

Chemical and cytotoxic analyses of three varieties of Brazilian propolis (green propolis, jataí propolis and brown propolis) and its anti-*Sporothrix brasiliensis* in vitro activity

[Análises químicas e citotóxicas de três variedades de própolis brasileiras (própolis verde, própolis jataí e própolis marrom) e sua atividade in vitro anti-*Sporothrix brasiliensis*]

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ABSTRACT

In this study, we described the antifungal activity of three Brazilian propolis extracts: brown, green and from jataí bees against *Sporothrix brasiliensis*. The extracts were obtained from ethanolic extraction and their chemical composition was determined by high-performance liquid chromatography coupled to mass spectrometry. The cellular toxicity was measured in MDBK (Madin-Darby Bovine Kidney) cells and quantified by the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H bromine tetrazolato). For antifungal activity, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined by broth microdilution. The results showed that cell toxicity was not observed at lower concentrations (0.097 to 0.39 µg/ml) for all extracts in comparison to cell control. Among the chemical compounds identified, caffeic acid, p-coumaric acid, chlorogenic acid, ferulic acid and rutin were quantified. In antifungal activity, green and jataí did not exhibit activity against the isolates (MIC and MFC greater than 0.78 mg/ml). However, all isolates of *S. brasiliensis* were sensitive to brown propolis (MIC of 0.09 to 0.78 mg/ml), including the standard strain (P<0.001). Among the Brazilian propolis studied, the brown propolis showed activity against the *S. brasiliensis* isolates and more studies should be undertaken in order to evaluate its promising use in the treatment of sporotrichosis.

Keywords: zoonosis, itraconazole, antifungal resistance, therapeutic alternative

RESUMO

Neste estudo, descreveu-se a atividade antifúngica de três extratos de própolis brasileiras: marrom, verde e de abelhas jataí (*Tetragonisca angustula*), contra *Sporothrix brasiliensis*. Os extratos foram obtidos de extração etanólica, e a sua composição química foi determinada por cromatografia líquida de alta eficiência, acoplada à espectrometria de massa. A toxicidade celular foi medida em células MDBK (*Madin-Darby Bovine Kidney*), avaliada por observação microscópica e quantificada pelo ensaio MTT (3-(4,5-dimetiltiazol-2-ilo)-2,5-difenil-2H bromo tetrazolato). Para a atividade antifúngica, determinou-se a concentração inibitória mínima (CIM) e a concentração fungicida mínima (CFM) por meio de microdiluição em caldo. Os resultados mostraram que a toxicidade celular não foi observada em concentrações menores (0,097 a 0,39 µg/ml). Entre os compostos químicos identificados, foram quantificados o ácido cafeico, ácido p-cumárico, ácido clorogênico, ácido ferúlico e a rutina. Na atividade antifúngica, as própolis verde e jataí não apresentaram atividade contra os isolados (CIM e CFM maior que 0,78 mg/ml), porém todos os isolados de *S. brasiliensis* foram sensíveis à própolis marrom (CIM de 0,09 a 0,78 mg/ml), incluindo a cepa padrão (P<0,001). Entre as própolis brasileiras estudadas, a marrom mostrou atividade contra *S. brasiliensis*, e mais estudos devem ser realizados para avaliar seu uso promissor no tratamento da esporotricose.

Palavras-chave: zoonoses, itraconazol, resistência antifúngica, alternativa terapêutica

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INTRODUCTION

Among the infectious diseases of public health importance, sporotrichosis is one of the emerging fungal infections occurring in several countries, mainly in India, Mexico, Colombia, Peru, Brazil, South Africa and Japan (Carrada-Bravo e Olvera-Macías *et al.*, 2013). The disease is characterized by nodular and ulcerative lesions limited to the subcutaneous tissue and may also involve the lymphatic system, as well as spreading to internal organs in a systemic form in severe forms of the disease, occurring especially in immunocompromised patients (Madrid *et al.*, 2012; Paixão *et al.*, 2015).

The transmission occurs through traumatic inoculation of conidia in skin tissue and may occur through the bites and scratches of sick cats (Romeo and Criseo, 2013). Pathogenic species of the *Sporothrix schenckii* complex (Marimon *et al.*, 2008) may provoke the disease, with *Sporothrix brasiliensis* being considered the most virulent species and with high prevalence in feline sporotrichosis outbreaks in Brazil (Chaves *et al.*, 2013; Rodrigues *et al.*, 2014). Several therapeutic protocols are recommended, and itraconazole is considered the antifungal of choice. However, the emergence of antifungal resistance has been reported in recent years (Marimon *et al.*, 2008; Chaves *et al.*, 2013; Stopiglia *et al.*, 2014; Waller *et al.*, 2015) which alerted researchers to the need to search for new antifungal compounds.

It is known that the use of plant extracts is one of the oldest practices of humanity and the use of traditional medicine is essential in basic health primary care in developing countries (Yigit *et al.*, 2009). Bee products such as honey, royal jelly, pollen, propolis, among others have been increasingly used in human and veterinary medicine as natural products (Bastos *et al.*, 2011). Individually, propolis stands out for its therapeutic properties, such as antimicrobial, anti-inflammatory, healing, anesthetic, and anticariogenic properties, in addition to be used in the pharmaceutical and food industries (Ortega *et al.*, 2011).

Resinous material from the collection of exudates and plant buds by bees, along with their produced enzymes and waxes (Bankova *et al.*, 2000), the propolis (from the Greek *pro*: "in

defense" and *polis*: "city") has great importance for bees since it prevents the entry of intruders into the hive and embalms dead bodies in order to avoid decomposition and the spread of odors (Simone-finstrom and Spivak, 2012). Among the present compounds, flavonoids are important for their anti-inflammatory and antimicrobial activities (Sonmez *et al.*, 2005) including against pathogenic fungi (Siqueira *et al.*, 2009).

Among the species of medical importance, *Candida albicans*, *C. krusei*, *C. glabrata*, *C. tropicalis*, *Cryptococcus neoformans*, *Malassezia pachydermatis*, *Paracoccidioides brasiliensis*, *Microsporium gypseum*, *Trichophyton rubrum*, *T. mentagrophytes* and *T. verrucosum* already have proven sensitive to propolis produced by different bees throughout the world, including Brazil (Siqueira *et al.*, 2009) Argentina (Agiéro *et al.*, 2014), United States (Chua *et al.*, 2014), Croatia (Jug *et al.*, 2014), Turkey (Çam *et al.*, 2009) and Iran (Khosravi *et al.*, 2013). The promising antimicrobial activity of propolis was also observed in fungi resistant to conventional drugs.

In sporotrichosis, antifungal resistance has been increasingly observed, including to itraconazole (Marimon *et al.*, 2008; Stopiglia *et al.*, 2014; Waller *et al.*, 2015), making therapeutic success difficult in human and animal cases. Although there are few studies regarding the control of sporotrichosis through natural products, *S. schenckii* has been sensitive to Brazilian Bulgarian propolis (Salomão *et al.*, 2008) however, there are no studies against *S. brasiliensis*, which is considered the most prevalent species in feline sporotrichosis in Brazil (Sanchonete *et al.*, 2015; Waller *et al.*, 2015). Due to the need to search for new antifungal compounds in the treatment of sporotrichosis, this work aimed to evaluate the anti-*Sporothrix brasiliensis* activity of three propolis varieties produced in Brazil by different bees, and analyze their chemical composition and cytotoxic effects.

MATERIALS AND METHODS

Samples of brown propolis were commercially purchased from *Cooperativa de Apicultores e Fruticultores da Região Sul* (Beekeeper and Fruit Grower Cooperative of the Southern Region), located in the city of Pelotas/ RS, Brazil; green

propolis was acquired from a commercial company (Nectar Farmacêutica[®] LTDA, Siderópolis/SC, Brazil), whereas propolis from *jataí* bees was obtained from an apiary of stingless bees located in the city of Morro Redondo/RS, Brazil.

The hydroalcoholic extracts were prepared as described by Paulino *et al.* (2002). The propolis samples were frozen at -70°C and, then were ground. The extraction was performed in a solution containing alcohol 96°GL in a ratio of 1:3 to propolis alcohol under stirring for 24 hours at 37°C. Subsequently, the solvent was evaporated and the resulting dry matter was dissolved in phosphate buffer (pH 7.2) and emulsified using EUMULGIN[®] HRE-40, in order to obtain the final concentration of 100mg/ml. Sterilization of the compound was conducted in hydrophilic filter with porosity of 22µm. Afterwards, the dilution was performed with sterile distilled water until achieving the concentration of use.

The chemical composition of propolis extracts was determined by liquid chromatography coupled to mass spectrometry. For this, 10µl of the extracts were injected into a liquid chromatograph of ultra high efficiency (Shimadzu Prominence) coupled to mass spectrometry of high resolution mass (quadrupole-time of flight type) (micrOTOF -Q, Bruker Daltonics). Phenolic compounds were separated using C18 pre-column (2.0 × 4mm) and C18 Luna column (2.0 x 150mm, 100 Å, 3mM) Phenomenex (Torrance, CA, USA), being formic acid solution in water (0.1% v/v, eluent A) and methanol (eluent B) used as mobile phase at a flow rate of 0.2ml/min and the column temperature at 40°C. The elution gradient was: 0-2min, 10% B; 2-15min, 10-75% B; 15-30min, 75% B; 30-32min 75-10% B; 32-40min, 10% B. The PDA detector was defined to scan in the range of 210-800nm. The mass spectrometer was operated in the negative ESI mode, with a voltage of the capillary in 4000V, nebulization gas pressure (N₂) of 2 bar, drying gas at 8L/min and source temperature of 180°C using the equipment default parameters. The equipment was calibrated with 10mM sodium formate, covering the entire range of acquisition (m/z of 50 to 1200). The mass spectrum was processed using the Data Analysis 4.0 software (Bruker Daltonics).

For quantitation of phenolic and flavonoid compounds, calibration curves were prepared using external standards of gallic acid, catechin, 4-hydroxybenzoic acid, chlorogenic acid, epitequina, caffeic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid, rutin, ellagic acid, myricetin, quercetin, hesperetin, kaempferol, luteolin, apigenin, pinocembrin, chrysin and galangin, in the concentrations ranging from 0 to 10µg/ml. Phenolic compounds present in the samples were characterized by their UV/Vis (220-800nm) retention times relative to external standards and mass spectrum.

Different concentrations of propolis extracts of 1000 and from 100 to 0.09µg/ml were added in MDBK (Madin-Darby Bovine Kidney) cell monolayers. Cell viability was assessed by daily microscopic observation of the morphological changes of cells and was measured by MTT assay [3-(4,5 dimethylthiazol-2yl)-2, 5-diphenyl-2H-tetrazolato bromine] after 72h of incubation (Amoros *et al.*, 1992). Cytotoxicity was expressed as cytotoxic concentration in 90% (CC90%), that is, concentration capable of inhibiting 90% of cell culture compared to untreated cells. The tests were performed in quadruplicate and repeated three times on alternate days.

For in vitro tests, clinical isolates of *Sporothrix brasiliensis* from cats (n= 8) and dogs (n= 4) with sporotrichosis in the cities of Pelotas and Rio Grande/RS, in southern Brazil, were used, as well as a standard strain of human sporotrichosis (n: 1; code: IPEC 16919 - Clinical Research Institute Evandro Chagas, Fiocruz, Rio de Janeiro / RJ, Brazil; FMR 8314 - Facultad of Medicine i Ciencies de la Salut, Reus, Spain). All isolates were previously molecularly identified by the *Medical and Molecular Mycology Laboratory*, Federal University of São Paulo (São Paulo/SP, Brazil). The total fungal isolates (n= 13) were stored in the mycological collection of the Center for Research and Diagnosis in Mycology Veterinary, Federal University of Pelotas (Pelotas/RS, Brazil).

The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were determined by microdilution technique in broth, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2008) and adapted to the use of natural products

(Waller *et al.*, 2015). Fungal inoculum were prepared from young colonies grown on potato dextrose agar for 7 days at 35°C and adjusted in a spectrophotometer (Spectrum Co. Instruments, Shanghai, China) in the transmittance of 80-82% at the wavelength of 530nm. The suspensions were diluted in RPMI 1640 (Roswell Park Memorial Institute medium, Sigma, Steinhiem, Germany) supplemented with 2% glucose and MOPS [3- (N-morpholino propanesulfonic acid)] (1:50, v/v). In order to obtain the concentrations of propolis from 0.78 to 0.02mg/ml, serial dilutions were prepared in RPMI-1640 medium supplemented with 2% glucose and MOPS in microplates of 96 wells. For negative control, 100µl of RPMI-1640 medium supplemented with 2% glucose and 100µl of MOPS were added. For positive control, 100µl of RPMI-1640 medium supplemented with 2% glucose and 100µl of MOPS and inoculum were prepared. For the use of itraconazole (Sporanox®, Janssen-Cilag Pharmaceutical Ltda., Lot TLL 089, São José dos Campos/SP, Brazil), the antifungal was prepared in dimethyl sulfoxide (DMSO), according to the CLSI guidelines. The microplates were incubated at 27°C for 72 hours for reading the MIC values. To obtain the values of MFC, 10µl of each well with no visible fungal growth was transferred to Petri dishes containing Sabouraud dextrose (Acumedia, Lansing, Michigan, USA) and incubated at 27°C for 72 hours in order to

visualize fungal growth. All experiments were performed in duplicate and repeated twice.

The variance analysis and comparison of geometric means were performed according to the Kolmogorov-Smirnov test for non-parametric data using the statistical program BioEstat® (version 5.3), and the value $P < 0.05$ considered significant.

RESULTS

The values obtained for cell toxicity in MDBK cells of the three hydroalcoholic extracts of propolis are expressed through the medium of the tested concentrations (Figure 1). In lower concentrations (0.097 to 0.39mg/ml), all extracts did not exhibit cell toxicity compared to control cell. From the concentration of 0.78g/ml, cytotoxicity was observed in brown propolis (PM) and green (PV), whereas for jataí propolis (PJ), the cytotoxicity was observed at 1.57mg/ml. The increasing order of cytotoxic activity was $PJ < PV < PM$.

By liquid chromatography coupled to mass spectrometry in negative mode (negative ESI), qualitative and quantitative analyses of the extracts are shown in Table 2.

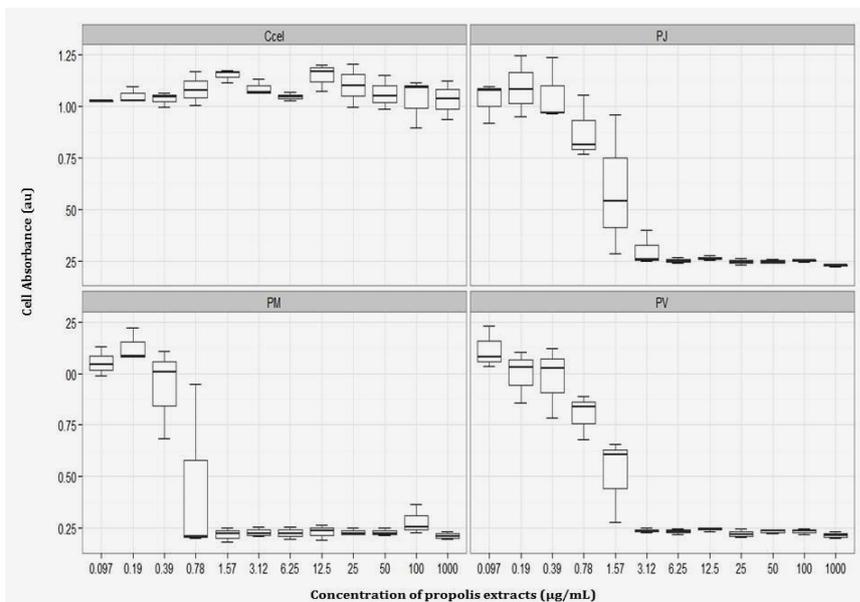


Figure 1. Cytotoxicity of hydroalcoholic extracts of brown propolis (PM), propolis (PV) and *jataí* bees (PJ) compared to MDBK (CCEL).

The results of the total phenolic and flavonoid contents of the three propolis varieties produced in southern Brazil are described in Table 1. The total phenolic content determined by the Folin-Ciocalteu method ranged from 3.93mg of gallic acid/g for *jataí* propolis to 41.84mg/g for green propolis. In total flavonoid content, the values

ranged from 2.5mg epicatechin/g to *jataí* propolis to 13.46mg for brown propolis. The results indicated a higher amount of flavonoids in brown propolis, followed by green propolis. Both samples showed higher phenolic content, highlighting green propolis.

Table 1. Total phenolic and total flavonoid contents (mg g⁻¹) identified in the three propolis varieties produced in Southern Brazil (mean±SD)

Total compounds	Green Propolis	Brown Propolis	Jataí Propolis
Phenolic content*	41.84±5.39	34.39±1.91	3.93±0.54
Flavonoids content**	11.40±1.49	13.46±0.74	2.05±0.53

* mg of gallic acid per gram of extract; ** mg of epicatechin per gram of extract.

Table 2. Quantitative and qualitative analyses (mg/g) of hydroalcoholic extracts of Green Propolis, Brown Propolis and *Jataí* Propolis using liquid chromatography coupled to mass spectrometry in negative mode (negative ESI)

Chemical Compound and respective group	m/z [M-H] ⁻	Retention time (minutes)	Propolis		
			Green	Brown	<i>Jataí</i>
Phenolic acid					
<i>Hydroxybenzoic acid derivative</i>					
Gallic acid	169.0132	5.41	n.d.	n.d.	n.d.
4-Hydroxybenzoic acid	137.0233	11.17	+	+	+
Syringic acid	197.0445	12.45	n.d.	+	n.d.
Vanillic acid	167.0339	12.12	n.d.	+	n.d.
<i>Hydroxycinnamic acid derivative</i>					
Caffeic acid	179.0338	12.05	0.61	0.17	+
Chrologenic acid	353.0867	11.27	0.96	0.36	0.26
<i>p</i> -Coumaric acid	163.0390	13.67	52.09	3.39	6.6
Ferulic acid	193.0495	14.01	0.64	0.20	0.09
<i>Hydroxydiphenic acid derivative</i>					
Ellagic acid	300.9978	15.21	+	+	+
Flavonoid					
<i>Flavanone</i>					
Catechin	289.0707	10.25	n.d.	n.d.	n.d.
Hesperetin	301.0707	17.33	+	+	+
Pinocembrine	255.0652	19.49	+	+	+
<i>Flavone</i>					
Apigenin	269.0445	18.24	+	+	+
Chrysin	253.0495	20.12	+	+	+
Luteolin	285.0394	17.30	+	+	+
<i>Flavonol</i>					
Epicatechin	289.0707	11.74	n.d.	n.d.	n.d.
Galangin	269.0444	20.53	+	+	+
Kaempferol	285.0393	18.00	+	+	+
Myricetin	317.0292	15.52	n.d.	n.d.	n.d.
Quercetin	301.0343	16.89	+	+	+
Rutin	609.1450	14.64	3.71	0.81	0.65

+ Detectable; n.d. Not detectable;

Among 21 standard compounds tested, 15 were identified in all propolis extracts, being vanillic acid and syringic acid only identified in brown propolis. Of the compounds detected, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid and rutin were quantified in all propolis extracts, except caffeic acid for jataí propolis. P-coumaric acid was the major compound in the three samples of propolis, being highly quantified in green propolis (52.09mg/ml), followed by rutin and chlorogenic acid for all extracts.

According to Table 3, green propolis and *jatai* propolis did not exhibit inhibitory or fungicidal activities at tested concentrations (MIC and MFC greater than 0.78mg/ml). In turn, all *S. brasiliensis* tested were sensitive to brown propolis in MIC values between 0.78 and 0.09mg/ml, which varied statistically from other propolis ($P < 0.001$). The MIC₅₀ for isolates from

dogs and cats was 0.19mg/ml for both animal species, while the MIC₉₀ was 0.39mg/ml and 0.78mg/ml, respectively, indicating a remarkable inhibitory activity of this bee product. Although MFC values ranged from 0.09 to greater than 0.78mg/ml for *S. brasiliensis* from cats, fungicidal activity was not observed in *S. brasiliensis* from dogs, showing weak fungicidal activity of brown propolis in the tested concentrations (MFC₉₀ > 0.78mg/ml).

In relation to the drug of choice in the treatment of sporotrichosis, the MIC values of itraconazole varied from 0.25 to greater than 16µg/ml, where sensitivity of *S. brasiliensis* found in dogs (MIC₅₀ of 4µg/ml and MIC₉₀ of 16µg/ml) was observed. However, this did not occur in *S. brasiliensis* from cats (MIC_{50,90} > 16µg/ml), indicating it in vitro resistance to itraconazole. This antifungal showed no fungicidal activity (MFC_{50,90} > 16µg/ml).

Table 3. Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of hydroalcoholic extracts of Green Propolis, Brown Propolis and Jataí Propolis against *Sporothrix brasiliensis* isolated from animal sporotrichosis

<i>Sporothrix brasiliensis</i> (No. samples)		Green Propolis (mg/ml)		Brown Propolis (mg/ml)		Jataí Propolis (mg/ml)		Itraconazole (µg/ml)	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Cats (8)	Range	>0.78	>0.78	0.09 – 0.39	0.09 – >0.78	>0.78	>0.78	0.25 – >16	>16
	50%	>0.78	>0.78	0.19	>0.78	>0.78	>0.78	>16	>16
	90%	>0.78	>0.78	0.39	>0.78	>0.78	>0.78	>16	>16
Dogs (4)	Range	>0.78	>0.78	0.19 – 0.78	>0.78	>0.78	>0.78	0.5 – >16	16 – >16
	50%	>0.78	>0.78	0.19	>0.78	>0.78	>0.78	4	>16
	90%	>0.78	>0.78	0.78	>0.78	>0.78	>0.78	16	>16
IPEC 16919 (1)	Range	>0.78	>0.78	0.39	>0.78	>0.78	>0.78	>16	>16
	Range	>0.78	>0.78	0.09 – 0.78	0.09 – >0.78	>0.78	>0.78	0.25 – >16	16 – >16
Overall (13)	50%	>0.78	>0.78	0.19	>0.78	>0.78	>0.78	>16	>16
	90%	>0.78	>0.78	0.78	>0.78	>0.78	>0.78	>16	>16

DISCUSSION

Although propolis is considered a natural product and the possible absence of toxicity to humans and animals is reported as one of its qualities (Vynograd *et al.*, 2001), the results showed toxicity in MDBK cells. In different cell cultures, the cytotoxicity has been demonstrated at concentrations of 5% (Pereira *et al.*, 2002) and 0.0003% (Sawaya *et al.*, 2004), being described as moderate to highly toxic. These values were different from the present results, because the non-cytotoxic dose of brown and green propolis was 0.097µg/ml, respectively, when compared to

the medium of the control cells, whereas for jataí propolis it was 0.39µg/ml.

Jataí propolis was shown to be less toxic (0.78µg/ml), whereas brown propolis was more toxic (0.097µg/ml), with these values being equivalent to the higher concentration without morphological alterations in the cells when compared to control cell. This toxicity may be derived from the extraction method, since, according to Paulino *et al.* (2002), hydroalcoholic extraction allows for removal of a greater amount of bioactive substances in propolis. In greater quantities and variability,

these substances would have greater toxic action, in addition to significant antifungal action, which was observed in brown propolis.

The identified phenolic and flavonoid compounds in the samples are in accordance with those described in the existing literature. The similarity in the qualitative chemical compositions among the three bee products is in accordance with the findings of Sawaya *et al.* (2004), which observed that propolis produced by *Apis mellifera* and *Tetragonisca angustula* from different regions of Brazil showed similarity in the chemical composition, even to those produced by different bee species. For this observation, the main plant source for the production of jataí propolis should be taken into consideration: *Schinus terebinthifolius*, popularly known as "red mastic" (Sawaya *et al.*, 2004). Widely distributed in Brazil, this plant also is used as source for the development of other propolis varieties, such as green propolis, which contributes to the similarity in the chemical composition between both samples.

Although green propolis and brown propolis produced by *A. mellifera* have presented chemical similarity, the detection of syringic acid and vanillic acid was observed only in brown propolis. This fact may be due to the geographical region of collection, since this factor may influence in the chemical composition of propolis produced by these bee species (Vynograd *et al.*, 2001). In jataí propolis, the presence of caffeic acid in the composition was not observed, and, in relation to quantitative analysis, this product showed lower values when compared to the other propolis varieties. This fact may be explained by the *T. angustula* species (Bankova *et al.*, 2000), as well as the findings of Pereira *et al.* (2002), that observed the higher concentration of phenolic acids in propolis produced by *A. mellifera* in comparison to propolis produced by *T. angustula*.

For the first time, the activity of three Brazilian propolis varieties was studied in contrast to the

main etiological agent of sporotrichosis in Brazil. Among the products, brown propolis has shown to be promising, because all *S. brasiliensis* isolates were sensitive, including itraconazole-resistant isolates. These results encourage further studies with this bee product, since it has shown potential applicability as an antifungal product. In addition to brown propolis, other products from bees have been described as promising in the treatment of sporotrichosis, such as red Brazilian propolis and Bulgarian propolis (Salomão *et al.*, 2008) against *S. schenckii*. These same products were active against others fungal species of interest, such as *Candida albicans* and *Paracoccidioides brasiliensis* (Salomão *et al.*, 2008).

Although the other propolis varieties have not shown to be active against *S. brasiliensis*, their antifungal activities were documented. Dermatophyte species of *Trichophyton* were susceptible to green propolis (Siqueira *et al.*, 2009), as well as yeasts of *Candida albicans* and *C. glabrata* to jataí propolis (Campos *et al.*, 2014), demonstrating that different propolis were active against pathogenic fungi of human and veterinary interest.

CONCLUSIONS

In the present study, the total phenolic and flavonoid contents of three Brazilian propolis varieties were determined, showing similarity to each other in their chemical composition. The presence of compounds with recognized antimicrobial activity were also determined. In the evaluation of cell toxicity, jataí propolis was the least cytotoxic of the studied products. In antifungal activity, brown propolis excelled as the only propolis with anti-*Sporothrix brasiliensis* activity. This study showed the promising use of brown propolis as an antifungal in the treatment of sporotrichosis caused by *Sporothrix brasiliensis*. However, further studies should be undertaken in order to understand its efficacy and security.

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