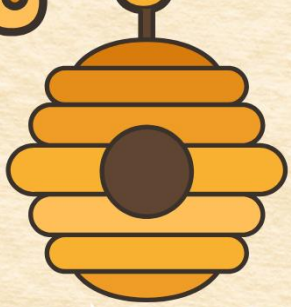

HOW HONEY COULD BEE THE KEY TO TACKLING ANTI-MICROBIAL RESISTANCE

MAX OTEIWI

ST EDWARDS COLLEGE EAST GOSFORD

SID-38268260

HSC SCIENCE EXTENSION REPORT

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Abstract

The rise of Antimicrobial Resistance (AMR) has increased demand for new and alternative therapeutic agents. Honey has long been used as a traditional ointment by Indigenous Australians and Ancient Egyptians due to its antibacterial properties derived from phenolic compounds and phytochemicals. This investigation evaluated the antibacterial activity of seven different honey types against *Escherichia coli (*gram-negative) and *Staphylococcus epidermidis* (gram-positive) bacteria. Antibacterial effects were assessed through zones of inhibition (ZOI, mm) using the Kirby Bauer disc diffusion method, with each honey tested in triplicate across three concentrations (25%, 50%, 75%) under controlled lab conditions. Contributing factors such as Brix% and water content were also measured using a honey refractrometer. Statistical analysis using a single-tail student t-test ($p<0.05$) revealed significant differences in antibacterial activity between honey extracts and control groups. Against E. coli, honeys 1-6 produced measurable zones of inhibition (ZOI), rejecting the null hypothesis, while honey 7 (Manuka MGO +100) showed no significant effect. In contrast S. epidermidis exhibited consistently smaller ZOI values, with the null hypothesis accepted in most cases. This study provides the underlying potential honey has as antibacterial agent where further research could assess honeys flavonoids, pH and floral origins influencing antibacterial compounds.

Literature Review

Importance of Antimicrobial Resistance research

Anti-Microbial Resistance (AMR) occurs when bacteria, viruses, fungi, and parasites no longer come to affect by antibiotics and antimicrobial medicines. Therefore, antibiotics become ineffective, and infection becomes more difficult to treat, increasing the risk of spreading disease, major illness, and death. (WHO. 2023). The development of antibiotics is considered among the most important advancements of science, saving millions of lives a year. However Anti-Microbial Resistance threatens this scientific progress, creating significant risks to human health (Hilary D. M 2016), killing at least one million people per year globally, and contributed to over five million deaths since 1990, and projected to cause thirty-nine million more fatalities between 2024 and 2050. (University of Oxford. (n.d.).

Traditional use of Honey as an antibiotic

Due to the urgent need for finding new antibiotics, caused by the uprise of AMR, the creation of new medications has included the investigation of the efficacy of traditional honey use, as used by the Australian Aborigines and Ancient Egyptians many thousands of years ago. The first written reference to honey use was a Sumerian tablet, dating back to 2100-2000BC (Mandal, 2011). In Indigenous heritage, Jelly Bush honey was a commonly used ointment to treat wounds using the honey from bees feeding on the nectar of Jelly Bush (Leptospermum polygalifolium) which is native to the Eastern regions of Australia and part of the same family as Manuka (Faltyn, 2020).

Importance of bees and the environment

Bees play a crucial role in building the future populations of flowering plants due to their attraction to nectar, resulting in the collection of pollen to be delivered and transported to a new plant combining with the stamen, creating new seedlings (Voeller. D, 2024). The composition of honey depends on the source of plants that the bees pollinate from (Eteraf- Oskouei. T & Najafi. M, 2013). The area that each honey comes from all have their own anti-microbial properties from plants that feed into the creation of honey, contributing to its antibiotic material. An example in studies shows methylglyoxal in New Zealand manuka honey has been shown to originate from dihydroxyacetone, which is present in the nectar of manuka flowers. Manuka honey, which was freshly produced by bees, contained low levels of methylglyoxal and elevated levels of dihydroxyacetone (Adams. C, 2009) showing the importance of surrounding environment on the effects of Antibiotic properties.

Phytochemical Activity

The antimicrobial activity of honey is overly complex and remains not fully recognised as to why. Honey has several phytochemical components that play a crucial role in its strong antimicrobial resistance. (Piotr. S, 2017).

Honey has high concentrations of sugar (80% the weight of this product) eliminating microorganisms such as bacteria that are proven sensitive to high osmotic pressure. This results in osmosis as an induced outcome causing water to passively flow out of the bacterial cells, causing the cells to shrink, dehydrate and die (Bakarm 2017).

Combining with its high osmotic pressure, honey's low pH levels, averaging between 3.4 to 6.1 which is known to be low enough to be inhibitory to animal pathogens. The minimum pH values for growth of some common wound-infecting species are Escherichia coli, 4.3; Salmonella sp., 4.0; Pseudomonas aeruginosa, 4.4; Streptococcus pyogenes, 4.5. Therefor in undiluted honey the acidity is a significant antibacterial factor due to its low Ph levels. But if honey is diluted, especially by body fluids

which are well buffered, the pH will not be so low, and the acidity of honey may not be an effective inhibitor of many species of bacteria. (P. C. Molan n/d).

Hydrogen peroxide (H_2O_2) is a disinfectant and a strong oxidizing agent (Ali 2004). It provides honey with its antibacterial efficacy and is produced enzymatically. The enzyme glucose oxidase is naturally present in an inactive state in honey due to the low pH conditions where the glucose oxidase enzyme is secreted from the hypopharyngeal gland of the bee into the nectar to assist in the formation of honey from the nectar (P. C. Molan n/d).

Flavonoids

When honey is diluted, glucose oxidase is activated and acts on endogenous glucose to produce Hydrogen Peroxide (H_2O_2). Methylglyoxal (Figure 1) is also found in most honeys, shown to originate from dihydroxyacetone, which is a major antibacterial component of honey (Saling. J, 2023). Honey is rich in Phenolic acids and flavonoids such as chrysin, quercetin, kaempferol, and galangin (Figure 2) which exhibit a wide range of biological effects and act as natural antioxidants containing a wide range of biological effects, including antibacterial, anti-inflammatory, anti-allergic and anti-thrombotic activities (Pyrzynska. K, 2009). Flavonoids are considered as an indispensable component of honey in a variety of nutraceutical, pharmaceutical, medicinal, and cosmetic applications (Panche et al., 2016).

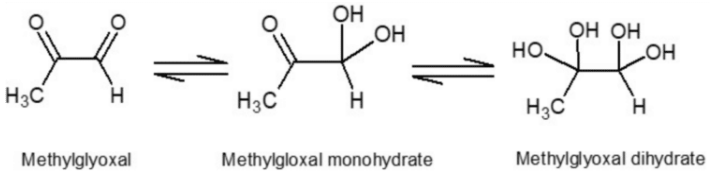


Figure 1: *Chemical structure of Methylglyoxal (source: Mauricio. (2021)*

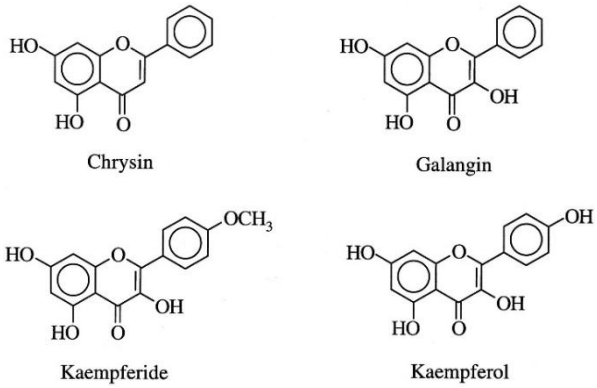


Figure 2: *Chemical structure of chrysin, quercetin, kaempferol, and galangin (Source: Yoko Otake, Thomas Walle (2002)*

Pathogens

The experiment on antimicrobial resistance of honeys on different pathogens will be tested on E. coli (gram negative) and S. aureus (gram positive) which are common pathogens tested in commonly used antimicrobial experiments. Gram-positive and Gram-negative bacteria differ in cell wall structure, influencing their response to osmotic pressure. Gram-positive bacteria have thick peptidoglycan layers, making them more resistant to osmotic stress, while Gram-negative bacteria are more vulnerable due to thin peptidoglycan and an outer membrane (Madigan et al., 2018; Beveridge, 2000; Silhavy et al., 2010). This structural contrast affects how each reacts to honey's high osmotic environment.

Kirby Bauer Method

The Kirby Bauer method is a cheap and trusted method to investigate the inhibition zones of honey against two bacterium and will be used in the construction of this experiment demonstrated in figure 3 below.

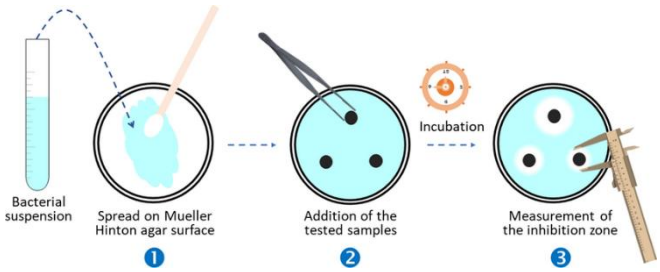


Figure 3: *Kirby Bauer disc diffusion method (Source: El Guerraf, 2022)*

Field of research publications

Recent studies show that researchers tested one hundred jelly bush honeys from a range of different areas and found that some had 1750mg/kg of the antimicrobial compound methylglyoxal, which was the highest concentration yet found of this kind (Nightingale. K, 2011)

Research question

Over thousands of years, honey has been used as a traditional ointment and resource of biological activity. What types of honey are more resistant to bacteria? And do different compounds correlate for this?

Research hypothesis

Extracts of multiple honeys will demonstrate antibacterial effects against a gram positive and gram-negative bacteria due to a range of phytochemical compositions.

Null Hypothesis

Multiple extracts of honey have no significant antimicrobial effects on different bacterium.

Method

Bacterial isolates

This primary study was conducted in multiple parts. Seven honeys were collected through a series of online and local purchases to accumulate a range of honeys, collected from European honeybees and native stinger-less bees, which have foraged for nectar on a variety of native floral regions. Antibacterial activity was tested through the disc diffusion method (Bauer et al. 1966).

Extraction/Dilutions

Consulting with the school lab technician, this primary study was conducted as a double-blind test to remove any observer bias by blindly labelling honeys 1-7 (Table 1). The honeys were then diluted to 75%, 50%, and 25% concentrations of honey (mg/mL) with distilled water (see Appendix B). All samples were collected into 100mL beakers labelled 1-7 and bathed in warm water for 24 hours to ensure the complete homogenous dilution of each honey with the distilled water to formulate the dependent variable. 3 Sterilised 5.5 mm filter paper discs were soaked in each solution for 24 hours.

Antibacterial assays

Eighteen total discs were created each with seven filter discs of each different honey, as well as a singular controlled disc containing 100% distilled water. This process was repeated creating 3 Muller Hilton agar plates for each percentage of dilution (25%,50%,75%) and for both bacteria used (9 for each bacteria total) using the cost effective and reliable Kirby Bauer method, whilst ensuring the sterilisation of all equipment using aseptic technique.

Bacterial cultures of Escherichia coli (gram-negative) and Staphylococcus epidermidis (gram positive) were used to determine the specific bacteria the antibiotics are most effective toward. All plates were then inoculated using the Drigalski Spatula Method (Diane Hartman, 2011) with each bacterium. The discs soaked in each solution were then placed onto the agar plates using sterile tweezers ensuring a rough even distribution of all eight discs around the agar. All plates were incubated at 37°C for 48 hours. The independent variable, Zone of inhibition (ZOI) was measured as the total diameter of inhibition including the 5.5mm discs with no zone reported as ‘none’ using digital callipers (+- 0.2mm accuracy) (See Appendix 3)

Refractrometer Analysis

Each of the seven honeys were collected and the purchase of a honey refractrometer was used to determine the Brix % (sugar contents) and water percentage in each of the honeys.

Dioptric oil was used to calibrate the refractrometer at its corresponding brix %

(71.5%) then using a flat head screwdriver, the calibration screw was slightly adjusted to suit the refractrometer until the line passed over 71.5%. Using a micropipette, two small drops of 100% honey was dropped onto the daylight plate and gently pressed flat until an even spread of honey surfaced around the plate. Raising the refractrometer to a suitable light source and keeping it, level then allowed the results to be seen though the eyepiece whilst adjusting the focus to create a clear image.

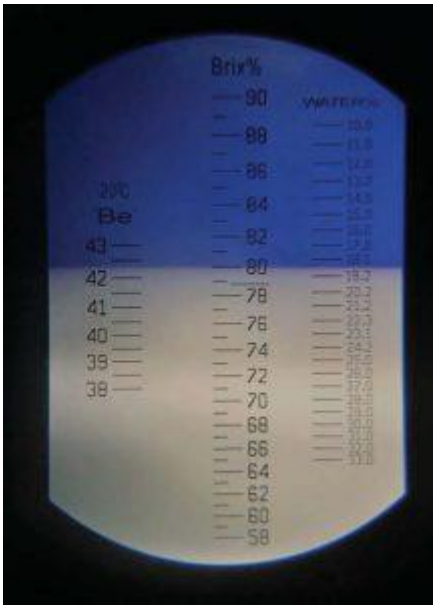


Figure 4: *Hony Refractometer eye piece readings/results (Source: Bäckmo, H. (2023, September 13)*

The brix% and water% data was collected by viewing the point where the line passed through the corresponding value. This was repeated three times for each honey were the results were recorded, and the mean was calculated.

Statistical Analyses

Independent sample student-T-Tests were conducted to compare the antibacterial activity of each honey sample and its corresponding concentration percentage. Statistical significance was observed through a critical t-value of 2.920 (α= 0.05) where values exceeding this threshold indicate significance in inhibition. T-values were found using the single tailed t-test formula shown below.

$$t = \frac{(x_1 - x_2)}{\sqrt{\frac{(s_1)^2}{n_1} + \frac{(s_2)^2}{n_2}}}$$

Figure 5*: Single Tail student t-test formula (Source: Biology for Life. (n.d)*

All analyses and data were found using Microsoft Xcel (2021) and results are detailed in the Discussion and Appendix.

The lab technician then revealed the names and types of honey after the data was collected, collated, and analysed as a successful completion of a double-blind test labelled 1-7 (Table 1).

Risk assessment

A risk assessment was conducted due to the use of live bacteria cultures, which pose potential health hazards. For example, Staphylococcus epidermidisa can cause skin and soft tissue infections, and in severe cases bloodstream infections (Bacteraemia) that can lead to hospitalisation (Tracey A., 2023). To minimise these risks, all culture tubes and agar plates were autoclaved. All equipment was either sterilised or properly disposed of following laboratory protocols. Personal protective equipment, including gloves, lab coats, and safety glasses were worn at all times. All other potential hazards outlined in the risk assessment (appendix) were identified and appropriately mitigated ensuring a safe environment.

Results

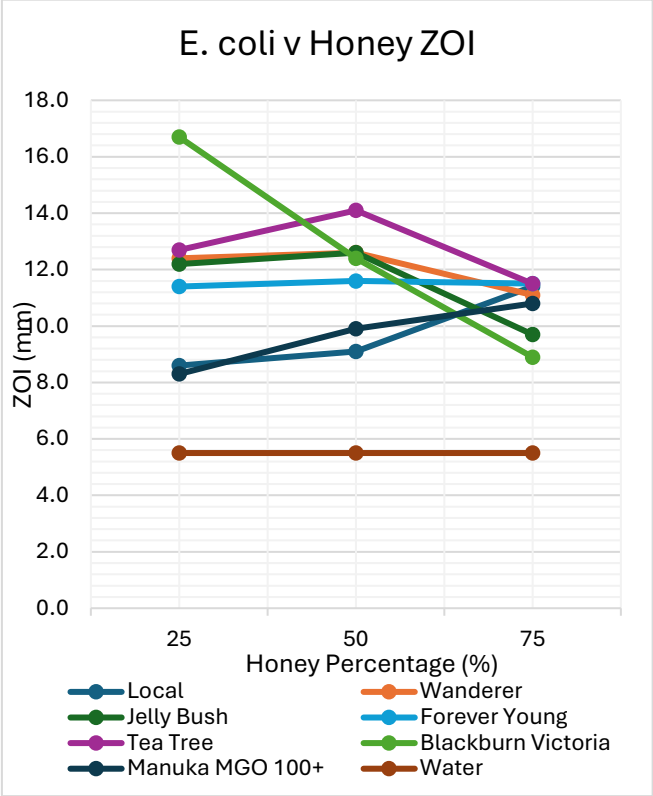


Figure 7: *Average zone of inhibition of each honey (1-7) including a distilled water control (8) against E. coli (Gram negative) applying the Kirby Bauer method (Bauer et al. 1966) using Muller Hilton agar plates.*

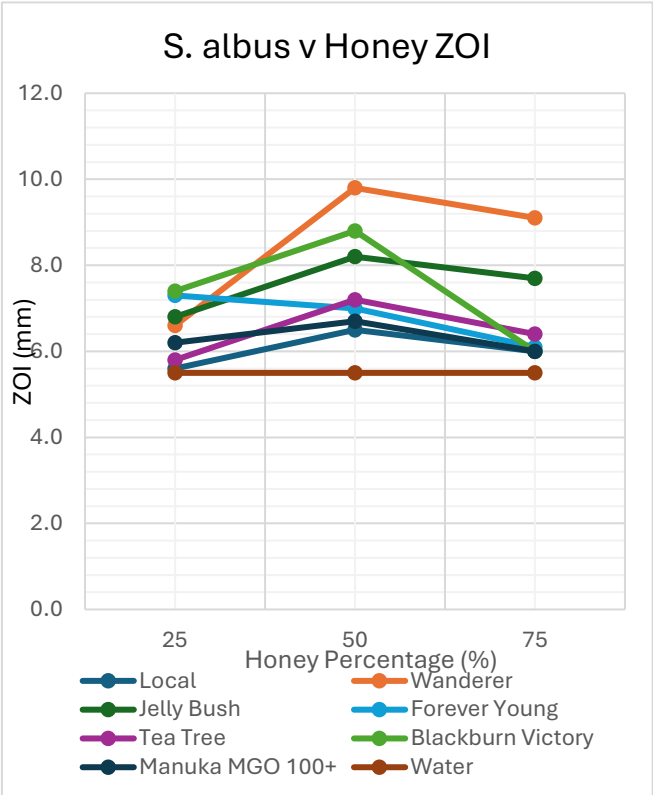


Figure 8: *Average zone of inhibition of each honey (1-7) including a distilled water control (8) against S. epidermidis (Gram positive) applying the Kirby Bauer method (Bauer et al. 1966) using Muller Hilton agar plates.*

Blindly Labelled	Type of Honey	Brix %	Water %
1	Mr Gentles Local honey	81.0	17.5
2	Wanderer pure raw Australian Honey	81.5	16.5
3	Jelly Bush Honey	81.5	17.0
4	Forever Young Australian Honey	82.0	16.5
5	Tea Tree Honey	81.0	17.5
6	Blackburn Victoria honey	82.5	16.0
7	Manuka MGO 100+	80.0	18.0

Table 1: *Displays the blind labelling (1-7) of each honey, the sugar concentration (Brix %) and corresponding water content (%) of seven different honey types.*

E. coli Honey No.	Concentration of honey (%)		
	25%	50%	75%
	Average Zone of Inhibition Diameter (mm)		
1	8.6	9.1	11.4
2	12.4	12.6	11.1
3	12.2	12.6	9.7
4	11.4	11.6	11.5
5	12.7	14.1	11.5
6	16.7	12.4	8.9
7	8.3	9.9	10.8
8	5.5	5.5	5.5

Table 2: *Mean ZOI scores of each honey for E. coli at every percentage dilution (25%, 50%, 75%).*

S. epidermidis Honey No.	Concentration of honey (%)		
	25%	50%	75%
	Average Zone of Inhibition Diameter (mm)		
1	5.6	6.5	6.0
2	6.6	9.8	9.1
3	6.8	8.2	7.7
4	7.3	7.0	6.1
5	5.8	7.2	6.4
6	7.4	8.8	6.0
7	6.2	6.7	6.0
8	5.5	5.5	5.5

Table 3: *Mean ZOI scores of each honey for S. epidermidis at every percentage dilution (25%, 50%, 75%).*

25% Concentration									
Honey No.	Bacteria	% variance	SD	t-value	Honey No.	Bacteria	% variance	SD	t-value
1	E. coli	9.404	3.067	3.067	1	S. epidermidis	0.009	0.094	0.067
2	E. coli	1.429	1.195	6.933	2	S. epidermidis	0.169	0.411	1.067
3	E. coli	3.727	1.930	6.700	3	S. epidermidis	0.382	0.618	1.333
4	E. coli	6.320	2.514	5.900	4	S. epidermidis	0.042	0.205	1.833
5	E. coli	4.507	2.123	7.200	5	S. epidermidis	0.056	0.236	0.333
6	E. coli	4.940	2.223	11.200	6	S. epidermidis	0.802	0.896	1.867
7	E. coli	0.420	0.648	2.800	7	S. epidermidis	0.056	0.236	0.667
8	E. coli	0	0	0	8	S. epidermidis	0	0	0

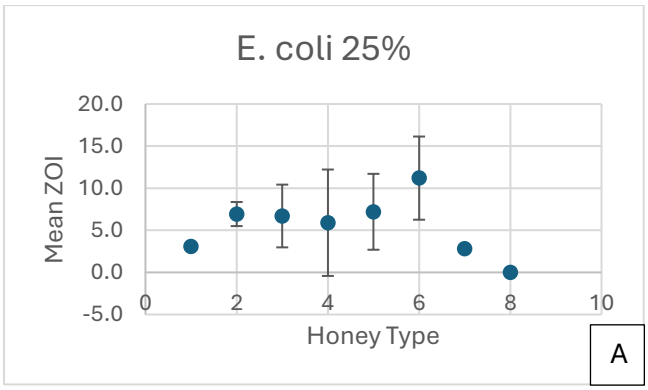
Table 4: *Comparison of the antibacterial activity of seven different honeys at 25% concentration against E. coli and S. epidermidis, shown by percentage variance, standard deviation (SD), and t-values. (Green= Rejection of Null hypothesis, Red= Acceptance of Null Hypothesis.*

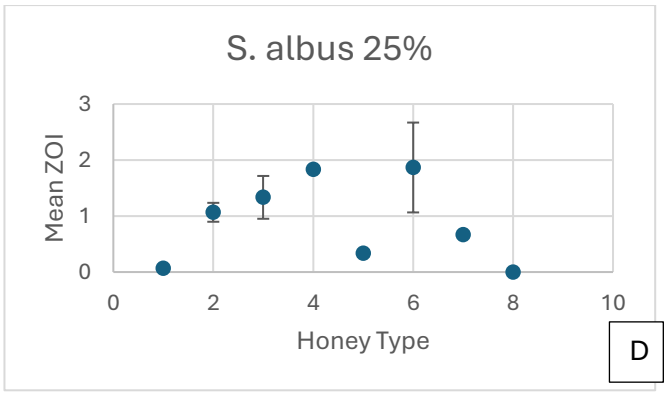
50% Concentration									
Honey No.	Bacteria	% variance	SD	t-value	Honey No.	Bacteria	% variance	SD	t-value
1	E. coli	2.069	1.438	3.633	1	S. epidermidis	0.036	0.189	0.967
2	E. coli	0.996	0.998	7.133	2	S. epidermidis	1.040	1.020	4.300
3	E. coli	1.742	1.320	7.067	3	S. epidermidis	0.616	0.785	2.733
4	E. coli	1.509	1.228	6.067	4	S. epidermidis	0.276	0.525	1.533
5	E. coli	0.429	0.655	7.252	5	S. epidermidis	0.380	0.616	1.700
6	E. coli	0.207	0.455	6.900	6	S. epidermidis	0.140	0.374	3.300
7	E. coli	1.182	1.087	0.512	7	S. epidermidis	0.069	0.262	1.233
8	E. coli	0	0	0	8	S. epidermidis	0	0	0

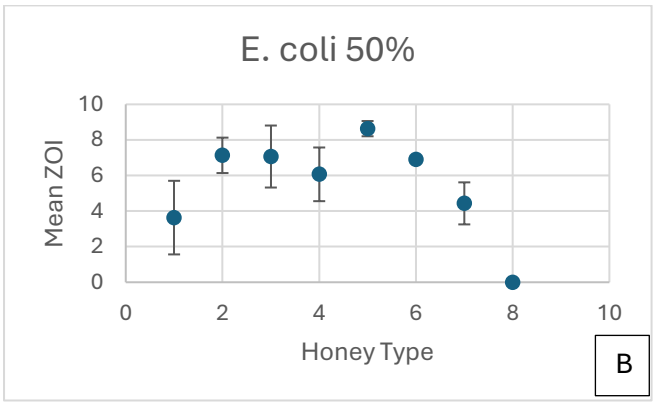
Table 5: *Comparison of the antibacterial activity of seven different honeys at 50% concentration against E. coli and S. epidermidis, shown by percentage variance, standard deviation (SD), and t-values. (Green= Rejection of Null hypothesis, Red= Acceptance of Null Hypothesis.*

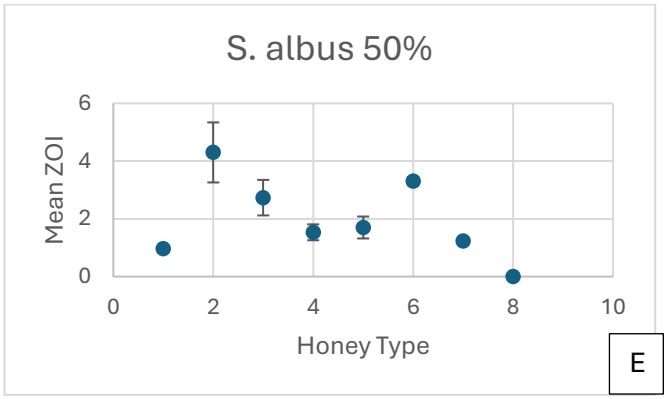
75% Concentration									
Honey No.	Bacteria	% variance	SD	t-value	Honey No.	Bacteria	% variance	SD	t-value
1	E. coli	0.887	0.942	5.900	1	S. epidermidis	0.002	0.047	0.533
2	E. coli	1.629	1.276	5.633	2	S. epidermidis	0.116	0.340	3.567
3	E. coli	0.029	0.170	4.233	3	S. epidermidis	0.327	0.572	2.200
4	E. coli	8.887	2.981	6.000	4	S. epidermidis	0.069	0.262	0.567
5	E. coli	0.962	0.981	5.026	5	S. epidermidis	0.136	0.368	0.933
6	E. coli	0.469	0.685	3.433	6	S. epidermidis	0.062	0.249	0.533
7	E. coli	0.240	0.490	0.238	7	S. epidermidis	0.016	0.125	0.533
8	E. coli	0	0	0	8	S. epidermidis	0	0	0

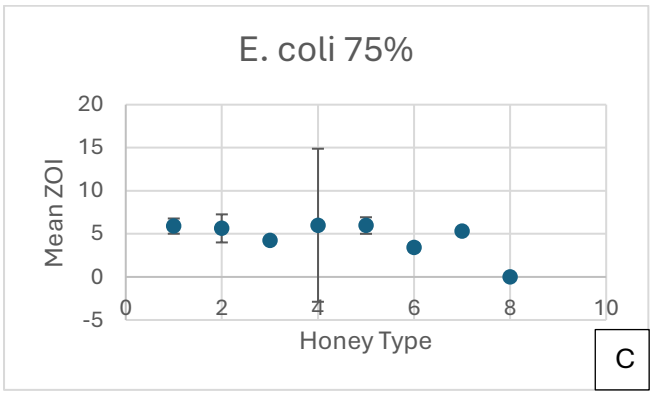
Table 6: *Comparison of the antibacterial activity of seven different honeys at 75% concentration against E. coli and S. epidermidis, shown by percentage variance, standard deviation (SD), and t-values (Green= Rejection of Null hypothesis, Red= Acceptance of Null Hypothesis.*











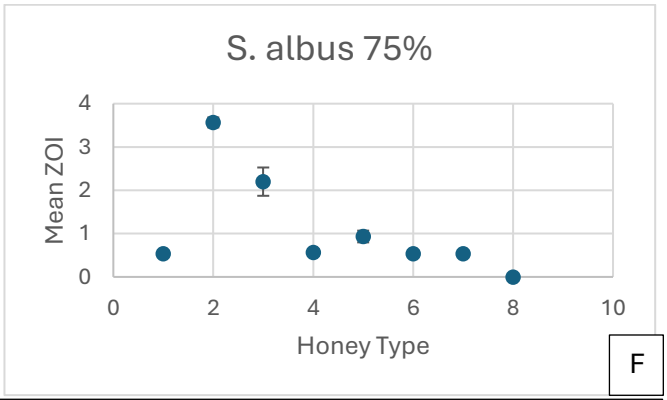
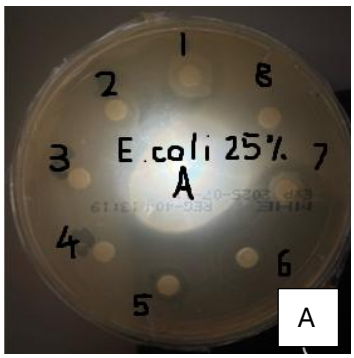
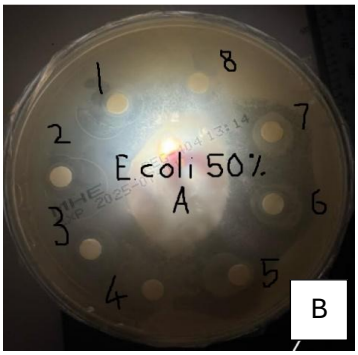
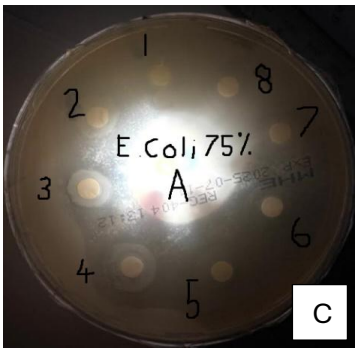


Figure 7: *Comparison mean ZOI of each honey type 1-7 including a distilled water control (8) at each percentage (25%,50%,75%) against each bacterium (E. coli and S. epidermidis) whilst demonstrating error bars of variance.* ***A:*** *25% E. coli,* ***B:*** *50% E. coli,* ***C:*** *75% E. coli,* ***D:*** *25% S. epidermidis,* ***E:*** *50% S. epidermidis,* ***F:*** *75% S. epidermidis*







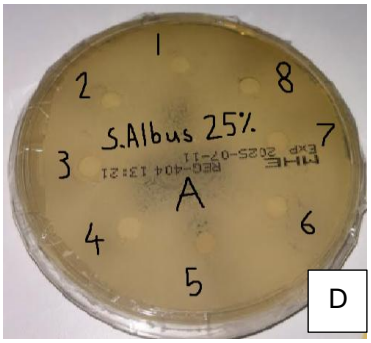


Figure 8: *Agar plates for ZOI samples as test A against E. coli (gram-negative) (A, B, C) and S. epidermidis (Gram Positive) (D, E, F). For Sample plates,* ***1:*** *Locally sourced.* ***2:*** *Wanderer pure raw Australian.* ***3:*** *Jelly Bush.* ***4:*** *Forever Young Australian.* ***5:*** *Tea Tree.* ***6:*** *Blackburn Victoria.* ***7:*** *Manuka MGO 100+.* ***8:*** *Distilled Water control*

Discussion

Disc Diffusion

Supportive of the hypothesis, all seven honeys exhibited modest antibacterial activity as indicated by the zones of inhibition (ZOI, Figure 7, Table 2). Overall, E. coli (gram-negative) showed a greater range of susceptibility compared to S. epidermidis (gram-positive) which consistently had smaller zones of inhibition. This aligns with existing literature stating that gram negative bacteria are more resistant, however in this experiment, E. coli was more effected due to the specific properties within the honeys used. Honey's 2-5 displayed peak antimicrobial effectiveness at 50% concentration (Figure 7) suggesting an optimal balance where reduced viscosity improves the diffusion of active compounds to be spread across a larger area. At 25% concentration, honey may be too diluted to be fully effective and at 75% concentration, increased thickness may limit diffusion producing smaller inhibition zones. Only honeys 1 and 7 showed a clear dose-response relationship supported by refractrometer results showing their higher water content (17.5% and 18% respectively, Table 1), which facilitated wider spread of antimicrobial compounds and larger inhibition zones.

Figure 8 shows that across all tests conducted on S. epidermidis, the difference in zones of inhibition (ZOI) between all honeys were minimal and remained within a narrow range. This suggests that the gram-positive cell wall of S. epidermidis may offer consistent protection against the honey's antibacterial compounds. Alternatively, it is possible that the specific phytochemicals or antibiotic compounds present within each honey were more effective against a gram-positive positive bacterium such as E. coli compared to S. epidermidis. This is visually presented in figure 8 which shows the disc diffusion assays of each honey at 50% concentration (1-7) and the control (8) against E. coli (Top row- A, B, C) and S. epidermidis (Bottom row- D, E, F).

Compound Analysis

Brix ranged from 80% to 82.5% and water content from 16% to 18%. Higher brix indicates elevated sugar concentrations, contributes to honey's antimicrobial ability through osmotic pressure and dehydration of microbes. Table 1 shows excessive viscosity linked to high Brix and low water content (e.g. Honey 5: Brix 82.5%, Water 16.0%). Honeys with higher water percentages (e.g. Honey 3 & 6 with 17.5% - 18.0%) demonstrated improved spread of compounds, especially at 50% dilution where viscosity levels were optimal for diffusion.

Statistical Significance

Across all concentrations tested (25%, 50%, 75%) of each honey (1-7) against *Escherichia coli* and *Staphylococcus epidermidis,* statistical significance was found using a critical t-value of 2.920 (a=0.05) where the values that exceeded this score indicate a significant difference between the sample's inhibition zones compared to the control (distilled water).

25% Honey Concentration

At 25% shown in table 4 and graph A and D, in figure 7, honeys 1-6 displayed significant statistical inhibition against E. coli, reaching t-values exceeding a threshold ≥ 2.920 (e.g., Honey 1 t= 3.067 and Honey 6= 11.200). Variance ranged from 1.4%-9.4% suggesting moderate reliability whilst SDs between 1.2 and 3.1 reflect some variance in ZOI measurements. S. epidermidis exhibited complete resistance of all honeys producing t-values below the significance threshold (e.g., Honey 4 t= 0.205) whilst presenting minimal variance (< 0.8%) and low Sd's (<0.9) suggesting consistency but ineffective antimicrobial activity.

50% concentration

As displayed in table 5 and graphs B and E in figure 7, increasing the concentration to 50% improved the inhibition zones of honey's 1-6 against E. coli, remaining significant above the threshold (e.g., Honey 6: t=6.900). Variances and SD decreased suggesting enhanced consistency of results. For S. epidermidis, honeys 2 and 6 achieved a significance above the threshold (t=4.300 and 3.300). Other honeys remained non-significant through small increases in ZOI and variability measurements.

75% concentration

At 75% concentration, Honeys 1-6 continued to remain effective in inhibiting growth of E. coli, with t-values reaching above the threshold (e.g., Honey 4= 6.00) with majority showing low variance in measurements except honey number 4 (8.887%) suggesting the reliability of results for most honeys. Against S. epidermidis, shown at 50% honey 2 again produced significant inhibition (t-values > 2.920) marking the first consistent activity of a honey against S. epidermidis suggesting a possible increased concentration dependent effect. Similarly portrayed in 25% and 50%, all other honeys remained statistically non-significant whilst showing low variance and SDs.

For all tests against E. coli, honey seven demonstrated and remained no significance reaching below the threshold. All water controls (8) at each concentration percentage and bacterial strain showed no inhibition zones suggesting that any inhibition is caused by the honey.

Previous studies

Previous studies show the consistent inhibition of *E. coli* across multiple honeys supports the antimicrobial potential of phenolic compounds, demonstrated in Australian native honeys where floral-derived components contributed to activity (Chen et al., 2012). In contrast, the limited activity against *S. epidermidis* at 25% concentration may reflect differences in bacterial cell wall permeability with Gram-positive species often exhibiting variable responses to honey-based antimicrobials (Kwakman et al., 2008).

Strengths and Limitations

This primary study saw all honeys tested show significant antimicrobial properties compared to control groups indicating the ZOI caused was purely from honey and its compounds. This provides a base for future further study into honeys, especially those of native Australian plants. The use of a double-blind test was successful to remove any bias that may have been influenced by existing literature sources. The consistency of controls and variables using different percentage concentrations of honey dilutions and distilled water controls was proven successful in formulating inhibition zones. Supporting this, formulating multiple effective tests for each percentage and bacteria allowed for validity within experiments conduction.

Several limitations may have impacted data reliability and overall validity of this experiment. The use of digital callipers (±0.2 mm) and visual estimation to measure the zones of inhibition (ZOI) which introduces possible systematic errors in measurements shown in the variance and SD in Tables 4,5 & 6 and figure 7. Other systematic limitations included the limited size of the study as time constraints resulted in the inability to conduct further supporting evidence. Random errors in the honey collection may have caused uncertainty as bees may visit multiple flowering species producing varying amounts of phenolic compounds in their honey. Methodological flaws include the lack of standardisation in honey as the purchasing of online and local sources introduces variability in freshness, storage condition and batch consistency. Using the Kirby Bauer disc diffusion method, soaking the filtration discs for 24 hours may have led to oversaturation or uneven absorption where excess liquid could

have caused residual moisture to diffuse differently across agar.

Summary, significance, and Future Research

It was hypothesised that various honeys would inhibit both Gram-positive (S. epidermidis) and Gram-negative (E. coli) bacteria due to differing phytochemical compositions. Extraction assays, refractrometer analysis and student t-tests rejected the null hypothesis for E. coli but accepted it for S. epidermidis. Overall, Honey 2 (Wanderer Pure Raw Australian) showed the highest antibacterial activity whilst honey 7 (Manuka MGO100+) was the least effective.

This study is significant as results support Honeys traditional medicinal use and highlights how it can be used in new drugs to combat antimicrobial resistance (AMR).

Future research could quantify flavonoid content of honey using the aluminium chloride ($AlCl_3$) colorimetric assay (Chang et al., 2002) to link phenolic compounds with antibacterial activity. Investigating regional and floral sources may explain variations in bioactive content. Future studies could also measure pH with a calibrated meter (Terrab et al., 2004) to assess its effectiveness in bacterial inhibition.

Conclusion

Honey has been used traditional as a medicinal ointment by Indigenous Australians, highlighting its long-standing role as a natural source of antimicrobial compounds and resistant activity in fighting contemporary drug-resistant pathogens. This study aimed to evaluate the antibacterial activity of seven honeys from different regions and examine how factors such as brix% and water content influenced bacterial inhibition. Statistical analysis using t-tests confirmed that the antibacterial activity of most honey extracts produced significant zones of inhibition against E. coli indicating strong antibacterial activity, while S. epidermidis did not show significant inhibition. These finding suggest that that honey may be against certain gram-negative bacteria, potentially offering a natural alternative in combatting antimicrobial resistance. The variation in antibacterial effectiveness between honeys and bacterial strains could be linked to the differences in phytochemical composition, viscosity and pH highlighting the importance of further phytochemical analysis, including flavonoid and phenolic content to better understand that causations of these effects. Due to systematic errors (e.g. small sample size and time constraints) and methodological limitations, future studies could also address the role of hydrogen peroxide, the floral and regional origin of honeys, and their applications against a broader spectrum of clinically studied and tested pathogens. The formation of this study supports the medicinal use of honey and underscores its potential as a source of bioactive compounds in treating future drug-resistant bacteria.

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Reference List (APA 7TH)

1. Adams, C. J., Boult, C. H., Deadman, B. J., Farr, J. M., Grainger, M. N. C., Manley-Harris, M., & Snow, M. J. (2009). The origin of methylglyoxal in New Zealand manuka (Leptospermum scoparium) honey. *Carbohydrate Research, 344*(8), 1050–1053. https://www.umf.org.nz/research-database/the-origin-of-methylglyoxal-in-new-zealand-manuka-leptospermum-scoparium-honey/

2. Ali, M. (2004). Hydrogen peroxide in honey. *Clinical Infectious Diseases*. *https://go.gale.com/ps/i.do?id=GALE%7CA122581710&sid=googleScholar&v=2.1&it=r&linkaccess=abs&issn=15254283&p=AONE&sw=w&userGroupName=anon%7Edd90f3a2&aty=open-web-entry*

3. American Academy of Paediatrics. (2022). How do antibiotics work? *HealthyChildren.org*. https://www.healthychildren.org/English/health-issues/conditions/treatments/Pages/How-Do-Antibiotics-Work.aspx

4. Banerjee, A., Kocarek, T. A., & Novak, R. F. (2024). Identification of a ubiquitination-target/substrate-interaction domain of cytochrome P-450 (CYP) 2E1. *Drug Metabolism and Disposition*. Advance online publication. https://dmd.aspetjournals.org/article/S0090-9556(24)04366-6/abstract

5. Brudzynski, K. (2020). Antibacterial compounds in honey. *Food Chemistry, 342,* 128376. https://doi.org/10.1016/j.foodchem.2020.12837

6. Chen, C., Campbell, L. T., Blair, S. E., & Carter, D. A. (2012). The effect of standard heat and filtration processing procedures on antimicrobial activity and hydrogen peroxide levels in honey. *Frontiers in Microbiology, 3,* 265. https://doi.org/10.3389/fmicb.2012.00265

7. El Guerraf, A., Ben Jadi, S., Bakirhan, N. K., Kiymaci, M. E., Bazzaoui, M., Ozkan, S. A., & Bazzaoui, E. A. (2022). Antibacterial activity and volatile organic compounds sensing property of polypyrrole-coated cellulosic paper for food packaging purpose. *Polymer Bulletin, 79*(12), 11543–11566. https://doi.org/10.1007/s00289-021-04041-w

8. Eteraf-Oskouei, T., & Najafi, M. (2013). Traditional and modern uses of natural honey in human diseases: A review. *Iranian Journal of Basic Medical Sciences, 16*(6), 731–742. https://pmc.ncbi.nlm.nih.gov/articles/PMC3758027/

9. Faltyn, C. (2020). Why we love Jelly Bush honey. *Apiary Made Blog*. https://apiarymade.com.au/blogs/blog/why-we-love-jelly-bush-honey

10. Felman, A., & Begum, F. (2019, January 18). Antibiotics: How they work, uses, side effects and how to use. *Medical News Today*. https://www.medicalnewstoday.com/articles/10278

11. Hartman, D. (2011). Perfecting your spread plate technique. *Journal of Microbiology & Biology Education, 12*(2), 204–205. https://europepmc.org/article/PMC/3577269

12. Irish, J., Blair, S., & Carter, D. A. (2011). The antibacterial activity of honey derived from Australian flora. *PLOS ONE, 6*(3), e18229. https://doi.org/10.1371/journal.pone.0018229

13. Israili, Z. H. (2014). Antimicrobial properties of honey. *American Journal of Therapeutics, 21*(4), 304–317. https://journals.lww.com/americantherapeutics/abstract/2014/07000/antimicrobial_properties_of_honey.13.aspx

14. Kwakman, P. H. S., te Velde, A. A., de Boer, L., Vandenbroucke-Grauls, C. M. J. E., & Zaat, S. A. J. (2008). Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS ONE, 3*(6), e1898. https://doi.org/10.1371/journal.pone.0001898

15. Mandal, M. D., & Mandal, S. (2011). Honey: Its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine, 1*(2), 154–160. https://www.researchgate.net/publication/236141166_Honey_Its_medicinal_property_and_antibacterial_activity

16. Marston, H. D., Dixon, D. M., Knisely, J. M., Palmore, T. N., & Fauci, A. S. (2016, September 20). Antimicrobial resistance. *JAMA, 316*(11), 1193–1204. https://jamanetwork.com/journals/jama/article-abstract/2553454

17. Mauricio. (2021, January 29). Identification and quantification of methylglyoxal in Manuka honey. *Magritek Blog*. https://magritek.com/2021/01/29/identification-and-quantification-of-methylglyoxal-in-manuka-honey/

18. Mohd Fadzelly Abu Bakar, S. B. Sanusi, F. I. Abu Bakar, O. J. Cong, & Zakbah Mian (2017). Physicochemical and antioxidant potential of raw unprocessed honey from Malaysian stingless bees. *Pakistan Journal of Nutrition, 16*(11), 888–894. https://doi.org/10.3923/pjn.2017.888.894

19. Molan, P. C. (n.d.). The antibacterial activity of honey. *University of Waikato Research Commons*. https://researchcommons.waikato.ac.nz/entities/publication/fffb2a5c-0c50-4fd3-a273-b53eab40efd8

20. Nightingale, K. (2011). Methylglyoxal in Jelly Bush honey. *Bulletin of the National Research Centre*. https://bnrc.springeropen.com/articles/10.1186/s42269-019-0044-7

21. Onyango, L. A., & Liang, J. (2024). Manuka honey as a non-antibiotic alternative against *Staphylococcus* spp. and their small colony variant (SCVs) phenotypes. *Frontiers in Cellular and Infection Microbiology, 14*. https://doi.org/10.3389/fcimb.2024.1380289

22. Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science, 5,* e47. https://www.cambridge.org/core/services/aop-cambridge-core/content/view/C0E91D3851345CEF4746B10406908F52/S2048679016000410a.pdf/flavonoids-an-overview.pdf

23. P.C Molan. (n.d.). Honey and human health. In *The Hive and the Honeybee*. https://link.springer.com/chapter/10.1007/978-1-4757-9371-0_3

24. Piotr, S. (2017). Antimicrobial activity of honey. In *Honey Analysis* (pp. 215–232). Springer. https://books.google.com.au/books?hl=en&lr=&id=T_iODwAAQBAJ&oi=fnd&pg=PA215

25. Pyrzynska, K., & Biesaga, M. (2009). Analysis of phenolic acids and flavonoids in honey. *Bulletin of the National Research Centre*. https://bnrc.springeropen.com/articles/10.1186/s42269-019-0044-7

26. Refractometer All You Need to Know | Bee Culture. (n.d.). Retrieved July 09, 2025, from https://beeculture.com/refractometer/

27. Saling, J. (2023). Flavonoids in honey. *Science direct*. *https://www.sciencedirect.com/science/article/abs/pii/S0165993609001216*

28. University of Oxford. (2024, September 17). Antibiotic resistance has claimed at least one million lives each year since 1990. *Oxford News*. https://www.ox.ac.uk/news/2024-09-17-antibiotic-resistance-has-claimed-least-one-million-lives-each-year-1990

29. Voeller, D. (2024). Why bees are climate heroes. *UC San Diego Library Digital Collections*. https://library.ucsd.edu/dc/object/bb2527121q/_1.pdf

30. World Health Organization. (2023). World AMR awareness week 2023. *WHO Campaigns*. https://www.who.int/campaigns/world-amr-awareness-week/2023

Images Used (APA 7th)

Source 1:

Dhar, I., & Desai, K. M. (2012). *Aging: Drugs to eliminate methylglyoxal, a reactive glucose metabolite, and advanced glycation endproducts* (Figure 1). In *Aging: Drugs to eliminate methylglyoxal…* [Chapter]. ResearchGate. https://www.researchgate.net/figure/Structure-of-methylglyoxal-MG-and-three-compounds-with-an-ability-to-bind-MG-or-inhibit_fig1_221928528

Source 2:

Otake, Y., & Walle, T. (2002). *Oxidation of the flavonoids galangin and kaempferide by human liver microsomes and CYP1A1, CYP1A2, and CYP2C9*. Drug Metabolism and Disposition, 30(2), 103–105. https://doi.org/10.1124/DMD.30.2.103

Source 3:

El Guerraf, A., Jadi, S. B., Karadas Bakirhan, N., & Bazzaoui, E. (2022). Schematic representation of the disk diffusion method of Kirby and Bauer [Figure]. In *Antibacterial activity and volatile organic compounds sensing property of polypyrrole-coated cellulosic paper for food packaging purpose* [ResearchGate figure]. Retrieved from https://www.researchgate.net/figure/Schematic-representation-of-the-disk-diffusion-method-of-Kirby-and-Bauer_fig5_358212661

Source 4:

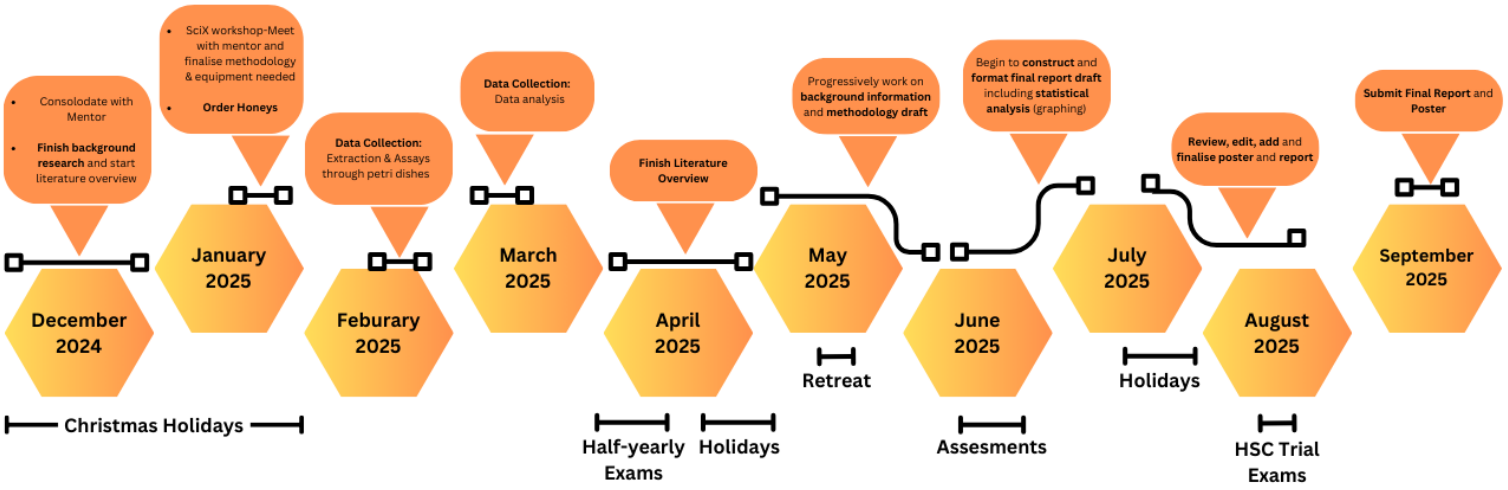
Bäckmo, H. (2023, September 13). *Honey refractometer – All you need to know*. Hanna's Bees. Retrieved from https://hannasbees.ie/honey-refractometer/

Source 5:

Biology for Life. (n.d.). *T-test*. Retrieved from https://www.biologyforlife.com/t-test.html

Appendix

Appendix A- Initial created Timetable



Appendix B- Raw Honey Dilution calculations

Honey no. 1	Concentration (%)		
	75	**50**	**25**
Honey (g)	7.74	5.10	2.69
Water (ml)	2.60	5.10	8.10

Honey no. 2	Concentration (%)		
	75	**50**	**25**
Honey (g)	7.90	5.39	2.57
Water (ml)	2.60	5.40	7.70

Honey no. 3	Concentration (%)		
	75	**50**	**25**
Honey (g)	7.51	5.02	2.53
Water (ml)	2.50	5.00	7.60

Honey no. 4	Concentration (%)		
	75	**50**	**25**
Honey (g)	7.79	5.27	3.00
Water (ml)	2.60	5.30	7.70

Honey no. 5	Concentration (%)		
	75	**50**	**25**
Honey (g)	7.76	5.04	3.18
Water (ml)	2.60	5.00	9.50

Honey no. 6	Concentration (%)		
	75	**50**	**25**
Honey (g)	8.03	5.85	2.60
Water (ml)	2.70	5.90	7.80

Honey no. 7	Concentration (%)		
	75	**50**	**25**
Honey (g)	7.96	5.34	2.54
Water (ml)	2.65	5.30	7.62

Appendix C: Raw ZOI measurements

E. coli @25% Honey					
Honey no.	Test a (mm)	Test b (mm)	Test c (mm)	Avg Circ (mm)	Accounted Filter Size ($\bar{x}$-5.5)
1	8.6	8.2	8.9	8.6	3.1
2	12.2	14.0	11.1	12.4	6.9
3	13.9	9.5	13.2	12.2	6.7
4	14.8	8.8	10.6	11.4	5.9
5	12.7	15.3	10.1	12.7	7.2
6	13.6	17.8	18.7	16.7	11.2
7	7.7	9.2	8.0	8.3	2.8
8	5.5	5.5	5.5	5.5	0.0

E. coli @50% Honey					
Honey no.	Test a (mm)	Test b (mm)	Test c (mm)	Avg Circ (mm)	Accounted Filter Size ($\bar{x}$-5.5)
1	8.9	7.5	11	9.1	3.6
2	11.3	13.7	12.9	12.6	7.1
3	14.3	12.3	11.1	12.6	7.1
4	10.6	10.8	13.3	11.6	6.1
5	14.2	14.9	13.3	14.1	8.6
6	11.8	12.5	12.9	12.4	6.9
7	9.6	11.4	8.8	9.9	4.4
8	5.5	5.5	5.5	5.5	0.0

E. coli @75% Honey					
Honey no.	Test a (mm)	Test b (mm)	Test c (mm)	Avg Circ (mm)	Accounted Filter Size ($\bar{x}$-5.5)
1	12.7	10.5	11	11.4	5.9
2	12.8	9.7	10.9	11.1	5.6
3	9.9	9.8	9.5	9.7	4.2
4	7.4	12.7	14.4	11.5	6.0
5	12.7	11.4	10.3	11.5	6.0
6	8.4	9.9	8.5	8.9	3.4
7	10.8	11.4	10.2	10.8	5.3
8	5.5	5.5	5.5	5.5	0.0

S. albus @25% Honey					
Honey no.	Test a (mm)	Test b (mm)	Test c (mm)	Avg Circ (mm)	Accounted Filter size ($\bar{x}$-5.5)
1	5.5	5.5	5.7	5.6	0.1
2	7.1	6.1	6.5	6.6	1.1
3	6.5	7.7	6.3	6.8	1.3
4	7.6	7.3	7.1	7.3	1.8
5	6	5.5	6	5.8	0.3
6	6.5	7	8.6	7.4	1.9
7	6	6	6.5	6.2	0.7
8	5.5	5.5	5.5	5.5	0.0

S. albus @50% Honey					
Honey no.	Test a (mm)	Test b (mm)	Test c (mm)	Avg Circ (mm)	Accounted Filter size ($\bar{x}$-5.5)
1	6.6	6.2	6.6	6.5	1.0
2	10.2	8.4	10.8	9.8	4.3
3	8.4	7.2	9.1	8.2	2.7
4	7.3	7.5	6.3	7.0	1.5
5	7.1	8.0	6.5	7.2	1.7
6	8.3	8.9	9.2	8.8	3.3
7	6.6	6.5	7.1	6.7	1.2
8	5.5	5.5	5.5	5.5	0.0

S. albus @75% Honey					
Honey no.	Test a (mm)	Test b (mm)	Test c (mm)	Avg Circ (mm)	Accounted Filter size ($\bar{x}$-5.5)
1	6.0	6.0	6.1	6.0	0.5
2	8.6	9.2	9.4	9.1	3.6
3	7.7	8.4	7.0	7.7	2.2
4	6.2	6.3	5.7	6.1	0.6
5	6.0	6.9	6.4	6.4	0.9
6	6.1	5.7	6.3	6.0	0.5
7	6.2	6.0	5.9	6.0	0.5
8	5.5	5.5	5.5	5.5	0.0

Appendix D: Risk Assessment

RISK ASSESSMENT **St Edward's College, East Gosford Students**

Science Extension 2025- write up

Written by: Max Oteiwi **Commenced on:** 18 Aug 2025 **Expires:** 18 Nov 2026

Classes for which experiment is required

Teacher: Mr Foster **Year Group:** 12 Extension Science

Procedure or reference, including variations

na

Equipment to be used

aluminium foil

glass beaker, 250 mL to 1 L

Potential hazards
Breakage of beaker. Cuts from chipped rims.

Standard handling procedures
Inspect and discard any chipped or cracked beakers, no matter how small the damage. Sweep up broken glass with brush and dustpan; do not use fingers.

match box

Potential hazards
Flammable.

Bunsen burner

Potential hazards
Roaring flame is very hot and can cause severe burns. Rapid passage of hand through fully luminous flame usually does not result in a burn. A BUNSEN 'ON SAFETY' (LUMINOUS FLAME) IS STILL AN IGNITION SOURCE. Roaring Bunsen burner may "burn back" at low gas flow, with flame emerging from air holes in base; this makes the base of the burner hot to touch and liable to cause burns. Gas from gas tap or from end of rubber tube burns with large luminous flame, likely to cause burns. Rubber hose is easily melted by flame from burner, e.g. if burner knocked over, resulting in fire from burn hole in tube. Ensure hair is tied back, so does not catch alight.

Standard handling procedures
NEVER USE A BUNSEN BURNER TO HEAT A FLAMMABLE LIQUID. NEVER BOIL METHYLATED SPIRITS USING A BUNSEN BURNER. Inspect and clean the jet and base of Bunsen burners regularly. Inspect and replace tube whenever any sign of wear or damage is noticed. Use only hoses of the correct size to ensure a comfortable fit on both Bunsen burner and gas tap.

dissection tray

Potential hazards
Tray may break, if made from glass.

Standard handling procedures
Sweep up broken glass with brush and dustpan; do not use fingers.

metal tweezers

Potential hazards
Can be used as a weapon if long and sharply pointed.

glass rod

Potential hazards
Glass rod may break in hand causing deep cuts.

Standard handling procedures
Inspect and discard any chipped or cracked rods, no matter how small the damage. Sweep up broken glass with brush and dustpan; do not use fingers.

hole puncher

incubator

Potential hazards
Possible source of electrical shock if not wired correctly. Possible ignition source.

Standard handling procedures
Check for electrical safety each time before use. Test and tag at regular intervals.

glass graduated pipette (glass graduated pipet)

Potential hazards
Possibility of ingestion of liquid if mouth is used to fill pipette. ALWAYS USE A PIPETTE FILLER. Possibility of breakage of glass and cuts. Inserting glass pipette into filler inappropriately may result in major hand injuries, if glass breaks.

Standard handling procedures
Provide a properly fitting pipette filler with every pipette. Inspect and discard any chipped or cracked pipettes, no matter how small the damage. Sweep up broken glass with brush and dustpan; do not use fingers.

plastic graduated pipette (plastic graduated pipet)

Potential hazards
Possibility of ingestion of liquid if mouth is used to fill pipette. ALWAYS USE A PIPETTE FILLER. Organic solvents may cause swelling of the surface layer of plastic, causing cracking and leaking.

Standard handling procedures
Provide a properly fitting pipette filler with every pipette. Do not use with organic solvents and do not clean with organic solvents.

digital calipers

Potential hazards
Pointed jaws may cause injury if misused.

nutrient agar plate

Potential hazards
Agar is harmless, but bacteria or fungi grown on agar may be pathogenic. Knowledge of microbiology and aseptic techniques is required to minimise risks to staff, students and the environment.

Standard handling procedures
It is generally not recommended to incubate agar at temperatures around 37°C, since this increases the likelihood of pathogenic organisms growing. Temperatures above 30°C should be avoided. Risk group 1 organisms, as described in AS/NZS 2243.3:2010, are generally regarded as suitable for supervised school experiments. Check the policy of your school authority.

filter paper

Potential hazards
Flammable. Used filter paper may contain harmful residues.

Standard handling procedures
After use, dispose of residue and filter paper appropriately.

Chemicals to be used

ethanol, pure liquid, absolute (ethyl alcohol) **CH_3CH_2OH**

UN: 1170 CAS: 64-17-5

Class: 3 PG: II Users: 7-12 Training: 1-5

GHS data:

DANGER

Highly flammable liquid and vapour
Causes serious eye irritation

Potential hazards
HIGHLY FLAMMABLE; DO NOT USE NEAR IGNITION SOURCES. Liquid irritates eyes. Prolonged contact with skin causes irritation. Low toxicity, if pure. May be highly toxic if prepared by azeotropic distillation with benzene, due to residual benzene. Forms violently explosive mixtures with nitric acid and other oxidising agents. Reaction of ethanol with acidified dichromate solution is highly exothermic. Potassium reacts violently with ethanol. Ethanol becomes less flammable as it is diluted with water; 50% ethanol is barely flammable at room temperature and <24% ethanol is not classified as a dangerous good.

Standard handling procedures
Store and use away from ignition sources. Do not heat ethanol in a container over an open flame; use a water bath that is sparkproof. Any experiments involving the combustion of ethanol are potentially hazardous. ETHANOL BURNS WITH A NEARLY COLOURLESS FLAME THAT IS DIFFICULT TO SEE IN STRONG LIGHT. Many serious injuries have resulted from "topping up" containers of burning ethanol used for heating purposes when it was thought that the flame was extinguished. If a fuel is required, consider using metaldehyde or hexamine tablets. Ethanol is a controlled substance, not usually available in schools. Methylated spirits is the usual form in which ethanol is obtained in schools; it contains methanol (5%), water (5%) and small amounts of pyridine and other coal-tar products to make the liquid unpalatable. Methylated spirits is adequate for most experiments carried out in schools.

Disposal

Retain in a flammable liquids cabinet for collection by a waste service. May be diluted with 20 times the volume of water and poured down the drain, provided no ecotoxic substances are present.

water <43.5 °C (cold-warm) **H_2O**

CAS: 7732-18-5

Class: nc PG: none Users: K-12 Training: 1-6

GHS data: Not classified as a hazardous chemical.

Potential hazards
Water below 43.5°C is generally considered safe for adults and children. Cold water causes numbness and hypothermia, if exposure is prolonged.

Standard handling procedures
Water in a laboratory should not be drunk, due to the possibility of chemical contamination. Water spilled on the floor may be a slip hazard.

Disposal
May be poured down the drain.

agarose, gel (polyagarobiose)

CAS: 9012-36-6

Class: nc PG: none Users: K-12 Training: 1-6

GHS data: Not classified as a hazardous chemical.

Potential hazards
Low toxicity.

Disposal
Gel may be placed in the garbage.

Biologicals and food to be used

Escherichia coli (E. coli)

Potential hazards
Possibility of infection during experiments with E. coli. Some strains are highly pathogenic.

Standard handling procedures
Some school authorities ban the culturing of unknown micro-organisms in experiments, due to the possibility of culturing pathogens. Commercially obtained pure non-pathogenic strains may be bought from reputable suppliers. Many school authorities do not permit sub-culturing of bacteria from wild cultures. Your school authority may allow commercially obtained pure strains of non-pathogenic bacteria to be subcultured from one plate to another, provided the appropriate safeguards are followed. Risk group 1 organisms, as described in AS/NZS 2243.3:2010, are generally regarded as suitable for supervised school experiments. Check the policy of your school authority.

Staphylococcus albus (S. albus)

Potential hazards
May cause infections in individuals who are immunocompromised or debilitated.

Standard handling procedures
Some school authorities ban the culturing of unknown micro-organisms in experiments, due to the possibility of culturing pathogens. Commercially obtained pure non-pathogenic strains may be bought from reputable suppliers. Many school authorities do not permit sub-culturing of bacteria from wild cultures. Your school authority may allow commercially obtained pure strains of non-pathogenic bacteria to be subcultured from one plate to another, provided the appropriate safeguards are followed. Risk group 1 organisms, as described in AS/NZS 2243.3:2010, are generally regarded as suitable for supervised school experiments. Check the policy of your school authority.

honey

Potential hazards
Rare allergies to honey are known. People with allergies or sensitivities to celery, pollen or other bee-related allergies may be allergic to honey.

Standard handling procedures
Do not eat in class, due to the possibility of contamination.