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Antimicrobial, antioxidant and cytotoxic activities of propolis from *Melipona orbignyi* (Hymenoptera, Apidae)

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ABSTRACT

Propolis from stingless bees is well known for its biologic properties; however, few studies have demonstrated these effects. Therefore, this study aimed to investigate the chemical composition and antimicrobial, antioxidant and cytotoxic activities of propolis from the stingless bee *Melipona orbignyi*, found in Mato Grosso do Sul, Brazil. The chemical composition of the ethanol extract of propolis (EEP) indicated the presence of aromatic acids, phenolic compounds, alcohols, terpenes and sugars. The EEP was active against the bacterium *Staphylococcus aureus* and the fungus *Candida albicans*. The EEP showed antioxidant activity by scavenging free radicals and inhibiting hemolysis and lipid peroxidation in human erythrocytes incubated with an oxidizing agent. Additionally, EEP promoted cytotoxic activity and primarily necrotic death in K562 erythroleukemia cells. Taken together, these results indicate that propolis from *M. orbignyi* has therapeutic potential for the treatment and/or prevention of diseases related to microorganism activity, oxidative stress and tumor cell proliferation.

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1. Introduction

Propolis is a natural product produced by bees, resulting from the addition of mandibular secretions to resins collected from different plant parts (Bankova et al., 2000; Teixeira et al., 2005). In the hive, propolis is used to narrow nest entrances, seal gaps and embalm dead organisms inside the hive, thus preventing decomposition and spreading of odors (Simone-Finstrom and Spivak, 2010).

Propolis has a very broad chemical diversity because its composition varies with the site of collection, plant materials and producing species of bee (Bankova et al., 2000; Liberio et al., 2011). Several species of bees produce propolis, including *Apis mellifera* and the tribe Meliponini, which are known as stingless bees (Velikova et al., 2000a; Bankova and Popova, 2007).

Products generated by stingless bees, including honey, beehive pollen and propolis, are well known for their nutritional and therapeutic properties (Choudhari et al., 2012). Propolis from stingless bees stands out among these products, and its antimicrobial (Velikova et al., 2000a; Farnesi et al., 2009; Choudhari et al., 2012), antioxidant (Sawaya et al., 2009) and antitumor (Franchi et al., 2012; Choudhari et al., 2013) activities have been described. Despite this, the majority of scientific reports that have described the chemical composition and biological activities of propolis were conducted with the species *A. mellifera* (Bankova et al., 2000; Pereira et al., 2003).

There are more than 200 species of stingless bees in Brazil (Velikova et al., 2000a), but studies on most of them are scarce. Of these, the species *Melipona* (*M.*) *orbignyi*, locally known as '*mandurí-de-Mato-Grosso*', is distributed throughout Argentina, Bolivia, Paraguay and Brazil. In Brazil, this species is restricted to the states of Mato Grosso and Mato Grosso do Sul (Camargo and Pedro, 2008). However, there have been no scientific reports regarding the

Abbreviations: AAPH, 2,2′-Azobis-(2-amidinopropane) dihydrochloride; *A. Mellifera*, *Apis mellifera*; BSTFA, bis-(trimethylsilyl) trifluoroacetamide; CAT, catalase; EEP, ethanol extract of propolis; *E. coli*, bacterium *Staphylococcus aureus*; *C. albicans*, fungus *Candida albicans*; FBS, fetal bovine serum; *M. orbignyi*, *Melipona orbignyi*; GAE, gallic acid equivalents; QE, quercetin equivalents; GSH-px, glutathione peroxidase; MIC, minimum inhibitory concentration; CFU, colony-forming units; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MDA, malondialdehyde; PI, propidium iodide; IC_{50}, half-maximal inhibitory concentration; SOD, enzymes superoxide dismutase; TBA, thiobarbituric acid; TMCS, trimethylchlorosilane.

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therapeutic properties of propolis from this species, and only some of its behavioral aspects have been described (Brizola-Bonacina et al., 2009). Therefore, this study describes for the first time the chemical composition and antimicrobial, antioxidant and cytotoxic properties of propolis produced by the stingless bee *M. orbignyi*.

2. Materials and methods

2.1. Preparation of the ethanol extract of propolis (EEP)

Propolis samples from *M. orbignyi* bees were collected from the state of Mato Grosso do Sul, in the Midwest region of Brazil. For this, one colony was identified and four collected of propolis were performed with approximately 3.8 g each. The ethanol extract of propolis (EEP) was prepared with 4.5 mL of 80% ethanol per 1 g of propolis, kept in a water bath at 70 °C in a sealed container until total dissolution and subsequently filtered to obtain the EEP (Alencar et al., 2007).

2.2. Determination of the polyphenol and total flavonoid levels

The total polyphenol concentration in the EEP was determined according to the Folin–Ciocalteu colorimetric method (Meda et al., 2005). For this purpose, 0.5 mL of EEP (100 μg/mL) was mixed with 2.5 mL of Folin–Ciocalteu reagent and 2.0 mL of 14% sodium carbonate (Na_2CO_3). The absorbance was read at 760 nm after a 2-h incubation at room temperature in the dark. Gallic acid (0.4–11 μg/mL) was used as a standard to produce a calibration curve. The average of 3 readings was used to determine the total polyphenol content, which was expressed as the mg of gallic acid equivalents (GAE)/100 g of propolis.

The flavonoid content in EEP was determined according to the method described by Liberio et al. (2011), with minor modifications. For this purpose, 0.5 mL of EEP (100 μg/mL) was mixed with 4.5 mL of 2% aluminum chloride hexahydrate ($AlCl_3{\cdot}6H_20$) in methanol. The absorbance was read at 415 nm after 30-min incubation at room temperature in the dark. Quercetin (0.4–11 μg/mL) was used as a standard to produce a calibration curve. The average of 3 readings was used to determine the flavonoid content, which was expressed as the mg of quercetin equivalents (QE)/100 g of propolis.

2.3. Chemical analysis

For this purpose, 1 mg of dry extract was reacted with 50 μl pyridine + 100 μl bis-(trimethylsilyl) trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) in a sealed glass tube for 30 min at 100 °C to prepare samples for gas chromatography (Greenaway et al., 1988). Sample volumes of 1 μl were injected and analyzed by GC–MS. The analysis was performed by capillary GC–MS. The GC–MS analyses were performed on a gas chromatograph (GC-17A, Shimadzu, Kyoto, Japan) equipped with mass spectrometer detector (QP 5050a), using DB-5 (J & W, 5% de phenyl-dimethylpolysiloxane), fused-silica capillary column (30 m in length × 0.25 mm i.d., 0.25 μm film thickness), under the following conditions: carrier gas helium (99.999% and flow rate 1.0 mL min^{-1}); 1 μL injection volume, split ratio (1:10), with initial oven temperature of 85 °C and heating from 85° to 315 °C at 4 °C min^{-1}. The injector temperature was 280 °C and quadrupole detector temperature was 315 °C. The MS scan parameters included electron impact ionization voltage at 70 eV, a mass range of 45–600 m/z and a scan interval of 0.5 s. Temperature-programmed retention indices (Zhao et al., 2005) were calculated using a mixture of normal paraffin (C_8–C_{30}) as external references. The identifications were completed by comparing the mass spectra obtained in the QP5050 Libraries. In some cases, when identified spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. If possible reference compounds were co-chromatographed to confirm GC retention times.

2.4. Antimicrobial activity

To determine the minimum inhibitory concentration (MIC) of EEP against the gram-negative bacterium *Escherichia coli* (ATCC8739), the gram-positive bacterium *Staphylococcus aureus* (ATCC25923) and the fungus *Candida albicans* (ATCC10231), a microdilution assay was performed according to the methods of Hammer et al. (1998) and the Clinical and Laboratory Standards Institute (CLSI, 2007). Briefly, 96-well plates were filled with Mueller–Hinton broth, and a serial dilution of the extract (0.78–100 mg/mL) was performed. Aliquots of different microbial inocula (1.5×10^8 colony-forming units (CFU)/mL) were added to the respective plates. The assays were performed in triplicate for each microorganism. The absorbances were read at 620 nm in a spectrophotometer (Thermo TP-Plate reader; Thermo Fisher Scientific, Inc., Waltham, MA, USA) after a 24-h incubation at 37 °C. A sample of 80% ethanol (final concentration: 0.3%) was used as a control. MIC was defined as the lowest concentration of EEP that could stabilize or reduce the growth of the inoculum.

To determine the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of EEP, 10-μl aliquots were collected from the wells corresponding to the MIC and the two concentrations exceeding the MIC and were subcultured on Mueller–Hinton agar. MBC and MFC were defined as the lowest concentration that resulted in the death of 99.9% of the inoculum. Three independent experiments were performed in triplicate for each EEP concentration.

2.5. Antioxidant activity

2.5.1. DPPH free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated according to the method described by Gupta and Gupta (2011), with minor modifications. Briefly, 200 μL of extract (0.1–200 μg/mL) were mixed with 1.8 mL of 0.11 mM DPPH in an 80% ethanol solution. The mixture was homogenized and incubated at room temperature in the dark for 30 min, and the absorbance was read at 517 nm on a spectrophotometer. Ascorbic acid was used as a reference antioxidant. The tests were performed in duplicate in 3 independent experiments. The percentage inhibition was calculated from the control with the following equation:

$$\text{Scavenging activity}(\%) = (1 - \text{Absorbance}_{\text{sample}}/\text{Absorbance}_{\text{control}}) \times 100$$

2.5.2. Antioxidant assay using a human erythrocyte model

2.5.2.1. Preparation of erythrocyte suspensions. Following approval by the Research Ethics Committee (Comitê de Ética em Pesquisa; CEP) of the University Center of Grande Dourados (Centro Universitário da Grande Dourados; UNIGRAN), Brazil (CEP process number 123/12), 5 mL of peripheral blood were collected from healthy donors into sodium citrate-containing tubes and were subsequently centrifuged at 2000 rpm for 5 min. After centrifugation, the blood plasma and leukocyte layers were discarded, and the erythrocytes were washed 3 times with saline solution and centrifuged at 3000 rpm for 5 min. Finally, 2.5% or 5% hematocrit erythrocyte suspensions were prepared in saline.

2.5.2.2. Oxidative hemolysis inhibition assay. The protective effect of the propolis extract was evaluated according to the method described by Valente et al. (2011), with minor modifications. The assays were conducted with erythrocyte suspensions (2.5% hematocrit). The erythrocytes were preincubated at 37 °C for 30 min in the presence of different concentrations of EEP or ascorbic acid (50–125 μg/mL), after which a 50 mM 2,2′-Azobis-(2-amidinopropane) dihydrochloride (AAPH) solution was added. This mixture was incubated at 37 °C for 4 h with periodical stirring. Erythrocytes incubated with 1% ethanol or ascorbic acid were used as the negative and positive controls, respectively. Hemolysis was determined spectrophotometrically after every 60 min of sample incubation for a total of 240 min; specifically, aliquots were removed, diluted in saline and centrifuged at 3600 rpm for 10 min, after which the absorbance of the supernatant was read.

The percentage hemolysis in the supernatants was measured with the formula A/B × 100, where (*A*) is the sample absorbance and (*B*) is the total hemolysis (erythrocytes with distilled water). The absorbance was read at 540 nm. Four independent experiments were performed in duplicate.

2.5.2.3. Inhibitory efficiency against lipid peroxidation. A 5% erythrocyte suspension was used to assess the protective effects of EEP against lipid peroxidation. Erythrocytes were preincubated at 37 °C for 30 min with different concentrations of EEP or ascorbic acid (50–125 μg/mL). A sample of 1% ethanol was used as a negative control. Next, 50 mM AAPH was added to the erythrocyte solution, which was then incubated at 37 °C for 2 h with periodical stirring. After this period, the samples were centrifuged at 2000 rpm for 5 min, and 500-μL aliquots of the supernatant were transferred to tubes with 1 mL of 10 nmol thiobarbituric acid (TBA). As a standard control, 500 μL of 20 mM malondialdehyde (MDA) solution were added to 1 mL of TBA. The samples were incubated at 96 °C for 45 min. The samples were then cooled, 4 mL of n-butyl alcohol were added and the samples were centrifuged at 3000 rpm for 5 min. The sample supernatants were removed, and the absorbance was read at 532 nm. Two independent experiments were performed in duplicate. MDA levels in the samples were expressed in nmol/mL, obtained with the following formula:

$$\text{MDA} = \text{Absorbance}_{\text{sample}} \times (20 \times 220.32/\text{Absorbance}_{\text{MDAstandard}})$$

2.6. Cytotoxic activity and cell death profile

The K562 erythroleukemia cell line was grown in suspension in RPMI 1640 media (Cultilab, Campinas, São Paulo, Brazil) supplemented with 10% fetal bovine serum (FBS; Cultilab), 100 U/mL of penicillin and 100 μg/mL of streptomycin in a humidified atmosphere at 37 °C in 5% CO_2. The cytotoxic activity and cell death profile were evaluated according to the method described by Paredes-Gamero et al. (2012), with minor modifications. Cells were seeded into 96-well plates (10^5 cells/mL) and cultured in medium with 10% FBS in the absence or presence of EEP (31–500 μg/mL) for 24 h. After this period, the K562 cells were washed with PBS and resuspended in annexin-labeling buffer (0.01 M HEPES, pH 7.4, 0.14 M NaCl

Table 1
Chemical composition of EEP from *M. orbignyi* (% of TIC: total ion current, GC–MS).*

Compounds	% TIC
Aromatic acid	
Benzoic acids	11.6
Phenolic acid	
Dihydrocinnamic acids	1.5
Cinnamic acids	3.2
Phenyl, benzyl, long chain caffeates	2.9
C-prenylated coumarinic acids	2.8
Alcohols and terpenes	
Diterpenic acids	24.4
Triterpenic alcohols	10.7
Others	
Sugars	1.6

* The generated ion current depends on the characteristics of the compounds.

Table 2
MIC and MBC/MFC values for EEP from *M. orbignyi*.

	EEP
MIC	
Staphylococcus aureus	3.1
Escherichia coli	–
Candida albicans	3.1
MBC/MFC	
Staphylococcus aureus	3.1
Candida albicans	50.0

The MIC and MBC/MFC are expressed in mg/ml; (–) absence of inhibition.

and 2.5 mM $CaCl_2$). The suspensions were stained with annexin-FITC and propidium iodide (PI) (Becton Dickinson, Franklin Lakes, NJ, USA), according to the manufacturer's instructions. The cells were incubated at room temperature for 15 min. Ten thousand events were collected per sample, and the analyses were performed on a FACSCalibur flow cytometer (Becton Dickinson) with CellQuest software (Becton Dickinson).

3. Results

3.1. Chemical composition

The total polyphenol and flavonoid concentrations present in the EEP from *M. orbignyi* were 211 ± 7.5 mg GAE/100 g of propolis and 23 ± 1.0 mg QE/100 g of propolis, respectively. The components of the *M. orbignyi* EEP have been identified and are listed in Table 1. The major components were aromatic acids, alcohols and terpenes. The minor compounds were phenolic acids and sugars.

3.2. Antimicrobial activity

The EEP showed antimicrobial activity against the gram-positive bacterium *S. aureus* and the fungus *C. albicans*. However, it was not effective against the gram-negative bacterium *E. coli*. Among the microorganisms evaluated, EEP exhibited bactericidal activity at a concentration of 3.1 mg/mL and fungicidal activity at 50.0 mg/mL (Table 2).

3.3. Antioxidant activity

3.3.1. DPPH free radical scavenging activity

The free radical scavenging activity of EEP is shown in Table 3. The standard antioxidant, ascorbic acid, exhibited a half-maximal inhibitory concentration (IC_{50}) of 3 ± 0.4 μg/mL and a maximum inhibition of 98 ± 0.4% of the DPPH free radical at a concentration of 10 μg/mL. The EEP exhibited an IC_{50} of 40 ± 4.8 μg/mL and a maximum inhibition of 96 ± 0.6% of DPPH at a concentration of 100 μg/mL.

3.3.2. Oxidative hemolysis inhibition assay

The EEP was also evaluated for its ability to protect erythrocytes against AAPH-induced hemolysis. Ascorbic acid exhibited antihemolytic activity throughout the entire experimental period in a concentration and time-dependent manner. EEP protected erythrocytes from the action of the hemolysis-inducing agent during the first 120 min of incubation (Fig. 1). When erythrocytes were incubated only with EEP or ascorbic acid, no hemolysis was observed at the times and concentrations tested (data not shown).

3.3.3. Efficiency of EEP on the inhibition of AAPH-induced lipid peroxidation

In an assessment of the EEP with regard to its efficiency in inhibiting lipid membrane peroxidation in erythrocytes incubated with the oxidizing agent AAPH, both ascorbic acid and EEP showed reduced MDA levels at all concentrations tested when compared to erythrocytes that were incubated only with the hemolysis-inducing agent (Fig. 2).

3.4. Cytotoxic activity and cell death profile

K562 erythroleukemia cells that had been treated with different concentrations of EEP were stained with annexin V-FITC/PI to determine the cell death profiles. EEP demonstrated concentration-dependent cytotoxicity. At the highest concentration tested (500 μg/mL), there was an 83 ± 0.4% in viable cells (Fig. 3A). The most effective cytotoxic propolis concentrations, 250 and 500 μg/mL, promoted cell death by necrosis (15 ± 1 and 63 ± 2%) and secondary necrosis (12 ± 1% and 21 ± 3%), respectively, indicating a concentration-dependent activity (Fig. 3B and C).

4. Discussion

This study determined the chemical composition and biological activities of *M. orbignyi* propolis, a compound popularly used for its medicinal properties in the absence of scientific evidence, which has experienced strong anthropic pressures in recent years.

Brazilian propolis, produced by stingless bees, is described as having a complex chemical composition that includes terpenes (monoterpenes, diterpenes, triterpenes and sesquiterpenes), phenolic compounds and carbohydrates (Velikova et al., 2000a; Bankova and Popova, 2007). Its therapeutic properties are directly related to its chemical composition. Among the polyphenols, flavonoids are described as responsible for the various biological activities of propolis, especially its antimicrobial action (Marcucci, 1995). Additionally, the diterpenic acid identified in *M. orbigny*

Table 3
DPPH free radical scavenging activity (%) of EEP at different concentrations (μg/ml) and IC_{50} values.

Sample	0.1	1	5	10	25	50	75	100	200	IC_{50}
Ascorbic acid	6 ± 2.6	12 ± 2.4	69 ± 3.2	98 ± 0.4	98 ± 0.3	98 ± 0.5	98 ± 0.4	97 ± 0.4	96 ± 0.4	3 ± 0.4
EEP	4 ± 1.1	3 ± 1.1	9 ± 1.5	17 ± 1.1	43 ± 2.6	75 ± 3.5	91 ± 1.3	96 ± 0.6	95 ± 0.2	40 ± 4.8

Values are shown as means ± SEM (n = 3).

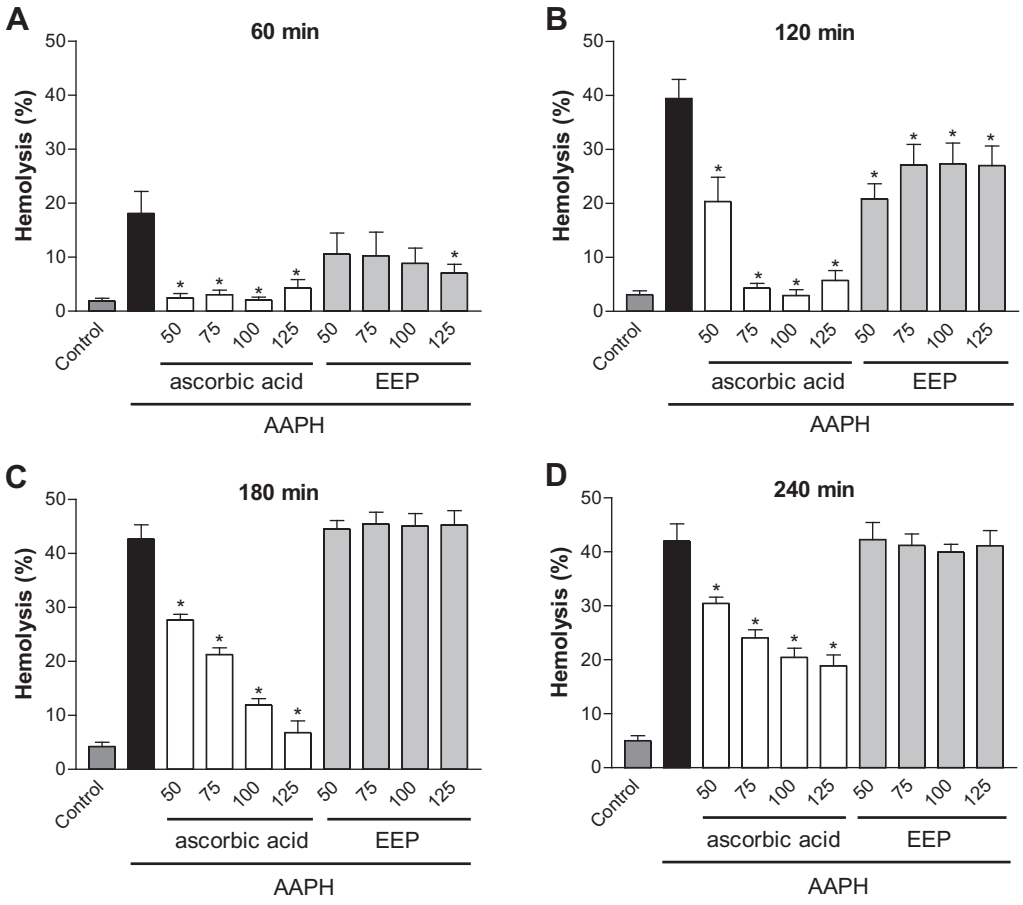

Fig. 1. Effects of different ascorbic acid (control antioxidant) and *M. orbignyi* EEP concentrations (50–125 μg/mL) on the AAPH-induced (50 mM) hemolysis of human erythrocytes. Erythrocytes were measured at (A) 60 min, (B) 120 min, (C) 180 min and (D) 240 min of incubation. The control represents erythrocytes incubated with vehicle only (ethanol without AAPH). Values are expressed as the mean ± SEM of 4 independent experiments conducted in duplicate. *Represents statistically significant results ($p < 0.05$) of a comparison between the treated group and the AAPH group (erythrocytes incubated only with oxidizing agent) at the respective times.

EEP was also described as the main antibacterial compound in Brazilian propolis from the stingless bee *Melipona quadrifasciata anthidioides* (Velikova et al., 2000b). The observed antimicrobial activities against the fungus *C. albicans* and gram-positive bacterium *S. aureus* indicated the antimicrobial potential of *M. orbigny* EEP, a result similar to that found in other stingless bee species and the species *A. mellifera*, also found in Brazil (Kujumgiev et al., 1999; Bankova and Popova, 2007).

The antioxidant properties of *M. orbignyi* EEP were evaluated for their ability to scavenge free radicals and protect against damage caused by oxidizing agents. The free radicals scavenging ability demonstrated by the *M. orbigny* propolis was similar to the results observed for propolis from the bee species *A. mellifera* (Lu et al., 2003; Mercan et al., 2006) and for certain plant extracts (Khan et al., 2013; Khatoon et al., 2013).

EEP exhibited anti-hemolytic action and protective actions against lipid peroxidation when incubated with human erythrocytes in the presence of an oxidizing agent. Phenolic compounds have been reported to be important antioxidants that act as hemolysis inhibitors in erythrocytes under conditions of oxidative stress (Asgary et al., 2005; Valente et al., 2011). The antioxidant role of polyphenols results from the donation of hydrogen atoms from an aromatic hydroxyl group to the free radical, leading to stabilization of the radical (Duthie et al., 2003).

In addition to electron donation from compounds present in propolis, this natural product can also act as an antioxidant by stimulating antioxidant enzyme activities in erythrocytes (Valente

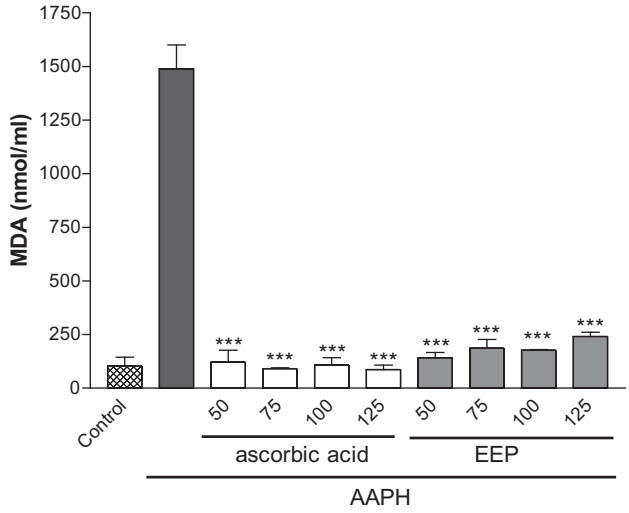

Fig. 2. Effects of different ascorbic acid (standard antioxidant) and *M. orbignyi* EEP concentrations (50–125 μg/mL) on AAPH-induced (50 mM) lipid peroxidation. The control represents erythrocytes incubated with vehicle only (ethanol without AAPH). Values are expressed as the mean ± SEM of 2 independent experiments conducted in duplicate. ***Represents statistically significant results ($p < 0.001$) when the treated group was compared to the AAPH group (erythrocytes incubated only with oxidizing agent).

et al., 2011). Zhu et al. (2011) evaluated the effects of Chinese and Brazilian propolis in diabetic rats and found that Brazilian propolis increased the levels of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-px) and catalase (CAT).

The antioxidant capacity of a compound can assist in the prevention of diseases related to oxidative stress, which is caused by an imbalance between the formation and neutralization of free radicals in the body through enzymatic and non-enzymatic antioxidants (Fang et al., 2002). An excess of free radicals in the body can result in cell membrane phospholipid oxidation, DNA and protein damage and tissue injury. Diabetes, cardiovascular disorders, atherosclerosis, rheumatoid arthritis, neurodegeneration and cancer are included among oxidative stress-related diseases (Fang et al., 2002; Zhu et al., 2011). Among these, cancer is the leading cause of death worldwide, accounting for 7.6 million deaths in 2008 or 13% of all deaths recorded (World Health Organization, 2013). The search for new drugs that could act as chemotherapeutic agents has increased in recent years, especially for drugs derived from natural products, as these are expected to have low side effects (Castaldo and Capasso, 2002). Thus, propolis has been the subject of many studies and has demonstrated satisfactory results, as evidenced by its antitumor activity in several cell lines (Watanabe et al., 2011; Franchi et al., 2012).

Therefore, this study sought to elucidate the cytotoxic activity of *M. orbignyi* EEP against the human K562 erythroleukemia cell line; these cells showed a decrease in viability when in contact

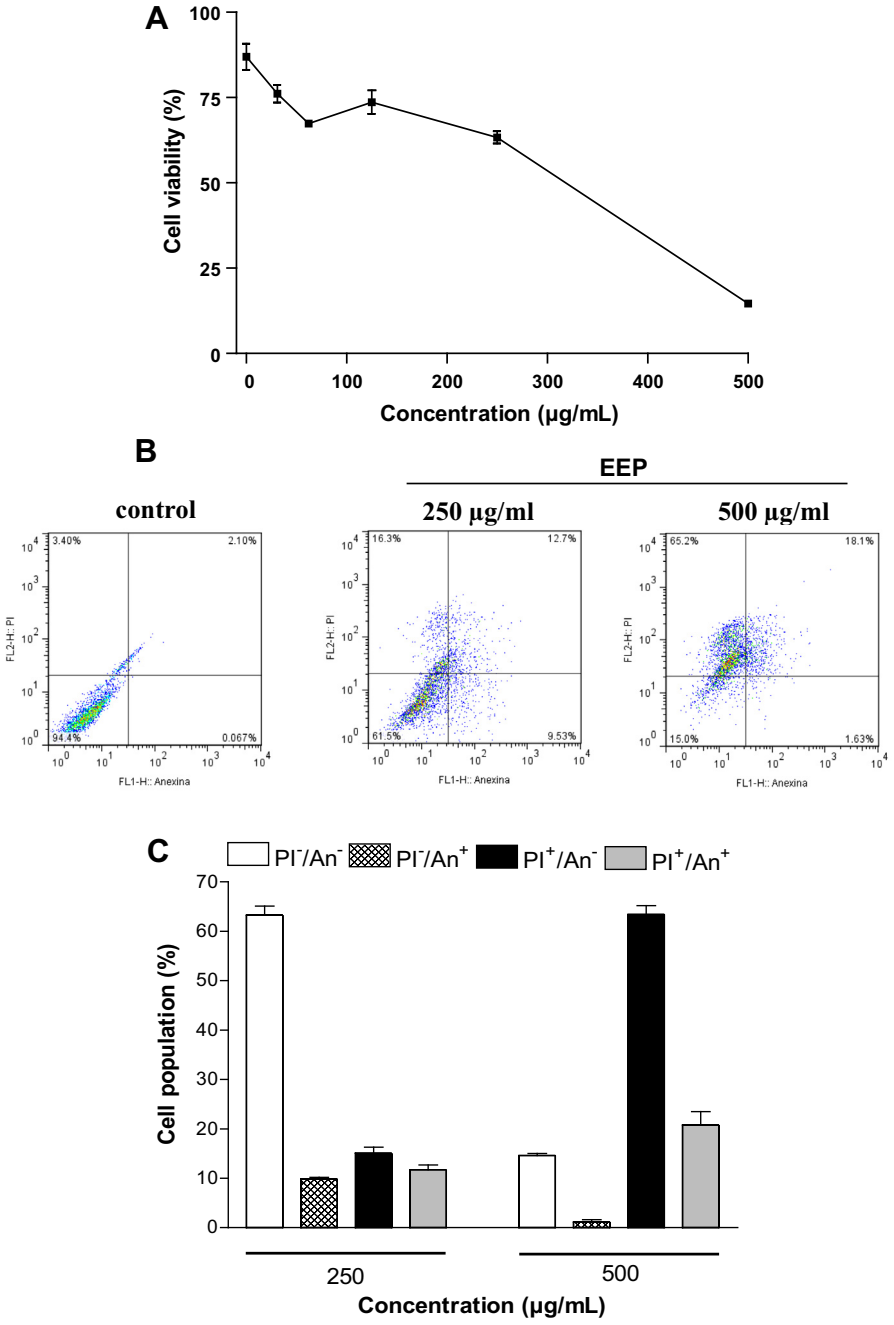

Fig. 3. Cytotoxic activity of *M. orbignyi* EEP against the K562 erythroleukemia cell line. (A) EEP concentration–response curve in K562 cells. (B) Representative flow cytometry diagrams of annexin V-FITC/PI-stained cells: left lower quadrant (PI^-An^-) represents viable cells, right lower (PI^-An^+) represents apoptotic cells, upper left (PI^+An^-) represents necrotic cells and upper right (PI^+An^+) represents cells in secondary necrosis. (C) Frequencies of cell death profiles obtained from the corresponding diagrams at concentrations of 250 and 500 µg/mL.

with the tested EEP, despite being resistant to some chemotherapeutic agents (Barbosa et al., 2006). The main compounds responsible for the anti-tumor activity of propolis include flavonoids, terpenes and caffeic acid phenethyl ester, and this activity could be attributed to synergism between the substances present in the resin (Valente et al., 2011; Watanabe et al., 2011).

While investigating the cell death profile promoted by EEP, evidence showed a predominantly necrotic death. This type of cell death is interesting with regard to therapy against tumor cells that are resistant to apoptotic cell death, which usually occurs with the use of conventional chemotherapy (Gu et al., 2012). Chemotherapeutic drug resistant cells present a major challenge for cancer treatment, and the discovery of new compounds that have different mechanisms of action is extremely important, as these compounds could stimulate the pathway of necrotic cell death (Han et al., 2007).

Together, these results showed that propolis from the stingless bee *M. obignyi* possesses broad biological activity, indicating that this natural product exhibits promise for the treatment and/or prevention of various diseases related to microorganisms, oxidative stress and tumor cell proliferation.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fct.2014.01.008.

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