed anti-leishmania activity since they were able to reduce the number of live promastigotes. HEG reduced the number of viable promastigotes of *L. amazonensis* in a dose-dependent manner with IC_{50} of 47 μg/ml, and at the concentration of 250 μg/ml was able to inhibit 100% of promastigote forms (Table 1). After fractionation, the EAF fraction was the most active (IC_{50} 29.89 μg/ml), inhibiting 100% of the promastigotes from 62.5 μg/ml. The other fractions also show inhibitory effect for *L. amazonensis* promastigotes at the tested concentrations (CF: IC_{50} 43.21 μg/ml and HAF: IC_{50} 49.48 μg/ml). FH showed no antileishmanial activity (Table 1).

The antileishmanial activity against promastigotes and cytotoxicity to HEG and the active fractions (CF, EAF, and HAF) was compared using the selectivity index. The HEG demonstrated CC_{50} of 292.7 μg/ml and EAF CC_{50} of 244.9 μg/ml, while HAF and CF 381.7 μg/ml and 78.34 μg/ml respectively. The selectivity index for promastigotes has shown that the most active fraction (EAF) also has lower cytotoxic activity for macrophages (Table 1).

The extract (HEG) and the active fraction (EAF) induced a significant reduction in the number of amastigotes within the macrophages compared to the control group, in the experimental infection *in vitro* with peritoneal macrophages infected with *L. amazonensis*.

Treatment of infection of macrophages with HEG and EAF reduced number of amastigotes inside macrophages. This

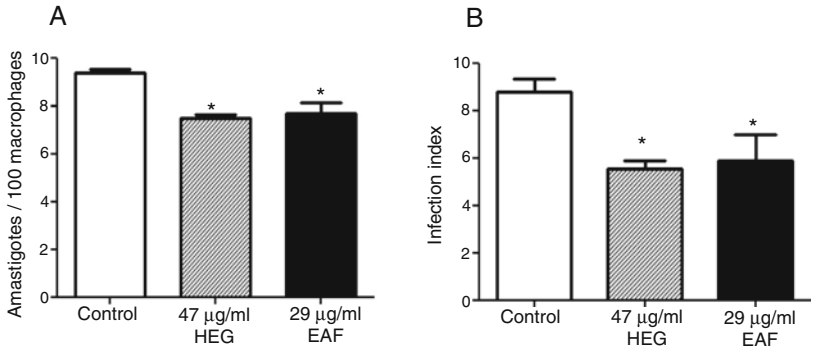

Fig. 1. *In vitro* effect of geopropolis extract (HEG) and fraction (EAF) on *Leishmania amazonensis* intracellular amastigotes. The number of amastigotes in infected macrophages (A), and infection index (B). The negative control is *L. amazonensis* intracellular amastigotes not treated. The results represent mean ± standard deviation of individual samples tested in triplicate (*): $p < 0.05$ in comparison to the negative control.

antileishmanial effect was associated with a reduction on the infection index. The percentage reduction in infection was 37% in the macrophages treated with HEG, whereas those treated with EAF reduced by 33% (Fig. 1).

The analysis of HEG and fractions by GC/MS, identified twenty three compounds by comparison of retention times, mass spectra using NIST Library and literature data. The compounds identified belong to the class of fatty acids, organic acids, phenolic acids, triterpenes, steroids, sugars and alcohols. Fatty acids were identified in the samples, with the exception of the EAF fraction, with a higher concentration of palmitic and stearic acids. In the hexane fraction the triterpenes β-amyrin, lupenone and steroids campesterol, stigmasterol and β-sitosterol were identified, while in the chloroform fraction oleanolic acid and β-sitosterol. Phenolic acids, sugars and alcohols were identified mainly in HEG and EAF fraction (Table 2).

According the results obtained by HPLC/UV it was possible to confirm the presence of gallic and ellagic acid (Fig. 2). The calibration curves obtained to quantify the concentration of gallic acid and ellagic acid presented correlation coefficient ≥0.998, confirming the linearity of the method. The detected concentration of gallic acid was 2.66 μg/mg and 9.85 μg/mg for HEG and EAF, respectively, while the ellagic acid concentration was 20.32 μg/mg for HEG and 27.73 μg/mg for the EAF fraction.

The fractionation of the extract by liquid–liquid partition increased the concentration of ellagic and gallic acids in EAF, mainly gallic acid since the concentration was about 3.7 times higher than HEG.

The geopropolis samples with antileishmanial activity showed the presence and higher concentrations of phenolic acids, mainly EAF fraction with gallic acid (40.5%) and ellagic acid (31.6%) (Table 2). The mass spectra obtained by the GC–MS analysis after silylation with BSTFA from the extract and fractions of the geopropolis showed the ions *m/z* 458, 443, 281, 73 for gallic acid (4TMS) and ions *m/z* 590, 575, 487, 281, 73 for ellagic acid (4TMS).

Considering the high concentration of gallic and ellagic acids in the most active fraction (EAF), analytical standards of these compounds were evaluated for antileishmanial activity against *L. amazonensis* promastigotes. The phenolic acids exhibited antileishmanial activity, however, gallic acid was more effective in inhibit against promastigotes forms (IC_{50} 14.48 μg/ml) than ellagic acid (IC_{50} 151.7 μg/ml) (Table 2).

Discussion

The extract and fractions of the geopropolis presented an anti-*Leishmania* activity, with the EAF fraction being more effective than the extract (HEG) in reduce the promastigotes numbers, and also to reduce the number of amastigotes in infected macrophages, despite the low cytotoxicity for uninfected murine macrophages.

The selectivity index (SI) found for geopropolis extract and its fractions indicate higher toxicity to promastigote forms and a moderate toxicity to uninfected macrophages (SI <10) (Bringmann et al., 2013). These results are important in the search for effective natural products for the treatment of leishmaniasis (Alves et al., 2017). According to Veiga et al. (2017) extracts with IC_{50} ≤100 μg/ml for promastigote forms are considered promising for the bioprospection of products with anti-*Leishmania* activity, especially when they show low toxicity (CC_{50} >200 μg/ml) to no infected cells values, as showed here to the EHG and EAF.

After the liquid chromatography tandem–mass spectrometry (LC–MS/MS) it was possible to identify eleven compounds present in HEG and EAF, including gallic and ellagic phenolic acids, as well as the hydrolysable tannins as: corilagin, HHDP-glucose, pedunculagin, trigalloyl glucose, tellimagrandin I, valoneic acid dilactone, casuarictin, tellimagrandin II e trisgalloyl-HHDP-glucose) (Dutra et al., 2014).

The presence of gallic acid in *M. fasciculata* geopropolis is expected since this compound has been identified as one of the main compounds in geopropolis from other species of the genus *Melipona* as: *M. quadrifasciata*, *M. scutellaris*, and *M. marginata* (Velikova et al., 2000; Dutra et al., 2014; Batista et al., 2016). Few studies have identified ellagic acid in propolis and geopropolis samples (Dutra et al., 2014; Araújo et al., 2016a; Batista et al., 2016). The qualitative and quantitative variations in phenolic compounds seen between different bee products are mainly influenced by the plant species visited by the bees, type of bee species, geographic region, and climatic factors (Gómez-Caravaca et al., 2006).

It is possible that the anti-*Leishmania* activity is related to the presence of the gallic and ellagic acids, identified in the extract and EAF, as confirmed by the mass spectra (Zoechling et al., 2009; Degani et al., 2014) since these two phenolic acids exhibited antileishmanial activity against promastigotes (Tasdemir et al., 2006; Shuaibu et al., 2008), but it is important to emphasizes that according our results the gallic acid was more effective.

Previous results also showed a lower anti-*Leishmania* effect against the promastigotes of *L. major* for the gallic acid in comparison to the ellagic acid (IC_{50} 16.4 and 9.8 μg/ml, respectively). The both phenolic acids were also effective against amastigotes, since they were able to reduce the infection and infectivity on Balb/c macrophages infected by *L. major* (IC_{50} values 5.0 and 0.9 μg/ml, respectively), with selectivity index higher than 20 (Alves et al., 2017).

In addition, several authors (Kolodziej et al., 2001; Kolodziej and Kinderlen, 2005) showed low toxicity to the gallic acid despite the anti-*Leishmania* activity to *L. donovani* promastigotes data that

Table 2
Chemical composition identified in the extract and fractions of the geopropolis after silylation by GC–MS.

Compound class	Identified compound[a]	HEG	HF	CF	EAF	HAF
				TIC (%)[b]		
Fatty acids	Palmitic acid	0.52	7.27	0.96	–	0.51
	Oleic acid	–	1.72	–	–	–
	Stearic acid	1.53	2.12	5.29	–	2.47
	Arachidic acid	–	0.33	–	–	–
	Behenic acid	–	0.44	–	–	–
	Lignoceric acid	–	0.25	–	–	–
Organic acid	Quinic acid	1.68	–	–	–	1.01
Phenolic acids	Gallic acid	22.30	–	4.80	40.5	5.76
	Ellagic acid	14.70	–	1.53	31.6	21.60
	Protocatechuic acid	0.10	–	0.14	–	–
Triterpenes	β-Amirin	–	0.42	–	–	–
	Lupenona	–	0.43	–	–	–
	Oleanolic acid	–	0.13	2.35	–	–
Steroids	Campesterol	–	0.07	–	–	–
	Stigmasterol	–	0.55	–	–	–
	β-Sitosterol	–	1.47	0.39	–	–
Sugars	Frutose	0.32	–	–	–	–
	Glucose	21.0	–	–	3.15	18.60
	Mannose	12.8	–	–	–	10.33
	Xylose	0.61	–	–	–	0.28
Alcohols	Glycerol	0.22	–	–	–	0.35
	Xylitol	0.92	–	–	–	1.22
	Inositol	0.10	–	–	–	0.10

[a] Name of the compounds without the trimethylsilyl (TMS) constituent.
[b] Expressed in percentage of total ion chromatograms (TIC %).
HEG, hydroalcoholic extract of geopropolis; HF, hexane fraction; CF, chloroform fraction; EAF, ethyl acetate fraction; HAF, hydroalcoholic fraction.

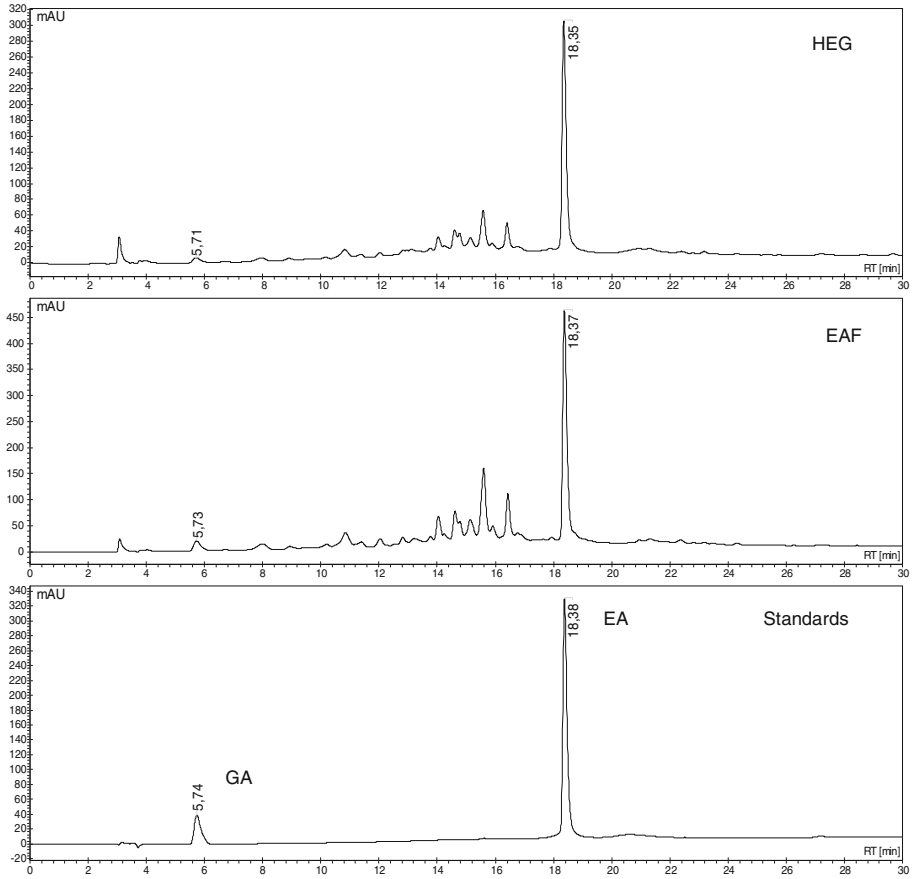

Fig. 2. HPLC chromatograms of hydroalcoholic extract of geopropolis (HEG), ethyl acetate fraction (EAF), standards of gallic acid (GA) and ellagic acid (EA) in 254 nm.

corroborate with those ones found in the present study with the extract of geopropolis and the gallic acid.

Conclusions

The antileishmanial activity of the hydroalcoholic extract of geopropolis from *M. fasciculata* and its ethyl acetate fraction is probably related to the presence of gallic acid, and ellagic acid, with high effect on the promastigotes and amastigotes form and with a moderate toxicity to murine macrophages what may open a new perspective in the research of new drugs with antileishmanial effect.

Authors' contributions

RPD, JLB and MCPS contributed to biological studies. RPD and MCAB contributed by collecting geopropolis samples, and performing chromatographic analysis. FJBP contributed to the cytotoxicity test. FRFN contributed to critical reading of the manuscript. MNSR and RNMG designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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