Brazilian Brown Propolis as a Natural Source against Leishmania amazonensis

Authors : Victor Pena Ribeiro, Caroline Arruda, Jennyfer Andrea Aldana Mejia, Jairo Kenupp Bastos Abstract : Leishmaniasis is a serious health problem around the world. The treatment of infected individuals with pentavalent antimonial drugs is the main therapeutic strategy. However, they present high toxicity and persistence side effects. Therefore, the discovery of new and safe natural-derived therapeutic agents against leishmaniasis is important. Propolis is a resin of viscous consistency produced by Apis mellifera bees from parts of plants. The main types of Brazilian propolis are green, red, yellow and brown. Thus, the aim of this work was to investigate the chemical composition and leishmanicidal properties of a brown propolis (BP). For this purpose, the hydroalcoholic crude extract of BP was obtained and was fractionated by liquidliquid chromatography. The chemical profile of the extract and its fractions were obtained by HPLC-UV-DAD. The fractions were submitted to preparative HPLC chromatography for isolation of the major compounds of each fraction. They were analyzed by NMR for structural determination. The volatile compounds were obtained by hydrodistillation and identified by GC/MS. Promastigote forms of Leishmania amazonensis were cultivated in M199 medium and then 2×106 parasites.mL-1 were incubated in 96-well microtiter plates with the samples. The BP was dissolved in dimethyl sulfoxide (DMSO) and diluted into the medium, to give final concentrations of 1.56, 3.12, 6.25, 12.5, 25 and 50 µg.mL⁻¹. The plates were incubated at 25°C for 24 h, and the lysis percentage was determined by using a Neubauer chamber. The bioassays were performed in triplicate, using a medium with 0.5% DMSO as a negative control and amphotericin B as a positive control. The leishimnicidal effect against promastigote forms was also evaluated at the same concentrations. Cytotoxicity experiments also were performed in 96-well plates against normal (CHO-k1) and tumor cell lines (AGP01 and HeLa) using XTT colorimetric method. Phenolic compounds, flavonoids, and terpenoids were identified in brown propolis. The major compounds were identified as follows: p-coumaric acid (24.6%) for a methanolic fraction, Artepelin-C (29.2%) for ethyl acetate fraction and the compounds of hexane fraction are in the process of structural elucidation. The major volatile compounds identified were β -caryophyllene (10.9%), germacrene D (9.7%), nerolidol (10.8%) and spathulenol (8.5%). The propolis did not show cytotoxicity against normal cell lines (CHO) with $IC_{50} > 100 \ \mu g.mL^{-1}$, whereas the $IC_{50} < 10 \ \mu g.mL^{-1}$ showed a potential against the AGP01 cell line, propolis did not demonstrate cytotoxicity against HeLa cell lines $IC_{50} > 100 \mu g.mL^{-1}$. In the determination of the leishmanicidal activity, the highest (50 μ g.mL⁻¹) and lowest (1.56 μ g.mL⁻¹) concentrations of the crude extract caused the lysis of 76% and 45% of promastigote forms of L. amazonensis, respectively. To the amastigote form, the highest (50 μ g.mL⁻¹) and lowest (1.56 μ g.mL⁻¹) concentrations caused the mortality of 89% and 75% of L. amazonensis, respectively. The IC₅₀ was 2.8 μ g.mL⁻¹ to amastigote form and 3.9 µg.mL⁻¹ to promastigote form, showing a promising activity against Leishmania amazonensis. **Keywords** : amastigote, brown propolis, cytotoxicity, promastigote

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