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Natural Antibiotic Effects of Honey, Garlic, Lemon, and
Ginger on Escherichia Coli

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Abstract

Antibiotic resistance is a serious issue with the overuse of synthetic antibiotics, which have been known to kill good bacteria and kill with one method, allowing bacteria to evolve and mutate, unlike natural antibiotics. The purpose of my project was to find out which natural antibiotics- Manuka Honey, Garlic, Lemon, and Ginger, is the most effective against inhibiting E.Coli growth. My hypothesis was that Garlic will be best with the biggest zone of inhibition. In this experiment, 20 Luria Broth Agar plates were swabbed with K-12 strain E.Coli bacteria and split into six parts each, consisting of: Negative control with nothing, Positive control with Ampicillin, and 4 different concentrations of the natural antibiotic on a disk (4000mg/ml, 2000mg/ml, 1000mg/ml, 500mg/ml), using a serial dilution with water. 5 samples per antibiotic (Honey, Garlic, Lemon and Ginger) were prepared, and the 20 plates were incubated for 24 hours at 37 degreesC. The zone of inhibition's diameter in millimeters for each disk was measured. I did 3 trials.

Based on the average zone of inhibition diameter for 15 samples in 3 trials, Garlic showed the largest zone at all concentrations (23.9mm at 4000mg/ml) and was better than Ampicillin. Manuka Honey (12.8mm at 4000mg/ml) and Lemon (11.4mm at 4000mg/ml) did inhibit the E. coli, but were not very effective. Ginger results were poor (8.6mm at 4000mg/ml), and dropped rapidly at lower concentrations. My hypothesis was correct, Garlic inhibits E.Coli best even at lowest concentrations of 500mg/ml (10.7mm) and was the best. Garlic is effective due to its 100+ active compounds from Allicin, and targets enzymes that are needed for the production of energy for the bacteria. These results will hopefully encourage people to take natural antibiotics instead of synthetic antibiotics, so that antibiotic resistance doesn't continue.

Background Research

- ❑ Antibiotic resistance is fast becoming a problem in the world, with the overuse of synthetic antibiotics, which have been known to kill good bacteria in your body. Since they are over-prescribed, the bacteria mutates and become resistant over time. Synthetic antibiotics also usually kill target bacteria with one method only, allowing bacteria to evolve and become resistant.
- ❑ Natural antibiotics instead are hard to become resistant to due to the many compounds that can counteract against bacteria. They do not destroy useful bacteria, and have many different ways of killing bacteria, making it very difficult for bacteria to become resistant, Some natural antibiotics that have shown to produce effective results are Manuka Honey, Garlic, Lemon, and Ginger.
- ❑ Manuka Honey uses its antibacterial compounds Methylglyoxal and Bee Defensin 1, its high concentration of sugar, and more to fight off bacteria. Manuka creates an osmotic effect, such that there is no water for bacteria so they dehydrate and die.
- ❑ Garlic uses its powerful enzyme Allicin, and its 100+ compounds to stop bacteria's production of energy. Garlic contains two key compounds Alliin and Alliinase, a protein-based enzyme. When the garlic is damaged, the Alliinase converts Alliin into a third compound Allicin which when oxidized, breaks down into 100+ antibacterial compounds. These compounds in turn can make many other effective defensive combination. Allicin in garlic targets enzymes that are needed for the production of energy and food for the bacteria cell. Hence bacteria are inhibited from functioning.
- ❑ Lemons contain alkaloids, which act by stopping DNA from being produced in a bacteria cell, causing it to die.
- ❑ Finally, Ginger contains special compounds called Gingerols, which are responsible for breaking down a bacteria's cell wall and interferes with cell membrane functions.

- **Purpose:** The goal of my project is to investigate in depth about natural antibiotics and how well they inhibit *Escherichia coli* (E. coli) bacteria, which is one of the bacteria known for causing food poisoning and others. In my experiment, I will be using the natural antibiotics Manuka Honey, Garlic, Lemon, and Ginger, which I have researched to be effective against various bacteria, and can also be consumed. I will also be using the synthetic antibiotic, Ampicillin, as a positive control. With these results, I hope to discover which of the 4 natural antibiotics inhibits E. coli growth the best with the largest zone of inhibition, by measuring the zone's diameter, so that people may learn how effective natural antibiotics are. This is becoming extremely important nowadays because synthetic antibiotics have been overused, and have shown to harbor antibiotic resistance in bacteria, hence we may be left with natural antibiotics as our ultimate weapon against these resistant bacteria. I hope to help people with their health and let people know how useful natural antibiotics are so they can eat them when they are sick or even include them in their diets to grow stronger immune systems.
- **Hypothesis:** My hypothesis is that if natural antibiotics are added to a disk placed in an agar plate with E. Coli, its growth will be inhibited within a circular zone of inhibition around the disk. I hypothesized that if garlic is added to E. Coli, the zone of inhibition will be the greatest against Manuka Honey, Lemon, and Ginger by about 100% of the time, constantly, even when it is diluted. I believe Garlic got the lead because it not only contains 3 main compounds, alliinase which converts alliin into allicin when garlic is oxidized, it further breaks down into more than 100 compounds. Neither Manuka honey, lemon, nor ginger contains more than 100 compounds. Although garlic becomes less effective when cooked, it still contains many antibacterial factors. Garlic is also not only antibacterial, but antifungal and antiviral as well. It is even known to be more effective than penicillin. Garlic is very useful for wounds, medicines, and as food. It contains many natural sugars, enzymes, amino acids, flavonoids, trace minerals, and much more. It also doesn't have any side effects of overuse, specifically antibiotic resistance, which makes it able to be used multiple times without being ineffective and is known to be a very powerful infection fighter. I selected the percentage of my prediction, 100%, because I think that garlic will be the most effective against the other three natural antibiotics.
- **Materials:** Black sharpie, K-12 Strain E.Coli (Ward Science), 60 Luria Broth agar plates, Manuka Honey (100% Raw Australian), Garlic cloves, Lemon, Ginger roots, Pipette and 50 ul pipette tips, Distilled water, Goggles, Gloves, Inoculating loop, Glass spreader, Homemade incubator, 6mm blank disks, 6mm Ampicillin disks, High Precision digital Weighing scale, Heat lamp, Tin foil, Digital Thermometer, Ruler, Tape

Method

- 1) Put on goggles, gloves, and any other protective clothing.
- 2) Weigh with digital weighing scale and put 4000 mg of Manuka honey and 1 ml of water in a test tube. Shake well and label C1 (concentration 1).
- 3) Pipette 0.5 ml of the first tube and put it in the second test tube with 0.5 ml of water. Shake well and label C2 (concentration 2).
- 4) Repeat step 3 for the third and fourth test tube and label C3 (concentration 3) and C4 (concentration 4) correspondingly.
- 5) Open agar plate and swab E. Coli with inoculating loop and glass spreader to even it out.
- 6) Close agar plate, flip over, and divide it into six sections with a sharpie. Label each section - (negative control), + (positive control), C1 (concentration 1), C2 (concentration 2), C3 (concentration 3), and C4 (concentration 4).
- 7) Flip over agar plate and open. Place an ampicillin disk in the + section, one blank disk in the – section and each concentration section. Pipette 20 microliters of concentration solution to each corresponding c1, c2, c3 and c4 disk and nothing in the - or + disks.
- 8) Close agar plate and tape it around. Repeat steps 2-7 for 4 more agar plates.
- 9) Repeat steps 2-8 for garlic, lemon, and ginger.
- 10) For garlic, crush garlic clove into fine pieces. For lemon, cut in half and squeeze juice. For Ginger, cut slices of ginger roots and crush them.
- 11) Place all 20 agar plates in the incubator with sharpie/agar side up. Turn on heat lamp and adjust tin foil for a heat of 37 degrees Celsius. Leave for 24 hours.
- 12) After 24 hours, take out agar plates. Measure and record diameter of zone of inhibition with ruler in mm.
- 13) Repeat steps 1-12 for 2 more trials.

Method (2)

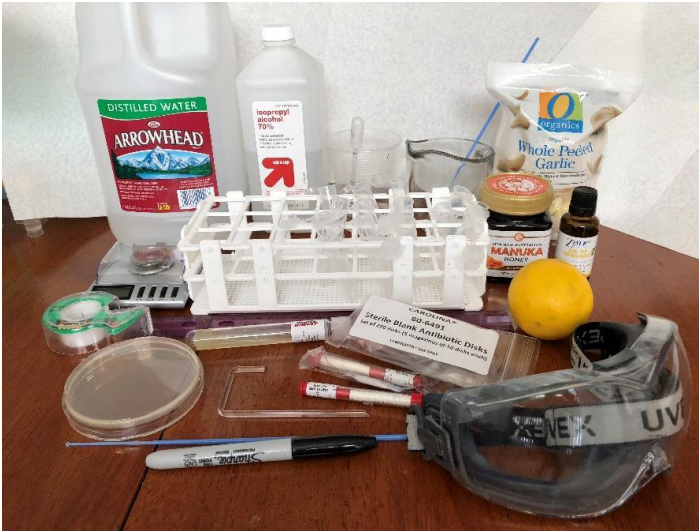
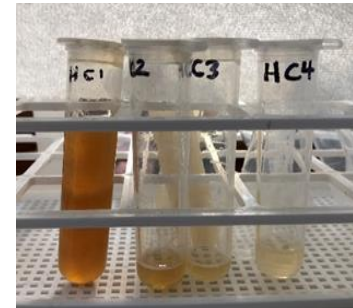
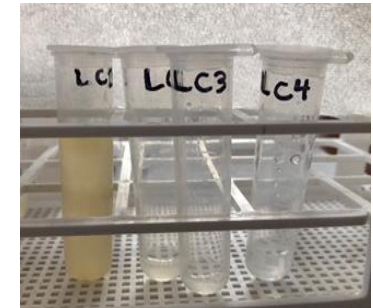


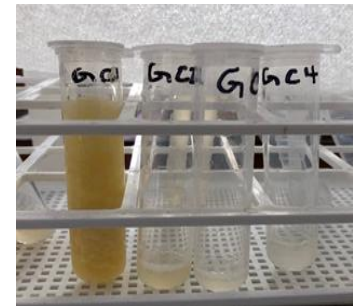
Fig1. Materials for the experiment



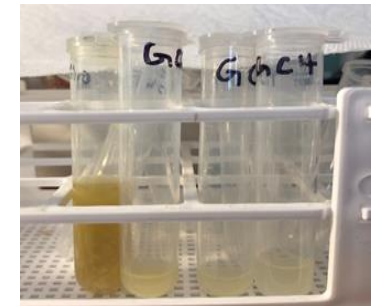
Honey



Lemon



Garlic



Ginger

Fig.3 Natural Antibiotic solutions in 4 different concentrations (4000mg/ml, 2000mg/ml, 1000mg/ml, 500mg/ml)



Fig2. E.Coli in slant tube, K-12 strain (WardScience)

Results

This section presents the results of the experiments, which consists of 3 trials of each Antibiotic (Honey, Garlic, Lemon, Ginger). In each trial, the diameter of the zone of inhibition is measured for 5 samples of the following 6 plates:

- (1) Positive Control (Ampicillin)
- (2) Negative Control (No antibiotic)
- (3) Concentration 1 (4000mg/ml of antibiotic solution)
- (4) Concentration 2 (2000mg/ml of antibiotic solution)
- (5) Concentration 3 (1000mg/ml of antibiotic solution)
- (6) Concentration 4 (500mg/ml of antibiotic solution)

For each antibiotic, the averages of the 15 samples (over 3 trials, 5 samples per trial) were calculated, and the standard deviation was derived as well.

Natural Antibiotics (Average Diameter of Zone of Inhibition - mm)						
	Positive Control (Ampicillin)	Negative Control (No Antibiotic)	Concentration 1 (C1 - 4000mg/ml)	Concentration 2 (C2 - 2000mg/ml)	Concentration 3 (C3 - 1000mg/ml)	Concentration 4 (C4 - 500mg/ml)
Honey	22.5	0.0	12.8	9.1	6.9	6.2
Std Dev	1.77	0.00	1.70	1.67	0.92	0.41
Garlic	22.4	0.0	23.9	18.1	14.1	10.7
Std Dev	1.64	0.00	1.62	1.96	1.25	1.71
Lemon	21.7	0.0	11.4	8.2	6.9	6.1
Std Dev	1.62	0.00	1.59	1.01	0.83	0.35
Ginger	21.1	0.0	8.6	7.3	6.5	6.1
Std Dev	1.13	0.00	2.06	0.84	0.38	0.13

Fig.4 The Average and Standard Deviations of the Zone of Inhibition diameters for all 4 Natural Antibiotics – Honey, Garlic, Lemon, and Ginger, for 15 samples over 3 trials.

Results (2)

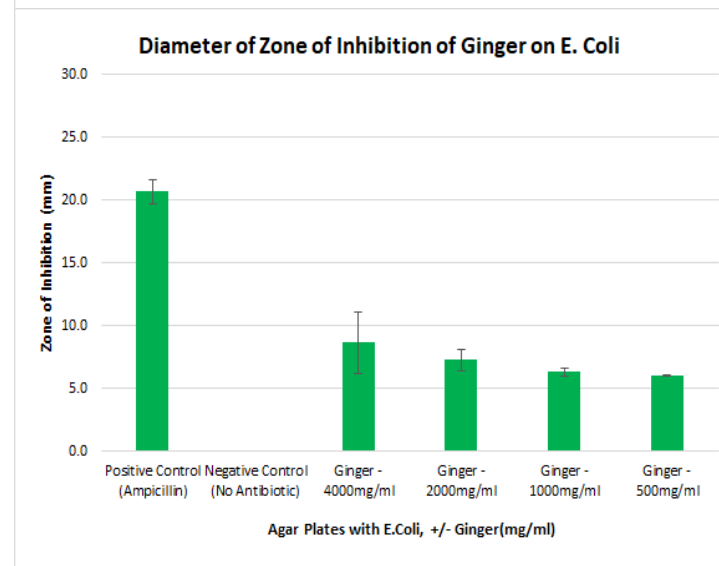
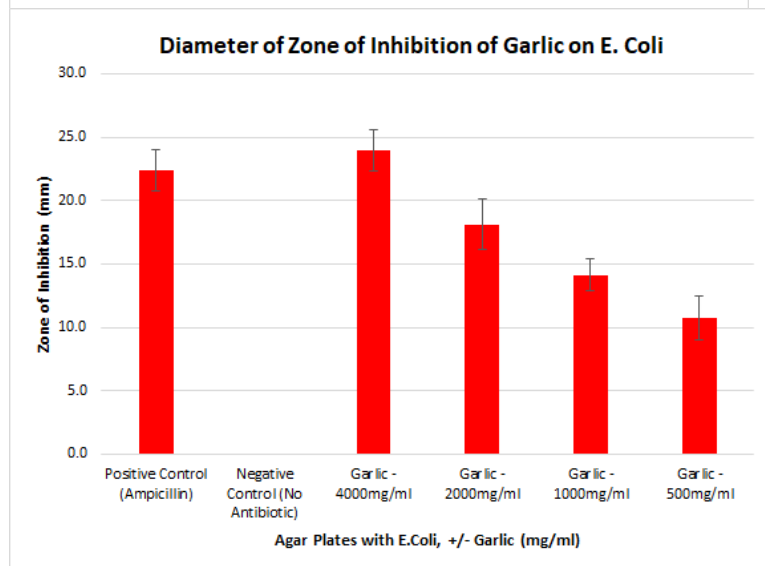
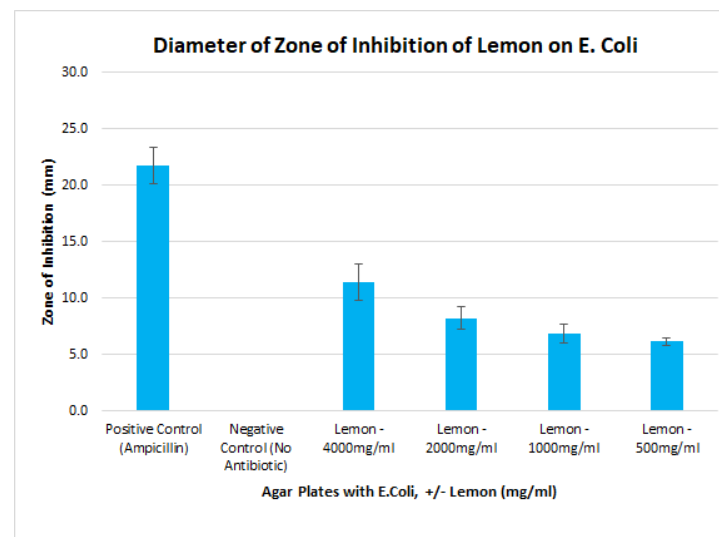
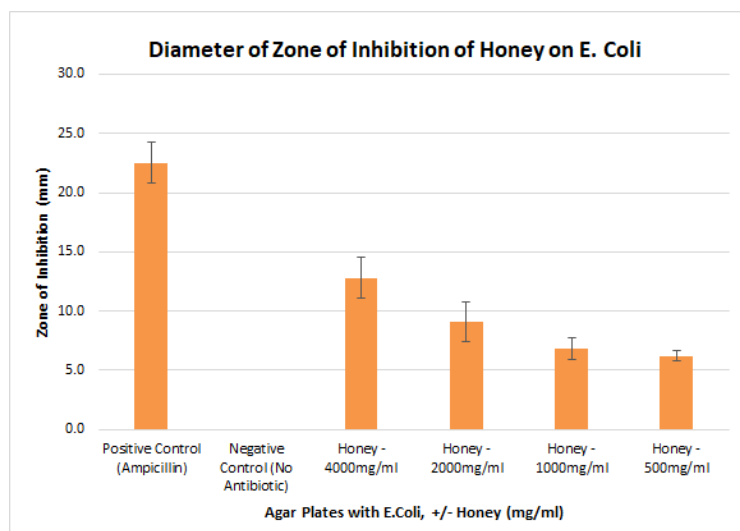


Fig.5 The Average and Standard Deviations of the Zone of Inhibition diameters for all 4 Natural Antibiotics – Honey, Garlic, Lemon, and Ginger, for 15 samples over 3 trials.

Results (3)

Comparing the Average Zone of Inhibition diameters for all 4 Natural Antibiotics (Honey, Garlic, Lemon, and Ginger), for 15 samples over 3 trials, with 4 different concentrations. Garlic has the largest zone of inhibition for all 4 concentrations, followed by Honey, Lemon and Ginger. Garlic is still effective at the lowest concentration⁴. The results are compared against positive control of Ampicillin, and a negative control.

Diameter of Zone of Inhibition of 4 Natural Antibiotics on E. Coli (Overall)

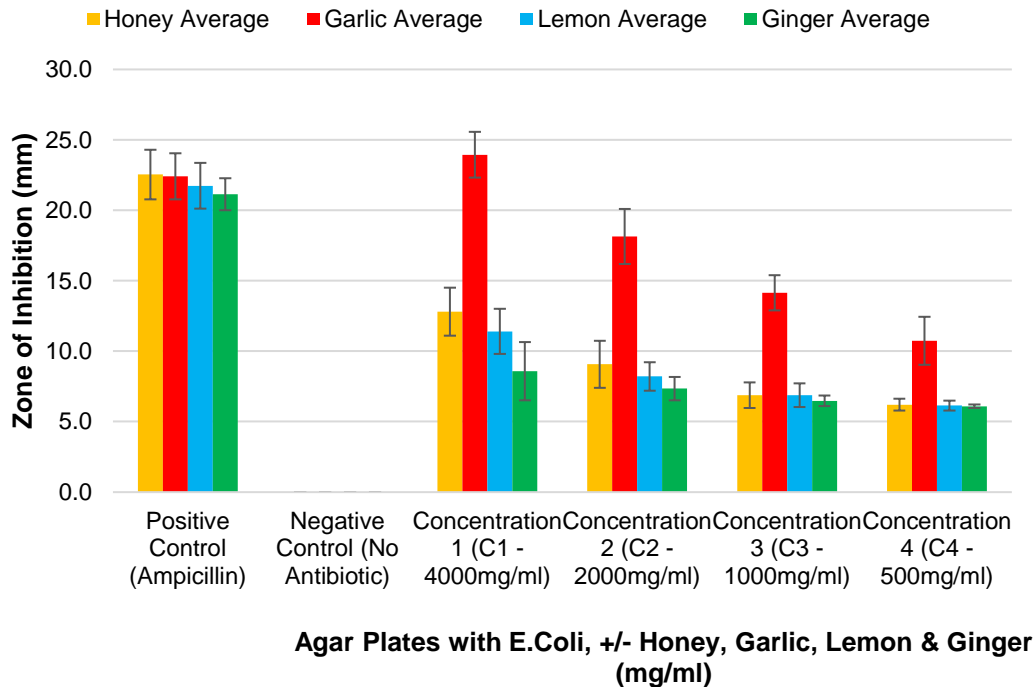


Fig.6 Comparing the Average and Standard Deviations of the Zone of Inhibition diameters for all 4 Natural Antibiotics – Honey, Garlic, Lemon, and Ginger, for 15 samples over 3 trials.



Fig.7 A Honey sample

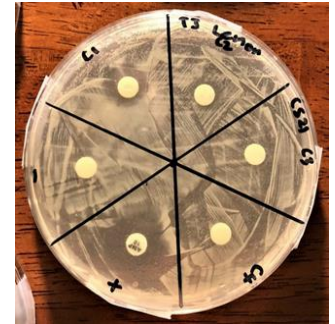


Fig.9 A Lemon sample



Fig.8 A Garlic sample



Fig.10 A Ginger sample

Conclusions

- My experiment tested out four natural antibiotics (Manuka Honey, Garlic, Lemon, and Ginger) and with one synthetic antibiotic, Ampicillin, as a positive control. My hypothesis was that Garlic would best inhibit E. coli growth compared to the other three natural antibiotics, since many studies have said that Garlic is very effective against bacteria because it has many antibacterial compounds. One of such is Allicin, which is Garlic's main reason of being so effective. Results showed that my hypothesis was correct and the zone of inhibition of **Garlic was the greatest – at 23.9 mm average diameter of zone of inhibition, at the highest concentration of 4000mg/ml, 18.1mm at 2000mg/ml, 14.1mm at 1000mg/ml, and 10.7mm at 500mg/ml**
- Manuka Honey (12.8mm at 4000mg/ml) and Lemon (11.4mm at 4000mg/ml) were almost the same amount of effectiveness, but the results were pretty low overall, with Manuka Honey in the slight lead. Ginger had the worst results (8.6mm at 4000mg/ml, to about 6.1mm at 500mg/ml). I had done research on Ginger, saying that it was a very health beneficial and strong natural antibiotic. However, this may be for certain and different types of bacteria, and may not be effective on E.Coli.
- All of the natural antibiotics were very consistent in that they became less effective the more they were diluted. **Garlic was in the lead in every concentration – being still quite effective (at 10.7mm zone of inhibition diameter) even at the lowest concentration of 500mg/ml.** This illustrates the superior efficacy of Garlic, followed by Manuka Honey, Lemon and Ginger.
- I hope to use these results to encourage people to use more natural antibiotics, such as Garlic, to prevent antibiotic resistance, which is a big issue all around the world today and have caused many people to suffer. Many people see synthetic antibiotics as their first choice mainly because it is fast to get and they trust it because it is made in a lab. Not many people know that natural antibiotics, such as Garlic, can be just as effective.

Recommendations

Overall, I think my experiment went pretty well. Three of the antibiotics, Manuka Honey, Lemon, and Ginger, were a lot less effective than I predicted, but it may just be because these 3 natural antibiotics are less effective against E.Coli bacteria. Hence one thing I could extend is to try different types of bacteria for these 4 natural antibiotics, because different natural antibiotics may be more effective for different types of bacteria. Another thing I would also definitely do is to try different natural antibiotics, including some essential oils (Oregano Oil, Tea Tree Oil. Eucalyptus oil, Lavender oil), and compare with synthetic antibiotics, against different types of bacteria.

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