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Antimicrobial activity of honeys from two stingless honeybee species and *Apis mellifera* (Hymenoptera: Apidae) against pathogenic microorganisms

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ABSTRACT

Honeys are described possessing different properties including antimicrobial. Many studies have presented this activity of honeys produced by *Apis mellifera* bees, however studies including activities of stingless bees honeys are scarce. The aim of this study was to compare the antimicrobial activity of honeys collected in the Amazonas State from *Melipona compressipes*, *Melipona seminigra* and *Apis mellifera* against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Chromobacterium violaceum*, and *Candida albicans*. Minimum inhibitory concentrations were determined using the agar dilution method with Müller-Hinton agar (for bacteria) or Saboraud agar (for yeast). *Staphylococcus aureus* and *E. faecalis* were inhibited by all honeys at concentrations below 12%, while *E. coli* and *C. violaceum* were inhibited by stingless bee honeys at concentrations between 10 and 20%. *A. mellifera* honey inhibited *E. coli* at a concentration of 7% and *Candida violaceum* at 0.7%. *C. albicans* were inhibited only with honey concentrations between 30 and 40%. All examined honey had antimicrobial activity against the tested pathogens, thus serving as potential antimicrobial agents for several therapeutic approaches.

KEYWORDS: *Melipona*, antibacterial, antifungal, natural products, functional food

Atividade antimicrobiana de méis de duas espécies de abelhas sem ferrão e *Apis mellifera* (Hymenoptera, Apidae) contra micro-organismos patogênicos

RESUMO

Méis são descritos possuindo diferentes propriedades, incluindo a antimicrobiana. Muitos estudos têm apresentado essa atividade de méis produzidos por abelhas *Apis mellifera*, no entanto estudos incluindo atividades de méis de abelhas sem ferrão são escassos. O objetivo deste estudo foi comparar a atividade antimicrobiana de méis de *Melipona compressipes*, *Melipona seminigra* e *A. mellifera*, coletados no Estado do Amazonas, contra *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Chromobacterium violaceum*, e *Candida albicans*. As concentrações inibitórias mínimas foram determinadas usando o método de diluição em ágar, com ágar Muller-Hinton (para bactérias) ou ágar Sabouraud (para a levedura). *S. aureus* e *E. faecalis* foram inibidos por todos os méis em concentrações inferiores a 12%, enquanto *E. coli* e *C. violaceum* foram inibidos por méis de abelhas sem ferrão em altas concentrações entre 10 e 20%. *A. mellifera* inibiu *E. coli* na concentração de 7% e *C. violaceum* em baixa concentração (0,7%). *C. albicans* foi inibida apenas em concentrações entre 30 e 40% dos méis. Assim, todas as variedades de mel testadas apresentaram atividade antimicrobiana sobre os patógenos testados, servindo assim como agente antimicrobiano potencial para diversas abordagens terapêuticas.

PALAVRAS CHAVE: *Melipona*, antibacteriano, antifúngico, produtos naturais, alimentos funcionais

For dietary purposes, honey is an excellent supplementary food with a high-energy component due to its sugar content (Evangelista-Rodrigues *et al*. 2005), mineral salts and other nutritional substances (Souza *et al*. 2004). Honey is composed primarily of the simple sugars glucose and fructose. It also contains sucrose and 17–20% of water, (Kamal and Klein 2011). In addition to being a natural sugar source, honey has been shown to have immunological, antibacterial, antifungal, antioxidant and anti-inflammatory properties (Bean 2012; Chen *et al*. 2012; Deravajan and Venugopal 2012). Honey is produced by numerous species of bees and their chemical composition may vary according to the habitat of each species. The Hymenoptera order includes approximately 2500 species of bees, primarily distributed in the southern continents (Almeida and Danforth 2009). Among the Hymenoptera, social bees are distributed in the Apidae family, which consists of 53 genera and approximately 300 species with a pantropical distribution (Michener 2007). In addition to the well-known honeybee (*Apis mellifera*), there are also stingless bees from the Apidae and Meliponini families that were the only species that produced honey in Brazil prior to the introduction of the European honeybee (Kerr *et al*. 2001). The interest in management and conservation of the stingless bees is justified because the ecological implications for the pollination of native crops, the high therapeutic value credited to their honey and pollen, and also the economic contributions of the honey industry (Kerr *et al*. 1996). Despite the extensive use of honey from stingless bees in home remedies, especially in traditional communities of the Amazon, a majority of previous studies have been conducted using honey from the *Apis* species (Carvalho-Zilse and Kerr 2006). As there are no studies evaluating the antimicrobial activity of honeys these species of stingless bee, the aim of this study was to compare the antimicrobial activity of honeys collected in the Amazonas State (Brazil) from *Melipona compressipes, M. seminigra* and *A. mellifera* against five different human pathogenic microorganisms: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli, Chromobacterium violaceum*, and *Candida albicans*.

For each stingless bee species included in the study, 300 mL of honey was obtained, in a single collection, using a disposable syringe to sample directly from the honey pots of bee colonies located at the National Amazon Institute of Research (INPA, Manaus, Brazil). Honey from *Apis mellifera*, which originated from a commercial apiary, was purchased at a local market in a single collection. Reference strains *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *C. violaceum* (ATCC 12472) and *C. albicans* (ATCC 90028) were maintained in Lignières slants at 4ºC and for use, they were streaked in the same culture media and incubated at 37 ºC. Lignières medium contained, per liter of distilled water, 8 g of nutrient broth (Oxoid, Basingstoke, United Kingdom), 5 g of gelatin (Fluka Analytical, Saint Gallen, Switzerland), and 7 g of agar (Himedia, Mumbai, India); pH was adjusted to 7.4 with NaOH. Following Clinical and Laboratory Standards Institute guidelines (CLSI, 2006), minimum inhibitory concentrations (MICs) were determined using the agar dilution method in Petri dishes filled with Müller-Hinton agar (for bacteria) (Himedia) or Sabouraud dextrose agar (for yeast) (Oxoid). One colony of each ATCC sample was seeded in tubes containing 3mL of Luria-Bertani (LB) (Acumedia, Michigan, United States) medium and incubated at 37ºC until reaching 1.5 x 10^8 UFC/mL. Culture medium was supplemented with decreasing concentrations of honey between 50% and 0,2% (v/v). We used as control a culture medium supplemented with sucrose in the same concentrations. Bacterial suspensions (10 µL) were then inoculated in spots onto media/honey plates and incubated at 37ºC for 18 hr. Plates containing Müeller-Hinton agar and Saboraud agar without honey were used as negative controls. Each experiment was performed in triplicate.

A summary of assay results is presented in Table 1. The antimicrobial assay showed the antimicrobial activity of honeys against different organisms. *S. aureus* and *E. faecalis* were inhibited by all honeys at concentrations less than 12% v/v, while *E. coli* and *C. violaceum* were inhibited by stingless bee honeys at high concentrations between 10 and 20% v/v. *A. mellifera* honey inhibited *E. coli* at a concentration of 7% v/v and *C. violaceum* at 0.7% v/v (Table 1). *C. albicans* was observed to be the most resistant microorganism, requiring honey concentrations between 30 and 40% v/v to inhibit growth (Table 1). In sensitivity tests, honey from *A. mellifera*, *M. seminigra* and *M. compressipes* inhibited the growth of the microorganisms included in the study (Table 1). Honey from *A. mellifera* showed a higher inhibitory capacity against growth of *E. coli* and *C. violaceum*, which was evident by the lower MIC (7.0% and 0.7% v/v, respectively). Conversely, honey from *M. compressipes* was had a higher inhibitory capacity against *S. aureus* and *C. albicans* (MIC 7.0% and 30% v/v, respectively) (Table 1). The honey from *M. seminigra* was the

Table 1 - Minimal inhibitory concentration (% v/v) of honeys from *Apis mellifera, Melipona compressipes* and *Melipona seminigra* against five human pathogens.

	S. aureus	*E. faecalis*	*E. coli*	*C. violaceum*	*C. albicans*
A. mellifera	7.0	10.0	7.0	0.7	40
M. seminigra	8.0	5.5	16.0	10.0	35
M. compressipes	7.0	7.5	20.0	20.0	30

most efficient only against the *E. faecalis* strain (MIC 5.5% v/v). Sucrose control mediums did not inhibit any strain used in this work in concentrations tested.

In this study, honey from *M. compressipes, M. seminigra* and *A. mellifera* had a wide range of antimicrobial activities against bacteria and yeast. All kind of honey examined displayed some antibacterial activity (Table 1). The lowest MIC observed was 0.7% v/v against *C. violacium*, while the highest was 40% v/v against *C. albicans*. The observed MIC value of 7% v/v against *E. coli* and *S. aureus* is displayed according Lusby *et al*. (2005), which reported a MIC of 5% against these same microorganisms using honey from *A. mellifera*. Chan-Rodrigues *et al*. (2012) reported inhibition of *S. aureus*, *E. coli* and *E. faecalis* with lower MICs using honey from *Melipona beecheii* compared to honey from *A. mellifera*. The results of the present study showed susceptibility of *S. aureus* to honeys from *M. seminigra* and *M. compressipes* with MICs between 7–8%, which are similar MIC value to the 5% MIC previously observed for honey from *M. beecheii*. However, results were different of previous studies against *E. coli* and *E. faecalis*. Specifically, honeys tested in the current study were more effective against *E. faecalis* with MICs between 5.5–7.5% compared to MICs between 10–15% in the study by Chan-Rodrigues *et al*. (2012). Although investigating different strains of *E. coli*, Chan-Rodrigues *et al*. (2012) observed MICs that were 5–15% lower than values reported for *E. coli* in the current study, which ranged from 16–20%. Furthermore, Chan-Rodrigues *et al*. (2012) reported higher MICs for honey from *A. mellifera*. Specifically, MICs of 15, 20 and 25% were observed for *S. aureus, E. coli* and *E. faecalis*, respectively. Whereas, MICs of 7.0, 7.0 and 10%, respectively, were observed in the current study. With MICs between 30–40%, *C. albicans* was determined to be the most resistant microorganism included in the current study. These results are not consistent with Demera and Angert (2004), who reported greater susceptibility of *C. albicans* to honeys from *A. mellifera* and *Tetragonisca angustula*. A possible explanation for these differences may be the variable composition of tested honey. In addition to the honeybee species, floral origin may also influence honey composition. General variations observed in overall antibacterial activity have been attributed to variation in the concentration of hydrogen peroxide in the honey and, in some cases, to the concentration of non-peroxide factors (Molan 1992). In some works the strains were different of those used in the current study. In these cases, other possible explanation may be that different bacterial strains possess different antimicrobial susceptibilities (Pieri *et al*. 2012). However, *S. aureus* strain (ATCC 25923) used in the current study was the same used by Chan-Rodrigues *et al*. (2012) indicating that differences in results for honey from *A. mellifera* likely occurred due different honey composition. Same conclusion could be related to differences between the results against *C. albicans* (ATCC 90028) by Demera and Angert (2004) and the findings of present study once the strains used were the same. Importantly *C. violaceum* has been reported as an infectious agent to humans and animals worldwide, including Amazon region, especially being transmitted by water (Perez *et al.* 2007). No previous studies have investigated the inhibition of *C. violaceum* by honeys from *Melipona* spp. and *Apis* spp. bees, with special reference to honey of *A. mellifera* who obtained very low MIC value and could be a promising antimicrobial agent to treat infections of these bacteria. Alves *et al.* (2008) demonstrated a practical therapeutic use for honey made by different bee species of the genus *Melipona* (*M. subnitida*) in cutaneous wound healing in rats. The mean healing time of infected wounds was lower in honey-treated groups compared to control groups and they reported 100% eradication of Gram-negative bacteria on wound, as well as a significant reduction of Gram-positive bacteria. Alves *et al*. (2008) suggest that this activity was mainly due to hydrogen peroxide produced by glucose oxidase and may also be related to other characteristics such as low pH presented by honey tested, osmotic pressure, or other unknown factors phytochemicals. The antimicrobial activity demonstrated *in vivo* by Alves *et al*. (2008) supports by the results of the current study, which showed significant antimicrobial activity of honeys produced by two species of *Melipona* spp. However in the present study the factor osmotic pressure could be excluded due to non-inhibition of strains by the sucrose control. Further research is needed to determine the specific compounds responsible for this antimicrobial activity. In conclusion, all tested honey varieties possessed antimicrobial activity against several human pathogenic microorganisms, thus serving as potential antimicrobial agents for use in several therapeutic approaches.

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