BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 123-127 | DOI: 10.14421/biomedich.2025.141.123-127 ISSN 2540-9328 (online)

Synergistic Anti-Biofilm Activity of Klanceng Honey and Probiotics against *Candida albicans*

Budhi Setiawan[1], Masfufatun[2,*], Lusiani Tjandra[3], Kartika Ishartadiati[4]
[1,3]Pharmacology Department; [2]Biochemistry Department; [4]Parasitology Deparment, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya
Jl. Dukuh Kupang XXV no 54 Dukuh Kupang 60225, Dukuh Pakis, Surabaya, Indonesia.

Corresponding author*
masfufatun@uwks.ac.id

Manuscript received: 03 January, 2025. Revision accepted: 08 May, 2025. Published: 14 May, 2025.

Abstract

Candida albicans is a common cause of fungal infections, especially in hospitals. Biofilm formation by this fungus is a significant problem as it leads to increased drug resistance and complications in treatment. The biofilm's extracellular matrix protects the fungal cells, making it difficult for antifungal drugs to penetrate and clear the infection. Aim: This study investigates the potential of honey and probiotic formulations to inhibit the biofilm matrix formation in *Candida albicans*. Result: Klanceng honey showed the strongest inhibition of *Candida albicans* biofilm formation, reducing it by 89.39% (OD = 0.34 ± 0.10), compared to yogurt (29.43%, OD = 2.25 ± 0.16) and Yakult (67.95%, OD = 1.02 ± 0.25) ($p < 0.05$). When mixed with yogurt in a 5:1:2 ratio (honey:yogurt: water), Klanceng honey reduced biofilm formation by 84.92% (OD = 0.50 ± 0.12). Manuka and Melifera honey in the same ratio also showed strong inhibition, reducing biofilm formation by 77.84% (OD = 0.73 ± 0.08) and 90.52% (OD = 0.31 ± 0.07), respectively. This performance exceeded fluconazole's, which achieved a 65.52% reduction (OD = 1.14 ± 0.02). For all three honey types, the 4:1:3 and 2:1:5 ratios (honey:Yakult: water) were more effective, with reductions of up to 80.96% for Melifera and 75.64% for Klanceng ($p < 0.05$). Conclusion: This research suggests that honey and probiotic formulations hold promise as natural alternatives in combating *Candida albicans* infections. The study found that the ratio of honey to probiotics in the formulations significantly influenced their effectiveness.

Keywords: Klanceng; honey; Yogurt; probiotic; Antibiofilm.

INTRODUCTION

The epidemiology of *Candida albicans* infections reveals their pervasive impact on immunocompromised and non-immunocompromised individuals globally. *Candida albicans* is notably the leading cause of invasive candidiasis, especially in nosocomial (hospital-acquired) settings, accounting for around 55% of cases in various healthcare facilities (Şanlı et al., 2024; Kreitmann et al., 2024). Predisposing factors for candidiasis include prolonged antibiotic use, central venous catheters, and immunosuppression from conditions such as cancer or post-surgery recovery (Oliva & Venditti., 2024; de Almeida et al., 2024). Studies also highlight an evolving epidemiology with an increasing prevalence of non-albicans Candida species due to antifungal resistance, particularly among long-term patients (Saeed et al., 2024). Understanding these epidemiological patterns is critical for advancing diagnostic and therapeutic interventions, given the heightened morbidity and mortality rates associated with *Candida albicans* infections in vulnerable patient populations.

Biofilm formation in *Candida albicans* significantly contributes to its pathogenicity and resistance to antifungal treatments. These biofilms, complex structures composed of yeast and hyphal cells embedded in a protective extracellular matrix, enhance resistance to antifungal agents by limiting drug penetration and fostering genetic adaptation (Chamtouri et al., 2024; Waykar & Kumarapillai, 2024). These biofilms complicate infections, particularly in clinical settings where C. albicans adhere to indwelling medical devices, leading to persistent infections and heightened resistance, especially against azoles and echinocandins (Alvarez et al., 2024). Biofilm-associated cells demonstrate increased expression of resistance genes and can endure host immune responses, making infections challenging to manage (Makled et al., 2024). Moreover, synergistic treatment strategies, such as combining antifungals with biofilm-disrupting agents, have shown promise but are not yet widespread in clinical practice (Sun et al., 2024). Understanding the mechanisms of biofilm formation and resistance is thus crucial for developing more effective therapeutic approaches.

Honey and probiotics demonstrate significant potential as antibiofilm agents against *Candida albicans*, effectively disrupting biofilm formation and enhancing antimicrobial effectiveness. Honey, with its high

osmolarity, acidity, and rich polyphenol content, creates an inhospitable environment for Candida biofilm development. Studies have shown that honey prevents Candida adhesion on surfaces and destabilizes existing biofilms (Acaroz et al., 2024). When combined with probiotic strains like Lactobacillus spp., honey's antibiofilm effects are amplified, as probiotics themselves release metabolites that interfere with Candida biofilm matrices (Machado et al., 2023). Probiotic bacteria also produce biosurfactants that disrupt biofilm integrity, preventing Candida cells from establishing resilient communities on host surfaces (Luca et al., 2024). These combined approaches offer promising, natural antibiofilm strategies that could reduce reliance on conventional antifungal drugs.

Klanceng honey, also known as stingless bee honey, is a unique native Indonesian honey produced by Tetragonula species (stingless bees). Klanceng honey is produced in limited quantities, making it rarer and often more valued. The lower honey yield and traditional harvesting techniques contribute to its exclusivity and cultural significance in Indonesia. While it is traditionally valued in Indonesian folk medicine for its health benefits, scientific research on Klanceng honey, especially regarding its antifungal efficacy, remains limited. Existing studies primarily focus on its antibacterial properties and bioactive components, like phenolic compounds, which suggest potential anti-fungal effects. However, detailed studies on its specific efficacy against fungal pathogens such as *Candida albicans* or dermatophytes are scarce. This gap underscores the need for further research to fully explore and validate the antifungal potential of Klanceng honey, which could reinforce its role as a natural alternative in managing fungal infections. Therefore, this study aims to provide a comparative analysis of the inhibition effect of Klanceng honey and probiotics formulation on the biofilm matrix of *Candida albicans*.

MATERIALS AND METHODS

Regeneration and Preparation of C. albicans Inoculum

A single isolate from an aged SDA medium was collected using an inoculating loop and streaked onto a fresh SDA medium. The SDA medium was then incubated at 37°C for 2–3 days to allow the formation of a single C. albicans colony.

The single C. albicans colony grown on the new SDA medium was inoculated into an Erlenmeyer flask containing 10 ml of SDB. The flask was shaken for 18–20 hours at a speed of 120 rpm, resulting in a turbid Candida suspension, referred to as the inoculum.

Preparation of C. albicans Suspension

The inoculum obtained was transferred into a centrifuge tube and centrifuged for 15 minutes to form a pellet. The pellet was separated from the filtrate and resuspended in PBS (Phosphate-Buffered Saline). This centrifugation and resuspension process was repeated twice. The pellet obtained from the third centrifugation was resuspended in RPMI medium, and the optical density (OD) was measured at 595 nm using a microplate reader. If the OD of the C. albicans suspension exceeded 0.5, dilution was performed until an OD of 0.5 (equivalent to 1×10^6 CFU/ml) was achieved. This C. albicans suspension was then ready for antibiofilm testing (Fauzan et al., 2023).

Biofilm Matrix Formation Test

100 µL of C. albicans suspension was added to each test and control well (except for blank wells, which were replaced with 100 µL of RPMI medium) and incubated at 37°C for 2 hours. After incubation, each well was washed twice with PBS, and 100 µL of the treatment solution was added to each test well. The treatment solutions were prepared with three different ratios of honey: yogurt or Yakult: medium, specifically 5:1:2, 4:1:3, and 2:1:4. For the blank and negative control wells, 100 µL of RPMI medium was added, while the positive control wells received 100 µL of fluconazole solution. The microplate was then incubated at 37°C for 48 hours.

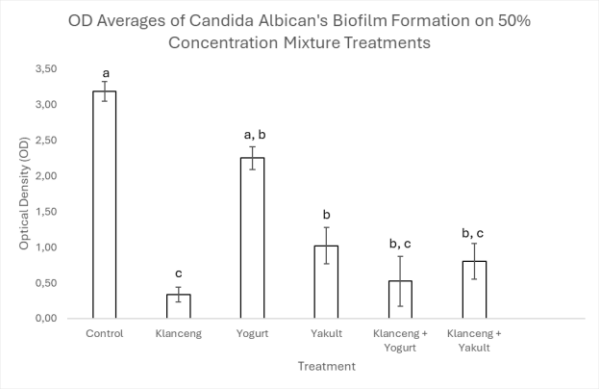
After the 48-hour incubation, the non-adherent planktonic cells were discarded, and the wells were rinsed with PBS. Next, 100 µL of methanol was added to each well and incubated for 15 minutes at room temperature. The remaining methanol was discarded, and the microplate was air-dried briefly. Then, 100 µL of 0.1% crystal violet (CV) was added to each well and incubated for 15 minutes. Excess CV was discarded, and the wells were rinsed with PBS. Finally, 100 µL of 96% ethanol was added to each well, and the optical density (OD) was measured at $\lambda = 595$ nm.

Data Analysis

The research data were analyzed using SPSS (Statistical Product and Service Solutions) statistical software. For sample sizes less than 50, the Shapiro-Wilk test was used for normality assessment. Homogeneity was tested using Levene's test if the data were normally or near-normally distributed. One-way ANOVA was employed to evaluate differences in C. albicans growth across variations in honey: yogurt or Yakult: medium (distilled water) composition. If the data were not normally distributed or not homogeneous, the non-parametric Kruskal-Wallis test was applied. Post hoc testing was conducted to identify which test groups exhibited significant differences.

RESULTS AND DISCUSSION

Result



Klanceng honey most effectively inhibited *Candida albicans* biofilm formation (0.34 ± 0.10), showing an 89.39% decrease compared to the control (3.19 ± 0.14). In comparison with control, Yogurt and Yakult also showed significant inhibition, with decreases of 29.43% (2.25 ± 0.16) and 67.95% (1.02 ± 0.25) respectively. In the same concentration of 50%, combining Klanceng honey with yogurt or Yakult (83.57% and 74.80% decreases, respectively) did not enhance its effect.

Table 1. Inhibition of *Candida albicans* Biofilm Formation by Klanceng Honey and Yogurt Formulations.

		Mixture (honey: probiotic: water)	OD Mean ± SD	Biofilm proportion (%)	Biofilm decrease (%)
Control			3.29 ± 0.08^{a}	100.00	0.00
Fluconazole			1.14 ± 0.02^{c}	34.48	65.52
Types of Honey	Manuka	(5:1:2)	0.73 ± 0.08^{d}	22.16	77.84
		(4:1:3)	1.34 ± 0.15^{b}	40.58	59.42
		(2:1:5)	1.11 ± 0.08^{c}	33.81	66.19
	Melifera	(5:1:2)	0.31 ± 0.07^{f}	9.48	90.52
		(4:1:3)	1.07 ± 0.02^{c}	32.56	67.44
		(2:1:5)	$0.39 \pm 0.04^{e,f}$	11.70	88.30
	Klanceng	(5:1:2)	0.50 ± 0.12^{e}	15.08	84.92
		(4:1:3)	0.73 ± 0.08^{d}	22.03	77.97
		(2:1:5)	0.71 ± 0.05^{d}	21.62	78.38

In combination with Yogurt, Klanceng honey most effectively inhibited *Candida albicans* biofilm formation, with the 5:1:2 (honey:yogurt:water) mixture achieving an 84.92% reduction (OD = 0.50 ± 0.12). This surpassed the antifungal fluconazole (65.52% reduction, OD = 1.14 ± 0.02). With the 5:1:2 ratio, Manuka and Melifera honey also showed relatively higher inhibition (77.84% reduction, OD = 0.73 ± 0.08; 90.52% reduction, OD = 0.31 ± 0.07 respectively) than other mixture ratios. Therefore, this combination of honey, yogurt, and water especially in the 5:1:2 mixture ratio, exhibited the most substantial inhibitory effect on *Candida albicans* biofilm formation, even surpassing the antifungal fluconazole in some instances.

Table 2. Inhibition of *Candida albicans* Biofilm Formation by Honey and Yakult Formulations.

		Mixture (honey: probiotic: water)	OD Mean ± SD	Biofilm proportion (%)	Biofilm decrease (%)
Control			3.19 ± 0.10^{a}	100	
Fluconazole			1.08 ± 0.08^{d}	34.04	65.96
Types of Honey	Manuka	(5:1:2)	0.73 ± 0.08^{d}	51.46	48.54
		(4:1:3)	1.34 ± 0.15^{b}	43.811	56.19
		(2:1:5)	1.11 ± 0.08^{c}	20.27	79.73
	Melifera	(5:1:2)	0.31 ± 0.07^{f}	27.70	72.30
		(4:1:3)	1.07 ± 0.02^{c}	19.04	80.96
		(2:1:5)	$0.39 \pm 0.04^{e,f}$	19.57	80.43
	Klanceng	(5:1:2)	0.50 ± 0.12^{e}	46.05	53.95
		(4:1:3)	0.73 ± 0.08^{d}	24.36	75.64
		(2:1:5)	0.71 ± 0.05^{d}	25.12	74.88

Across all three honey types (Manuka, Melifera, and Klanceng), the 4:1:3 and 2:1:5 (honey:Yakult:water) ratios consistently demonstrated significantly greater inhibition of *Candida albicans* biofilm formation compared to the 5:1:2 ratio. This trend was observed for Manuka honey (56.19% and 79.73% reduction for 4:1:3 and 2:1:5 vs. 48.54% for 5:1:2), Melifera honey (80.96% and 80.43% reduction for 4:1:3 and 2:1:5 vs. 72.30% for 5:1:2), and Klanceng honey (75.64% and 74.88% reduction for 4:1:3 and 2:1:5 vs. 53.95% for 5:1:2). This suggests that a lower proportion of honey in the mixture, relative to Yakult and water, enhances the inhibitory effect on biofilm formation.

Discussion

The study demonstrates the inhibitory potential of Klanceng honey combined with probiotics on the biofilm matrix of *Candida albicans*, highlighting a natural approach to addressing anti-fungal resistance associated with biofilm formation. Among the honey types tested, Melifera honey exhibited the highest biofilm inhibition, reducing biofilm formation by up to 90.52% at the 5:1:2 ratio in combination with water and Yogurt. This effect surpassed the inhibition of fluconazole, the positive control, which achieved a 65.52% reduction. The results confirm the synergy between honey's inherent bioactive compounds and probiotic metabolites in destabilizing the extracellular biofilm matrix.

The biofilm matrix's resistance to antifungal treatments is primarily attributed to its ability to limit drug penetration and foster genetic resistance mechanisms (Chamtouri et al., 2024; Waykar & Kumarapillai, 2024). Honey's rich polyphenolic content and acidity create a hostile environment for *Candida albicans*, disrupting adhesion and biofilm integrity (Acaroz et al., 2024). Klanceng honey, although less effective than Melifera, demonstrated significant inhibition (84.92%) at the 5:1:2 ratio, suggesting its potential as a natural antifungal agent despite its lower yield and limited scientific exploration. The findings emphasize the importance of optimizing honey-to-probiotic ratios to maximize antifungal efficacy.

Probiotics play a critical role by producing biosurfactants and organic acids that interfere with biofilm formation and enhance the antimicrobial effects of honey. Lactobacillus strains are known for their ability to disrupt biofilms and prevent the establishment of resilient microbial communities (Luca et. al., 2024). These findings align with previous studies that demonstrating that combining natural antimicrobials can offer a dual approach: directly inhibiting biofilm formation and reducing the dependence on conventional antifungal agents.

This research investigated the effectiveness of different honey types and mixtures with yogurt or Yakult in inhibiting *Candida albicans* biofilm formation. Klanceng honey alone showed the strongest inhibitory effect (89.39% reduction of control), followed by the combination with water and yogurt in a 5:1:2 ratio (84.92%) and the mixture with water and Yakult in a 4:1:3 ratio (75.54%). Interestingly, combining Klanceng honey with yogurt or Yakult with the same 50% concentration did not significantly enhance its inhibitory effect.

Further investigation explored the impact of varying honey: yogurt: water ratios. When combined with yogurt, Klanceng honey again demonstrated significant inhibition, particularly with the 5:1:2 ratio, achieving an 84.92% reduction in biofilm. This even surpassed the antifungal fluconazole (65.52% reduction). Notably, this 5:1:2 ratio also proved most effective for Manuka and Melifera honey, yielding 77.84% and 90.52% reductions respectively.

These findings suggest that: Klanceng honey is a potent inhibitor of *Candida albicans* biofilm. The ratio of honey to yogurt and water significantly influences the inhibitory effect. A 5:1:2 ratio of honey: yogurt: water maximizes biofilm inhibition for Klanceng and Melifera honey. This highlights the potential of honey, especially Klanceng honey in combination with yogurt, as a natural alternative or adjunct to conventional antifungal treatments. Further research is needed to elucidate the underlying mechanisms and clinical implications.

Notably, the study underscores the variability in the efficacy of honey and probiotic formulations based on the ratio of the components. For instance, Melifera Honey’s performance peaked at the 5:1:2 ratio but diminished with lower concentrations, indicating the critical role of honey's active compounds in achieving optimal results. The lower efficacy of Klanceng honey at higher dilution ratios highlights the need for further research to understand the impact of its specific bioactive components and their interactions with probiotics.

Although the results indicate that Melifera honey demonstrated the highest inhibition of *Candida albicans* biofilm formation at the 5:1:2 ratio (90.52% reduction), Klanceng honey remains a primary focus due to its unique attributes and broader implications for antifungal therapy. Klanceng honey, derived from stingless bees native to Indonesia, has significant cultural and medicinal value, making it a relevant candidate for local natural remedies. Furthermore, Klanceng honey demonstrated remarkable consistency across various formulations, achieving an 89.39% inhibition and up to 84.92% in combination with yogurt, comparable to or exceeding those of fluconazole. This positions Klanceng honey as an accessible and effective alternative for antifungal applications, particularly in resource-limited settings where it is locally available. Klanceng honey's rare status and limited scientific exploration highlight its importance as a candidate for further research and development. By focusing on Klanceng honey, the study not only underscores its potential but also advocates for

exploring underutilized natural resources in combating anti-fungal resistance.

CONCLUSIONS

This study highlights the promising potential of Klanceng honey and probiotics as natural, effective alternatives to conventional antifungal therapies. Future investigations should focus on elucidating the molecular mechanisms underlying their synergistic effects, evaluating their efficacy in vivo, and exploring the potential for scaling these formulations for clinical application. By addressing the growing challenge of antifungal resistance, such natural approaches could complement existing therapies and improve outcomes in managing *Candida albicans* infections

***Acknowledgements*:** Special gratitude goes to the Department of Biochemistry, University of Wijaya Kusuma Surabaya, and the Infectious Hospital University of Airlangga.

***Authors' Contributions*:** Masfufatun designed the study. Lusiani Tjandra carried out the laboratory work. Kartika Ishartadiati analyzed the data. Budhi Setiawan wrote the manuscript. All authors read and approved the final version of the manuscript.

***Competing Interests*:** The authors declare that there are no competing interests.

***Funding*:** The author would like to express his deepest gratitude to Universitas Wijaya Kusuma Surabaya for the support and funding provided for the implementation of this research with contract number 87/LPPM/UWKS/III/2024

REFERENCES

Acaroz, U., Kurek-Gorecka, A., Olczyk, P., Tas, N., Ali, A., Paramanya, A., ... & Jin, X. (2024). The role of bee products in the control of antimicrobial resistance and biofilm formation. Kafkas Universitesi Veteriner Fakultesi Dergisi, (2). Link.

Alvarez, L., Kumaran, K. S., Nitha, B., & Sivasubramani, K. (2024). Evaluation of biofilm formation and antimicrobial susceptibility (drug resistance) of *Candida albicans* isolates. Brazilian Journal of Microbiology, 1-12. Link

Chamtouri, M., Merghni, A., Miranda-Cadena, K., Sakly, N., Gaddour, N., de Los Reyes-Gavilán, C. G., ... & Quindós, G. (2024). Characterization of Yeast Isolated from the Gut Microbiota of Tunisian Children with Autism Spectrum Disorder. Journal of Fungi, 10(11), 730. Link

de Almeida, B. L., Arcieri, V. C., Razente, D. M., Freire, M. P., Guimarães, T., Araújo, E. D. M. P. A. D., ... & Magri, M. M. C. (2024). Intra-Abdominal Candidiasis in Cancer Patients: A 10-Year Experience in a Middle-Income Country. Mycoses, 67(10), e13807. Link

Kreitmann, L., Blot, S., & Nseir, S. (2024). Invasive fungal infections in non-neutropenic patients. Intensive Care Medicine, 1-5. Link

Luca, L., Pauliuc, D., & Oroian, M. (2024). Honey microbiota, methods for determining the microbiological composition and the antimicrobial effect of honey–A review. Food Chemistry: X, 101524. Link.

Machado, A., Zamora-Mendoza, L., Alexis, F., & Álvarez-Suarez, J. M. (2023). Use of plant extracts, bee-derived products, and probiotic-related applications to fight multidrug-resistant pathogens in the post-antibiotic era. Future Pharmacology, 3(3), 535-567. Link.

Makled, A. F., Ali, S. A., Labeeb, A. Z., Salman, S. S., Shebl, D. Z., Hegazy, S. G., & Sabal, M. S. (2024). Characterization of Candida species isolated from clinical specimens: insights into virulence traits, antifungal resistance and molecular profiles. BMC microbiology, 24(1), 388. Link

Oliva, A., & Venditti, M. (2024). Invasive Candidiasis in Surgery. In Infections in Surgery: Prevention and Management (pp. 107-116). Cham: Springer Nature Switzerland. Link

Saeed, N. K., Almusawi, S., & Al-Beltagi, M. (2024). Candidemia chronicles: Retrospective analysis of candidemia epidemiology, species distribution, and antifungal susceptibility patterns in Bahrain. World Journal of Virology, 13(4). Link

Şanlı, K., Arslantaş, E., Ceylan, A. N., Öncel, B., Özkorucu, D., & Özkan Karagenç, A. (2024). Candidemia in Pediatric-Clinic: Frequency of Occurrence, Candida Species, Antifungal Susceptibilities, and Effects on Mortality (2020–2024). Diagnostics, 14(20), 2343. Link

Sun, C., Zhu, L., Yang, L., Tian, Z., Jiao, Z., Huang, M., ... & Guo, G. (2024). Antimicrobial peptide AMP-17 induces protection against systemic candidiasis and interacts synergistically with fluconazole against *Candida albicans* biofilm. Frontiers in Microbiology, 15, 1480808. Link

Waykar, R., & Kumarapillai, S. Antifungal Drug Resistance in *Candida albicans*: Identifying Novel Targets for the Development of Effective Antifungal Agents. Link

THIS PAGE INTENTIONALLY LEFT BLANK