Determining the Antifungal Activity of Local Medical Grade Honey against *Candida albicans*

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Abstract

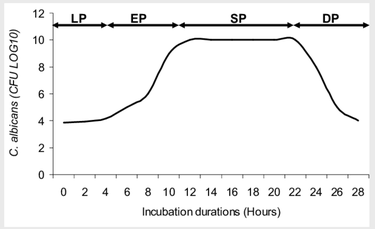
The challenge of developing effective over-the-counter antifungal treatments remains a focal point within the scientific community. With fungal species increasingly becoming resistant, concern within the clinical settings and individual well-being continue to be emphasized. This strain is commonly found in oral, vaginal, and gastrointestinal tracts, with its ability to thrive in various pH levels. This prompts concerns regarding its susceptibility to natural remedies like medicinal honey. Through a series of four standardized experiments, we sought to comprehend and acknowledge the biological dimorphic characteristics exhibited by this isolate. This study utilized a spectrophotometer set to OD 600 nm to determine the optimal growth phase of *C. albicans*, measuring absorbance at 600 nm wavelength in 8-hour intervals. A 1:2 serial dilution was conducted to capture the growth dynamics. Data collected facilitated the estimation of cell concentration/density using 96-well plates. Adjustments were made to the transmission percentage of inoculated *C. albicans* to achieve the desired 1 x 10^6 CFU/mL, 74-75% transmittance, 0.5 McFarland standard. Prior to subsequent procedures, a 1:1000 serial dilution was performed. The zone of inhibition (ZOI) well diffusion method involved applying 100 uL of inoculated solution onto 3 YPD plates, followed by punching holes for 15 different medicinal honey samples. Sterilized toothpicks facilitated the transfer of each honey sample into designated wells, with approximately two drops of honey added to each. Minimum inhibitory concentration (MIC) using RPMI 1640 medium and minimum fungicidal concentration (MFC) assessments were conducted after composing this abstract. Evidence displays that *C. albicans* optimal growth period resides in the 6-7-hour mark of incubation at 37°C. After 5 trials of zone of inhibition with slight changes made to the concentrations and methods of transferring, it is accepted that the *C. albicans* isolate resists medicinal honey. It is predicted that the minimum inhibitory concentration with RMPI 1640 should display a high MIC concentration value and a high MFC value, due to the overgrowing nature of the fungus displayed by the ZOI trials.

Introduction (Background)

The use of honey within the clinical setting has been one of the most effective and preventative ways to treat various diseases and alignments for centuries. Recently, honey stands as a promising biological property that includes antimicrobial, antibiofilm, antioxidant, and anti-inflammatory wound healing activities. Further, medicinal honey has been used for wound dressing with notable successful reduction in wound size in recent patient cases. Although medicinal honey poses an extreme potential as one of the most effective pathogenic remedies, an increase in resistance to antifungal agents for skin infections caused by *C. albicans* has been reported. Within the last decades, Candida albicans has served as the leading causality agent nearing 40% of invasive infections (Chang, 2020). Viscous and thick in nature, the component of honey is composed of 81% sugar (fructose), 17% water and 2% of other compounds on average. Honey compounds contain non-volatile phenolic compounds, enzymes, flavonoids, and volatile compounds containing alcohols, ketones, and acids. These chemical properties influence the pharmacological compositions of honey and its effects on microorganisms. The hydrogen peroxide activity possessed in all honey types, chiefly exhibits antimicrobial reactions, placing a direct effect on the pH of the environment and the clinical isolate of choice. This study challenges the antifungal properties of *C. albicans* against medicinal grade honey within a controlled environment. The species *Candida* are recognized to develop various mechanisms that confer resistance towards antifungal treatment. When growing and reproducing, this opportunistic organism establishes a complex community of microbial cells surrounding a self-made polymeric matrix which adheres to cells at the surface level forming a biofilm. Fungal biofilms capture and colonize inanimate objects thereby contributing to many infections. The varying characteristics of biofilms and its ever changing structural and microbial association is extremely adaptable to different environments. During development, the hyphae phase exudes vast extensions adhering to many surfaces. This distinctive character poses an extreme challenge for clinicians and researchers to effectively control and inhibit its rapid spread.

Figure 1. Growth Curve of Candida albicans

(LP: Lag growth phase, EP: Exponential growth phase, SP: Stationary growth phase, DP: Decline growth phase).



Experimental condition	Cell growth phases			
	Lag	Exponential	Stationary	Decline
Static	-0.993**	-0.982**	-0.991**	-0.979**
Dynamic	0.499	-0.957**	-0.944**	-0.967**

**: P <0.01 ; ddl=11

Methods

Optimizing Growth Phase of *C. albicans:* Growth period of C. albicans within a specific timeframe, a 1:16 serial dilution was conducted with isolated C. albicans cultures early morning. This allowed to determine the precise period and conditions conducive for the proliferation of the fungi. The growth period was assessed using a spectrophotometer set to measure its optical density at 600 nm (OD 600). Using the spectrophotometer, we were able to estimate the concentration of cells per volume within the liquid sample encompassing both living and dead cells.

Another trial of serial dilutions were made at 1:100,000,000 fold. Once again, RMPI was the chosen medium to inoculate 1 colony. The dilution factors would be as followed:

1 mL RMPI with C. albicans (1:1,000 10^3)
900 uL RMPI (1:10,000 10^4)
900 uL RMPI (1:100,000 10^5)
900 uL RMPI (1:1,000,000 10^6)
900 uL RMPI (1:10,000,000 10^7)
900 uL RMPI (1:100,000,000 10^8)

Transmittance: To identify the McFarland standard (0.5) for fungal growth, we employed the method of transmittance to identify the appropriate cell count of 1 x 10^6 CFUs/mL within a sterile water medium. Transmittance (T) represents the fraction of incident light that passes through a substance. Put simply, it measures the light that successfully traverses material and emerges on the other side of the container. Achieving a 0.5 McFarland of fungal solution signifies a 1 x 10^6 CFU/mL cell count. This suggests a transmission rate of 75-77% achieved within the fungal medium.

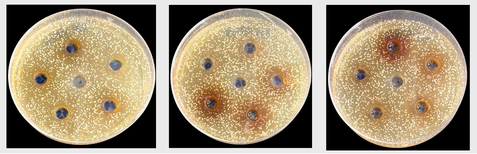
Zone of Inhibition (ZOI): Zone of Inhibition agar well-diffusion method was conducted across 15 honey samples. 5 trials were done with different methods of transferring and at varying concentration levels.

Minimum Inhibition Concentration (MIC): The Minimum Inhibitory Concentration test the lowest concentration of C. albicans capable of inhibiting visible growth. RMPI-1640 medium was used to culture 32 mL of a stock solution containing 1-2 colonies of C. albicans. Four distinct honey dilutions were prepared in separate tubes for each honey sample. The concentrations for these dilutions were as follows:

50% (1.0 g honey + 2 mL RMPI + C. albicans)
40% (0.8 g honey + 2 mL RMPI + C. albicans)
30% (0.6 g honey + 2 mL RMPI + C. albicans)
20% (0.4 g honey + 2 mL RMPI + C. albicans)

Results

Figure 3. Well-Diffusion Zone of Inhibition (ZOI) Plates



Measure tomw and input data here.

Figure 4. Honey Sample PA-5 Minimum Inhibitory Concentration (MIC) Reading

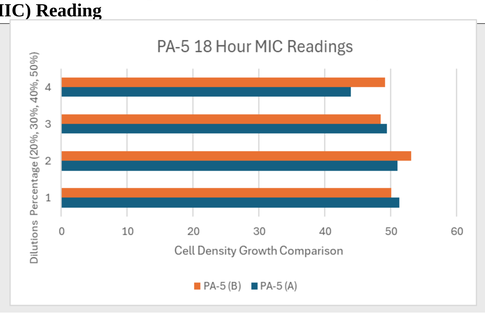
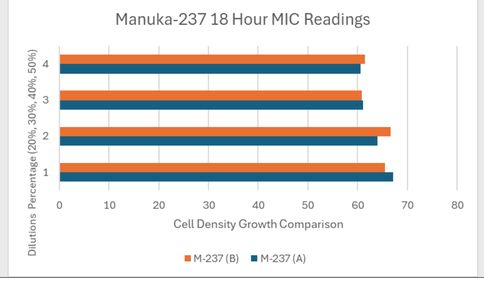


Figure 5. Honey Sample Manuka 237 Minimum Inhibitory Concentration (MIC) Reading



Conclusions

- The 15 honey samples demonstrated minimal to negligible antimicrobial activity in both zone of inhibition trials and MIC experiments. The underlying reasons for this observation require further investigation, as the authors and adjacent instructors have scrutinized factors such as the method of transfer, concentration from the stock solution, and the type of solution used for stock culture.
- Among the honey samples assessed, two are known for their notably high antimicrobial activity and subjected to the Minimum Inhibitory Concentration (MIC) protocol. After transitioning the culture medium to RMPI, MIC results were evaluated based on OD 600 readings following 18 hours of incubation at 32 degrees Celsius. These results revealed no discernible reduction in growth across triplicated honey dilutions.
- To optimize fungal growth, a serial dilution of 1:100,000 was performed, resulting in dilutions ranging from 10^3 to 10^8. The aim was to identify the minimal dilution necessary to achieve the least amount of growth. Following an 18-hour incubation period, it was noted that a dilution factor of 10^6 exhibited minimal growth.
- For treating *C. albicans* with medicinal-grade honey, early intervention is recommended to impede overgrowth that could lead to exacerbated health conditions. The combination of medicinal honey with antifungal drugs holds promise in gradually inhibiting the growth of highly opportunistic, extremely resistant pathogens like *C. albicans*.

Broader Impacts:

It is evident that the biological characteristics of C. albicans is one that requires countless hours of research and time dedicated to understanding and preventing its growth. For future direction of this study, it is recommended that obtaining the lowest serially diluted concentration before performing any experiment, is necessary in order to determine if the chosen medicinal honey samples truly have an impact against it. Further, obtaining known:

- MBC
- Check every 2 hour for a total of 10-18 hours for optimal zoi appearance and catch when over growth begins.
- Manuka works for C.acnes but not as effective on C. albicans but this does not mean the honey samples used in the experiement were any less suitable.
- Test other honeys as studies show Agastache, Tea Tree and Jarrah honeys exhibited the largest zone of inhibition at 40% concentration (19.5 mm) followed by tea-tree honey at 80% honey (14 mm).
- Further fungal infection in the body studies

References

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