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Atividade antimicrobiana de méis de cinco espécies de abelhas brasileiras sem ferrão

Antimicrobial activity of honey from five species of Brazilian stingless bees

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- NOTA –

RESUMO

*A atividade antimicrobiana de méis produzidos por **Melipona asilvai**, **Melipona quadrifasciata anthidioides**, **Friseomelita doederleinei**, **Tetragonisca angustula** e **Plebeia** sp. foi investigada. O teste de difusão em poço demonstrou que todos os méis tem ação antibacteriana frente a **Staphylococcus aureus**, mas somente as amostras produzidas por **M. quadrifasciata anthidioides** e **F. doederleinei** inibiram o crescimento de **Escherichia coli**. No ensaio de determinação da concentração inibitória mínima, os méis de **M. asilvai**, **M. quadrifasciata anthidioides**, **F. doederleinei** e **T. angustula** foram mais ativos que os de **Plebeia** sp. frente a **S. aureus** e **E. coli**. Os micro-organismos **Pseudomonas aeruginosa** e **Candida albicans** foram resistentes a todos os méis em ambos ensaios. Os méis foram mais efetivos contra as bactérias do que frente a uma solução de açúcar, sugerindo que o mecanismo de inibição do crescimento bacteriano não está somente relacionado ao efeito osmótico. Os resultados obtidos podem explicar o uso medicinal desses méis em doenças bacterianas.*

Palavras-chave: *abelha sem ferrão, atividade antibacteriana, efeito osmótico, mel.*

ABSTRACT

*The antimicrobial activity of honey produced by **Melipona asilvai**, **Melipona quadrifasciata anthidioides**, **Friseomelita doederleinei**, **Tetragonisca angustula** and **Plebeia** sp. were investigated. The agar well diffusion assay demonstrated that all honeys had antibacterial activity against **Staphylococcus aureus**, but only the samples from **M. quadrifasciata anthidioides** and **F. doederleinei** inhibited the growth of **Escherichia coli**. In the Minimum Inhibitory Concentration determination assay, **M. asilvai**, **M. quadrifasciata anthidioides**, **F. doederleinei** and **T. angustula** honeys were more active than that from **Plebeia** sp. for **S. aureus** and **E. coli**. The microorganisms **Pseudomonas aeruginosa** and **Candida albicans** were resistant to the all native stingless bee honeys in both assays. Honeys were more effective against bacteria than a sugar solution, suggesting that the mechanism for bacterial growth inhibition is not only related to the osmotic effect. The results of antimicrobial activity may explain the popular medicinal use of these honeys in bacterial diseases.*

Key words: *stingless bee, antibacterial activity, osmotic effect, honey.*

Bees from the ***Meliponinae*** subfamily (Hymenoptera: ***Apidae***), known as "stingless bees", are inhabitants of the tropics, with about 600 species distributed across 56 genera (VIT et al, 2004). The ***Meliponinae*** subfamily is subdivided into two tribes: Meliponini, formed only by the genus ***Melipona***; and ***Trigonini***, comprising a large number of genera, such as ***Friseomelita***, ***Tetragonisca*** and ***Plebeia*** (NOGUEIRA-NETO, 1997). Bees of the genus ***Melipona*** are found exclusively in the Neotropical region (Central and South America, and Caribbean Islands), but the tribe ***Trigonini*** are distributed throughout Central and South America, Asia, the Pacific Islands, Australia, New Guinea and Africa (VIT et al., 2004).

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In Brazil stingless bees are abundant in number and species. Until the mid-19th century, before the introduction of ***Apis mellifera*** from Europe and Africa, stingless bees along with some native wasp species were the only sources of honey in Brazil (NOGUEIRA-NETO, 1997). Exploited in the past especially by Indians, stingless bees are now kept through meliponiculture, developed mainly in North-eastern Brazil (CORTOPASSI-LAURINO et al., 2006). These honeys have great acceptance in production areas for their distinctive sweetness, aroma and flavour when compared to ***Apis mellifera*** honey, but they are used much more as medicines (RODRIGUEZ-MALAVER et al., 2009). Honeys from stingless bees are used in popular therapies to combat a broad spectrum of health problems, such as cataract, eye infections, lack of appetite, sores, lung and upper respiratory tract diseases (POSEY, 1987), many of which related to microorganisms.

The medicinal use of honeys from stingless bees has been the subject of only a few studies published in the literature (CORTOPASSI-LAURINO & GELLI, 1991; MIORIN et al., 2003; DEMERA & ANGERT, 2004; RODRIGUEZ-MALAVER et al., 2009; SGARIGLIA et al., 2010). Therefore, the aim of this study was to assess the *in vitro* antimicrobial properties of honeys from ***Melipona asilvai***, ***Melipona quadrifasciata anthidioides***, ***Friseomelita doederleinei***, ***Tetragonisca angustula*** and ***Plebeia*** sp. produced in the state of Bahia, Brazil. The honey samples were collected directly from beekeepers in the municipalities of Serrinha (***M. asilvai***, ***M. quadrifasciata anthidioides*** and ***Friseomelita doederleinei***) and Cruz das Almas (***T. angustula***) in May 2006, and in Canudos (***Plebeia*** sp.) in August 2006.

The antimicrobial activity was evaluated against strains from the Culture Collection of Microorganisms of Bahia (CCMB): ***Escherichia coli*** CCMB261 sulphonamide resistant, ***Staphylococcus aureus*** CCMB262 streptomycin and dihydrostreptomycin resistant, ***Pseudomonas aeruginosa*** CCMB268 and ***Candida albicans*** CCMB266. The determination of antimicrobial activity of honey was carried out using the agar well diffusion (AWD) and the minimum inhibitory concentration (MIC) determination assays in triplicate. Honey samples were prepared by dissolving a weighed amount of honey in distilled water, resulting in 50% (v v^{-1}) solutions, which were filtered in a sterile cellulose membrane (0.22µm). In the AWD assay (CLSI, 2003b) the microorganisms were grown on Mueller-Hinton broth. After growth periods of 18h (bacteria) and 36h (yeast), the inoculums were adjusted approximately to $5x10^3$UFC mL^{-1} and $1.5x10^6$UFC mL^{-1} in saline solution at 0.45%, respectively. Microorganism suspensions (100µL) were mixed with 100mL of Mueller-Hinton Agar and immediately poured into Petri plates (15x150mm). Wells of 6mm diameter were made, and a 75µL sample of diluted honey was released in each well. The plates were incubated at 28°C for 36h (yeast) and 37°C for 18h (bacteria). A positive result was considered with inhibition zones larger than 9mm (well diameter included). The inhibition zones diameters are expressed as means±standards deviation. Nystatin, chloramphenicol and gentamicin (0.01mg mL^{-1}) were used as positive controls.

For the MIC determination in 96-well plates (CLSI, 2002; 2003a), 150µl of diluted honey solution and 40µL of Mueller-Hinton broth (4X concentrated) were conditioned in the first well and the serial dilutions were carried out in all subsequent wells. Cultures of 18h (bacteria) and 36h (yeast) were collected in saline solution 0.45% and 5µL of microorganism suspension was added in each well. The microorganism concentration in the well was $7.5x10^4$UFC mL^{-1} and 2.5x10UFC mL^{-1} (for bacteria and yeast, respectively). The plates were incubated at 28°C for 48h (yeast) and 37°C for 24h (bacteria), then 50µL of 2,3,5-triphenyltetrazolium chloride (0.5% w v^{-1}) was added in each well. The result was observed after 3h of incubation and red colours were indicative of microbial growth. The MIC was defined as the lowest concentration of honey in which there was no visible growth after incubation. Controls with nystatin for the yeast, and chloramphenicol and gentamicin for the bacteria, microbial strains viability, sample sterility and water were performed. A solution with 3g of sucrose, 35g of fructose and 45g of glucose in 17g of sterile deionised water (COOPER et al., 1999) was also tested to determine whether inhibitory effects were due to the sugar content of the honey samples.

The antimicrobial effects are summarised in table 1. In the AWD assay, all honeys exhibited antibacterial activity against ***S. aureus*** with mean zones of inhibition between 17.5 and 32.7mm. However, only honeys produced by ***M. quadrifasciata anthidioides*** and ***F. doederleinei*** showed antibacterial activity against ***E. coli***, with inhibition zones of 20mm. In the MIC assay, all honey samples were active against ***E. coli*** and ***S. aureus***, but ***M. asilvai***, ***M. quadrifasciata anthidioides***, ***F. doederleinei*** and ***T. angustula*** honeys were more active than that from ***Plebeia*** sp. In general MIC methodology was more sensitive than AWD for detection of antimicrobial action of honey of stingless bees.

Table 1 - Antimicrobial activity of honeys and controls by well diffusion method (inhibition zones diameters in mm) and MIC determination (mg mL^{-1}).

	Microorganisms							
	Escherichia coli		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Candida albicans*	
	AWD	MIC	AWD	MIC	AWD	MIC	AWD	MIC
	Bee species							
MA	NS	72.0	22.0±0.0	72.0	NS	NS	NS	NS
FD	20.0±0.0	74.0	30.0±0.0	19.0	NS	NS	NS	NS
MQ	20.0±0.0	70.0	32.7±1.5	35.0	NS	NS	NS	NS
TA	NS	132.0	28.0±2.0	33.0	NS	NS	NS	NS
PS	NS	254.0	17.5±3.6	254.0	NS	NS	NS	NS
	Controls							
GE	17.5±0.5[1]	0.005	25.3±3.5[1]	0.009	18.3±2.1[1]	<0.005	-	-
CL	NS	10.00	NS	< 0.078	NS	1.25	-	-
NY	-	-	-	-	-	-	24.2±0.0[1]	<0.006
SS	-	622.0	-	622.0	-	622.0	-	NS

[1]Concentration = 0.01mg mL^{-1}, AWD = Agar Well Diffusion (inhibition zones diameters in mm, mean±standard deviation), MIC = Minimum Inhibitory Concentration (mg mL^{-1}), NS = Not sensitive, MA = ***Melipona asilvai,*** FD = ***Friseomelita doederleinei***, MQ = ***Melipona quadrifasciata anthidioides***, TA = ***Tetragonisca angustula***, OS = ***Plebeia*** sp., GE = Gentamicin, CL = Chloramphenicol, NY = Nystatin, SS = Sugar solution.

The microorganisms ***P. aeruginosa*** and ***C. albicans*** were resistant to all honeys in both assays. This increased resistance of Gram-negative bacteria to honey samples may be related to the low permeability of their outer membrane and the specialised mechanism to expel foreign substances from the cell, called efflux pump (SLAMA, 2008).

The sugar solution was active against ***E. coli***, ***P. aeruginosa*** and ***S. aureus*** with high MIC value (622.0mg mL^{-1}). The results suggested that the mechanism by which the honeys inhibited the bacterial growth is not only originated from the osmotic effect, since the carbohydrate solution was around 2.5 to 32.5 times less effective than honey samples tested.

To our knowledge, no studies have reported the antimicrobial activity of honey produced by ***M. asilvai*** and ***F. doederleinei***. On the other hand, previous evaluations of antibacterial activity of honeys from ***M. quadrifasciata anthidioides***, ***T. angustula*** and some ***Plebeia*** species were carried out (CORTOPASSI-LAURINO & GELLI, 1991; MIORIN et al., 2003; DEMERA & ANGERT, 2004; RODRIGUEZ-MALAVER et al., 2009; SGARIGLIA et al., 2010). Direct comparisons with the results obtained by other authors cannot be made due to the methodological differences in testing and microbial strains, but the tendencies in the biological behaviour of each group may be related. Honey samples from ***T. angustula*** from Costa Rica were more effective against ***C. albicans*** than ***S. aureus*** (DEMERA & ANGERT, 2004), contrary to the results observed here. However, honeys from ***M. quadrifasciata anthidioides*** from South-eastern Brazil (CORTOPASSI-LAURINO & GELLI, 1991) and ***T. angustula*** from Peru (RODRIGUEZ-MALAVER et al., 2009) were also more active against ***S. aureus*** than ***E. coli***, as verified here.

The broad activity of the samples against ***S. aureus*** explains the popular use of stingless bee honeys on clinical manifestations where this bacterium is usually involved, such as wounds, skin diseases, eye infections, lung and upper respiratory system problems. The observed results of antibacterial action cannot be merely attributed to an osmotic effect, therefore a detailed investigation of the parameters responsible for the antimicrobial activity is necessary to characterise the mechanism of action of stingless bee honeys with medicinal potential, produced in the state of Bahia.

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