# INVESTIGATING THE ANTIBACTERIAL PROPERTIES OF AUSTRALIAN PROPOLIS & ASSESSING PHARMACEUTICAL POTENTIAL

By Chiko Katsande

ST EDWARDS COLLEDGE EAST GOSFORD

## Abstract

Antimicrobial resistance is a growing threat to the treatment of various infectious diseases especially in the case of bacterial infections, driving research into natural compounds and antibacterial activity. This investigation examined the significance of in vitro antibacterial activity of propolis produced by Australian honeybees. 6 samples from bee species Tetragonula carbonaria and Apis mellifera subspecies, from different locus, had their chemical contents extracted into ethanol tinctures. They were tested using the standardised Kirby-Bauer disc diffusion method against gram-positive bacteria: *Staphylococcus epidermidis* and *Micrococcus luteus* as well as the gram-negative bacterium *Escherichia coli*. Subsequent zones of inhibition were measured and analysed in a one-tailed student's t-test ($p = 0.05$). Analysis revealed statistical significance in antibacterial activity for all samples against gram-positive bacteria, with inhibition zones ranging from 7.7–26.2 mm, while gram-negative inhibition was weaker i.e. 5.5–10.2 mm, significant in only half of the samples. Additionally, variation was observed in results between bee species and geographic location. The results display propolis' potential as a natural antibacterial agent and suggest further study into species and region specific chemical variations to inform specific applications of propolis.

# 1. Literature Review

## *1.1 The Importance of Pharmacological Research in Natural Compounds*

Antimicrobials are a class of medicines that target microorganisms. In this classification of medicines are antibiotics, antivirals, antifungals and antiparasitics. Antibiotics specifically are used to remedy infections caused bacteria (Australia, 2024). Antibiotics treat bacterial infections by inhibiting growth (Bacteriostatic) or killing them (Bactericidal) (Bernatová et al., 2013).

Drug resistant microbes or superbugs are the product of antimicrobial resistance (AMR) a phenomenon that occurs when microbes develop resistance to antimicrobials, notably super bacteria are a more immanent threat due to misuse of antibiotics (WHO, 2023).

Antibiotics have side effects especially within the microbe dependent digestive system (NHS, n.d.) one such side effect is organ damage, the most prevalent form being drug induced liver injury (DILI) (Björnsson Es, n.d.). Antibiotics are prescribed over a planned course to ensure that these effects are minimised, and all infectious bacteria are killed. AMR causes complications as infections become harder to treat requiring longer courses of antibiotics causing more serious implications e.g. DILI, increasing the rates of death by infection. The IMHE estimates that by 2050 AMR could be the direct or contributing factor to approximately 8 million deaths each year (*IHME - The Lancet*, n.d.).

Naturally occurring compounds are reported to have antimicrobial potential against drug resistant bacteria due to their unique chemical composition (Álvarez-Martinez f J et al., 2020). Such ‘natural antibiotics’ were discovered even before antibiotics were conceptualised. Historical records indicate ancient civilisations like the Ancient Greeks and Romans utilised propolis for its bioactive properties as an antiseptic, disinfectant, and healing agent for wound treatment (Kegode t M et al., 2022). The potential of organic compounds has driven further research in beehive products like propolis, a well known remedy with reported antimicrobial activity. Today propolis has seen many medicinal uses to treat upper respiratory tract infections, common cold, and flu-like infections, wounds, burns, acne, herpes and neurodermatitis *(Wagh, 2013).* These are only a few uses of propolis which suggest that further research and development into propolis could yield potential counters for AMR.

## *1.2 Propolis phytochemical composition*

Propolis is a resinous compound that is made from natural substances collected by worker honeybees from various plants (Pasupuleti et al., 2017). A study of the foraging range of the bee species *Apis mellifera* (Madeleine Beekman & FL.W. Ratnieks., 2000) has shown the range in which honeybees forage depends on the environment of the landscape they are in, a botanically diverse region in which vegetation is abundant near the nest the foraging range tends to be within 1 km but in areas with more spread-out vegetation the foraging range can increase up to 11km if necessary. Further study is required to determine a maximum foraging range, but the highest observed range reported in the study was 11km dependent upon flora, the surveyed landscape, and the species of the honeybee. Typically, raw propolis is composed of 50% plant resins, 30% waxes, 10% essential and aromatic oils, 5% pollens and 5% other organic substances (Bankova et al., 2000). Plants antimicrobial properties can be attributed to the secondary metabolites or phytochemicals that the plant produces for protection (Kumar et al., 2023). As bees collect plant resins, they are collecting phytochemicals which then influence the chemical composition of propolis providing it with bioactive properties. This is reflected by the 111 flavonoids, 58 terpenes and 70 phenolics identified during years 2000-2014, only adding to the 300 plus chemical compounds already identified (Huang et al., 2014).

Research has shown that the environment propolis samples are collected from have a significant impact not only on the antimicrobial activity, but

the phytochemical concentrations of the sample highlighting the correlation between plant environment and antimicrobial activity (Kegode t M et al., 2022).Within propolis' vast chemical composition, flavonoids play an important role in antibacterial properties, specifically, flavonols and dihydroflavonoids, which are present in propolis in high amounts. *(Jing et al., 2022).* Due to high antibacterial potency, majority of the antibacterial properties of propolis are attributed to the flavonoids collected from the plant resins around the hives. In a study of propolis as a potential component in antibiotic pharmaceutical products (*Wieczorek et al., 2022*) some flavonoids were identified in samples of propolis during the study.

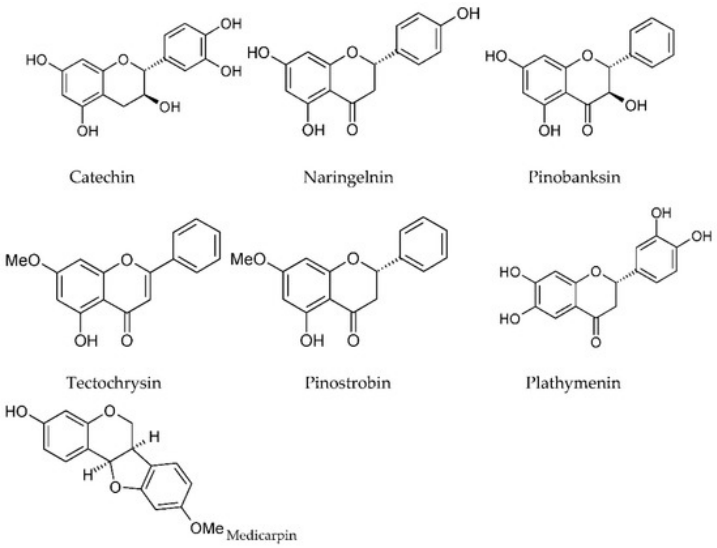



***Figure 1 -*** *The structures of some flavonoids identified in propolis samples* (*Wieczorek et al., 2022*)

These are only some of the hundreds of flavonoids that have been identified in propolis samples (Huang et al., 2014). The flavonoids antibacterial properties are the result of their polyphenolic structure. It includes a benzopyranone scaffold and two aromatic rings connected by a three-carbon chain (Ying et al., 2025). This structure (figure 2) produces bactericidal and/or bacteriostatic effects by damaging the cytoplasmic membrane, blocking the synthesis of nucleic acids through the inhibition of topoisomerase and/or DNA gyrase, inhibiting the respiratory chain and ATP synthase, and preventing cell wall and membrane synthesis by inhibiting enzymes. (Gallegos et al., 2015)

### *1.3 Investigating propolis antibacterial properties*

Due to the variety of antibiotic effects, the combination of flavonoids, polyphenols and other secondary metabolites found in propolis imitates the behaviour of a broad spectrum antibiotic.

Antibacterial activity is typically determined through the in vitro Kirby-Bauer Disc diffusion method. The disc diffusion method refined by William Kirby and Alfred Bauer and standardised by the World Health Organisation (WHO) in 1961, is a low cost, effective method used to estimate the antimicrobial properties of preclinical antimicrobials (Libretexts, 2016). The disc diffusion method is used to determine the susceptibility of microbials against antimicrobials of interest by inoculating an agar plate with a cultured strain and placing filter paper impregnated with a concentration of the antimicrobial and measuring the zone of inhibition (Bayot m L & Bragg, 2024). Previous studies of propolis against cultured bacteria have shown propolis' ability to inhibit bacterial growths against both gram positive and negative bacteria (Marghitas et al., 2010).

Propolis has typically been more effective against gram positive bacteria compared to gram negative bacteria likely due to the difference in the structure of their cell walls. Gram positive bacterium have a peptidoglycan cell wall 20-80nm thick, whereas gram negative bacterium cell walls are only 2-3nm thick and are covered by an additional outer lipid bilayer membrane (Sizar et al., 2023). This additional outer membrane can prevent antibiotic substances from reaching target structures like proteins within the cell greatly increasing gram negative bacteria's resistance to a wide range of antibiotics especially those that function by inhibiting intracellular processes (Breijyeh et al., 2020).

### *1.4 Bee species & propolis*

Previous studies show that different bee species, sub-species and varieties influence the composition of propolis due to the difference in plants they collect resins from. Research on propolis is typically cannot be universal as the

complex influences on propolis composition result in variable results unique to each sample depending on geographic location, species, plant sources and even the time of collection. This is shown in a study that compared bees from various locations including the study of propolis from stingless bees (Tetragonula carbonaria). In which the study concluded that the propolis contained terpenic acids and phenolic acids however, the characteristic flavonoids and prenylated phenolics found in propolis from other Australian honeybee species were notably lacking (Huang et al., 2014).

Chemical variation between Australian native stingless bee (Tetragonula carbonaria) and other commonly kept bees e.g. European honeybee (Apis mellifera) or the Italian honeybee (Apis mellifera ligustica) is influenced heavily not only by geographical location but bee species as well. Further data and study of the extent at which species and geography influence chemical composition is not heavily studied and more research is required to develop a universally grounded understanding of propolis.

### 2. Scientific research question

Does propolis produced by honeybees kept in Australia exhibit statistically significant in vitro antibacterial activity and is it affected by geographic location?

### 3. Scientific Hypotheses

Alternate Hypothesis:

Propolis extracts will exhibit a larger zone of inhibition and hence greater antibiotic activity than the negative control for both gram negative and positive bacteria. The ZOI will be influenced by the geographical origin of the sample.

Null hypothesis:

There will be no difference between the propolis samples and negative controls indicative of no bioactivity in the extract. The ZOI will not be influenced by geographical origin.

## 4. Methodology

### *4.0 Summary*

The investigation was a blind experiment conducted in multiple parts. First, samples were bought from apiarists based on the eastern coast of Australia from Victoria through to the Sunshine coast in Queensland and local samples from regions of the Central Coast were sourced and collected through apiarist of the Central Coast Beekeeping Association.

The raw propolis then had its phytochemicals extracted into a tincture using a modified version of the University of Sydney propolis ethanol maceration methodology (*Provided by Dr Kenya Fernandes*). A Kirby-Bauer disc diffusion experiment was then performed using a modified version of the methodology detailed in Andresa P.N et al., 2013. A students t-test was conducted on the analysed data to deterimine significance of inhibition and a two factor ANOVA test was conducted to determine the signifcance of geographic location on antibacterial activity.

### *4.1 Collection Procedure*

Initially samples were intended to be collected from apiarists on the central coast from varying microclimatic zones, however, the 2022 NSW Varroa mite infestation that was deemed to be incapable of eradication in 2023 reduced the number of available samples, a wider search and collection of samples from Queensland to Victoria with varying species was required. Three propolis samples were commercially sourced through experienced beekeepers from Queensland and Victoria from experienced apiarists, the remaining three were sourced from varying areas of the Central Coast 2 from experienced commercial apiarist and one from an amateur beekeeper. For all six of the samples the location of the hive and, if known, the species of bee was recorded.

### *4.2 Phytochemical Extraction*

Initially any debris or contaminants was manually removed from the propolis samples. Each sample of propolis was weighed to the nearest 0.01g. mass of the sample was recorded. The weighed

propolis was then pulverised using cryogenic grinding where the propolis is frozen typically using liquid nitrogen (dry ice was used as a cheap alternative) and then mechanically broken down into a fine powder using a grinder.

Each sample of propolis powder was moved to a beaker. The appropriate volume of ethanol was added and recorded (10 ml of ethanol per 1g of propolis). Each beaker was covered with foil and a magnetic stirrer was placed inside to continuously extract the phytochemicals of each sample through mechanical maceration extraction method for 48 hours resulting in an ethanol solution.

After 48 hours of extraction, the samples were moved into a centrifuge tube and spun down using a centrifuge to collect the solid propolis at the bottom of the tube. The ethanol supernatant extract was poured into a flat, wide-mouth container (In this investigation another beaker was used for simplicity) and the remaining solid propolis was disposed of accordingly. The weight of the sample (not including the beaker) was measured to the nearest 0.01g.

The beaker was placed into a fume hood and left until the ethanol completely evaporated (usually within a couple of hours to overnight for ~10 ml). Following the complete evaporation of the ethanol the remaining sampled was collected from the beaker and the mass was measured to the nearest 0.01g.

The appropriate amount of DMSO (1 ml of DMSO per 0.1g of sample to make a final concentration of 100 mg/ml) was added into the specimen sample beaker and a small spatula was used to scrape and mix until the propolis was dissolved.

For storage samples and extracts were stored inside a container inside a fridge or freezer to prevent damage to the sample.

### *4.3 Disc Diffusion Assays*

For each strain of bacteria, three Mueller – Hinton agar plates were inoculated using the Drigalski spatula (hockey-stick) method. In each propolis extract, nine filter paper disks were placed in a beaker (or petri dish) containing one sample of propolis extract, there was also an additional control beaker containing DMSO.

One soaked disk from each sample was removed using sterile forceps and gently pressed on the edge of the dish to remove any excess solution. Each filter paper disk was then placed inside of the agar plate, the sterile DMSO filter paper disk functioned as a negative control for growth inhibition.

The plates were then be incubated for 48 hours at a temperature of 37°C and the zone of inhibition was recorded using electronic vernier callipers and recorded.

This process was repeated with three different bacteria cultured to a McFarland standard of 0.5: 2 gram-positive (Micrococcus luteus and Staphylococcus epidermidis) one gram-negative (Escherichia coli) bacterium.

The measured zones of inhibition (ZOI) were recorded in mm including the 5.5mm filter paper discs, where no inhibition was recorded as a measurement of ‘0mm’. Data was then tabulated in Microsoft excel for efficient statistical analysis.

### *4.4 Statistical analyses*

A one tailed students t-test was used to determine the significance of antibacterial activity between samples and the negative control. The t- value was calculated using the formula:

$$t = \frac{\bar{x} - \mu}{\frac{\sigma}{\sqrt{n}}}$$

Where $\bar{x}$ is the sample mean, $\mu$ is the expected mean for in the case of the null hypothesis which is ‘0’, $\sigma$ is the standard deviation of the sample and n is the number of trials. The p values was set at 0.05 for a one tailed test resulting in a critical value of 2.920 that was used to determine whether the null was rejected.

Additionally, a two-factor ANOVA tests was conducted between samples from the species Tetragonula carbonaria and the inhibition zones against the three bacteria to assess the interaction between geographic location and antibacterial activity whilst the species is controlled the was done to a significance of $p < 0.05$, which was then

confirmed by a post hoc Bonferroni correction to check for Type 1 error and reject or accept the null hypothesis.

### *4.5 Ethical Concerns & Risk assessment*

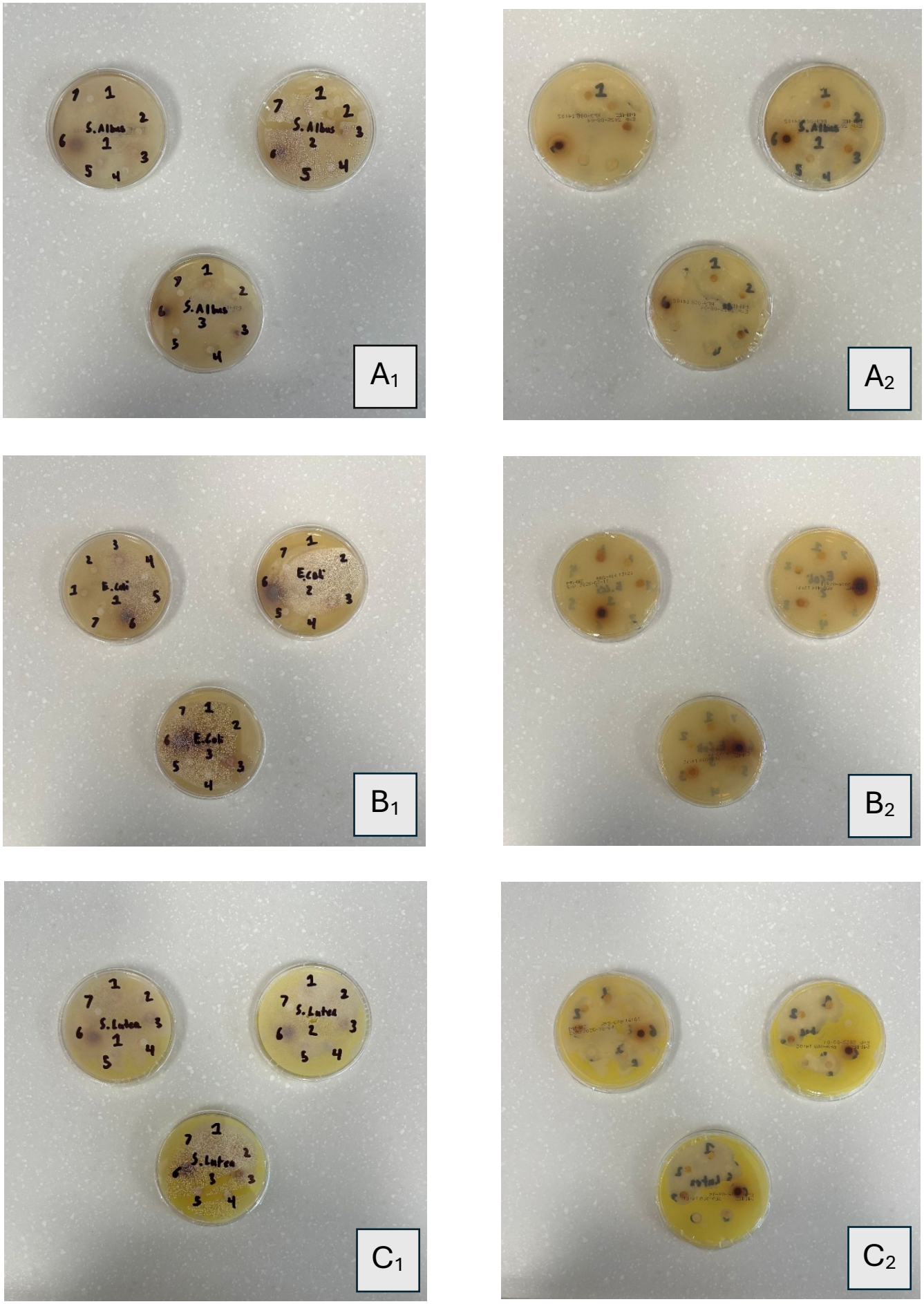
Ethical considerations were made when discussing the impact of collecting propolis from bee hives especially those of the endemic Australian stingless bee species as their hives are constructed from propolis and bee wax. The improper collection of propolis from the hive threatens the security of the nest which can endanger the bees. As such, Native beehive propolis was collected only when suitable for the hive which was determined for locally collected samples by the Central Coast Beekeepers Association. Similarly, in the case of bee species where propolis collection is not detrimental to the hive, a simple non-invasive propolis trap was used to collect local samples as propolis is typically discarded as a waste biproduct of hive activity due to its tackiness and handling. Other samples were purchased from experienced apiarist from Queensland and Victoria who own commercial hives and sell propolis.

A risk assessment (appendix 4) was also made when determining which strains of bacteria to use during the investigation. Bacteria were specifically chosen from the ASSIST guidelines for best practice for microbiology in Australian schools (Barnett D, 2017), to reduce risk by selecting less threating bacterium in Risk Group 1 (Escherichia coli, Staphylococcus epidermidis, Micrococcus luteus) which is unlikely to cause human or animal disease in healthy individuals.

Live bacteria as pathogens can cause disease and sickness e.g. diarrhoea is caused by an E. coli infection (Mueller & Tainter, 2023). To prevent the spread of the bacteria used in the investigation equipment was continuously sterilised and appropriate PPE was used to limit exposure to the pathogens. Once the investigation was over all cultures and agar plates were autoclaved and PPE was disposed of accordingly. (See appendix 4)

## 5. Results

Figure 2 – 10% concentration filter paper disc Against S. Epidermidis (A), E. Coli (B), M. Luteus (C).




For sample plates 1: Sunshine Coast - Tetragonula carbonaria, 2: Brisbane - Tetragonula carbonaria, 3: Springfield - Tetragonula carbonaria, 4: Umina - Apis mellifera ligustica, 5: MacMasters Beach - Apis mellifera Linnaeus, 6: Victoria – N/A

Figure 3 – The mean ZOI for each propolis sample Staphylococcus epidermidis.

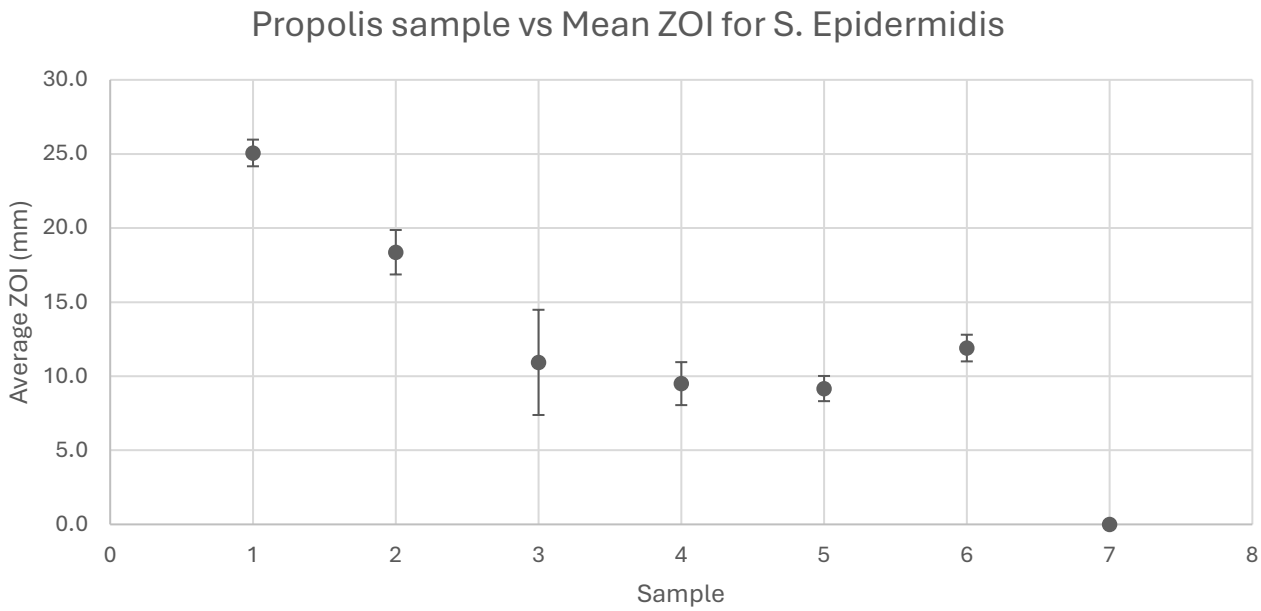



Figure 4 – The mean ZOI for each propolis sample against Micrococcus luteus (Outlier in red).

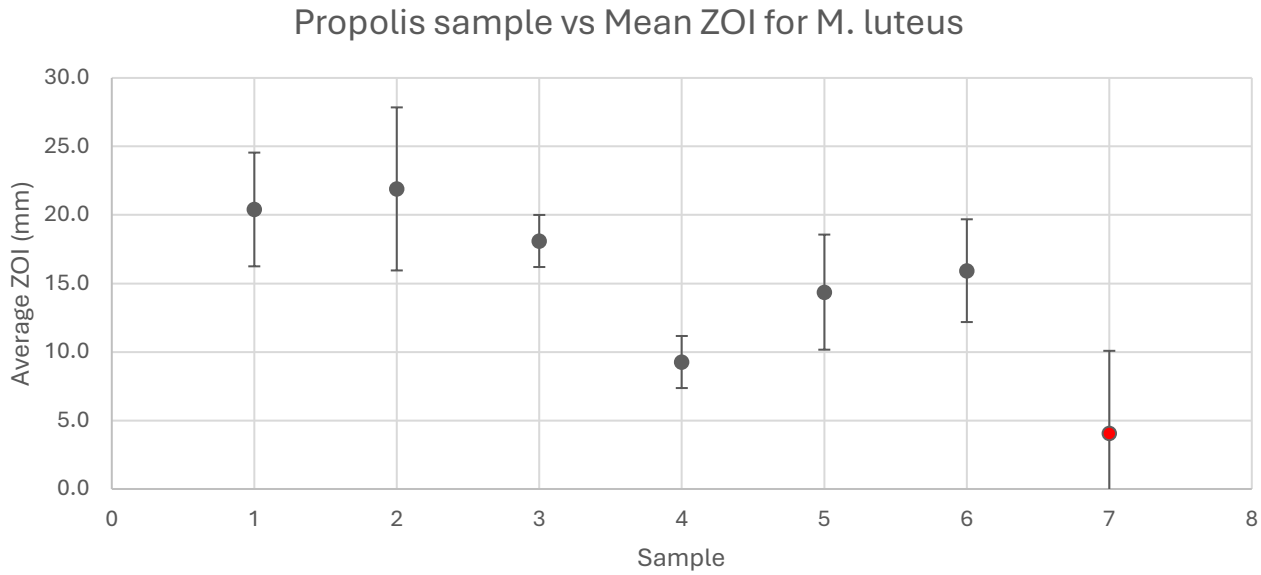
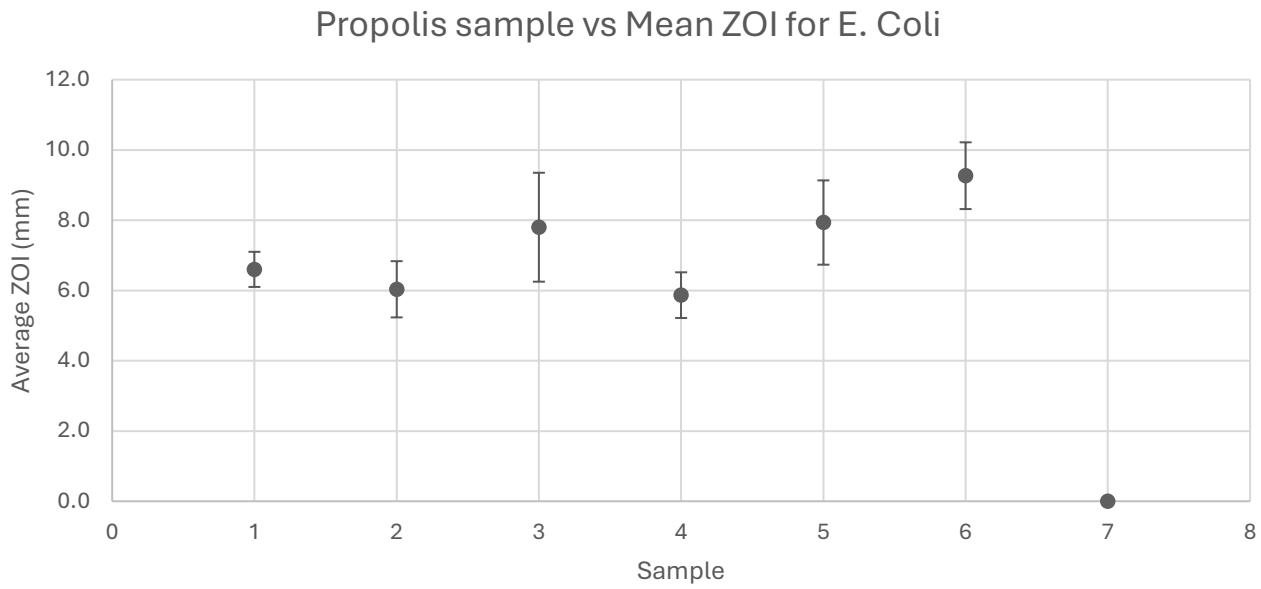



Figure 5 – The mean ZOI for each propolis sample against Escherichia coli.



Figures 3 through 5 were plotted with error bars calculated using the $\frac{Range}{2}$ to determine a potential range of values.

Table 1 – Results from the two factor ANOVA test between geographic location and ZOI against various bacteria

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| **Location** | 118.73850 | 2 | 59.36926 | 5.94419 | 0.010422 | 3.55456 |
| **Zone of Inhibition** | 920.20960 | 2 | 460.10480 | 46.06679 | 8.32E-08 | 3.55456 |
| **Interaction** | 207.29700 | 4 | 51.82426 | 5.18877 | 0.005853 | 2.92774 |
| **Within** | 179.78000 | 18 | 9.98778 | | | |
| **Total** | 1426.02500 | 26 | | | | |

In this table the alpha value was set to 0.05, in the post hoc corrected Bonferroni correction the comparison was between geographic locations within a single bacterium producing an adjusted significance level of $p < \frac{0.05}{6}, hence\ p < 0.00833$ for the interaction. For the location and zone of inhibition adjusted significance level was $p < \frac{0.05}{63}, hence\ p < 0.01667$.

Table 2 – The t-values from a one tailed students t-test for each bacterium.

| | **E. coli** | **S. epidermidis** | **M. luteus** |
|---|---|---|---|
| 1 | 4.667 | 42.072 | 7.080 |
| 2 | 2.441* | 16.982 | 5.148 |
| 3 | 2.812* | 3.229 | 12.801 |
| 4 | 1.225* | 5.399 | 3.782 |
| 5 | 3.876 | 8.371 | 4.270 |
| 6 | 8.407 | 15.085 | 5.245 |
| 7 | N/A | N/A | N/A |

In this table, values >2.920 correspond to statistical significance (null was rejected) statistical insignificance (null was accepted) is denoted by the marker (*).

Figure 6 – Map of Geographic location and species of bee for each sample


Note: In this map details are listed as Sample - Location - Species

## 6. Discussion

The results of this investigation aimed to examine propolis' performance as an antibacterial agent and further study into the impacts of species and geographic location on propolis antibacterial activity.

### *6.1 Antibacterial observations against gram positive bacteria*

The results of the disc diffusion assay against gram positive bacteria *S. epidermidis* and *M. luteus* (see Figure 2, A and C) demonstrate moderate to strong inhibitive qualities for all samples. For *S. epidermidis* the ZOI ranged from 7.7mm to 26.2mm and for *M. luteus* the ZOI ranged from 7.9mm to 26mm. This implies that there are bioactive compounds in the sample that are either bacteriostatic or bactericidal which matches results from two other studies (Marghitas et al., 2010; Abdullah et al., 2020). In the disc diffusion assay for *M. luteus* Trial 3 (see Figure 2, C, S. Luteus 3) there is an evident ZOI surrounding the negative control which was likely due to a failure in the sterilisation process in which ethanol was not completely burned off the tweezers used to place the sample. This outlier has been accounted for and is reflected in the comparison of each samples mean ZOI (see Figure 3 and Figure 4). Ignoring the outlier in the control for *M. Luteus*, marked in red, the data shows a relationship between the samples and produced bacterial inhibition.

### *6.2 Antibacterial observations against gram negative bacteria*

Contrastingly the results of the disc diffusion assay against the gram negative bacterium *E. coli* (see Figure 2, B) exhibit minimal inhibitive qualities for all samples. The ZOI ranged from 5.5mm to 10.2mm which suggest that the samples are not as effective against gram negative bacteria as opposed to gram positive which is also affirmed by the results in *Marghitas et al. (2010)* in which the range of ZOI values for E. coli was 7mm to 12mm. Similar results were also found in Abdullah et al. (2020) which emphasised variation between species.

The lack of inhibitory effect is also reinforced by the mean ZOI of inhibitions (see Figure 5) which highlights the minimal antibacterial activity of the samples in vitro against gram negative bacteria. Indicating stronger resistance to the sample which is supported by the ANOVA test (Table 1). Results between the zones of inhibition and bacteria which showed that the variation in results between bacteria was significant as the F-value was substantially greater than the F-critical ($46.06679 > 3.55456$) and in post hoc the p value was significantly less than the corrected Bonferroni value ($8.32E\text{-}08 < 0.01667$) further supporting the notion that the variation between significance in bacteria is substantial.

### *6.3 Statistical significance of bacterial inhibition*

The results from the students t-test against gram positive bacteria (see Table 2, *S. epidermidis* and *M. luteus*) rejects the null hypothesis for a level of significance set at $p = 0.05$ with a critical value of 2.920. Majority of the t-values exceed this level of significance ($t > 2.920$) by a notable margin (marked in green) which supports the conclusion to reject the null hypothesis and accept the alternate. Furthermore, in the t-test results for the gram negative bacteria *E. coli* (see Table 2) the null hypothesis was rejected by the results at a rate of 50% as only 3 out of 6 samples exhibited statistically significant results ($p < 0.05$). This is consistent with the notion that antibacterial agents are less effective against gram negative bacteria, which indicates that propolis may lack strong bactericidal effects against the double membrane structure of gram negative bacterium cell walls which is known to be more resistant to antibiotic agents (Breijyeh et al., 2020) which is consistent with the findings in 6.2. The results for samples 2, 3 and 4 support the null hypothesis as their t-values; 2.44, 2.81 and 1.22 respectively, show statistically insignificant inhibition ($p > 0.05$).

### *6.4 Variations between geographical locations and antibacterial activity*

The results of samples 1, 2 and 3 from demonstrated variation (see figure 3, 4 and 5)

where the independent variable was the geographical location (see Figure 6). The results from the two-factor ANOVA test (see Table 1) produced an F-value for the sample location equal to 5.94419 with an F-critical value of 3.55459 in support of the alternate hypothesis that between the ZOI and the sample location there was a statistically significant variation due to the location of sample collection in antibacterial activity of propolis from the bee species *Tetragonula carbonaria*. Furthermore, a post hoc Bonferroni correction between ANOVA values confirmed the significance as the p-value, 0.01042 was less than that of the corrected significance p-value 0.01667 reducing the possibility of false positives (Type 1 Error). The results of the interaction between geographic location and antibacterial activity produced an F-value of 5.18877 and an F-critical of 2.92774 which further reinforced the alternate hypothesis that effectiveness against different bacteria was influenced by location, post hoc analysis confirmed the positive result ($p<0.00833$). These varying levels of antimicrobial activity are consistent with trends in the study of variations in phenolic content in propolis from separate locations (Saral et al., 2019) and the study of European propolis samples (Al-ani I et al., 2018) and its comparison to the varying antimicrobial properties of samples from other locations. Where, propolis from London had antimicrobial effectiveness against Methicillin-resistant *Staphylococcus aureus* unlike Turkish propolis which had anti-tuberculosis activity and was effective against multiple mycobacteria.

### *6.5 Observations between species and antibacterial activity*

The results demonstrated a notable difference between the species of bee from what the sample propolis was collected from. Typically, the highest ZOI came from propolis made by *Tetragonula carbonaria* (Samples 1, 2 and 3), however, 2 of the 3 statistically substantial ZOI's produced against gram negative bacteria *E. coli* were produced by honeybees of the species *Apis mellifera*. This suggest that there is variation in antibacterial activity due to the species of bee that produced the sample which is affirmed by the conclusion reached in the study by Huang et al. (2014). The conclusion proposed that this difference was due to differing botanical preferences across species. The same study also depicted a lack of flavonoids and other phenolic compounds in *Tetragonula carbonaria* propolis which had been found in the propolis of other species of bee within Australia. This variation could be the impact of a behavioural difference in favoured plants e.g. the introduced populus *nigra 'Italica'* and the *Eucalyptus* species endemic to Australia both of which are known for possessing antimicrobial properties. This could be a potential reason for the difference in antibacterial activity between the species however, more study is required to determine the differences in behaviours and their impact of chemical composition.

### *6.6 Experimental design and limitations*

The experiment was designed based on the standardised Kirby-Bauer disc diffusion method as it is cost effective, simple, and efficient. The experimental method though supplemented with sufficient controls in the disc diffusion method and extraction process was limited by the equipment available. For example, the centrifuge used in the methodology was a hand cranked model as opposed to the more effective and efficient automated centrifuge to reduce cost. This led to minimal variation in the results as there was increased room for random error which could skew the results e.g. variable centrifuge speed. Similarly, the manual removal of debris was difficult and inefficient due to the sticky and tarry nature of propolis which was hard to manage. This also allowed for increased random error in the results as contaminants like pollen may have been present in the samples. The impact of these random errors and contaminants was minimised through the rigorous extraction process. Similarly, any variation in the disc diffusion was minimised through the Drigalski spatula method which involved the sterilisation of potential bacteria on equipment.

### *6.7 Future testing and applications*

Further studies of the antimicrobial properties of propolis samples on the eastern coast of Australia should aim to investigate the influence of bee species on propolis samples e.g. *Tetragonula carbonaria* vs a specific European species like *Apis mellifera ligustica.* Additionally, study of the variations in botanical disposition of differing geographic locations and phytochemical compositions would provide necessary information to determine what flora produces the strongest broadest antimicrobial properties in propolis samples. Though the antibacterial activity of propolis is significant, this information is necessary to assess the specific applications of east coast Australian propolis in pharmaceutical products.

## 7. Conclusion

The investigation revealed there to be a relationship between each sample and antibacterial activity that was evaluated to be statistically significant ($p < 0.05$, $t > 2.920$). Against gram negative bacteria *E. coli* observed antibacterial activity in the form of an inhibition zone was less compared to that of gram positive bacteria *S. epidermidis* and *M. luteus*. The bacterial activity however was still notable and greater than that of the negative control as predicted in the hypothesis. Comparison between antibacterial activity of samples produced by the stingless bee, *Tetragonula carbonaria* and the *Apis mellifera* subspecies *Linnaeus* and *Ligustica* revealed substantial variation between species where samples produced by *Tetragonula carbonaria* exhibited the strongest ZOI. Similarly, comparison between geographical location also revealed differences between geographical location where samples from Queensland exhibited more potent inhibitory effects than that of any other sampled region. However, further study of geographical and species influence on propolis is required to determine the nature of the relationship between geographical location, species, and antibacterial activity. Previous studies however have suggested that this variation is due to the presence of certain flora (Kegode t M et al., 2022) and differing botanical preferences between species (Huang et al., 2014).

The results of the investigation of this report reject the null hypothesis and accept the alternate hypothesis that propolis from the eastern coast of Australia does possess antibacterial properties that are influenced by geographical location and hence does possess potential as a natural compound to be used in pharmaceutical research.

## 8. References

Cited in APA 7th edition format:


1. Barnett D. (2017). GUIDELINES for best practice for microbiology in Australian schools. [Online document]. ASSIST. https://assist.asta.edu.au/printpdf/4196

2. Australia, H. (2024, October 01). Antibiotics. Retrieved November 21, 2024, from https://www.healthdirect.gov.au/antibiotics

3. Bernatová, S., Samek, O., Pilát, Z., Šerý, M., Ježek, J., Jákl, P., Šiler, M., Krzyžánek, V., Zemánek, P., Holá, V., Dvořáčková, M., & Růžička, F. (2013, Oct 24). Following the Mechanisms of Bacteriostatic versus Bactericidal Action Using Raman Spectroscopy. Molecules. Retrieved November 21, 2024, from https://pmc.ncbi.nlm.nih.gov/articles/PMC6270526/

4. Cowan, M. M. (1999, Oct). Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. Retrieved November 21, 2024, from https://pmc.ncbi.nlm.nih.gov/articles/PMC88925/

5. Yeshi, K., Turpin, G., Jamtsho, T., & Wangchuk, P. (2022, Jun 15). Indigenous Uses, Phytochemical Analysis, and Anti-Inflammatory Properties of Australian Tropical Medicinal Plants. Molecules. Retrieved November 21, 2024, from https://pmc.ncbi.nlm.nih.gov/articles/PMC9231311/

6. WHO. (2023, November 21). *Antimicrobial resistance.* World Health Organisation (WHO). Retrieved August 20, 2025, from https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

7. Björnsson Es. (n.d.). Drug-induced liver injury due to antibiotics. Scandinavian Journal of Gastroenterology. Retrieved November 21, 2024, from https://pubmed.ncbi.nlm.nih.gov/28276834/

8. Álvarez-martínez f J, Barrajón-catalán, E, & Micol, V. (2020, Oct 11). Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. Biomedicines. Retrieved November 21, 2024, from https://pmc.ncbi.nlm.nih.gov/articles/PMC7601869/

9. Abdullah n A, Zullkiflee, N, Zaini, S. N. Z, Taha, H, Hashim, F, & Usman, A. (2020, Sep 12). Phytochemicals, mineral contents, antioxidants, and antimicrobial activities of propolis produced by Brunei stingless bees Geniotrigona thoracica, Heterotrigona itama, and Tetrigona binghami. Saudi Journal of Biological Sciences. Retrieved November 21, 2024, from https://pmc.ncbi.nlm.nih.gov/articles/PMC7569112/

10. Libretexts. (2016, March 08). Microbiology Labs I. Biology Libretexts. Retrieved November 25, 2024, from https://bio.libretexts.org/Learning_Objects/Laboratory_Experiments/Microbiology_Labs/Microbiology_Labs_I/

11. The Lancet. (n.d.). Institute for Health Metrics and Evaluation. Retrieved November 25, 2024, from https://www.healthdata.org

12. Bayot m L, & Bragg, B. N. (2024, May 27). Antimicrobial Susceptibility Testing. Retrieved November 25, 2024, from https://www.ncbi.nlm.nih.gov/books/NBK539714/

13. Marghitas, L. A., Flore, C., Mihai, C. M., Dezmirean, D. S., & Fit, N. L. (2010). *The Study of the Antimicrobial Activity of Transylvanian (Romanian) Propolis.* Research Gate. Retrieved August 20, 2025, from https://www.researchgate.net/publication/49591565_The_Study_of_the_Antimicrobial_Activity_of_Transylvanian_Romanian_Propolis

14. Kegode t M, Bargul, J. L, Mokaya, H. O, & Lattorff, H. M. G. (2022, Jul 27). Phytochemical composition and bio-functional properties of Apis mellifera propolis from Kenya. Royal Society Open Science. Retrieved November 25, 2024 from https://pmc.ncbi.nlm.nih.gov/articles/PMC9326266/

15. Madeleine Beekman, & F.L.W. Ratnieks. (2000). Long-range foraging by the honey-bee, Apis mellifera L. Research Gate. Retrieved November 25, 2024 from https://www.researchgate.net/publication/227499273

16. Alisdair R. Fernie. (2007, July 7th). The future of metabolic phytochemistry: Larger numbers of metabolites, higher resolution, greater understanding | ScienceDirect. Retrieved November 30, 2024 from https://www.sciencedirect.com/science/article/abs/pii/S0031942207004608

17. Patra a K. (2012, Feb 18). An Overview of Antimicrobial Properties of Different Classes of Phytochemicals. Dietary Phytochemicals and Microbes. Retrieved November 30, 2024 from https://pmc.ncbi.nlm.nih.gov/articles/PMC7121617/

18. Al-ani I, Zimmermann, S, Reichling, J, & Wink, M. (2018, Jan 3). Antimicrobial Activities of European Propolis Collected from Various Geographic Origins Alone and in Combination with Antibiotics. Medicines. Retrieved November 30, 2024 from

https://pmc.ncbi.nlm.nih.gov/articles/PMC5874567/

19. Šuran J, Cepanec, I, Mašek, T, Radić, B, Radić, S, Gajger, I. T, & Vlainić, J. (2021, May 14). Propolis Extract and Its Bioactive Compounds From Traditional to Modern Extraction Technologies. Molecules. Retrieved December 01, 2024 from https://pmc.ncbi.nlm.nih.gov/articles/PMC8156449/

20. Andresa P.N, Nathália U.F, Edna A.B, Bruno A.R, Mirela M.O.L.L.V, & Andresa, B. (2013, April 26th). Methodologies for the evaluation of the antibacterial activity of propolis. Research Gate. Retrieved December 01, 2024 from https://www.researchgate.net/publication/258448255

21. Bernklau, E., Bjostad, L., Hogeboom, A., Carlisle, A., & S, A. H. (2019, Jan 8). Dietary Phytochemicals, Honey Bee Longevity and Pathogen Tolerance. Insects. Retrieved December 02, 2024 from https://pmc.ncbi.nlm.nih.gov/articles/PMC6359238/

22. Al-ani, I., Zimmermann, S., Reichling, J., & Wink, M. (n.d.). Antimicrobial Activities of European Propolis Collected from Various Geographic Origins Alone and in Combination with Antibiotics. Medicines (Basel. Switzerland). Retrieved December 02, 2024 from https://pubmed.ncbi.nlm.nih.gov/29301368/

23. Castaldo, S., & Capasso, F. (2002, Nov). Propolis, an old remedy used in modern medicine. Fitoterapia. Retrieved April 27, 2025 from https://pubmed.ncbi.nlm.nih.gov/12495704/

24. Kiziltas, H., & Erkan, C. (2020, September 28). The effects of different beehives on propolis production and quality. Food Science and Technology. Retrieved April 27, 2025 from https://www.scielo.br/j/cta/a/QftfNVXHV4Bkb779KXpYV8h/

25. Pasupuleti, V. R., Sammugam, L., Ramesh, N., & Gan, S. H. (2017, Jul 26). Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. Oxidative Medicine and Cellular Longevity. Retrieved April 27, 2025 from https://pmc.ncbi.nlm.nih.gov/articles/PMC5549483/

26. Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., K, S., & Oz, F. (2023, Jan 16). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules. Retrieved April 28, 2025 from https://pmc.ncbi.nlm.nih.gov/articles/PMC9862941/

27. Wagh, V. D. (2013, Dec 9). Propolis: A Wonder Bees Product and Its Pharmacological Potentials. Advances in Pharmacological Sciences. Retrieved April 28, 2025 from https://pmc.ncbi.nlm.nih.gov/articles/PMC3872021/

28. Ying, L., Jiajia, Z., Zhenyi, L., Yan, Z., Chen, M., & Hongjun, W. (2025, March 10). Flavonoids as Promising Natural Compounds for Combating Bacterial Infections. MDPI. Retrieved April 28, 2025 from https://www.mdpi.com/1422-0067/26/6/2455

29. Mueller, M., & Tainter, C. R. (2023, July 13). Escherichia coli Infection. Retrieved July 29, 2025, from https://www.ncbi.nlm.nih.gov/books/NBK564298/

30. Breijyeh, Z., Jubeh, B., & Karaman, R. (2020, Mar 16). Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to

Resolve It. Molecules. Retrieved July 31, 2025, from https://pmc.ncbi.nlm.nih.gov/articles/PMC7144564/

31. Sizar, O., Leslie, S. W., & Unakal, C. G. (2023, May 30). Gram-Positive Bacteria. Retrieved July 31, 2025, from https://www.ncbi.nlm.nih.gov/books/NBK470553/

32. Wieczorek, P. P., Hudz, N., Yezerska, O., Horčinová-Sedláčková, V., Shanaida, M., Korytniuk, O., & Jasicka-Misiak, I. (2022, February 28). Chemical Variability and Pharmacological Potential of Propolis as a Source for the Development of New Pharmaceutical Products. MDPI. Retrieved August 18, 2025, from https://www.mdpi.com/1420-3049/27/5/1600

33. Kumar, A., Nirmal, P., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., Sneha, K., & Oz, F. (2023, January 16). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. MDPI. Retrieved August 18, 2025, from https://www.mdpi.com/1420-3049/28/2/887

34. Saral, O., Kiliçarslan, M., Şahin, H., & Yildiz, O. (2019). *Evaluation of antioxidant activity of bee products of different bee races in Turkey.* Research Gate. Retrieved August 18, 2025, from https://www.researchgate.net/publication/335036849_Evaluation_of_antioxidant_activity_of_bee_products_of_different_bee_races_in_Turkey

35. Gallegos, M., Vargas, P., & Rodriguez-Garcia, I. (2015). Antibacterial Actions of Flavonoids. [PDF]. https://digital.csic.es/bitstream/10261/275709/1/antiflavo6.pdf

36. Huang, S., Zhang, C. P., Wang, K., Li, G. Q., & Hu, F. L. (2014, November 26). *Recent Advances in the Chemical Composition of Propolis.* MDPI. Retrieved August 20, 2025, from https://www.mdpi.com/1420-3049/19/12/19610

36. Bankova, V. S., Castro, S. L. D., & Marcucci, M. C. (2000, January 01). *Propolis: Recent advances in chemistry and plant origin.* Apidologie. Retrieved August 20, 2025, from https://www.apidologie.org/articles/apido/abs/2000/01/M0105/M0105.html

### *8.1 Acknowledgements*

I would like to thank and acknowledge the Central Coast Beekeepers Association, especially Secretary Sherrie Smith for helping with sourcing of and apiary officer Michael Graham for helping with the ethical collection of local propolis samples. I'd also like to thank my teachers Mr. Lynch and Mr. Foster as well as the St Edwards lab technicians who helped me organise and conduct the investigation.

# 9. Appendices

Raw Data & Descriptive statistics

## Appendix 1 – Raw data and descriptive statistics for Escherichia coli

| E. coli | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample** | **Trial 1** | **Trial 2** | **Trial 3** | **Mean** | **Range** | **Standard deviation** | **Variance ($mm^2$)** |
| 1 | 6.6 | 7.1 | 6.1 | 6.6 | 1.0 | 0.4 | 0.2 |
| 2 | 6.5 | 6.6 | 5.5 | 6.2 | 1.1 | 0.5 | 0.2 |
| 3 | 6.9 | 6.7 | 9.8 | 7.8 | 3.1 | 1.4 | 2.0 |
| 4 | 5.5 | 5.5 | 6.7 | 5.9 | 1.2 | 0.6 | 0.3 |
| 5 | 6.4 | 8.8 | 8.6 | 7.9 | 2.4 | 1.1 | 1.2 |
| 6 | 8.3 | 10.2 | 9.3 | 9.3 | 1.9 | 0.8 | 0.6 |
| 7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

## Appendix 2 – Raw data and descriptive statistics for Staphylococcus epidermidis

| S. albus | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample** | **Trial 1** | **Trial 2** | **Trial 3** | **Mean** | **Range** | **Standard deviation** | **Variance ($mm^2$)** |
| 1 | 24.6 | 24.4 | 26.2 | 25.1 | 1.8 | 0.8 | 0.6 |
| 2 | 17.2 | 17.7 | 20.2 | 18.4 | 3.0 | 1.3 | 1.7 |
| 3 | 7.6 | 10.5 | 14.7 | 10.9 | 7.1 | 2.9 | 8.5 |
| 4 | 10.2 | 7.7 | 10.6 | 9.5 | 2.9 | 1.3 | 1.6 |
| 5 | 9.8 | 8.1 | 9.6 | 9.2 | 1.7 | 0.8 | 0.6 |
| 6 | 11.0 | 11.9 | 12.8 | 11.9 | 1.8 | 0.7 | 0.5 |
| 7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

## Appendix 3 – Raw data and descriptive statistics for Micrococcus luteus

| M. luteus | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample** | **Trial 1** | **Trial 2** | **Trial 3** | **Mean** | **Range** | **Standard deviation** | **Variance ($mm^2$)** |
| 1 | 15.3 | 22.3 | 23.6 | 20.4 | 8.3 | 3.6 | 13.3 |
| 2 | 14.1 | 26.0 | 25.6 | 21.9 | 11.9 | 5.5 | 30.4 |
| 3 | 15.7 | 19.1 | 19.5 | 18.1 | 3.8 | 1.7 | 2.9 |
| 4 | 11.7 | 8.2 | 7.9 | 9.3 | 3.8 | 1.7 | 3.0 |
| 5 | 15.9 | 17.8 | 9.4 | 14.4 | 8.4 | 3.6 | 12.9 |
| 6 | 20.8 | 13.3 | 13.7 | 15.9 | 7.5 | 3.4 | 11.9 |
| 7 | 0.0 | 0.0 | 12.1 | 4.0 | 12.1 | 5.7 | 32.5 |

# Appendix 4 – Risk assessment

**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.

**hand centrifuge**

*Potential hazards*
Rotating head may disintegrate, with pieces flying tangentially outwards. The outer container of the centrifuge should be designed to safely retain the pieces, so that no person is injured. A centrifuge may vibrate violently if the opposite tubes are not of equal mass.

*Standard handling procedures*
Check centrifuge for corrosion or damage before each use. The outer container, responsible for retaining flying pieces in the event of a failure of the head, should be examined carefully. If the containment is damaged in any way, e.g. by corrosion, the centrifuge should be replaced. Ensure that opposite tubes are always closely balanced before starting a centrifuge. Glass centrifuge tubes may break in the centrifuge, especially if damaged in any way; broken glass and solution must be cleaned carefully.

**plastic centrifuge tube**

*Potential hazards*
Organic solvents may affect the plastic, leading to leaks. Carefully balance the weights of opposite centrifuge tubes before inserting them into the centrifuge.

*Standard handling procedures*
Inspect and discard any damaged or deformed centrifuge tubes, no matter how small the damage, since they may break during use. Do not use with organic solvents.

**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.

**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.

**disposable plastic pipette** (disposable plastic pipet)

*Potential hazards*
Organic solvents may dissolve or swell the plastic, causing it to leak.

*Standard handling procedures*
Do not use with organic solvents.

**magnetic stirrer**

*Potential hazards*
May cause an electric shock if conducting liquid spilled or power cord damaged. Magnetic field may damage sensitive electronic equipment.

*Standard handling procedures*
If mains powered, inspect regularly for signs of damage to cord, and check if cord loose in plug or cord loose at entry to stirrer. Check for any signs of corrosion or other damage; test and tag at regular intervals.

**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

**carbon dioxide, solid** (dry ice) $CO_2$

**Class:** 9 **PG:** III **Users:** Tch **Training:** 1-5 UN: 1845 CAS: 124-38-9

**GHS data:**

**WARNING**

Causes severe skin burns and eye damage
May cause drowsiness and dizziness

*Potential hazards*
Causes frostbite or "burns" on contact with skin. Evolved gas is toxic at high concentrations in air due to absorption through lungs into blood, lowering the pH. Magnesium burns violently in a block of dry ice to form magnesium oxide and carbon.

*Standard handling procedures*
DO NOT PLACE DRY ICE IN A CLOSED CONTAINER SINCE THE CONTAINER MAY EXPLODE. Wear heavy insulating gloves when handling dry ice. Do not transport in a lift with people due to possibility of lift failure and asphyxiation. If transporting dry ice by car, ensure windows are open. Students should not touch dry ice due to the possibility of burns.

*Disposal*
May be placed in a well-ventilated location to evaporate.

**ethanol 16-17 M (95-100% wt/wt)** (ethyl alcohol) $CH_3CH_2OH$

**Class:** 3 **PG:** II **Users:** 7-12 **Training:** 1-5 UN: 1170 CAS: 64-17-5

**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.

**agarose, gel** (polyagarobiose)

**Class:** nc **PG:** none **Users:** K-12 **Training:** 1-6 CAS: 9012-36-6

**GHS data:** Not classified as a hazardous chemical.

*Potential hazards*
Low toxicity.

*Disposal*
Gel may be placed in the garbage.

**dimethyl sulfoxide, pure liquid** $H_3CSOCH_3$

CAS: 67-68-5

**Class:** nc **PG:** none **Users:** 7-12 **Training:** 1-5

**GHS data:** Not classified as a hazardous chemical.

*Potential hazards*
Generally considered non-toxic. May cause skin or eye irritation. May decompose explosively at the boiling point, especially in the presence of acids or bases. May react violently with acyl chlorides, metal nitrides, metal perchlorates, sodium hydride, periodic acid and fluorinating agents.

*Standard handling procedures*
Miscible with water. Suitable for use as a safe low-volatility (boiling point 187°C) aprotic solvent.

*Disposal*
May be poured down the drain, in a stream of water, to the limit of 100 mL/day.

## 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.

**Micrococcus luteus** (M. luteus)

*Potential hazards*
May be mixed in the wild with other bacteria that are pathogenic. May be an infection risk to immunocompromised individuals.

*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.

**pollen**

*Potential hazards*
ALLERGY ALERT. Pollens of numerous plant species cause an allergic reaction in ~10% of people. Seasonal allergic rhinitis is common in the population. "Thunderstorm asthma" may cause hospitalisation or death (e.g. Melbourne, Nov 2016) and occurs in Spring when rye grass pollen becomes wet and is broken in a storm into small particles which penetrate deeply into lungs. In the future, a different pollen could cause thunderstorm asthma.

*Standard handling procedures*
Avoid inhalation of pollen from flowers or grasses. People with pollen allergies should avoid strenuous outdoor activites on days when the pollen count is high. Remain indoors with windows closed, or in a vehicle with recirculating air conditioning, in the event of thunderstorm asthma period; even individuals not prone to asthma or allergies may be affected.

## Appendix 5 – Two factor ANOVA raw test results

| SUMMARY | E. coli | S. epidermidis | M. luteus | Total |
|---|---|---|---|---|
| ***Sunshine Coast*** | | | | |
| **Count** | 3 | 3 | 3 | 9 |
| **Sum** | 19.8 | 75.2 | 61.2 | 156.2 |
| **Average** | 6.6 | 25.06667 | 20.4 | 17.35556 |
| **Variance** | 0.25 | 0.973333 | 19.93 | 74.44278 |
| ***Brisbane*** | | | | |
| **Count** | 3 | 3 | 3 | 9 |
| **Sum** | 18.6 | 55.1 | 65.7 | 139.4 |
| **Average** | 6.2 | 18.36667 | 21.9 | 15.48889 |
| **Variance** | 0.37 | 2.583333 | 45.67 | 63.03111 |
| ***Springfield*** | | | | |
| **Count** | 3 | 3 | 3 | 9 |
| **Sum** | 23.4 | 32.8 | 54.3 | 110.5 |
| **Average** | 7.8 | 10.93333 | 18.1 | 12.27778 |
| **Variance** | 3.01 | 12.74333 | 4.36 | 25.93694 |
| ***Total*** | | | | |
| **Count** | 9 | 9 | 9 | |
| **Sum** | 61.8 | 163.1 | 181.2 | |
| **Average** | 6.866667 | 18.12222 | 20.13333 | |
| **Variance** | 1.4275 | 41.56194 | 20.2375 | |

**ANOVA**

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| **Sample** | 118.7385 | 2 | 59.36926 | 5.944191 | 0.010422 | 3.554557 |
| **Columns** | 920.2096 | 2 | 460.1048 | 46.06679 | 8.32E-08 | 3.554557 |
| **Interaction** | 207.297 | 4 | 51.82426 | 5.188768 | 0.005853 | 2.927744 |
| **Within** | 179.78 | 18 | 9.987778 | | | |
| **Total** | 1426.025 | 26 | | | | |