Title : Effects of *Apis mellifera* Honey on Balancing Probiotic Growth and Inhibiting Gastrointestinal Pathogenic Bacteria.

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ABSTRACT

Honey derived from *Apis mellifera* is a natural product rich in bioactive constituents. This study aimed to determine the levels of bioactive compounds in monofloral honeys, including longan, lychee, and sunflower honeys, and to evaluate their potential to enhance probiotic growth and inhibit pathogenic bacteria under *in vitro* conditions. The results demonstrated that lychee honey contained significantly higher total phenolic and flavonoid contents than the other honey samples ($p < 0.05$), with values of 226.15 ± 20.12 mg GAE/kg extract and 5.602 ± 0.199 mg QE/kg extract, respectively. Antimicrobial activity was assessed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against probiotic strains, including *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, and *Bifidobacterium animalis subsp. lactis*, as well as pathogenic bacteria *Escherichia coli* O157:H7 and *Vibrio cholerae*. The findings indicated that antimicrobial effects varied depending on both the bacterial species and the honey type. Furthermore, the growth-promoting effects of honey on probiotic strains were evaluated at different time points by measuring optical density at 600 nm. Longan and sunflower honeys at 15.63 mg/mL significantly enhanced the growth of *L. paracasei* compared with the untreated control. Based on these results, longan and sunflower honeys at 15.63 mg/mL were further investigated for their ability to modulate the balance between probiotics and pathogenic bacteria in co-culture experiments. Probiotic and pathogenic strains were co-cultivated at a ratio of 1:2 in the presence of honey for 48 hours. Total plate count analysis revealed that *L. paracasei* grew significantly in co-culture with *E. coli* O157:H7, whereas no viable *E. coli* O157:H7 cells were detected ($p < 0.05$). In contrast, co-

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culture with *V. cholerae* showed no statistically significant difference in bacterial growth. This outcome may be attributed to honey components that support the growth of both microorganisms and to the ability of *V. cholerae* to tolerate acidic conditions and certain inhibitory compounds, resulting in microbial equilibrium rather than clear suppression. Overall, these findings highlight the potential of honey as a source of bioactive compounds that may be applied in functional foods or health-promoting products aimed at modulating gut microbiota balance.

Keywords: *Apis mellifera* honey; Bioactive compounds; Gut microbiota; Prebiotics; Probiotics

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