Term paper

2024-03-04

Introduction

Natural antibiotics are a popular natural alternatives to pharmaceutical antibiotics, especially with increasing drug resistance among pathogenic bacteria. Honey and tea tree oil are known for their medicinal and antibacterial properties. This experiment aims to investigate the antibacterial effects of these two natural substances against bacterial growth.

Honey is a supersaturated solution of sugars produced by bees and its antimicrobial effects come from the osmotic effect, its acidity, hydrogen peroxide, and phytochemical factors (Aurongzeb & Azim, 2011). Honey has been found to possess antibacterial activities even in situations where pharmaceutical antibiotics were ineffective (Aurongzeb & Azim, 2011). Tea tree oil is distilled from the leaves of Melaleuca alternifolia and has the unique terpinen-4-ol compound which has been shown to kill bacteria by disrupting their cell walls (Mumu & Hossain, 2018). By examining the antibacterial effects of honey and tea tree oil, this experiment contributes a deeper understanding of how these natural substances can be effectively integrated into modern infection prevention strategies.

To test the antibacterial effects of honey and tea tree oil, they will be applied separately to petri dishes at different concentrations, 0%, 50%, and 100% and subjected to different temperatures, warm and cold. It is expected that the petri dish with the the highest amount of natural antibiotic in cold temperature should form the least amount of colonies with the control in warm temperature having the highest amount. I hypothesize that natural antibiotics significantly inhibit the growth of bacteria compared to a control group; also higher concentrations of the antibiotic will have stronger effects. This study will also compare tea tree oil and honey's effectiveness.

Methods

To acquire a bacterial colony to test on, I started by using the first catch of
urine which guarantees the presence of gram negative bacteria. I then used a sterile inoculating loop to inoculate a petri dish. The petri dishes used favored gram negative bacterial growth so this experiment only tests the affects of natural antibiotics on gram negative bacteria. In a small bathroom, I left the petri dish to grow for a few days with the help of a portable heater that kept the room warm.

I prepared the concentration samples by heating up filtered water and letting it cool to make it as distilled or as pure as possible. I used distilled water for the control. To make the 50% concentration sample, I mixed equal parts of the antibiotics and distilled water. For the 100% concentration sample, I used the antibiotics on their own.

Once the bacterial colony grew and was ready to be used, I obtained a small colony and suspended it in a drop of distilled water. In order of the randomized table generated below (Table 1), I inoculated the petri dish with the suspended bacteria by using the inoculation loop that carries exactly 1 microlitre of bacteria so that everything remained consistent. I then used a new sterile loop to add the antibiotic with the same amount. Everything was done near an open flame to avoid introducing new bacteria with a different loop per swab. Each petri dish was split into three sections, with one section used as the control (0% concentration). There are a total of 24 observations to account for each combination twice.

Once all the inoculations were performed, the petri dishes were placed in either the bedroom (warmer) or the basement (cooler). After 4 days, the colony forming units (CFUs) were counted.

```r
set.seed(123)

Petri_ID <- 1:16

Antibiotic <- rep(c("Honey", "Tea Tree Oil"), each = 4, times = 2)

Location <- rep(c("Basement", "Bedroom"), each = 8)

CFU <- rep(c("Data"), each = 16)

Concentration <- sample(rep(c("50%", "100%"), each = 4, times = 2))

Exp <- data.frame(Petri_ID, Location, Antibiotic, Concentration, CFU)

Exp_randomized <- Exp[order(Exp$Location, sample(nrow(Exp))), ]
rownames(Exp_randomized) <- NULL

Exp_randomized$Petri_Dish_Number <- rep(1:8, each = 2)
Exp_randomized$Petri_ID <- factor(seq_len(length(Antibiotic)))
```

Table 1: Code generated to guarantee true randomization of experiment. The control (0% concentration) is not shown in this table but is later added. It should be there after every two inoculations which is shown in table 3. The 3 levels are 0%, 50%, and 100% concentrations. The two blocks are basement and bedroom location. CFU is left blank for now.

```r
print(Exp_randomized)
```

```
##    Petri_ID Location   Antibiotic Concentration  CFU Petri_Dish_Number
## 1         1 Basement        Honey          100% Data                 1
## 2         2 Basement Tea Tree Oil          100% Data                 1
## 3         3 Basement        Honey           50% Data                 2
## 4         4 Basement        Honey          100% Data                 2
## 5         5 Basement        Honey          100% Data                 3
## 6         6 Basement Tea Tree Oil           50% Data                 3
## 7         7 Basement Tea Tree Oil           50% Data                 4
## 8         8 Basement Tea Tree Oil          100% Data                 4
## 9         9  Bedroom        Honey           50% Data                 5
## 10       10  Bedroom        Honey           50% Data                 5
## 11       11  Bedroom Tea Tree Oil          100% Data                 6
## 12       12  Bedroom Tea Tree Oil           50% Data                 6
## 13       13  Bedroom Tea Tree Oil           50% Data                 7
## 14       14  Bedroom        Honey           50% Data                 7
## 15       15  Bedroom Tea Tree Oil          100% Data                 8
## 16       16  Bedroom        Honey          100% Data                 8
```

Results

```r
Petri_ID_ <- factor((1:24))

Location_ <- factor(rep(c("Basement", "Bedroom"), each = 12),
                    levels = c("Basement", "Bedroom"))
```

```r
Antibiotic_ <- factor(c("Honey", "Tea Tree Oil", "Control", "Honey", "Honey",
                 "Control","Honey", "Tea Tree Oil", "Control","Tea Tree Oil",
                 "Tea Tree Oil", "Control",
                "Honey", "Honey", "Control", "Tea Tree Oil", "Tea Tree Oil",
                "Control", "Tea Tree Oil", "Honey", "Control", "Tea Tree Oil",
                "Honey", "Control"), levels = c("Honey", "Tea Tree Oil",
                                                "Control"))

Concentration_ <- factor(c("100%", "100%", "0%", "50%", "100%", "0%", "100%",
                           "50%",
                           "0%", "50%", "100%", "0%", "50%", "50%", "0%",
                           "100%", "50%", "0%", "50%", "50%",
                           "0%", "100%", "100%", "0%"),
                         levels = c("0%", "50%", "100%"))

CFU_ <- c(5, 7, 6, 6, 4, 5, 7, 5, 8, 8, 6, 7, 5, 4, 7, 7, 6, 6, 4, 10, 9, 6,
          7, 6)

Petri_Dish_Number_ <- factor(rep(1:8, each = 3), levels = c(1, 2, 3, 4, 5,
                                                            6, 7, 8))

Exp_dataset <- data.frame(Petri_ID_, Location_, Antibiotic_, Concentration_,
                          CFU_, Petri_Dish_Number_)
```

Table 2: Colony-forming units (CFU) recorded from various treatments of honey and tea tree oil at different concentrations, compared to control groups, across two distinct locations (Basement and Bedroom).

```r
print(Exp_dataset)
```

```
##    Petri_ID_ Location_  Antibiotic_ Concentration_ CFU_ Petri_Dish_Number_
## 1          1  Basement        Honey            100%    5                  1
## 2          2  Basement Tea Tree Oil            100%    7                  1
## 3          3  Basement      Control              0%    6                  1
## 4          4  Basement        Honey             50%    6                  2
## 5          5  Basement        Honey            100%    4                  2
## 6          6  Basement      Control              0%    5                  2
## 7          7  Basement        Honey            100%    7                  3
## 8          8  Basement Tea Tree Oil             50%    5                  3
## 9          9  Basement      Control              0%    8                  3
## 10        10  Basement Tea Tree Oil             50%    8                  4
## 11        11  Basement Tea Tree Oil            100%    6                  4
## 12        12  Basement      Control              0%    7                  4
## 13        13   Bedroom        Honey             50%    5                  5
## 14        14   Bedroom        Honey             50%    4                  5
## 15        15   Bedroom      Control              0%    7                  5
## 16        16   Bedroom Tea Tree Oil            100%    7                  6
## 17        17   Bedroom Tea Tree Oil             50%    6                  6
## 18        18   Bedroom      Control              0%    6                  6
## 19        19   Bedroom Tea Tree Oil             50%    4                  7
## 20        20   Bedroom        Honey             50%   10                  7
## 21        21   Bedroom      Control              0%    9                  7
## 22        22   Bedroom Tea Tree Oil            100%    6                  8
```

```
## 23        23   Bedroom        Honey           100%    7                 8
## 24        24   Bedroom      Control             0%    6                 8
```

```r
means_data <- aggregate(CFU_ ~ Antibiotic_ + Concentration_,
                        data = Exp_dataset, mean)

barplot(means_data$CFU_,
        names.arg = paste(means_data$Antibiotic_,
                          means_data$Concentration_,  sep = "\n"),
        main = "Average CFU by Antibiotic and Concentration",
        ylab = "Average CFU",
        col = c("blue", "red", "green"))
```

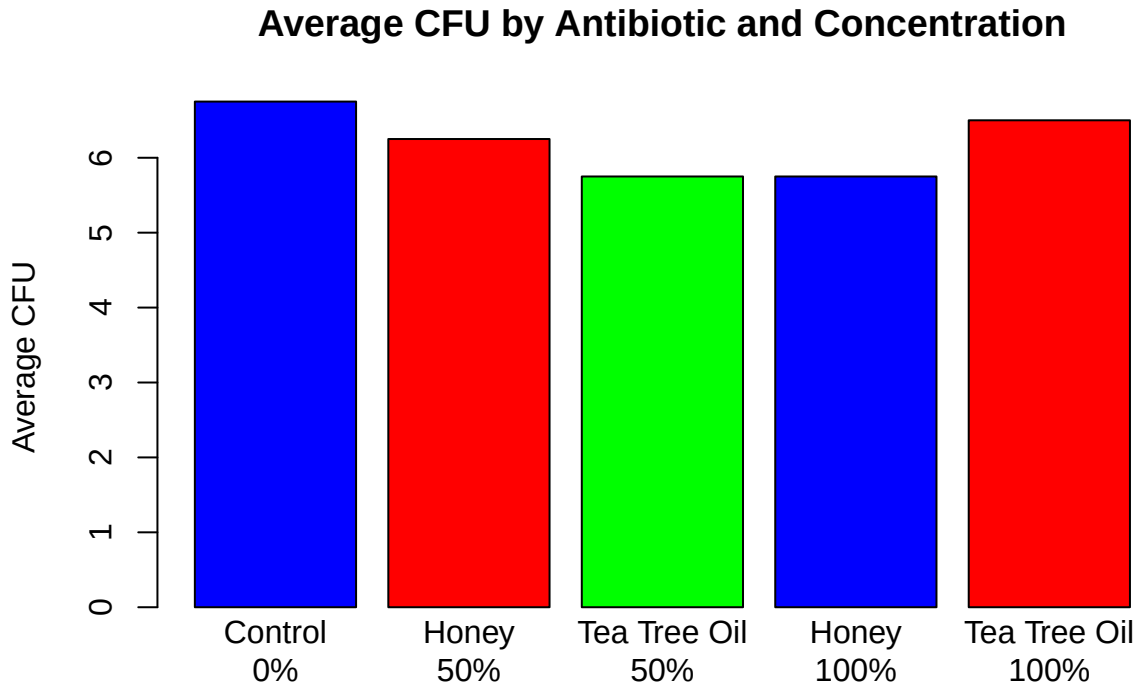


Figure 1: Average Colony-Forming Units (CFU) by Antibiotic and Concentration.

Analysis

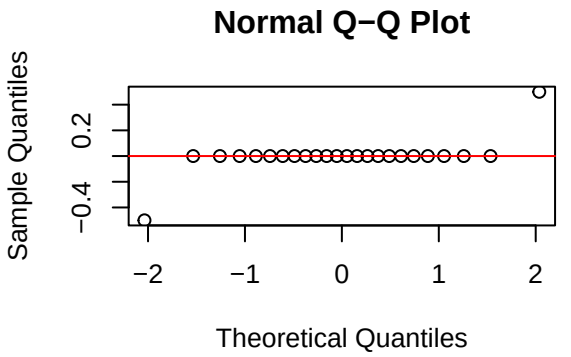
```r
# Model
model_bacteria_interac <- lm(CFU_ ~ Location_ * Antibiotic_ * Concentration_ *
                        Petri_Dish_Number_, data = Exp_dataset)

model_bacteria <- lm(CFU_ ~ Location_ + Antibiotic_ + Concentration_ +
                        Petri_Dish_Number_, data = Exp_dataset)
# ANOVA
```

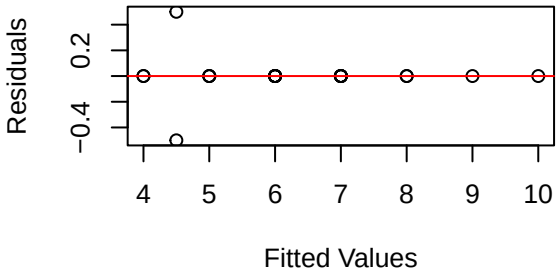
```r
anova_result <- anova(model_bacteria_interac)
print(anova_result)
```

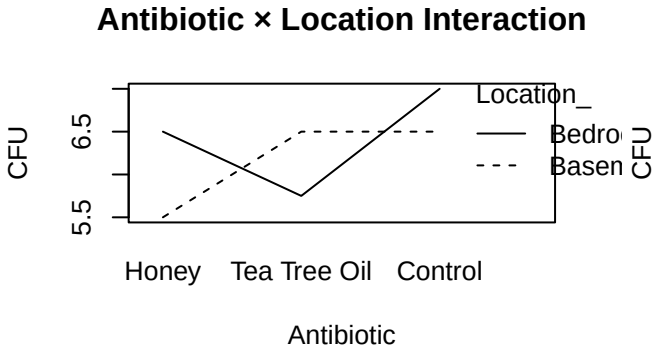
```
## Analysis of Variance Table
##
## Response: CFU_
##                                Df  Sum Sq Mean Sq F value Pr(>F)
## Location_                       1  0.3750  0.3750  0.7500 0.5456
## Antibiotic_                     2  2.5833  1.2917  2.5833 0.4027
## Concentration_                  1  0.1467  0.1467  0.2935 0.6839
## Petri_Dish_Number_              6 17.6625  2.9437  5.8875 0.3054
## Location_:Antibiotic_           2  3.0254  1.5127  3.0254 0.3766
## Location_:Concentration_        1  4.8151  4.8151  9.6303 0.1985
## Antibiotic_:Concentration_      1  2.8722  2.8722  5.7445 0.2516
## Antibiotic_:Petri_Dish_Number_  8 20.9780  2.6222  5.2445 0.3261
## Residuals                       1  0.5000  0.5000
```

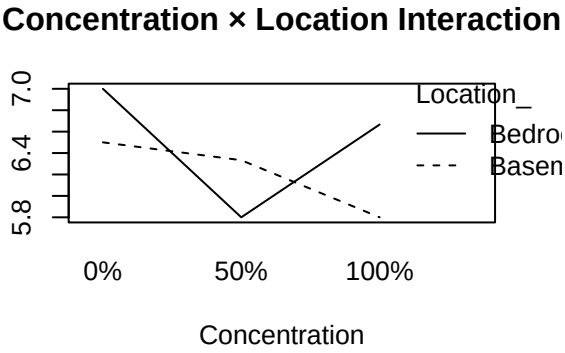
The anova shows that there is no significant difference between the factors or blocks.

```r
par(mfrow=c(2,2))

qqnorm(residuals(model_bacteria_interac))
qqline(residuals(model_bacteria_interac), col = "red")

# Residuals vs Fitted values
plot(fitted(model_bacteria_interac), residuals(model_bacteria_interac),
     xlab = "Fitted Values", ylab = "Residuals")
abline(h=0, col="red")

# Interaction plots
with(Exp_dataset, interaction.plot(Antibiotic_, Location_, CFU_,

                                   main = "Antibiotic × Location Interaction",
                                       xlab = "Antibiotic", ylab = "CFU"))

with(Exp_dataset, interaction.plot(Concentration_, Location_, CFU_,

                                main = "Concentration × Location Interaction",
                                       xlab = "Concentration", ylab = "CFU"))
```

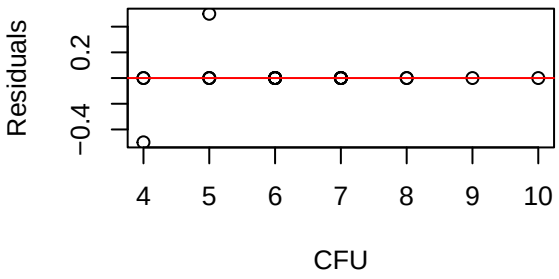









```
# Diagnostic plot: Force vs. Residuals
plot(CFU_, residuals(model_bacteria_interac), xlab = "CFU", ylab = "Residuals")
abline(h = 0, col = "red")
```



The points on the normal plot are exactly on the red line and there are no significant deviations.

In the residuals vs fitted values plot, the values are dispersed on the line with two outliers.

Both interaction plots have two crossing points. This signifies that there is an interaction between the antibiotics and concentrations.

The diagnostic plot points are mostly on the line with two outliers.

I removed the highest and lowest data points and it made no significant change so I kept them for further analysis.

```
# Contrasts
library("gmodels")
```

```
## Warning: package 'gmodels' was built under R version 4.2.3
```

```
# Compare Antibiotics
contrast1 <- fit.contrast(model_bacteria, "Antibiotic_", coeff = c(-1, 1, 0))
contrast1
```

```
##                             Estimate Std. Error    t value Pr(>|t|)
## Antibiotic_ c=( -1 1 0 ) -0.8968254  0.9712468 -0.9233754 0.372633
## attr(,"class")
## [1] "fit_contrast"
```

```
contrast2 <- fit.contrast(model_bacteria, "Antibiotic_", coeff = c(-1, 0, 1))
contrast2
```

```
##                             Estimate Std. Error    t value  Pr(>|t|)
## Antibiotic_ c=( -1 0 1 ) 0.07638889  0.9364791 0.08157031 0.9362309
## attr(,"class")
## [1] "fit_contrast"
```

```
contrast3 <- fit.contrast(model_bacteria, "Antibiotic_", coeff = c(0, - 1, 1))
contrast3
```

```
##                            Estimate Std. Error   t value  Pr(>|t|)
## Antibiotic_ c=( 0 -1 1 ) 0.9732143  0.9974508 0.9757015 0.3470199
## attr(,"class")
## [1] "fit_contrast"
```

There is no significant difference between the antibiotics.

```
# Compare Concentrations
contrast4 <- fit.contrast(model_bacteria, "Concentration_", coeff = c(-1, 1, 0))
contrast4
```

```
##                               Estimate Std. Error  t value  Pr(>|t|)
## Concentration_ c=( -1 1 0 ) -0.6507937   1.942494 -0.33503 0.7429469
## attr(,"class")
## [1] "fit_contrast"
```

```
contrast5 <- fit.contrast(model_bacteria, "Antibiotic_", coeff = c(-1, 0, 1))
contrast5
```

```
##                             Estimate Std. Error    t value  Pr(>|t|)
## Antibiotic_ c=( -1 0 1 ) 0.07638889  0.9364791 0.08157031 0.9362309
## attr(,"class")
## [1] "fit_contrast"
```

```
contrast6 <- fit.contrast(model_bacteria, "Antibiotic_", coeff = c(0, - 1, 1))
contrast6
```

```
##                            Estimate Std. Error   t value  Pr(>|t|)
## Antibiotic_ c=( 0 -1 1 ) 0.9732143  0.9974508 0.9757015 0.3470199
## attr(,"class")
## [1] "fit_contrast"
```

There is no significant difference between any of the concentrations.

```
# Compare Location
contrast7 <- fit.contrast(model_bacteria, "Location_", coeff = c(-1, 1))
contrast7
```

```
##                         Estimate Std. Error   t value Pr(>|t|)
## Location_ c=( -1 1 ) 0.3333333   1.284839 0.2594359 0.799362
## attr(,"class")
## [1] "fit_contrast"
```

There is no significant difference between the locations.

```
#Tukey Test

tukey_result <- TukeyHSD(aov(CFU_ ~ Location_ + Antibiotic_ + Concentration_ +
                               Petri_Dish_Number_, data = Exp_dataset))
print(tukey_result)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = CFU_ ~ Location_ + Antibiotic_ + Concentration_ + Petri_Dish_Number_, data = Exp_
##
## $Location_
##                   diff       lwr      upr    p adj
## Bedroom-Basement 0.25 -1.137863 1.637863 0.703462
##
## $Antibiotic_
##                         diff       lwr      upr     p adj
## Tea Tree Oil-Honey   0.125 -1.952495 2.202495 0.9861963
## Control-Honey        0.750 -1.327495 2.827495 0.6177626
## Control-Tea Tree Oil 0.625 -1.452495 2.702495 0.7128942
##
## $Concentration_
##               diff       lwr      upr    p adj
## 50%-0%   -0.09375 -2.171245 1.983745 0.992208
## 100%-0%   0.09375 -1.983745 2.171245 0.992208
## 100%-50%  0.18750 -1.889995 2.264995 0.969252
##
## $Petri_Dish_Number_
##             diff       lwr      upr     p adj
## 2-1 -0.89311594 -5.480328 3.694096 0.9956576
## 3-1  0.73188406 -3.855328 5.319096 0.9987312
## 4-1  1.02355072 -3.563662 5.610763 0.9903054
## 5-1 -0.77717391 -5.364386 3.810039 0.9981498
## 6-1  0.07427536 -4.512937 4.661488 1.0000000
## 7-1  1.51449275 -3.072720 6.101705 0.9246349
## 8-1  0.05072464 -4.536488 4.637937 1.0000000
## 3-2  1.62500000 -2.962212 6.212212 0.8966029
## 4-2  1.91666667 -2.670546 6.503879 0.7991995
## 5-2  0.11594203 -4.471270 4.703154 1.0000000
## 6-2  0.96739130 -3.619821 5.554604 0.9930164
## 7-2  2.40760870 -2.179604 6.994821 0.5880162
## 8-2  0.94384058 -3.643372 5.531053 0.9939608
## 4-3  0.29166667 -4.295546 4.878879 0.9999973
## 5-3 -1.50905797 -6.096270 3.078154 0.9258782
## 6-3 -0.65760870 -5.244821 3.929604 0.9993593
## 7-3  0.78260870 -3.804604 5.369821 0.9980677
```

```
## 8-3 -0.68115942 -5.268372 3.906053 0.9991967
## 5-4 -1.80072464 -6.387937 2.786488 0.8416489
## 6-4 -0.94927536 -5.536488 3.637937 0.9937522
## 7-4  0.49094203 -4.096270 5.078154 0.9999062
## 8-4 -0.97282609 -5.560039 3.614386 0.9927831
## 6-5  0.85144928 -3.735763 5.438662 0.9967496
## 7-5  2.29166667 -2.295546 6.878879 0.6400641
## 8-5  0.82789855 -3.759314 5.415111 0.9972627
## 7-6  1.44021739 -3.146995 6.027430 0.9405300
## 8-6 -0.02355072 -4.610763 4.563662 1.0000000
## 8-7 -1.46376812 -6.050981 3.123444 0.9357458
```

The tukey test shows no significant difference.

Conclusion

Overall, there were no significant differences observed when using honey or tea tree oil at different concentrations or locations. Despite the theoretical benefits and antimicrobial properties reported in literature, the experimental findings did not demonstrate a significant impact

Several factors may contribute to the lack of significant data acquired in the study. Bacteria, especially gram negative bacteria, may exhibit a high degree of adaptability to natural antibiotics, especially since the bacteria acquired was from a urinary sample. On the other hand French et al. (2005) found that honey applied topically may have a role in the treatment or prevention of gram negative bacteria. Additionally, the amount of 1 microlitres used could have been too small or been exposed for a short duration of time. The study was performed at home without consistent and controllable laboratory conditions. Therefore, the differences in temperature between the bedroom and basement were most likely negligible.

Some literature supports that natural antibiotics do not function efficiently as an antibiotic. A study by Arweiler et al. (2000) looking at the effectiveness of tea tree oil as a mouth wash shows that it does not reduce the formation of plaque flora. With the increase in antibiotic resistance in bacteria, finding an effective natural antibiotic has become difficult. There is currently a lot of discourse in literature on the effectiveness of natural antibiotics. While this study did not find significant effects under tested conditions, it does not negate the potential of honey and tea tree oil as effective antibacterial agents. It only highlights the need for further research on different types of bacteria, combining with other agents, or testing different conditions.

Works Cited

Arweiler, N. B., Donos, N., Netuschil, L., Reich, E., & Sculean, A. (2000). Clinical and antibacterial effect of tea tree oil - a pilot study. Clinical Oral Investigations, 4(2), 70–73. https://doi.org/10.1007/s007840050118

Aurongzeb, M., & Azim, K. M. (2011). Antimicrobial properties of natural honey: a review of literature. International Center for Chemical and Biological Sciences, 44(3): 118-124. http://www.pjbmb.org.pk/images/PJBMBArchive/2011/PJBMB_44_3_Sep_2011/08.pdf

French, V. M., Cooper, R. A., & Molan, P. C. (2005a). The antibacterial activity of honey against coagulase-negative staphylococci. Journal of Antimicrobial Chemotherapy, 56(1), 228–231. https://doi.org/10.1093/jac/dki193

Mumu, K., & Hossain, M. (2018). Antimicrobial activity of tea tree oil against pathogenic bacteria and comparison of its effectiveness with eucalyptus oil, lemongrass oil and conventional antibiotics. American Journal of Microbiological Research, 6(3), 73–78. https://doi.org/10.12691/ajmr-6-3-2