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ORIGINAL ARTICLE

Antibacterial and leishmanicidal activity of Bolivian propolis

N. Nina[1,2], B. Lima[3], G.E. Feresin[3], A. Giménez[4], E. Salamanca Capusiri[4] and G. Schmeda-Hirschmann[1]

1 Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile
2 Facultad de Ciencias de la Salud, Programa de Magister en Ciencias Biomédicas, Universidad de Talca, Talca, Chile
3 Facultad de Ingeniería, Instituto de Biotecnología, Universidad Nacional de San Juan, San Juan, Argentina
4 Facultad de Ciencias Químico Farmacéuticas y Bioquímicas, Instituto de Investigaciones Fármaco Bioquímicas – IIFB, Universidad Mayor de San Andrés – UMSA, La Paz, Bolivia

Significance and Impact of the Study: Propolis is used in Bolivia as an antimicrobial agent. Bolivian propolis from the main production areas was assessed for antibacterial and leishmanicidal effect and the results were compared with the propolis chemical composition. The active antibacterial propolis samples were phenolic-rich while those containing mainly triterpenes were devoid of activity or weakly active. A similar picture was obtained for the effect on *Leishmania*, with better effect for the phenolic-rich samples. As propolis is used for the same purposes regardless of the production area and composition, our findings indicate the need for the standardization of this natural product as antimicrobial.

Keywords
antibacterial activity, bacteria, Bolivia, disinfectant, leishmanicidal activity, phenolics, propolis, triterpenes.

Correspondence
Guillermo Schmeda-Hirschmann, Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, 3460000 Talca, Chile.
E-mail: schmeda@utalca.cl

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Abstract

The antimicrobial activity of Bolivian propolis was assessed for the first time on a panel of bacteria and two endemic parasitic protozoa. Ten samples of Bolivian propolis and their main constituents were tested using the microbroth dilution method against 11 bacterial pathogenic strains as well as against promastigotes of *Leishmania amazonensis* and *L. braziliensis* using the XTT-based colorimetric method. The methanolic extracts showed antibacterial effect ranging from inactive (MICs > 1000 μg ml^{-1}) to low (MICs 250–1000 μg ml^{-1}), moderate (62·5–125 μg ml^{-1}) and high antibacterial activity (MIC 31·2 μg ml^{-1}), according to the collection place and chemical composition. The most active samples towards *Leishmania* species were from Cochabamba and Tarija, with IC_{50} values of 12·1 and 7·8, 8·0 and 10·9 μg ml^{-1} against *L. amazonensis* and *Leishmania brasiliensis* respectively. The results show that the best antibacterial and antiprotozoal effect was observed for some phenolic-rich propolis.

Introduction

Propolis is a natural product obtained by bees from resins, bud and plant exudates. Propolis is used in Bolivian traditional medicine as an antimicrobial agent to treat respiratory illnesses, skin and gastric infections (Nina *et al.* 2015). It is commercialized in ethanolic solution (70–90% ethanol : water mixtures). However, there is no information on the effect of Bolivian propolis on micro-organisms of clinical relevance neither on the endemic protozoa *Leishmania* spp. Leishmaniasis is a group of neglected tropical disease transmitted to humans by phlebotominae sandflies (García *et al.* 2009). Cutaneous leishmaniasis is caused mainly by *Leishmania braziliensis* and visceral leishmaniasis is caused by *Leishmania infantum*. The disease is of high prevalence in Bolivia and the country has the highest prevalence of leishmaniasis in Latin America (García *et al.* 2009).

According to the World Health Organization (WHO 2000), the infectious diseases were the primary cause of mortality prior to the discovery and use of antimicrobials. In much of the developing world, healthcare-associated

infections with resistant micro-organisms such as *Escherichia coli*, *Salmonella* ssp. and *Staphylococcus aureus* methicilin resistant are a major cause of death. The clinical relevance and importance on human health was a parameter for selecting the bacteria assayed.

There has been an increasing interest on the potential antimicrobial properties of propolis, based on studies from samples collected in several places of the world. The antibacterial activity of propolis is related with the chemical composition which can differ according to the plant sources, collection area and season (Moreno *et al.* 1999; Vardar-Ünlü *et al.* 2008; Salatino *et al.* 2011). The antimicrobial effect of propolis has been attributed to the content of lignans, flavonoids and esters of phenolic acids. However, the activity can be better explained by synergistic mechanisms involving several biological pathways (Al-Waili *et al.* 2012). The leishmanicidal effect has been less explored because leishmaniasis is mainly confined to tropical and subtropical countries. There are reports on the leishmanicidal effect of propolis from Turkey on *L. tropica* (Duran *et al.* 2008) and samples from Portugal on *L. infantum* (Falcão *et al.* 2014). Propolis from Brazil has been investigated for antileishmanial effect against *Leishmania brasiliensis* (Pontin *et al.* 2008; da Silva *et al.* 2013) and *L. amazonensis* (Santana *et al.* 2014).

A recent work disclosed the chemical diversity and antioxidant effect of Bolivian propolis and showed the occurrence of at least two different chemical types of propolis in the country, namely triterpene-rich and phenolic-rich propolis (Nina *et al.* 2015). The aim of this work was to investigate the antibacterial and leishmanicidal activity of Bolivian propolis and its relation with the chemical composition.

Results and discussion

Antibacterial activity

Ten Bolivian propolis samples were assessed for antimicrobial activity in a panel of 11 bacteria and two endemic protozoa. Results are presented as MIC values for the antibacterial activity, while the antiprotozoal effect is reported as IC_{50} values.

The micro-organism selection was based on the traditional uses of propolis and included the Gram-positive *Staph. aureus* as well as several Gram-negative bacteria. Besides, these bacteria are of the clinical importance worldwide. The results of the antibacterial study (as MIC values in $\mu g\ ml^{-1}$) are presented in Table 1. The effect of the extracts on the selected bacteria ranged from inactive (MICs > 1000 $\mu g\ ml^{-1}$) to low (MICs 250–1000 $\mu g\ ml^{-1}$), moderate (62·5–125 $\mu g\ ml^{-1}$) and high (MICs 31·2 $\mu g\ ml^{-1}$). According to Ríos and Recio (2005), an extract is promising as an antimicrobial if the activity (as MIC values) is below 100 $\mu g\ ml^{-1}$.

The most active antibacterial sample was that from Valle Alto (Cochabamba), with MICs of 31·2 $\mu g\ ml^{-1}$ against *Escherichia coli* strains ATCC 25922, 121 and 122, *Pseudomonas* sp., *Yersinia enterocolitica* and *Proteus mirabilis*. It was also active against *E. coli* LM_2 (MIC: 62·5 $\mu g\ ml^{-1}$). The samples from Sucre (Chuquisaca) and San Andrés (Tarija) were also active against several of the bacteria while propolis from Santa Cruz (Camiri) and La Paz (El Sillar and Villa Coroico) were the less active. Propolis from Chapare was only active towards *Pseudomonas* sp. and *E. coli* 121 with MICs of 31·2 and 62·5 $\mu g\ ml^{-1}$ respectively. The samples from San Andrés were interesting because the MIC values were between 62·5 to 125 $\mu g\ ml^{-1}$ against eight of the 11 bacteria, with a MIC value of the 31·2 $\mu g\ ml^{-1}$ towards *Y. enterocolitica*. Overall, Bolivian propolis was more effective against Gram-negative bacteria than against the Gram-positive *Staph. aureus*.

Antimicrobial studies on Latin American propolis include work carried out using different and noncomparable methods, often without clear criteria of activity and using clinical isolates. Propolis extracts were evaluated for antimicrobial activity in Mexico (Velasquez *et al.* 2007; Carrillo *et al.* 2011) and from Colombia (Samara-Ortega *et al.* 2011). The minimal bactericidal concentration (MBC) for the two samples from Colombia, (Totoró and Buenos Aires) was of 15·39 and 17·03 mg ml^{-1} against *Staph. aureus* and 30·78 and 17·03 mg ml^{-1} for *Pseudomonas aeruginosa* respectively (Samara-Ortega *et al.* 2011). The ethanol extract from the Mexican propolis presented MBC in the range of 0·93–5 mg ml^{-1} for Gram-positive and 7·5–10 mg ml^{-1} for Gram-negative bacteria respectively (Carrillo *et al.* 2011). Silva *et al.* (2012) reported MIC values (in $\mu g\ ml^{-1}$) ranging from 590 to 1720 for *Staph. aureus*, 1560–2810 for *Ps. aeruginosa* and 3190–4860 for *E. coli* respectively. Campos *et al.* (2014) found antimicrobial activity in 80% ethanol extract of Brazilian stingless bees with MICs of 3100 $\mu g\ ml^{-1}$ for *Staph. aureus* and the yeast *Candida albicans*. A study by Sandle *et al.* (2014) reported the minimum inhibitory concentration of three disinfectants widely used by the health care and pharmaceutical sector against 112 fungal isolates. The compounds were active as fungicides with MICs <32 $\mu g\ ml^{-1}$.

According to Ríos and Recio (2005) for potential sources of antimicrobial agents, all the mentioned samples should be considered as inactive as the effect was found at exceedingly high, unrealistic concentrations. Seidel *et al.* (2008) compared the antimicrobial activity of ethanol extracts of propolis from tropical, subtropical and

Table 1 Antimicrobial activity of methanol extract of phenolic- and triterpenoid-rich propolis from Bolivia. Results are presented as MIC_{100} values in $\mu g\ ml^{-1}$ for bacteria and as IC_{50} in $\mu g\ ml^{-1}$ for *Leishmania* strains

	Micro-organisms												
	Gram (+) bacteria		Gram (−) bacteria									Protozoa	
Propolis sample	1	2	3	4	5	6	7	8	9	10	11	12	13
Phenolic-rich													
Valle Alto	125	125	31·2	31·2	31·2	62·5	31·2	31·2	125	125	31·2	9·7 ± 1·1	59·9 ± 4·8
Chapare	250	500	250	62·5	125	125	31·2	125	125	125	125	8·0 ± 0·8	10·9 ± 0·8
Sucre	125	125	125	125	31·2	125	31·2	62·5	125	31·2	62·5	13·9 ± 0·4	39·2 ± 2·2
San Andres 1	125	250	62·5	62·5	125	62·5	62·5	31·2	125	62·5	62·5	40·3 ± 5·2	52·5 ± 1·0
San Andres 2	250	250	62·5	62·5	62·5	62·5	62·5	31·2	125	125	31·2	12·1 ± 0·5	7·8 ± 1·1
Triterpenoid-rich													
El Sillar	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	81·6 ± 1·1	75·6 ± 1·9
Villa Coroico	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	69·1 ± 1·1	24·1 ± 1·9
Okinawa	500	500	125	1000	500	250	250	125	250	250	250	54·5 ± 2·1	14·8 ± 0·5
Camiri 1	>1000	>1000	>1000	250	>1000	>1000	>1000	>1000	>1000	>1000	>1000	59·6 ± 5·2	84·6 ± 0·3
Camiri 2	>1000	>1000	500	500	500	>1000	>1000	>1000	>1000	>1000	>1000	54·5 ± 3·7	43·4 ± 9·1
Isolated compounds													
Kaempferol 3-methyl ether	50	50	50	50	50	50	25	25	50	25	25	–	–
Drupanin	>50	>50	50	12·5	50	50	50	>50	50	>50	50	–	–
Kaempferol 7-methyl ether	>50	>50	50	50	50	>50	>50	50	>50	>50	50	–	–
Lupeol	>50	>50	50	50	50	50	>50	>50	50	>50	>50	–	–
Cefotaxime	0·5	0·5	0·5	0·5	0·5	0·5	7·5	0·5	12·5	0·5	0·05	–	–
CTA *Galipea longiflora*												21·8 ± 0·2	20·0 ± 0·4
Amphotericine B												0·25 ± 0·05	0·07 ± 0·01

Micro-organisms: 1: methicillin-sensitive *Staphylococcus aureus* ATCC 25923; 2: methicillin-resistant *Staph. aureus* ATCC 43300; 3: *Escherichia coli* ATCC 25922; 4: *E. coli* 121; 5: *E. coli* 122; 6: *E. coli* LM_2; 7: *Pseudomonas* sp.; 8: *Yersinia enterocolítica*- PI; 9: *Salmonella enteritidis* MI; 10: *Salmonella sp.* (LM); 11: *Proteus mirabilis* 94-2; 12: *Leishmania amazonensis* Lma; 13: *Leishmania braziliensis* M2904. CTA: crude total alkaloid.

temperate zones on a panel of Gram-positive and Gram-negative bacteria using the broth microdilution assay. The authors found more activity against Gram-positive bacteria, and categorized the samples according to the MIC values as strong (MIC range 3·9–31·25 $\mu g\ ml^{-1}$), moderate (MIC range 31·25–≤500 $\mu g\ ml^{-1}$), weak antibacterial activity or inactive (MIC ≥ 500 $\mu g\ ml^{-1}$). Moreno *et al.* (1999) reported antibacterial activity in propolis from Tucumán, Argentina, using the agar diffusion technique with MIC values between of 15·3–49·1 $\mu g\ ml^{-1}$ for *Staph. aureus*, 7·8–107·9 $\mu g\ ml^{-1}$ for *Streptococcus piogenes*, 7·5–77·1 $\mu g\ ml^{-1}$ for *Streptococcus agalactiae* and 14·0–210·0 $\mu g\ ml^{-1}$ for *Enterococcus faecalis* respectively. The data cannot be compared with our work as the methods used are different.

Vardar-Ünlü *et al.* (2008) described the antimicrobial activity of poplar-type propolis and *Populus* buds resin, with similar composition and high antibacterial effect against Gram-positive bacteria. The chemical composition of the poplar-type propolis is very different than that of Bolivian propolis. Bolivian propolis was more active against Gram-negative bacteria. Lopez *et al.* (2015) found that Brazilian red propolis displayed a better activity against most Gram-negative bacteria with MIC in the range between 6·25–500 $\mu g\ ml^{-1}$, but red propolis presents isoflavonoids in its composition.

Antiprotozoal activity

The antiprotozoal effect of Bolivian propolis was assessed towards promastigotes of *Leishmania amazonensis* and *L. brasiliensis*. The results are summarized in Table 1. The most active samples against both *Leishmania* species were from Tarija and Cochabamba (Chapare), with IC_{50} values of 12·1 and 7·8, 8·0 and 10·9 $\mu g\ ml^{-1}$ against *L. amazonensis* and *L. brasiliensis* respectively. The propolis from Cochabamba (Valle Alto) and Chuquisaca (Sucre) showed some selectivity against *L. amazonensis* (IC_{50} values of 9·7 and 13·9 $\mu g\ ml^{-1}$ respectively). For the same samples, the IC_{50} values for *L. brasiliensis* were 59·9 and 39·2 $\mu g\ ml^{-1}$

respectively. The Santa Cruz (Okinawa) sample presented the best selectivity against *L. brasiliensis* (IC_{50} values of 14·8 μg ml^{-1} against *L. brasiliensis* and 54·5 μg ml^{-1} towards *L. amazonensis*).

Propolis from Turkey reduced proliferation of *L. tropica* promastigotes at concentrations higher than 250 μg ml^{-1} (Duran *et al.* 2008). The sample was rich in diterpenes, sesquiterpenes and fatty acids. Brazilian green propolis from the Minas Gerais State, contain as main compounds artepillin C, drupanin, caffeic acid, *p*-coumaric acid and flavonoids (Pontin *et al.* 2008). The Bolivian phenolic-rich propolis contains artepillin C and drupanin in different ratios but with much higher drupanin than the Brazilian samples. In addition, Bolivian propolis presents a more complex flavonoid composition. *In vitro* assays of the Brazilian green propolis showed IC_{50} values of 18·13 μg ml^{-1} against promastigote forms of *L. brasiliensis*. The value is higher than that observed for three of the most active antiprotozoal Bolivian samples which were in the range 7·8–14·8 μg ml^{-1} towards the M2904 strain of *L. brasiliensis*. Propolis from Sao Paulo State, Brazil, showed antiproliferative activity on *L. brazilensis* promastigotes at 100 μg ml^{-1}. Its botanical source was the Asteraceae *Baccharis dracunculifolia*, and the chemical composition includes phenolic compounds, diterpenes, triterpenes and essential oils (da Silva *et al.* 2013).

Santana *et al.* (2014) reported antileishmanial activity of brown propolis from the semi-arid region of Brazil. This propolis showed growth inhibition of *L. amazonensis* promastigotes with IC_{50} values ranging from 4·96 to 36·95 μg ml^{-1}, depending on the extraction solvent of the samples.

The chemical composition of the Bolivian propolis investigated has been recently reported (Nina *et al.* 2015). Samples with high antibacterial activity from Cochabamba (Valle Alto), Chuquisaca and Tarija showed mainly phenolic compounds while propolis containing mainly triterpenes were weakly active or inactive. It has been reported that the antibacterial activity of propolis is due to a high content of phenolics and flavonoids, suggesting a possible synergy between naturally occurring flavonoids and other antibacterial agents for its effect (Kujumgiev *et al.* 1999; Días *et al.* 2012). The phenolic-rich propolis were the most active antiprotozoal samples. When the antileishmanial activity is associated with geographic origin, the most promising samples were those from the eastern Andean slopes of Cochabamba and Tarija. Highest effect against *L. amazonensis* was for samples from Cochabamba, Chuquisaca and Tarija, collected between the Andes highland and the tropical biome. The propolis from Cochabamba, Tarija and Santa Cruz (Okinawa) with better effect against *L. brasiliensis* includes two samples from the valley and one from the eastern tropical zone. This information is relevant when looking for the botanical sources of propolis.

The phenolics identified in the active Bolivian propolis comprised caffeoylquinic acids, cinnamic- and *p*-coumaric acid derivatives, the prenylcoumaric compounds drupanin, artepillin C and baccharin, flavonoids and ellagic acid. The prenylcoumaric acid derivatives, drupanin and artepillin C, were reported as constituents of *Baccharis grisebachii*, and were evaluated against *Staph. aureus* and *E. coli* (Feresin *et al.* 2003). The effect observed was weak with MICs values equal to 250 μg ml^{-1} against *Staph. aureus* methicillin-sensitive and methicillin-resistant and >250 μg ml^{-1} for *E. coli*. Two Portuguese propolis were evaluated against *Staph. aureus* and showed MIC_{50} values of 24·6 and 25·7 μg ml^{-1} respectively. The same samples showed IC_{50} values of 8·1 μg ml^{-1} towards *L. infantum* amastigotes (Falcão *et al.* 2014). The Portuguese propolis contained methyl ethers of kaempferol and quercetin but not prenylcoumaric acid derivatives.

It has been reported that poplar-type propolis is more active against Gram-positive bacteria and present as main compounds flavonoids such as pinobanksin, naringenin, quercetin, galangin, pinocembrin and chrysin as well as esters of phenolic acids (Vardar-Ünlü *et al.* 2008). According to Velazquez *et al.* (2007), caffeic acid phenethyl ester (CAPE), a propolis constituent, has very high growth-inhibitory activity towards Gram-positive bacteria, particularly against *Staph. aureus* (MIC = 0·1 mmol l^{-1}). However, Bolivian propolis presents different selectivity against bacteria, and shows distinctive chemical profiles (Table 1).

Constituents of Bolivian propolis kaempferol-3-methyl ether, kaempferol-7-methyl ether, drupanin and lupeol were assessed against the same panel of micro-organisms in concentrations ranging from 5 to 50 μg ml^{-1} to determine its MIC values (Table 1). Compounds with MIC values >50 μg ml^{-1} were considered inactive. The most active single constituent in this study was the flavonol kaempferol-3-methyl ether, with MICs of 25 μg ml^{-1} against *Pseudomonas* sp., *Yersinia enterocolitica*, *Salmonella* sp. and *Pr. mirabilis*. The same compound presented a MIC value equal to 50 μg ml^{-1} against four Gram-negative strains of *E. coli* and *Salmonella enteritidis* and the methicillin-sensitive and methicillin-resistant *Staph. aureus*. Drupanin showed a selective effect against *E. coli* 121 (MIC value of 12·5 μg ml^{-1}). However, the effect of the crude propolis against micro-organisms cannot be explained as the effect of a single constituent, and is probably associated to synergistic effect. Our findings encourages further work on the relation of native flora and propolis composition as well as the need for standardization of this natural product, used for the same

purposes regardless of the chemical composition and bioactivity.

Material and methods

Chemicals and reagents

Dimethyl sulfoxide (DMSO), phenazine methosulphate (PMS), 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT), phosphate-buffered saline (PBS) and Amphotericine B were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cefotaxime was from Argentia® (Pharmaceutica, Buenos Aires, Argentina), Mueller–Hinton broth (Laboratorio Britania, Buenos Aires Argentina) and DMSO for antibacterial testing (Merck, Darmstadt, Germany).

Samples and extracts preparation

Propolis was collected from the beehives during the years 2013–2014 using propolis screens. The resinous substance was obtained by scraping the screens. The collection places were as follows, Yungas from La Paz (El Sillar 15°24′S, 67°09′W; Villa Coroico 15°27′S, 67°15′W), Cochabamba (Chapare 17°00′S, 65°40′ W; Valle Alto, 17°33′S, 65°50′W), Chuquisaca (Sucre, 19°03′S, 65°16′W), Tarija (San Andres, 21°38′S, 64°51′W, two samples) and Santa Cruz (Camiri, 20°03′S, 63°31′W, two samples and Okinawa, 17°12′S, 62°53′W). Samples of crude propolis are stored as reference material at the IIFB, La Paz, Bolivia. The same samples were used for a study on antioxidant activity and chemical profiling (Nina *et al.* 2015). The crude propolis were extracted three times with MeOH at a 1 : 10 w/v ratio at room temperature. After filtration and concentration at reduced pressure, extracts were lyophilized for assays. The main compounds isolated from the propolis were assessed for antimicrobial effect.

Antibacterial activity

The antibacterial activity of the extracts and compounds was assessed against Gram (+): methicillin-sensitive *Staph. aureus* ATCC 25923 (American Type Culture Collection, Manassas, Virginia, US), methicillin-resistant *Staph. aureus* ATCC 43300, and Gram (−): *Escherichia coli* ATCC 25922, the clinical isolated *Escherichia coli*-121, *E. coli* 122 (Laboratorio Hospital Marcial Quiroga, San Juan, Argentina) (LHMQ), *E. coli* LM2 (LM: Laboratorio de Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina), *Salm. enteritidis* MI (MI-Instituto Malbrán, Buenos Aires, Argentina), *Salmonella* sp. (LM), *Yersinia enterocolítica*-PI (PI: Pasteur Institute); *Pseudomonas* sp. and *Pr. mirabilis* 94-2 (LHMQ). Bacteria were grown on Mueller–Hinton broth. The minimal inhibitory concentration (MIC) values were determined using the micro-broth dilution method according to the protocols of the CLSI (2012). All tests were performed in Mueller–Hinton broth, and cultures of each strain were prepared overnight. Microorganism suspensions were adjusted in a spectrophotometer with sterile physiological solution to give a final organism density of 0·5 Mc Farland scale ($1–5 \times 10^5$ CFU ml^{-1}). Stock solutions of extracts in DMSO were diluted to give serial twofold dilutions that were added to each medium to obtain final concentrations ranging from 16 to 1000 μg ml^{-1}. The final concentration of DMSO in the assay did not exceed 1%. Cefotaxime was included in the assays as positive control. The plates were incubated for 24 h at 37°C. Activity was evaluated at 620 nm using a Multiskan FC instrument (Thermo Scientific, Waltham, MA, USA). The MIC values were defined as the lowest extract/compound concentrations showing no bacterial growth after the incubation time. The assay were done in triplicate and repeated in two independent experiments.

Antileishmanial activity

The effect of the different propolis towards *Leishmania* was assessed according to Domínguez-Carmona *et al.* (2010) and Williams *et al.* (2003) with some modifications. The activity was measured on *in vitro* cultures of promastigote forms of *Leishmania amazonensis* (clon 1: Lma, MHOM/BR/76/LTB-012) and *L. braziliensis* (strain M2904 C192 RJA), cultivated at 26°C in Schneider medium (pH 6·8) supplemented with inactivated (56°C × 30 min) calf bovine serum (10%). Propolis samples were dissolved in DMSO (maximal final concentration 1%). Parasites in logarithmic phase of growth, at a concentration of 1×10^6 parasites ml^{-1}, were distributed on a 96 micro-well plates and different concentrations of the propolis (100, 25, 5 and 1 μg ml^{-1}) were added. The micro-well plates were incubated for 72 h at 26°C. After incubation, a solution of XTT (1 mg ml^{-1}) in PBS (pH 7·0 at 37°C) with PMS (0·06 mg ml^{-1}) was added (50 μl/well) and incubated again for 4 h at 26°C. Amphotericin B (0·5 μg ml^{-1}) was used as reference drug as well as a crude alkaloid extract (CTA) of the Bolivian plant *Galipea longiflora* (Calla-Magariños *et al.* 2009, 2013). Assays were carried out in triplicate. Optical density of each well was obtained using a Synergy HT microplate reader (Biotek, Winooski, VT, USA) employing 450-nm test wavelength and 650 nm as reference filter. The analyses of data were performed according to Williams *et al.* (2003). The IC_{50} values were calculated using The Gen5 program (Biotek) and expressed as IC_{50} in μg ml^{-1}.

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Conflict of Interest

The authors report no conflict of interest.

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