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Original Article

Partial characterization of ethanolic extract of *Melipona beecheii* propolis and *in vitro* evaluation of its antifungal activity

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ABSTRACT

The ethanolic extract of *Melipona beecheii* Bennett 1831, propolis from Yucatán Mexico was evaluated *in vitro* for the determination of its phenolic compound content, antioxidant capacity and antifungal activity. The results were compared against those of the ethanolic extract of *Apis mellifera* propolis. The total phenolic content, flavonoid content and flavanones–dihydroflavonols content were assessed by colorimetric methods. The antifungal activity was assessed *in vitro* against *Candida albicans*. For the ethanolic extract of *M. beecheii* propolis; total phenolic content, was 263.25 ± 8.78 μg/ml, total flavonoid content was 768 ± 204 μg/ml and flavanones–dihydroflavonols content was 335.42 ± 15.08 μg/ml. For antioxidant activity assessed as DPPH scavenging and iron reducing power ethanolic extract of *M. beecheii* propolis reported IC_{50} of 32.47 and 1.60 μg/ml of gallic acid equivalent respectively. Regarding antifungal activity against *C. albicans*, the minimal inhibitory concentration and minimal fungicidal concentration for ethanolic extract of *M. beecheii* propolis were 1.62 ± 0.33 and 2.50 ± 0.22 μg/ml of dry extract; For both minimal inhibitory concentration and minimal fungicidal concentration, ethanolic extract of *M. beecheii* propolis required 30% less concentration of dry extract than ethanol extract of *A. mellifera* propolis to exert the same antifungal actions against *C. albicans*. To the best of our knowledge, this is the first report about the flavonoid and flavanones–dihydroflavonols content of *M. beecheii* propolis. Due to the lack of information available about the stingless bee's honeycomb products, the study and conservation of endemic honeybees should remain as an active focus of research.

Introduction

The genus *Candida* represents a set of yeast-like fungi belonging to the *ascomycete* phylum. The species of the genus *Candida* are mostly free-living organisms, and may exist as commensals in the genitourinary and gastrointestinal tracts of various species of animals, including humans. Under certain conditions, several species of *Candida* behave as opportunistic pathogens, both for humans and other animals (Rodrigues et al., 2014). The pathologies generated by *Candida* range from superficial mycoses to life-threatening systemic infections (de Oliveira Santos et al., 2018).

The fact that a particular species of *Candida* behaves as an opportunistic pathogen or commensal depends on a complex series of interactions between the microorganism, the host and the microbial flora present at the interaction site. Infections caused by *Candida* are more frequent in immunocompromised populations (*e.g.* children, the elderly and patients with immunodeficiency), individuals with skin or mucous membranes breaches (*e.g.* intravenous drug users, intravenous catheters), or those in whom the usual microbiota is found to be altered (*e.g.* hormonal changes, use of broad-spectrum antibiotics) (Turner and Butler, 2014).

Among the *Candida* species *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* stand out as being frequently identified as opportunistic pathogens. These five species are grouped in what is known as the *Candida* pathogenic species complex (Turner and Butler, 2014). Although there are drugs with activity against several species of *Candida*, their use is limited due to the emergence of resistant strains and the narrow safety margin for many of these compounds. This makes candidiasis an important health concern, especially for hospitalized populations (Srivastava et al., 2018).

Due to the continuous emergence of strains resistant to classical antifungal agents, it is necessary to investigate new compounds with activity against this opportunistic pathogen, preferably with

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fewer side effects than those currently available (Srivastava et al., 2018).

Propolis, together with wax, pollen and honey is a product of the bee hive. It is produced by bees by mixing plant resins with secretions from the bee's digestive tract. Propolis is presented at room temperature as a malleable solid and is used by bees as a material for sealing and waterproofing the interior of the hive (Pasupuleti et al., 2017). Propolis contains diverse compounds with biological activity, in particular anthocyanins, flavonoids, tannins, saponins, terpenoids, polypeptides and lecithins. Among the biological activities attributed to propolis are antibacterial, antifungal, antioxidant and anti-inflammatory capacity (Pazin et al., 2017).

Most of the available information about the properties of propolis comes from the analysis of the one produced by the European bee (*Apis mellifera*) (Farooqui and Farooqui, 2012), with relatively little information regarding those produced by stingless bees (Oliveira et al., 2006).

In the present investigation we carried out a partial characterization of the propolis produced by a stingless bee endemic of the Yucatan Peninsula in Mexico (*Melipona beecheii*) through the evaluation of an ethanolic extract elaborated from it. The measured parameters concerned two of the most relevant biological activities of the propolis; the antioxidant activity and the antifungal activity. The antifungal activity was evaluated *in vitro* using the opportunistic pathogen *C. albicans* as a biological model. As a point of comparison, the propolis generated by *A. mellifera* was evaluated by the same procedure.

Materials and methods

Biological samples

The propolis of *Melipona beecheii* was collected directly from the hive. The collection was carried out in April 2015 in the municipality of Mani Yucatan Mexico (20.3931° N, 89.3918° W). The same procedure was carried out with the propolis of *Apis mellifera*, which was collected in March 2015 from the municipality of Tizimin Yucatan Mexico (21.1455° N, 88.1496° W). Propolis was protected from light and immediately transferred to the laboratory where it was stored at −80 °C until further analysis.

The standardized *Candida albicans* strain ATCC 10231 and a clinical isolate from the same species (Y01), were used. The clinical isolate was identified according to the standardized protocols of O'Horan Hospital in the state of Yucatan, Mexico. The microorganisms were stored at −80 °C and activated immediately prior to use.

Reagents and laboratory equipment

The following reagents were used: ethanol, methanol, distilled water (Hycel), gallic acid, $NaNO_2$, $AlCl_3{\cdot}6H_2O$ (Fermont), catechin, naringenin, trichloroacetic acid (Sigma-Aldrich), 2,4-dinitrophenyl hydrazine (Baker) and Sabouraud Dextrose Agar culture medium (SDA), Sabouraud Dextrose broth (SDB) (Bioxon). Spectrometric measurements were carried out in a Perkin Elmer Lamb-bio spectrophotometer, each evaluation was performed in triplicate.

Ethanolic extract from propolis

Each type of propolis (100 g) was milled at room temperature until a uniform particle size was achieved. Subsequently, 10 g of the sample was suspended in 100 ml of 80% (v/v) ethanol. The samples were incubated under agitation for 48 h at 37 °C protected from light, followed by centrifugation for 10 min at 5000 × *g* at 4 °C. The supernatant was recovered and filtered with a pore size of 0.45 μm. The result of the extraction was labeled as ethanol extract of *M. beecheii* (EEMB) and ethanol extract of *A. mellifera* (EEAM). The extracts were stored at −20 °C until use. The yield of the extraction was evaluated by comparing the dry weight of the extract with respect to the initial amount of propolis used for the extraction. In order to avoid the interference of ethanol in the determination of the antifungal activity, the extract was dried at 45 °C for 24 h followed by resuspension in DMSO 1% in a 1:1 ratio with respect to the initial volume.

Determination of total phenolic content (TPC), total flavonoids content (TFC), total flavanones and dihydroflavonols content (TFDC)

The quantification of total phenols was carried out according to the methodology described by (Brat et al., 2006) using gallic acid as standard. 500 μl of the Folin–Ciocalteu reagent (1:10) was added to 100 μl of each of the extracts under study. The samples were homogenized for 30 s and incubated at room temperature for 2 min. The absorbance was determined at 760 nm. The content of flavonoids was determined according to the methodology proposed by Lee et al. (2003) using catechin as standard. 250 μl of $NaNO_2$ (5%, w/v) was added to 250 μl of each of the extracts. The samples were incubated at room temperature for 5 min, followed by the addition of 125 μl of an aqueous solution of $AlCl_3{\cdot}6H_2O$ (10%, w/v). The samples were incubated for 10 min at room temperature, followed by the addition of 125 μl of 1 M NaOH. The determination of the absorbance was performed at 490 nm. The total content of flavanones and dihydroflavonols was determined using the methodology described by Popova et al. (2004), using naringenin as standard. 2,4-Dinitrophenyl hydrazine (500 μl, 1%, w/v) was added to 200 μl of each of the extracts followed by incubation at 50 °C for 50 min. The samples were allowed to cool at room temperature for 10 min, followed by the addition of potassium hydroxide in methanol (10%, w/v) in the amount needed to reach a final volume of 2.5 ml. The former solution (200 μl) was mixed with methanol (800 μl) to obtain a final volume of 1 ml. Spectroscopic readings were performed at a wavelength of 486 nm.

Antioxidant capacity

The antioxidant capacity was evaluated by DPPH free radical scavenging activity and the reducing power of iron (III). DPPH free radical scavenging activity was evaluated according to the methodology proposed by da Silva et al. (2013). In this assay, 1 ml of 0.1 mM solution of DPPH in ethanol was added to 100 μl of each of the samples followed by homogenization and incubation at room temperature for 30 min. The control consisted of distilled water following the same steps as for the samples. Gallic acid was used as a standard. The absorbance was measured at 517 nm.

For the determination of the reducing power of Fe(III), the methodology proposed by Coelho et al. (2017) was used. In this test 250 μl of phosphate buffer (0.2 M pH 6.6) and 250 μl $K_4Fe(CN)_6{\cdot}3H_2O$ (1%, w/v) were added to 250 μl of each extract followed by homogenization and incubation for 20 min at 50 °C, then 250 μl of 10% CCl_3CO_2H were added. Subsequently, the mixtures were centrifuged at 4000 × *g* for 10 min. The supernatant (500 μl) was transferred to a new vial followed by the addition of 400 μl of $FeCl_3$. The samples were incubated for 10 min at 50 °C. The samples were allowed to cool for 10 min at room temperature. The absorbance at 700 nm was determined. The determination of the percentage of uptake of free radicals and the reduction of Fe(III) to Fe(II) was made using the formula: $((TA - SA)/TA) \times 100$, where TA = target absorbance, SA = sample absorbance (Coelho et al., 2017).

Table 1
Comparison between the amount of compounds of phenolic origin and antioxidant activity of the ethanol extract of the propolis of two different species of bees *Apis mellifera* (EEAM) and *Melipona beecheii* (EEMB).

	Ethanolic extract of propolis	
	EEAM	EEMB
Total phenols (μg/ml gallic acid)	798.74 ± 20.76	263.25 ± 8.58
Total flavonoids (μg catechin/ml)	1723.47 ± 531	768 ± 204
Total flavanones–dihydroflavonols (μg naringenin/ml)	382.38 ± 86.23	335.42 ± 15.08
IC_{50} of DPPH (μg equivalents of gallic acid/ml)	49.53 ± 3.91	32.47 ± 3.13
IC_{50} of reduce Fe(III) (μg equivalents of gallic acid/ml)	4.3 ± .03	1.671 ± .02

IC_{50}: Half-maximal inhibitory concentration; DPPH: 2,2-diphenyl-1-picrylhydrazyl-hydrate.

Evaluation of the antifungal activity of EEMB and EEAM propolis

The antifungal activity of ethanolic extract against *C. albicans* was evaluated *in vitro* for both the ATCC 10231 strain and a clinical isolate (Y01). Disk diffusion antibiogram was performed as a qualitative test. For this, Petri dishes containing SDA were massively sowed with *C. albicans* inoculum standardized at 1×10^6 colony-forming units/ml (CFU). Disks for antibiogram (13 mm) were impregnated with the substances to be evaluated and placed on the agar surface. A range of concentrations of the ethanolic extract of propolis were evaluated (1–15 μg/ml of dry base), the negative control being carried out with the solvent (DMSO 1%), the positive control consisting of 1.35 μg of nystatin. All samples were incubated at 37 °C and evaluated after 24 h.

The determination of the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) was carried out using the microdilution method in 1.5 ml microcentrifuge tubes (Silici et al., 2005; Espinel-Ingroff et al., 2007; Keskin et al., 2017). Starting from 0.5 Mc Farland inoculum, the necessary volume was added to 250 μl of Sabouraud Dextrose Broth (2×) to reach a final concentration of 10^6 CFU in a volume of 500 μl. Immediately, the necessary amount of propolis extract resuspended in 1% DMSO was added to reach the concentration to be evaluated (1–15 μg/ml of dry extract), followed by the addition of distilled water in the amount necessary to reach an end volume of 500 μl. The samples were incubated under agitation at 150 rpm for 48 h at 37 °C. Fungal growth was assessed by measuring absorbance at 600 nm at 12, 24, 36 and 48 h (Silici et al., 2005; Espinel-Ingroff et al., 2007; Keskin et al., 2017). The solvent (1% DMSO) was used as a control (total growth) and 2 μg of nystatin an inhibition control. The percent inhibition was calculated by the formula: $((SA - NCA)/PCA) \times 100$, where SA = sample absorbance, NCA = negative control absorbance and PCA = positive control absorbance (Alsop et al., 1980; Kaya and Özbilge, 2014). MIC was considered as that concentration of propolis extract that showed an 80% reduction in growth at 24 h and MFC the concentration that produced a 99% reduction in growth compared to control at 48 h.

Statistical analysis

Multivariate regression analysis, one way ANOVA with Tukey range test was performed using the IBM SPSS statistics V23 for assessing the differences in the antifungal effect of both propolis.

Results

Determination of total phenolic content (TPC), total flavonoids content (TFC), and total flavanones and dihydroflavonols Content (TFDC).

The yield of the extraction was 4.70 (0.47%) and 5.10 (0.51%) mg dry weight of extract per 1 g of propolis from *M. beecheii* and *A. mellifera* respectively. EEMB and EEAM had a total phenolic content (measured as gallic acid equivalents) of 263.25 ± 8.78 μg/ml and 798.74 ± 20.76 μg/ml, respectively. The above represents a yield of 2.76 mg/g of gallic acid equivalents per gram of raw propolis in the case of *M. beecheii* 7.80 and mg/g of raw propolis of *A. mellifera* (Table 1).

The total flavonoids content expressed as catechin equivalents were 768 ± 204 μg/ml and 1723.47 ± 531 μg/ml for EEMB and EEAM, respectively. The flavonoid extraction yield was 7.68 mg/g and 17.23 mg/g in the case of propolis of *M. beecheii* and *A. mellifera* respectively (Table 1).

The total flavanones and dihydroflavonols content expressed as equivalents of naringenin was 335.42 ± 15.08 μg/ml and 382.38 ± 86.23 μg/ml for EEMB and EEAM, respectively. The yield of the extraction was 3.35 mg/g of crude propolis in the case of *M. beecheii* and 3.82 mg/g for *A. mellifera* (Table 1).

Antioxidant capacity

In the DPPH free radical scavenging activity, the EEMB and EEAM showed an IC_{50} of 32.47 μg/ml and 49.53 μg/ml of gallic acid equivalents respectively. With respect to the reducing power of Iron III, an IC_{50} of 1.60 μg/ml in gallic acid equivalents for *M. beecheii* and 4.30 μg/ml for *A. mellifera* (Table 1) was obtained.

Antifungal activity

Disk diffusion antibiogram. Using ATCC 10231 strain 50 μl of EEMB generated an inhibition halo of 21.12 mm, 50 μl of EEAM generated an inhibition halo of 16.16 mm. Using the clinical isolate (Y01) 50 μl of EEMB generated a 20 mm inhibition halo, EEAM showed no apparent inhibition. The solvent (1% DMSO) showed no apparent antifungal activity (Fig. 1).

Determination of the MIC and MFC

Based on the dry weight of the extract, the MIC for *C. albicans* ATCC 10231 was determined as 1.62 ± 0.33 μg/ml for EEMB and 2.30 ± 0.15 μg/ml for EEAM. The MFC for the same strain was 2.50 ± 0.22 μg/ml and 3.31 ± 0.28 μg/ml for EEMB and EEAM, respectively (Fig. 2 and Table 2). In the case of the clinical isolate (Y01) the MIC was determined at 2.25 ± 0.2 μg/ml and 2.75 ± 0.15 μg/ml for EEMB and EEAM, respectively. The MFC for the same isolate was determined in 2.90 ± 0.55 μg/ml and 3.75 ± 0.11 μg/ml for EEMB and EEAM, respectively. The solvent (1% DMSO) showed no inhibition of fungal growth (Fig. 2 and Table 2).

Multiple regression analysis was carried out to assess the doses-response effect of the EEMB and EEAM propolis against *C. albicans* (ATCC 10231 and Y01). For each of tested concentration the inhibitory effect of EEMB propolis was higher than the same concentration of EEAM propolis until both reached MFC, these differences showed statistical significance ($p = 0.031$ and $p = 0.011$ for *C. albicans* ATCC 10231 y Y01, respectively (Fig. 2). One way ANOVA with Tukey range test was used to evaluate the exerted effects by both EEMB and EEAM propolis resulting in significant differences ($p < 0.05$), except for concentrations above 3.50 μg/ml.

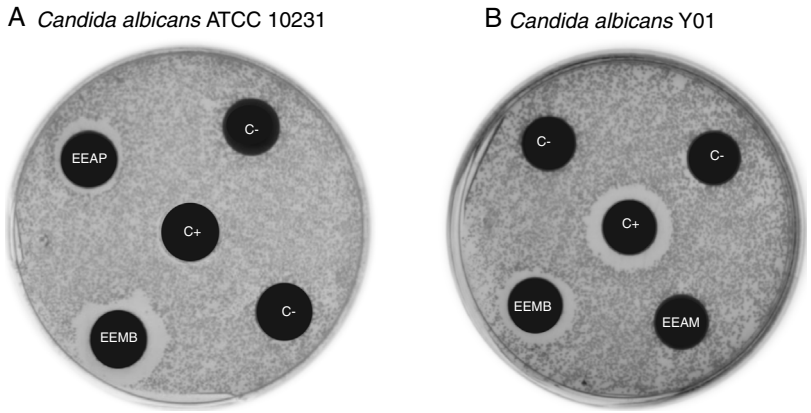

Fig. 1. Qualitative assay of the antifungal activity of the EEMB and EEAM propolis by disk diffusion assay against *Candida albicans* ATCC 10231 and a clinical isolate (Y01). EEMB, ethanol extract of *Melipona beecheii* propolis; EEAM, ethanol extract of *Apis mellifera* propolis; C+–, positive control (nystatin); C––, negative control (DMSO 1%).

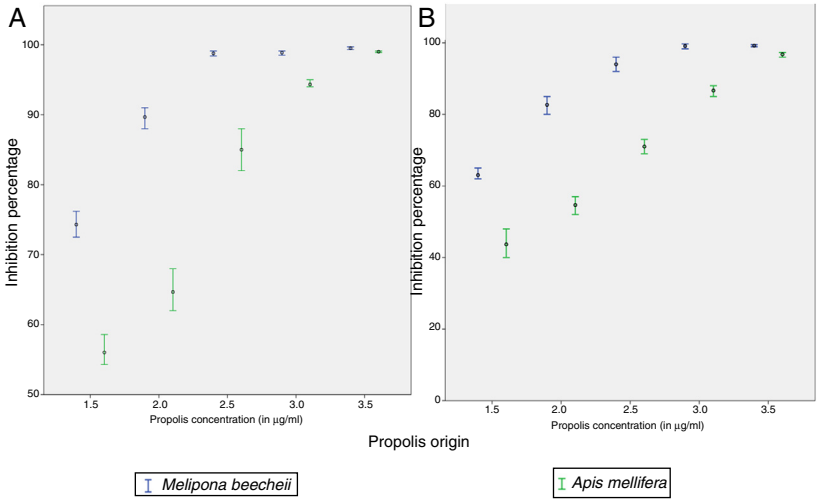

Fig. 2. Dose–response curve of the antifungal activity of propolis against *C. albicans*. Left (A) comparison of the activity of EEMB and EEAM propolis against *Candida albicans* ATCC 10231. Right (B) comparison of the activity of EEMB and EEAM propolis against a clinical isolate of *C. albicans* (Y01). Notice that in both cases a given concentration of *Melipona beecheii* propolis extract induced a more intense suppression of growth of *C. albicans* than the same concentration of *Apis mellifera* propolis extract until both reached the MFC. The difference between the treatments was regarded as statistically significant ($p = 0.031$ for ATCC 10231 and $p = 0.011$ for Y01). EEMB, ethanol extract of *M. beecheii* propolis; EEAM, ethanol extract of *A. mellifera* propolis.

Table 2
Compression of the antifungal activity against *Candida albicans* ATCC 10231 of the ethanol extract of propolis from *Melipona beecheii* and *Apis mellifera* and its relationship with the content of phenolic compounds present in the sample.

Propolis origin		µg dry extract/ml	µl extract (1% DMSO)/ml	Total phenols (µg/ml gallic acid)	Total flavonoids (µg catechin/ml)	Total flavanones–dihydroflavonols (µg naringenin/ml)
M. beecheii	MIC	1.62	3.44	0.90	2.64	1.15
	MFC	2.5	5.31	1.39	4.07	1.78
A. mellifera	MIC	2.2	4.3	3.43	7.4	1.44
	MFC	3.3	6.49	5.18	8.92	2.17

DMSO: Dimethyl Sulfoxide; MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration.

Discussion

Phenolic compounds are a diverse group of secondary metabolites produced by multiple plant species and represent the most diverse class of compounds present in propolis. Substances identified as phenolic compounds include phenolic acids, tannins, flavonoids, curcuminoids, coumarins, lignans, among others (Huang et al., 2010). In plant species, these substances fulfill various functions: intracellular signaling, defense against predators (*e.g.* toxins, antinutritional factors *etc.*), protection against phytopathogens, and attraction of pollinating species, among others (Huang et al., 2010). Antioxidant capacity is one of the most relevant biological activities attributed to propolis. It has been proposed that various antioxidants of natural origin can modify the natural history of diverse pathologies such as hypertension, diabetes mellitus, obesity, systemic inflammatory diseases, among others (Sforcin, 2016). It has been documented that much of the antioxidant activity of propolis occurs due to its phenolic compounds content (Huang et al., 2010; Liben et al., 2018).

In the present study, it was confirmed that both the propolis of *M. beecheii* and *A. mellifera* possess at least two mechanisms of antioxidant activity: the uptake of free radicals and the

reduction of metal ions in solution. It was also possible to demonstrate a significant difference in the total content of phenolic compounds and flavonoids in propolis elaborated by *M. beecheii* and *A. mellifera* ($p < 0.05$). Nevertheless, EEMB propolis required less concentration of gallic acid equivalents than EEAM propolis to exert the same antioxidant effect both in DPPH test and iron reducing power. This effect can be explained due to the differences in the phenolic compounds in each propolis.

The content of phenolic compounds present in propolis is highly variable because it depends on the diversity of plant species to which the bees have access during its elaboration (Zhang et al., 2016). Additionally, each bee species has a unique habit in its resin collection that increases the regional and interspecific variations in the chemical composition of the propolis (Bakchiche et al., 2017). The antioxidant capacity is not the same for each of the diverse phenolic compounds (Tabart et al., 2009). It has been shown that there are significant differences in the composition of the propolis generated by *A. mellifera* with respect to that produced by different species of stingless bees (*meliponini* tribe) (Huang et al., 2010).

The ability to reduce metal ions is especially relevant in food products since the absorption of many minerals only occurs at certain oxidation states. The above is demonstrated in the case of iron, which can only be absorbed as iron (II), lacking a significant bioavailability as iron (III). The use of antioxidants represents an alternative to improve the bioavailability of this class of minerals (Davidsson, 2003; Hurrell and Egli, 2010).

The findings of this study coincide with that described by the literature for propolis of *A. mellifera*, as well as with those reported for other stingless bee species (Manrique and Santana, 2008; da Silva et al., 2013). The compounds present in the ethanolic extracts of the propolis of *A. mellifera* and *M. beecheii* demonstrated activity against *C. albicans*. This coincides with reports of the literature regarding the propolis of *A. mellifera* and several species of stingless bees (Campos et al., 2015; Capoci et al., 2015; Shehu et al., 2016; Maureira et al., 2017; Dezmirean et al., 2017). However, information regarding the biological activities of propolis of *M. beecheii* continues to be scarce (Popova et al., 2005; Fonte-Carballo et al., 2016; Ibrahim et al., 2016).

As far as we know, this is the first report regarding the content of flavonoids and dihydroflavonols, as well as the antioxidant activity of the ethanolic extract of the propolis of *M. beecheii*.

Conclusion

The ethanol extract of the propolis of *A. mellifera* and *M. beecheii* contains significant amounts of phenolic compounds, which exert an antioxidant activity by at least two different mechanisms. The total content of phenolic compounds was superior in the case of the ethanol extract of *A. mellifera* to that of *M. beecheii*. The ethanolic extract of *M. beecheii* presented a higher antifungal activity against *C. albicans* than that of *A. mellifera*, reaching MIC with less than 3 μg of dry extract per ml, containing a concentration less 1 μg/ml gallic acid equivalents.

Authors contributions

JRS and EPL equally contributed to running the laboratory work, analysis of the data and drafting the paper. RRB contributed to the collection of propolis and running the laboratory work. AYP contributed in analysis of data and drafted the paper. TMS contributed in critical review of the manuscript. EOV designed the study, supervised the laboratory work and contributed in critical review 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.

References

Alsop, G.M., Waggy, G.T., Conway, R.A., 1980. Bacterial growth inhibition test. J. Water Pollut. Control Fed. 52, 2452–2456.

Bakchiche, B., Habati, M., Benmebarek, A., Gherib, A., 2017. Total phenolic, flavonoid contents and antioxidant activities of honey and propolis collected from the region of Laghouat (South of Algeria). World News Nat. Sci. 11, 91–97.

Brat, P., Georgé, S., Bellamy, A., Du Chaffaut, L., Scalbert, A., Mennen, L., Arnault, N., Amiot, M.J., 2006. Daily polyphenol intake in France from fruit and vegetables. J. Nutr. 136, 2368–2373.

Campos, J.F., Santos, U.P. dos, Rocha, P. dos S. da, Damião, M.J., Balestieri, J.B.P., Cardoso, C.A.L., Paredes-Gamero, E.J., Estevinho, L.M., de Picoli Souza, K., Santos, E.L.dos., 2015. Antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of propolis from the stingless bee *Tetragonisca fiebrigi* (Jataí). Evid. Based Complement. Altern. Med., http://dx.doi.org/10.1155/2015/296186.

Capoci, I.R.G., Bonfim-Mendonça, P. de S., Arita, G.S., Pereira, R.R. de A., Consolaro, M.E.L., Bruschi, M.L., Negri, M., Svidzinski, T.I.E., 2015. Propolis is an efficient fungicide and inhibitor of biofilm production by vaginal *Candida albicans*. Evid. Based Complement. Altern. Med., http://dx.doi.org/10.1155/2015/287693.

Coelho, J., Falcão, S.I., Vale, N., Almeida-Muradian, L.B., Vilas-Boas, M., 2017. Phenolic composition and antioxidant activity assessment of southeastern and south Brazilian propolis. J. Apic. Res. 56, 21–31.

da Silva, I.A.A., da Silva, T.M.S., Camara, C.A., Queiroz, N., Magnani, M., de Novais, J.S., Soledade, L.E.B., Lima, E. de O., de Souza, A.L., de Souza, A.G., 2013. Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. Food Chem. 141, 3552–3558.

Davidsson, L., 2003. Approaches to improve iron bioavailability from complementary foods. J. Nutr. 133, 1560S–1562S.

de Oliveira Santos, G.C., Vasconcelos, C.C., Lopes, A.J.O., de Sousa Cartágenes, M. do S., Filho, A.K.D.B., do Nascimento, F.R.F., Ramos, R.M., Pires, E.R.R.B., de Andrade, M.S., Rocha, F.M.G., de Andrade Monteiro, C., 2018. *Candida* infections and therapeutic strategies: mechanisms of action for traditional and alternative agents. Front. Microbiol. 9, http://dx.doi.org/10.3389/fmicb.2018.01351.

Dezmirean, D.S., Mărghitaş, L.A., Chirilă, F., Copaciu, F., Simonca, V., Bobiş, O., Erler, S., 2017. Influence of geographic origin, plant source and polyphenolic substances on antimicrobial properties of propolis against human and honey bee pathogens. J. Apic. Res. 56, 588–597.

Espinel-Ingroff, A., Arthington-Skaggs, B., Iqbal, N., Ellis, D., Pfaller, M.A., Messer, S., Rinaldi, M., Fothergill, A., Gibbs, D.L., Wang, A., 2007. Multicenter evaluation of a new disk agar diffusion method for susceptibility testing of filamentous fungi with voriconazole, posaconazole, itraconazole, amphotericin B, and caspofungin. J. Clin. Microbiol. 45, 1811–1820.

Farooqui, T., Farooqui, A., 2012. Beneficial effects of propolis on human health and neurological diseases. Front. Biosci. 4, 779–793.

Fonte-Carballo, L., Milián-Rodríguez, Y.E., Díaz-Solares, M., 2016. Potencialidad antimicrobiana y caracterización de propóleos de *Melipona beecheii* provenientes de dos fincas agroenergéticas. Pastos Forrajes 39, 149–156.

Huang, W.-Y., Cai, Y.-Z., Zhang, Y., 2010. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr. Cancer 62, 1–20.

Hurrell, R., Egli, I., 2010. Iron bioavailability and dietary reference values. Am. J. Clin. Nutr. 91, 1461S–1467S.

Ibrahim, N., Zakaria, A.J., Ismail, Z., Mohd, K., 2016. Antibacterial and phenolic content of propolis produced by two Malaysian stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica*. Int. J. Pharmacogn. Phytochem. Res. 8, 156–161.

Kaya, E., Özbilge, H., 2014. Determination of the effect of fluconazole against *Candida albicans* and *Candida glabrata* by using microbroth kinetic assay. Turk. J. Med. Sci. 42, 325–328.

Keskin, D., Ceyhan-Guvensen, N., Uygun, M., Uygun, D.A., 2017. Comparison of the antimicrobial and antioxidant activity of four different regions of Anatolia. Eur. J. Biotechnol. Biosci. 5, 20–24.

Lee, K.W., Kim, Y.J., Kim, D.-O., Lee, H.J., Lee, C.Y., 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. J. Agric. Food Chem. 51, 6516–6520.

Liben, T., Atlabachew, M., Abebe, A., 2018. Total phenolic, flavonoids and some selected metal content in honey and propolis samples from South Wolo zone, Amhara region, Ethiopia. Cogent Food Agric. 4, 1475925.

Manrique, J.A., Santana, W., 2008. Flavonoids, antibacterial and antioxidant activities of propolis of stingless bees, *Melipona quadrifasciata*, *Melipona compressipes*, *Tetragonisca angustula*, and *Nannotrigona* sp. from Brazil and Venezuela. Zootec. Trop. 26, 157–166.

Maureira, N., Viera, P., Fernandez, A., Urrejola, M., Bravo, C., Mardones, F., Vines, E.D., Haidar, Z.S., Maureira, N., Viera, P., Fernandez, A., Urrejola, M., Bravo, C., Mardones, F., Vines, E.D., Haidar, Z.S., 2017. Susceptibilidad de cepas de *Candida* oral a extracto etanólico del propóleo chileno de Olmué. Int. J. Odontostomatol. 11, 295–303.

Oliveira, P.C., Shinobu, M.C., Longhini, R., Franco, S., Svidzinski, T., 2006. Antifungal activity of propolis extract against yeasts isolated from onychomycosis lesions. Mem. Inst. Oswaldo Cruz 101, 493–497.

Pasupuleti, V.R., Sammugam, L., Ramesh, N., Gan, S.H., 2017. Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. Oxid. Med. Cell. Longev., http://dx.doi.org/10.1155/2017/1259510.

Pazin, W.M., Mônaco, L., da, M., Soares, A.E.E., Miguel, F.G., Berretta, A.A., Ito, A.S., 2017. Antioxidant activities of three stingless bee propolis and green propolis types. J. Apic. Res. 56, 40–49.

Popova, M., Bankova, V., Butovska, D., Petkov, V., Nikolova-Damyanova, B., Sabatini, A.G., Marcazzan, G.L., Bogdanov, S., 2004. Validated methods for the quantification of biologically active constituents of poplar-type propolis. Phytochem. Anal. 15, 235–240.

Popova, M., Silici, S., Kaftanoglu, O., Bankova, V., 2005. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomed. Int. J. Phytother. Phytopharm. 12, 221–228.

Rodrigues, C.F., Silva, S., Henriques, M., 2014. *Candida glabrata*: a review of its features and resistance. Eur. J. Clin. Microbiol. 33, 673–688.

Sforcin, J.M., 2016. Biological properties and therapeutic applications of propolis. Phytother. Res. 30, 894–905.

Shehu, A., Ismail, S., Rohin, M.A.K., Harun, A., Aziz, A.A., Haque, M., 2016. Antifungal properties of Malaysian Tualang honey and stingless bee propolis against *Candida albicans* and *Cryptococcus neoformans*. J. Appl. Pharm. Sci. 6, 44–50.

Silici, S., Koç, N.A., Ayangil, D., Çankaya, S., 2005. Antifungal activities of propolis collected by different races of honeybees against yeasts isolated from patients with superficial mycoses. J. Pharmacol. Sci. 99, 39–44.

Srivastava, V., Singla, R.K., Dubey, A.K., 2018. Emerging virulence, drug resistance and future anti-fungal drugs for *Candida* pathogens. Curr. Top. Med. Chem. 18, 759–778.

Tabart, J., Kevers, C., Pincemail, J., Defraigne, J.O., Dommes, J., 2009. Comparative antioxidant capacities of phenolic compounds measured by various tests. Food Chem. 113, 1226–1233.

Turner, S.A., Butler, G., 2014. The *Candida* pathogenic species complex. Cold Spring Harb. Perspect. Med. 4, http://dx.doi.org/10.1101/cshperspect.a019778.

Zhang, J., Shen, X., Wang, K., Cao, X., Zhang, C., Zheng, H., Hu, F., 2016. Antioxidant activities and molecular mechanisms of the ethanol extracts of *Baccharis* propolis and *Eucalyptus* propolis in RAW64.7 cells. Pharm. Biol. 54, 2220–2235.