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PP-359 [Apitherapy]

"Inhibitory effect of *Melipona beecheii* honey on the morphological transition of *Candida albicans*"

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Keywords: Candida albicans, Stingless bees, honey, morphological transition

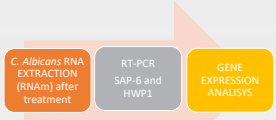
INTRODUCTION

Candida albicans is a polymorphic commensal fungus that is part of the microbiota in humans, as well as in other mammals and birds. Under certain conditions where the host's immune system fails, it becomes pathogenic. One of its main virulence factors is its morphological transition from yeast to hyphae. Recently, *C. albicans* has been classified as a critical priority fungus on the WHO's priority fungal pathogen list due to its high mortality rates, the severity of the disease in immunocompromised patients, and the development of antifungal resistance. The honey of *Melipona beecheii* has been reported to have inhibitory effects on the growth of *C. albicans* yeasts. The objective of this study was to evaluate the effect of *M. beecheii* honey on the morphological transition of *C. albicans*, specifically its transition from yeast to hyphae.

METHODOLOGY

Samples were collected in Maní, Yucatán, Mexico, and processed in the laboratory to evaluate antifungal activity. The minimum inhibitory concentration (MIC) was determined using both the agar dilution method and broth microdilution assay. To assess inhibition of morphological transition, *C. albicans* cells were incubated in RPMI medium buffered with MOPS and supplemented with 10% fetal bovine serum, in the presence of honey at 5, 10, 15, and 20% (w/v). After 3 hours of incubation, morphological changes were examined by light microscopy.

Figure 1. From left to right: (1) Meliponary, (2) *Melipona* honey extraction, (3) antimicrobial assay, and (4) microscopy observation and quantification of morphological changes.



Additionally, assays were performed to determine the expression of two genes involved in *C. albicans* virulence: *SAP6* and *HWP1*. mRNA was extracted, followed by RT-PCR, and gene expression was analyzed.

RESULTS

Figure 2. Antifungal activity of *M. beecheii* honey. Agar dilution assay at 5, 10, and 15% (w/v) and negative control (without honey).

The antifungal activity of the honey of *M. beecheii* was assessed by agar dilution (5, 10, 15% w/v), with complete inhibition observed at 10% w/v. Broth microdilution confirmed the MIC between 10-12% W/V.

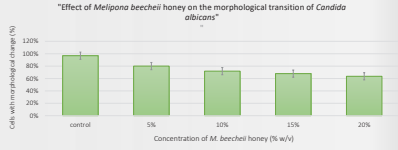


Figure 3. Evaluation of the inhibition of morphological transition in *C. albicans* with treatments corresponding to 5, 10, 15, and 20% of honey.

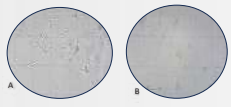


Figure 4. Representative images obtained by light microscopy. A. Control without honey shows all cells in hyphal morphology. B. Treatment with 20% of *M. beecheii* honey shows fewer hyphae and higher proportion of yeast cells.

The effect of *M. beecheii* honey on the inhibition of morphological transition in *C. albicans* was evaluated. Significant differences were observed among treatments ($p < 0.05$), with higher honey concentrations resulting in greater inhibition. In the 20% honey treatment, hyphal cells were reduced by 33.64% compared to the control (figure 4).

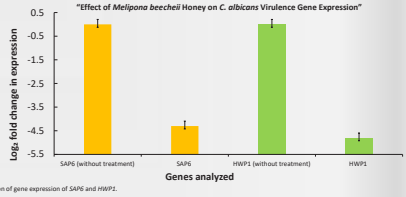


Figure 5. Evaluation of gene expression of *SAP6* and *HWP1*.

SAP6 expression decreased up to –4.5 and *HWP1* up to –4 relative to the control, showing strong inhibition of key virulence genes by *Melipona beecheii* honey.

CONCLUSIONS

M. beecheii honey exhibited effects on the morphological transition of *C. albicans* as well as on the expression of two key genes involved in adhesion, proteolytic activity, and biofilm formation. This study highlights the importance of these findings, as morphological transition represents one of the earliest stages of *C. albicans* virulence. The results demonstrate the potential of this honey as an alternative therapeutic agent.

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Candida albicans is a polymorphic commensal fungus that is part of the microbiota in humans, as well as in other mammals and birds. Under certain conditions where the host's immune system fails, it becomes pathogenic. One of its main virulence factors is its morphological transition from yeast to hyphae. Recently, C. albicans has been classified as a critical priority fungus on the WHO's priority fungal pathogen list due to its high mortality rates, the severity of the disease in immunocompromised patients, and the development of antifungal resistance.

The honey of Melipona beecheii has been reported to have inhibitory effects on the growth of C. albicans yeasts. The objective of this study was to evaluate the effect of M. beecheii honey on the morphological transition of C. albicans, specifically its transition from yeast to hyphae. The minimum inhibitory concentration (MIC) of honey was determined using the macrodilution method. To assess the effect of honey on filamentation, susceptibility assays were performed using culture medium supplemented with honey at concentrations of 8%, 10%, and 12% (w/v), and the percentage of filamentation inhibition was determined. Additionally, the effect of honey on the expression of the SAP6 and HWP1 genes, which are involved in C. albicans virulence and morphological transition, was evaluated using qRT-PCR.

The MIC of M. beecheii honey against C. albicans ranged between 10% and 12% (w/v). A percentage reduction in filamentation of 81% was observed in treatments with M. beecheii honey concentrations of 10% or higher compared to the control. Molecular analysis revealed that honey repressed the expression of the SAP6 gene, which encodes the aspartic protease Sap6 involved in tissue invasion and expressed during hyphal formation, and the HWP1gene, which encodes the adhesion protein Hwp1 of the hyphal cell wall and is induced during the yeast-to-hypha transition. Both genes were repressed by a factor of 4.8-fold.

This study highlights the importance of stingless bee honey in the field of microbiology as a potential alternative for the treatment of diseases caused by microorganisms resistant to currently available antibiotics.