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-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.

Introduction

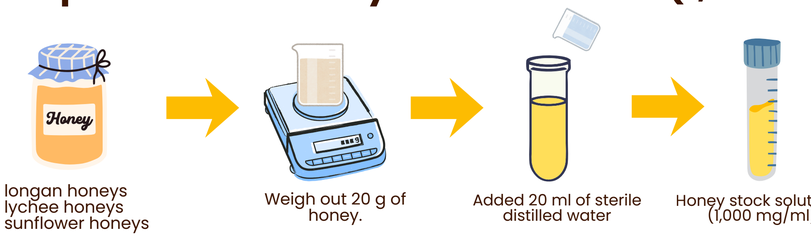
The human intestine harbors a large and diverse population of microorganisms that play crucial roles in digestion, nutrient absorption, and the maintenance of immune balance. Beneficial microorganisms, or probiotics, such as *Lactobacillus* and *Bifidobacterium*, produce various metabolites that help reduce inflammation, strengthen the intestinal barrier, and inhibit the growth of pathogenic bacteria. Prebiotics are non-digestible substances that serve as substrates for probiotics, leading to the production of short-chain fatty acids (SCFAs) that support gut health and microbial balance. Honey is a natural source of prebiotics, rich in oligosaccharides and phenolic compounds, which can promote probiotic growth, enhance SCFA production, and suppress pathogenic microorganisms. Therefore, honey shows strong potential for development as a functional ingredient in health-promoting foods and dietary supplements.

Objectives

- **To determine the contents of phenolic and flavonoid in longan, lychee, and sunflower honeys.**
- **To evaluate the ability of longan, lychee, and sunflower honeys for balancing probiotic growth and inhibiting gastrointestinal pathogenic bacteria.**

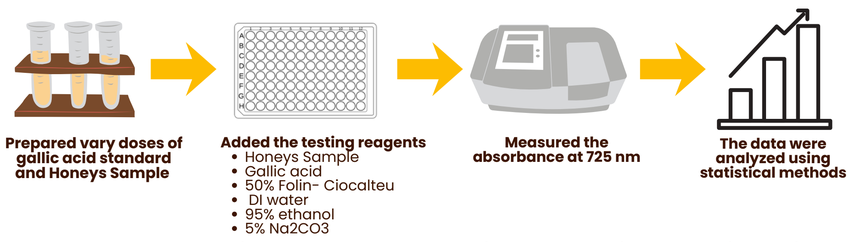
Methodology

1 Preparation of Honey Stock Solution (1,000 mg/mL)

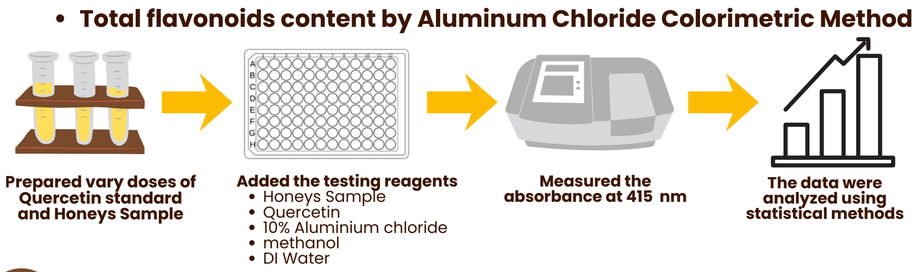


2 Chemical Analysis

- **Total phenolic compound by Folin-Ciocalteu assay**

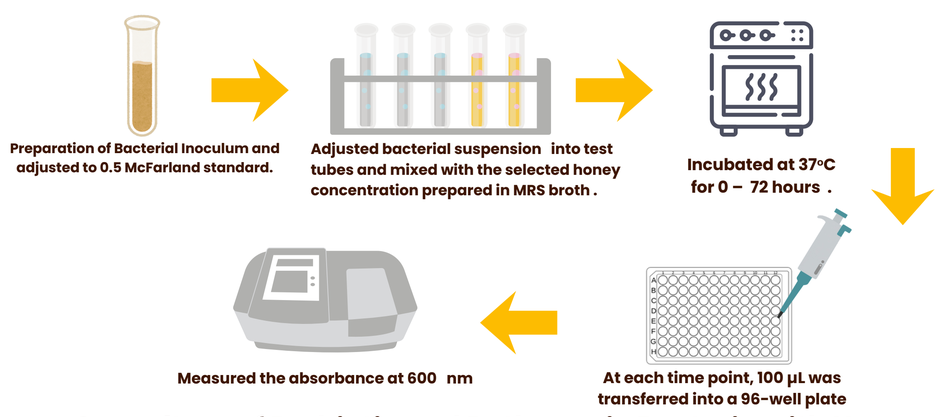


- **Total flavonoids content by Aluminum Chloride Colorimetric Method**

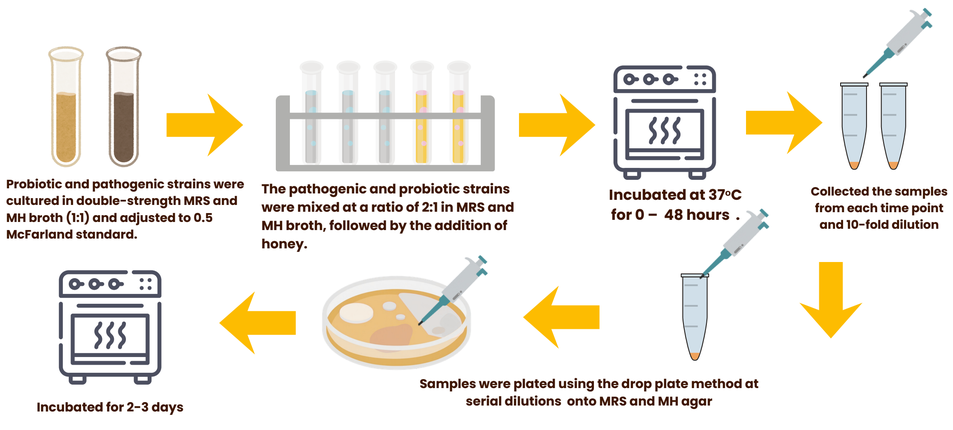


3 Biological Analysis

- **Growth-Promoting Activity of Honey on Probiotics**



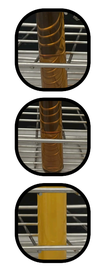
- **Co-culture of Probiotic and Pathogenic Bacteria with Honey**



Results

1.Physicochemical properties of honey samples

Honeys	Color	Viscosity	pH
Longan	Amber, moderately intense, clear	Low	4.14
Lychee	Dark amber, intense, and transparent	Moderate	3.46
Sunflower	Light golden yellow to pale amber slightly opaque	High	3.35



2.Total phenolic compound and Total Flavonoids content

Honeys	Total phenolic compound (mg GAE/kg)	Total flavonoids Content (mg QE/kg)
Longan	193.09 ±14.63^{b}	3.842 ± 0.131^{c}
Lychee	226.15 ± 20.12^{a*}	5.602 ± 0.199^{a*}
Sunflower	157.07 ± 22.05^{c}	5.122 ± 0.390^{b}

^{a,b,c} The statistical values were compared among the different honey samples. ($P < 0.05$). *The results showed the highest content ($P < 0.05$).

3.Growth-Promoting Activity of Honey on Probiotics

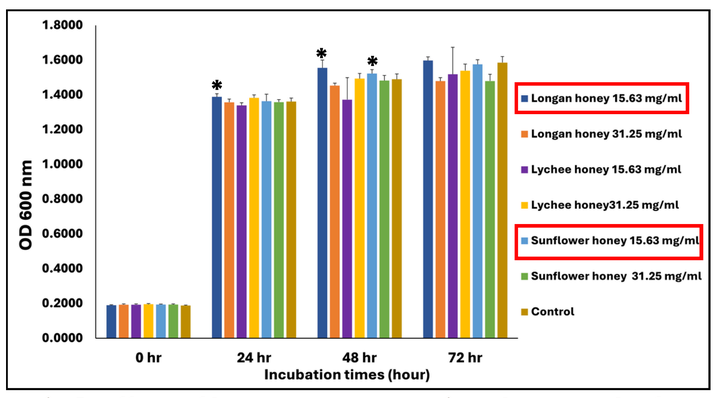


Fig.1 : Effect of honeys on promoting the growth of *L. paracasei* M137 (*$P < 0.05$)

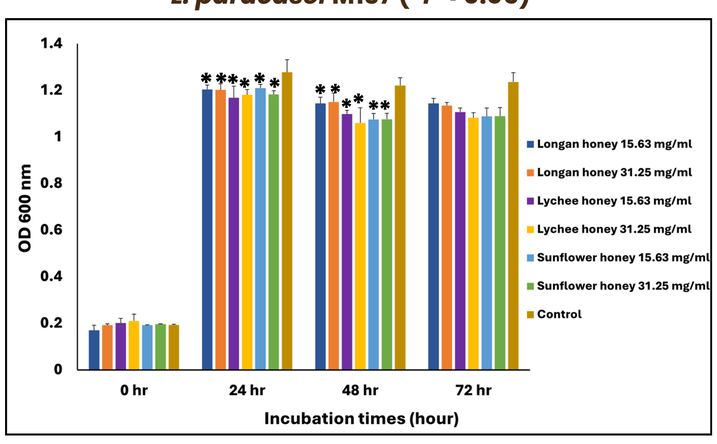


Fig.2 : Effect of honeys on promoting the growth of *L. plantarum* TISTR 2070 (*$P < 0.05$)

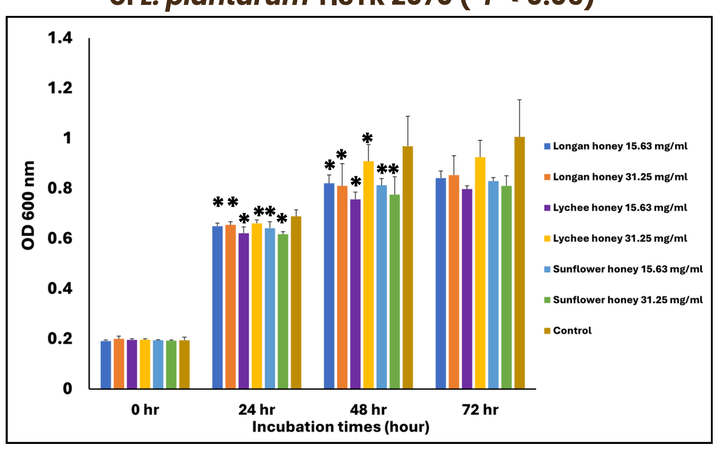


Fig.3 : Effect of honeys on promoting the growth of *B. animalis* subsp. *lactis* CP-9 (*$P < 0.05$)

4.Co-culture of Probiotic and Pathogenic Bacteria with Honey

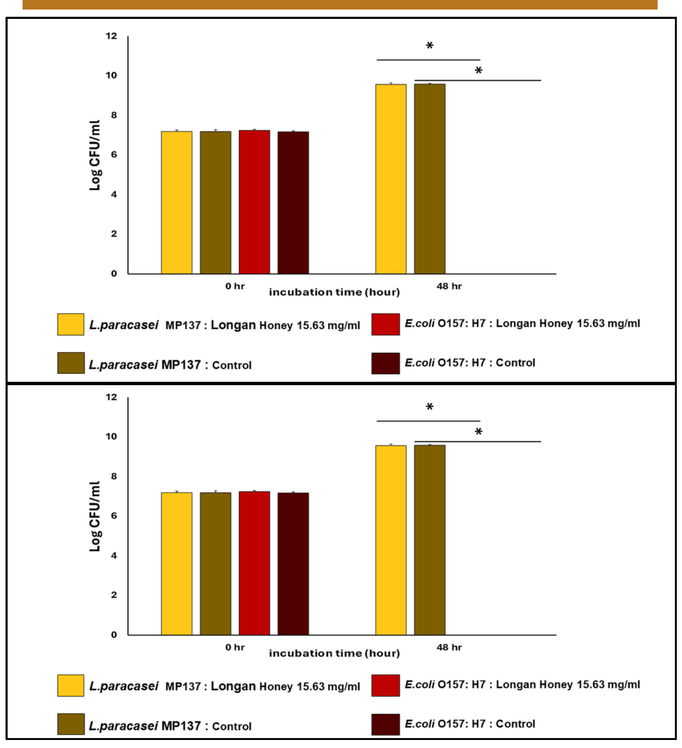


Fig.4 : Effect of honeys on *L. paracasei* MP137 and *E.coli* O157:H7 in a co-culture system (*$P < 0.05$)

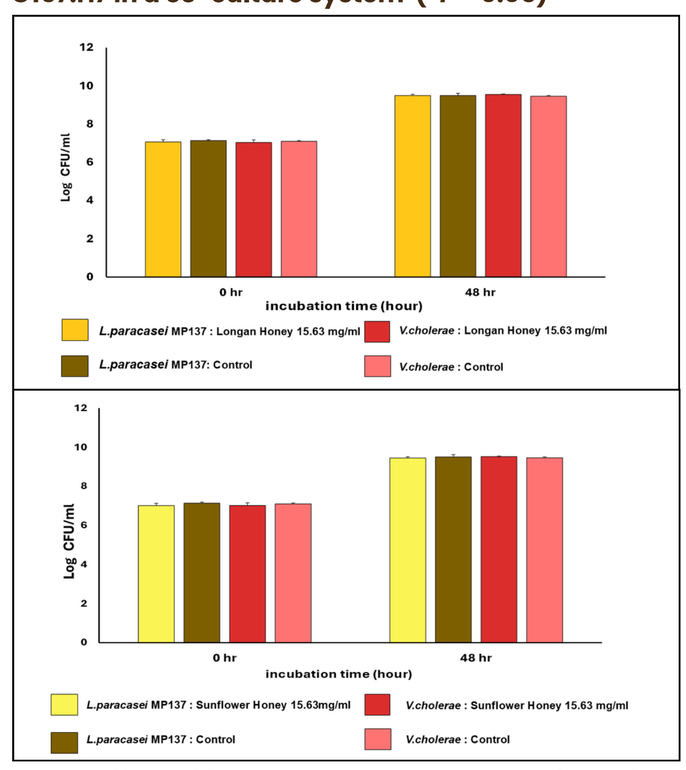


Fig.5 : Effect of honeys on *L. paracasei* MP137 and *V. cholerae* in a co-culture system (*$P < 0.05$)

Conclusion

- **Lychee honey exhibited the highest total phenolic and total flavonoid contents among the tested honey samples.**
- **Both longan and sunflower honeys at 15.63 mg/mL significantly promoted the growth of *L. paracasei* MP137, indicating their potential prebiotic effects.**
- **In co-culture experiments, *E. coli* O157:H7 did not grow when cultured together with *L. paracasei* MP137 in the presence of longan and sunflower honeys, suggesting an inhibitory effect against the pathogenic strain.**
- **When *L. paracasei* MP137 co-cultured with *V. cholerae*, the probiotic strain contributed to maintaining microbial balance by preventing the overgrowth of the pathogen.**

Acknowledgements

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References

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