The Antibacterial Effect of Ohi'a Lehua Blossom Honey on *E. coli* and *B. cereus*

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Program / Major: Running start

Abstract

Ohi'a Lehua blossoms (OLB), which are critical to honey production and habitats for endangered birds in Hawaii, are currently in danger of extinction due to fungal infection. Honey has been traditionally used as medicine, and providing insight on the potential of OLB honey could help increase awareness for protecting the trees in the future. This investigation evaluates the antimicrobial potential of OLB honey in comparison to Manuka honey (positive control). First, agar well diffusion method was used in triplicate to measure the clear zone of inhibition (ZOI), and evaluate Minimum Inhibitory Concentrations (MIC) with 100, 75, 50 and 25% concentrations of honeys and agave syrup (positive control). Manuka honey showed better antibacterial activity than OLB honey with about an average of 2 mm bigger ZOI for *B. cereus* in 100% concentration. OLB honey showed no ZOI for *E. coli*, while Manuka honey showed an average of 9.3 mm ZOI for 100% concentration (p value = 0.001). Manuka honey showed an MIC of 75% concentration for both bacteria, and OLB honey showed an MIC of 100% for *B. cereus*. Next, broth dilution method using dilutions 1:1, 1:2, 1:4, and 1:8 was done with Manuka and OLB honeys to assess MIC and Minimum Bactericidal Concentrations (MBC). MIC for Manuka honey for both types of bacteria was seen in 1:4 dilution. OLB honey showed an MIC of 1:2 for both bacteria, with more inhibition for *E. coli*. There were no bactericidal effects. Both honeys can be considered as a good candidate for alternate antimicrobial agents in medicine, however OLB honey had less antibacterial ability than Manuka honey. Phytochemical properties of both honeys should be further researched, along with antifungal and antioxidant properties.

Objectives

- Research alternatives for antibiotics and expand on our knowledge of honey to be used as an antibiotic. Many honeys, such as Manuka honey, has been thoroughly researched, yet Ohi'a Lehua Blossom honey is yet to be researched.
- This honey comes from the Ohi'a trees that are in the risk of a rapidly spreading disease called Rapid Ohi'a Death. This fungal disease can spread through the soil that is attached to unaware tourists' boots. This tree is also essential to the habitat of endangered bird species in Hawaii. Therefore, raising awareness for this tree by providing a further incentive to protect it - such as finding its biomedical potential – is high in significance.

Metrosideros polymorpha, also known as 'ōhi'a lehua tree

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Methods

Agar Disk Diffusion → Agar Well Diffusion → Broth Dilution

Wedderspoon's Manuka honey used for investigation

Ohi'a Lehua blossom (OLB) honey used for investigation

Initially, **agar disk diffusion method** was used to test for the antibacterial activity of OLB honey in comparison to the control compounds, Manuka honey, Agave syrup, and sterile distilled water. The results yielded were inconsistent, so agar well diffusion method was used to assess antibacterial potential.

Conclusion

Both OLB and Manuka honeys **showed antibacterial effect** against Gram positive and Gram negative bacteria, therefore supporting our hypothesis that OLB honey has antimicrobial properties. More research is required to confirm findings for OLB honey. Honey was able to prevent the spread and growth of bacteria due to the synergistic effect of its compounds, molarity, and pH value. Honey can kill cells or prevent bacterial growth even without its sugar components. Results show that when honeys are used at the appropriate therapeutic level, they can aid with our fight against antibiotic resistant bacteria and provide us alternative options for wound treatment. Additionally, the importance of Ohi'a Lehua trees for the environment and human society has increased, as the honey from the tree is valuable for medical progression.

Further research should be conducted to confirm findings, evaluate other medical potential, and develop the OLB honey into medicine or treatment. Additionally, the properties of the honey should be identified.

2 mL of each **concentration (100, 75, 50, 25%)** were prepared with compound and sterile distilled H2O (v/v):

OLB honey

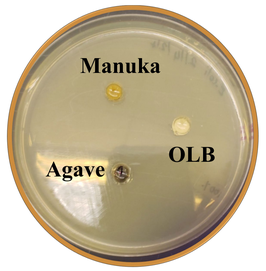
Manuka honey

Agave syrup

Agar well diffusion method

1. Swab bacterial lawn on Luria Agar
2. Punch 6 mm wells in agar
3. Fill wells with 200µL of appropriate compound
4. Incubate for 24 hours in 30ºC

After incubation, clear zone of inhibition was recorded around the wells (margin of error: ±1mm) (Table 2).



Example petri plate with compounds filled in the wells. Upper left has Manuka honey, upper right has OLB honey, and bottom has Agave syrup of 75% concentration.

Broth dilution method

1. Prepare McFarland solution and determine bacteria dilution that matches spectrometer reading
2. Prepare 1:1, 1:2, 1:4, 1:8 dilutions (w/v) of Manuka and OLB honeys with Luria Broth (Table 1)
3. Inoculate 0.1 mL of bacteria per 5 mL with appropriate dilution
4. Incubate solutions for 24 hours at 30 degrees Celsius
5. Spread bacterial lawn with 100 µL of each solution on Luria Agar media
6. Incubate plates for 21 hours at 30 degrees Celsius
7. Count and record number of cultures grown on plates

After incubation, visible inhibition was determined as the MIC value. Number of cultures per plate were counted and recorded (Table 3). 300 or more cultures in a plate were recorded to be too many to count (TMTC).

Table 1. Measurements used for each tube for broth dilution method.

Solution dilution	Honey weight (g)	Luria broth (mL)
1:1	8.72	8.72
1:2	8.34	16.67
1:4	6.25	18.75
1:8	3.125	21.875

Results

When agar well diffusion antimicrobial susceptibility testing is done with both types of honey, they showed antibacterial properties. For E. coli and B. cereus, Manuka honey showed a MIC of 75%. OLB honey showed an MIC value of 100% for B. cereus.

Table 2. Raw data of the ZOI results (mm) for each type of compound, concentration, and replicate when agar well diffusion method was used.

E. coli												
Concentrations	100%			75%			50%			25%		
Replicates/Compound	1	2	3	1	2	3	1	2	3	1	2	3
Agave	0	0	0	0	0	0	0	0	0	0	0	0
Manuka	9	9	10	0*	10	11	0	0	0	0	0	0
OLB	9*	0	0	0	0	0	0	0	0	0	0	0
B. cereus												
Concentrations	100%			75%			50%			25%		
Replicates/Compound	1	2	3	1	2	3	1	2**	3	1	2	3
Agave	0	0	0	*9**	0	0	0	0**	0	0	0	0
Manuka	12	13	10	10	10	8	0	10**	0	0	0	0
OLB	10	10	8	8**	*10***	0	*9**	11**	0	0	0	0

The results were statistically significant (p-value: 0.001) between Manuka honey and OLB honey for *E. coli* as seen by the post-hoc t-test. During data processing, data was averaged (*) or omitted (**). Italicized data signifies there was inhibition around the well present, but there was a point in the ellipse that the ZOI was zero.

Visible minimum inhibition was achieved at 1:4 dilution for Manuka honey for both bacteria, and 1:2 for OLB honey for both bacteria (Fig. 2). In comparison to the control bacterial lawn, plating results supports conclusions of minimum inhibitory concentrations (Fig. 3).

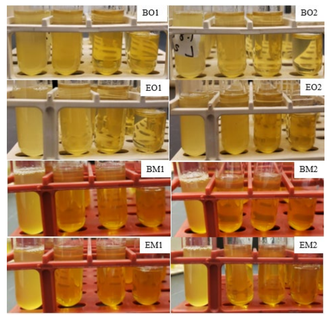
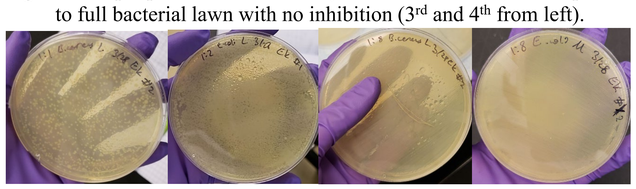


Fig. 2. Results of incubation for each tube used for broth dilution method. Photos are labeled with bacteria and honey types (EO: *E. coli* with OLB honey, BO: *B. cereus* with OLB honey, EM: *E. coli* with Manuka honey, BM: *B. cereus* with Manuka honey), and replicate number.

Fig. 3. Example plate with inhibition (1st and 2nd from left) in comparison to full bacterial lawn with no inhibition (3rd and 4th from left).



There was no Minium Bactericidal Concentration seen after plating, as almost all the plates had cultures.

Honey type	Manuka honey				OLB honey			
Bacteria type	E. coli		B. cereus		E. coli		B. cereus	
Replicate #	1	2	1	2	1	2	1	2
1:1	3	1	0	2	TMTC	TMTC	TMTC	TMTC
1:2	2	2	2	1	TMTC	TMTC	TMTC	TMTC
1:4	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC
1:8	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC

Table 3. Number of cultures on Luria agar media after plating the incubated tubes.

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