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Testing the Antibiotic Properties of Natural Substances

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Abstract: ***This research paper aims to test the antibiotic properties of natural substances to see if they can be viable replacements for antibiotic skin medications. The aim was to determine if these commonly used natural remedies could effectively inhibit bacterial growth on the skin, simulating a bacterial infection in a controlled lab setting using agar plates. It therefore offers alternatives to people who may not have access to antibacterial medications and to avoid the overuse of medications. Through incubation of E.coli bacteria, it found that hand sanitiser and garlic both had antibacterial properties and inhibited the growth of the bacteria. It also found that Triben, an antifungal medication can not be used to replace antibacterial medications. Further experimentation is recommended to test the antibiotic properties of garlic and apple cider vinegar as no concrete conclusions were drawn for these substances. The findings highlight that while certain home remedies might have potential antibacterial properties, their effectiveness is not universally reliable, and further research is needed to explore their use against various bacterial infections.***

Keywords: Antibacterial, natural, remedy, E.coli

1. Introduction

It is a well-known fact that overusing medications is detrimental to one's health, often leading to complications that are more severe than what one had initially attempted to treat. Although medications are made to help people, their regular use can be dangerous, giving rise to the search for alternative treatments often using natural ingredients. Often, natural remedies are used to try and treat colds, muscle pain, and skin infections. Lack of access to medication also makes natural remedies necessary. Many countries' healthcare systems are not advanced enough to provide medication and care to their entire population. Even if the medications are available, many people cannot afford to buy them. These are just a few of the many barriers people worldwide must face in order to get necessary healthcare, leaving no other option than natural remedies. However, are these remedies that are so commonly used effective?

This experiment tests four home remedies against E. coli bacteria: garlic, ginger, hand sanitiser, and apple cider vinegar, and one antifungal: Triben as well as a positive control using Fucicort and a negative control. It aims to discern whether the home remedies are effective in stopping the growth of the bacteria, specifically on the skin. This will be done by applying various substances to E. coli cultures representing a bacterial infection, and observing the growth of the bacteria after incubation.

2. Research

Fucicort contains fusidic acid, an antibiotic, and betamethasone valerate, a steroid. Betamethasone 'inhibits specific immune responses' (“Immune System Remodelling by Prenatal Betamethasone: Effects on β-Cells and Type 1 Diabetes”) which helps make the medication more aggressive. However, as this experiment is being performed on agar plates, there is no immune system to be suppressed therefore it will not affect the experiment.

The antifungal medication used is Triben. Triben contains the active ingredient clotrimazole which can be used for various fungal infections such as athlete's foot or yeast infections (“About clotrimazole cream, spray and solution”). I thought it would be interesting to see if an antifungal can be used against bacterial infections so I decided to include Triben in the experiment.

The natural remedies used were chosen as they are easily accessible, cheap, and they are the first results when you search for home remedies against bacterial skin infections online, therefore making them likely to be used.

Garlic contains the active compound allicin C6H10OS2 which was found to exhibit antibacterial properties and 'various bacterial strains resistant to antibiotics [...] were all found to be sensitive to allicin'. (“Antimicrobial properties of allicin from garlic (Ankri and Mirelman)) As allicin is a known antibacterial, it is interesting to see if the small amount found in garlic (5-18mg in one clove) will work against the bacteria, keeping in mind that only 200mg of garlic will be used in the experiment.

Ginger is also commonly used in households and is easily accessible. Ginger contains active ingredients such as gingerol, zingerone, and shogaols. (“Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities”) Shogaols have many biological activities such as anti-inflammatory, and anti-microbial functions. They are found in small quantities in fresh ginger and in larger quantities in cooked or dried ginger. They are also what give ginger its characteristic smell and have similar chemical structures to gingerol. Shogaols have shown anti-bacterial properties as they interfere with cell membranes and bacterial efflux.

Bacterial efflux is the process in which bacteria transport compounds outside the cell. (Barrett) This is done by efflux pumps found in the cell membranes of bacterial cells. The pumps remove toxic materials, such as antibiotics, from the

cell maintaining the concentration of the substances below toxic levels. This leads to the development of antibiotic resistance in the bacteria. This means that the bacteria has methods of surviving after antibiotics have been administered, making it harder to destroy them. However, a solution is to use various antibiotics that attack the bacteria differently and are therefore able to kill it. Furthermore, chemicals that interfere with bacterial efflux stop the removal of the antibiotic from the bacterium, increasing the toxicity within the bacterium and killing it.

Apple cider vinegar has the active ingredient acetic acid which is a by-product of fermentation and is produced during the production of the vinegar. Apple cider vinegar is high in acetic acid at about 5%, and it is what gives the vinegar its smell. Acetic acid is known to have antibacterial activity and it kills bacteria by altering their proteins and fats and destroying cell structures. (Evangeli and Thanh)

The final home remedy used is hand sanitiser which is commonly used as a fast replacement for washing your hands. It is supposed to kill germs and bacteria therefore I found it to be interesting to see if it inhibit the bacterial growth in this scenario as it is easy to access and cheap. Hand sanitiser contains high concentrations of alcohol, also a common antimicrobial that is effective against fungi, viruses and bacteria and is used in various cleaning products.

To understand what will happen in the experiment, it is important to understand how antibacterials work. Antibacterials work similarly to antibiotics, they target specific processes in the cells. For example, some antibacterials make holes in the cell membrane, causing the cytoplasm and cell organelles to leak out of the cell, therefore killing it. Other antibacterials inhibit the reproduction of bacteria by destroying their DNA or affecting the proteins that convert DNA into RNA. Fusidic acid, found in Fucicort, inhibits protein synthesis in specific proteins in the bacterial cell. (Fernandes)

Escherichia coli (E. coli) was used in this experiment as a model organism due to its fast reproduction time (of 20 minutes in ideal conditions), its ability to grow easily in various media, and the fact that it commonly affects humans. It is found in the intestine of warm-blooded organisms and it can aid digestion and protect the organisms from harmful microbes. (“FAQ: E. Coli: Good, Bad, & Deadly”) Specific strains of E. coli can be harmful to humans such as O157:H7. They disrupt bodily functions causing gastrointestinal issues and diarrhoea. Sometimes, dangerous strains of E. coli can cause damage to the kidneys and nervous system. Furthermore, E. coli can be transmitted between organisms, especially when those infected do not maintain proper hygiene.

3. Materials

The following materials were used for each iteration of the experiment:

1) 7 agar plates
2) E. coli sample
3) Incubator
4) Bunsen burner
5) 200mg Garlic
6) 200mg Ginger
7) 200mg Fucicort
8) 200mg Triben
9) 200mg Hand sanitiser
10) 200mg Apple cider vinegar

4. Method

1) Prepare the E.coli sample in a liquid medium
2) Throughout the experiment, maintain a sterile environment by keeping a Bunsen Burner lit with a blue flame close to the work area.
3) Use the mortar and pestle to crush the ginger. Strain it, measure 200mg of the liquid and pour onto a prepared petri dish. Thoroughly clean the mortar and pestle and repeat with the garlic.
4) Measure 200mg of Fucicort, Triben, hand sanitiser, and apple cider vinegar into individual petri dishes. Keep the petri dishes close to the Bunsen burner when they are open.
5) Use a pipette to pour about 1 ml of E. coli into each petri dish keeping the open agar pointed towards the Bunsen burner.
6) Sterilise a cell spreader by moving it over the flame of the Bunsen burner. Allow it to cool.
7) Use the cell spreader to coat the agar evenly with the bacteria. Make sure to sterilise the cell spreader for each dish to avoid contamination.
8) Incubate upside down at 37°c for 48 hours.

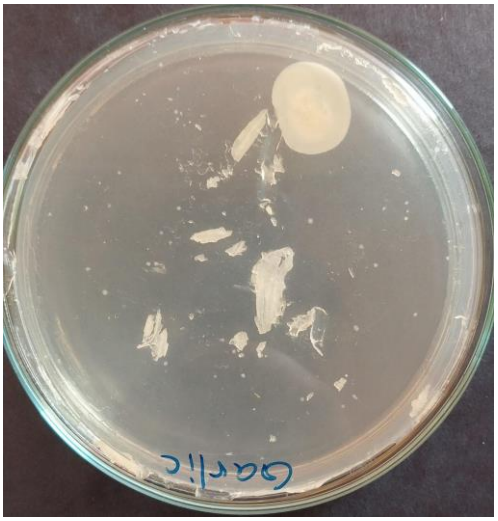

Figure 1: Petri dish containing garlic with contamination

I performed this experiment twice to confirm the results. After the first attempt, I noticed there was contamination in the petri dish containing garlic (see Figure 1). Therefore, the result was not viable as this likely affected the growth of the E. coli. This contamination possibly came from the garlic itself as it was not sterile, or from me not working in a sterile environment. Therefore, I decided to repeat the experiment while paying more attention to the observation of the sterile environment.

5. Results

Photos of the plates after 48 hours incubation:

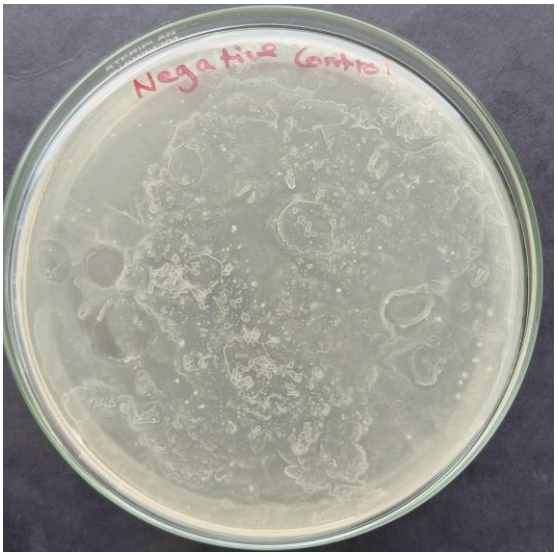

Figure 2: Petri dish containing negative control

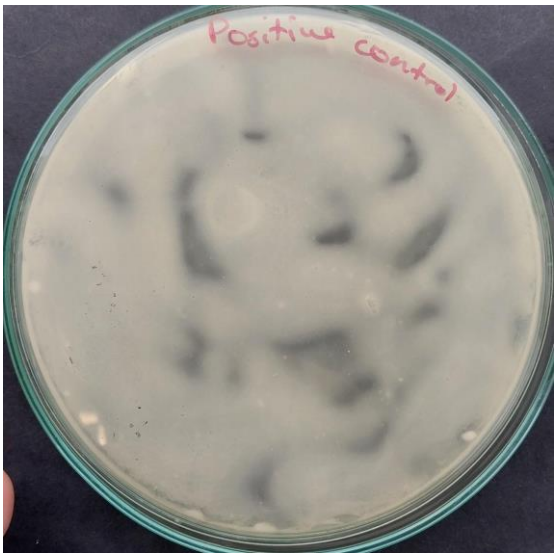

Figure 3: Petri dish containing positive control

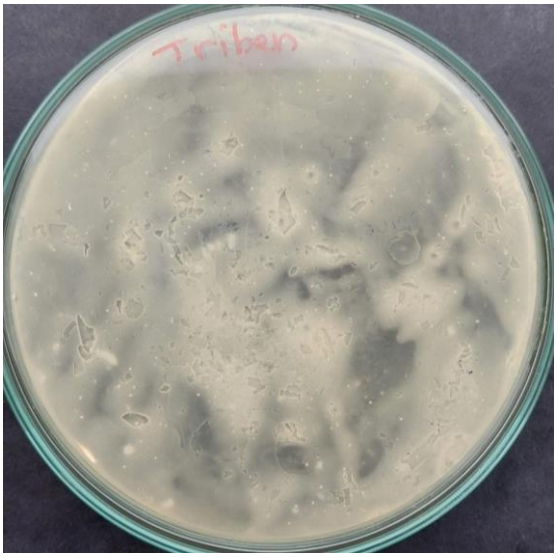

Figure 4: Petri dish containing Triben

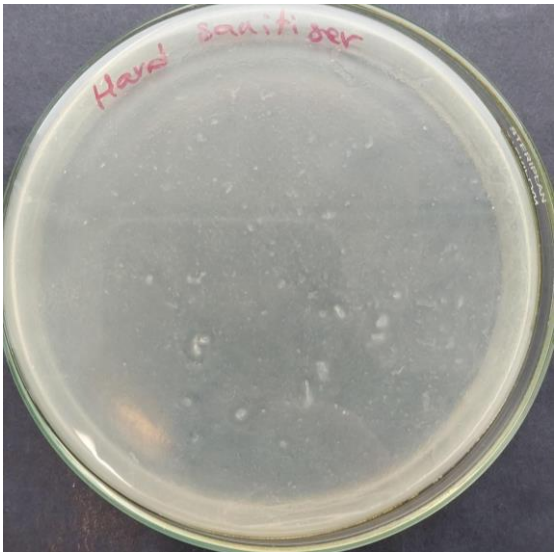

Figure 5: Petri dish containing Hand sanitiser

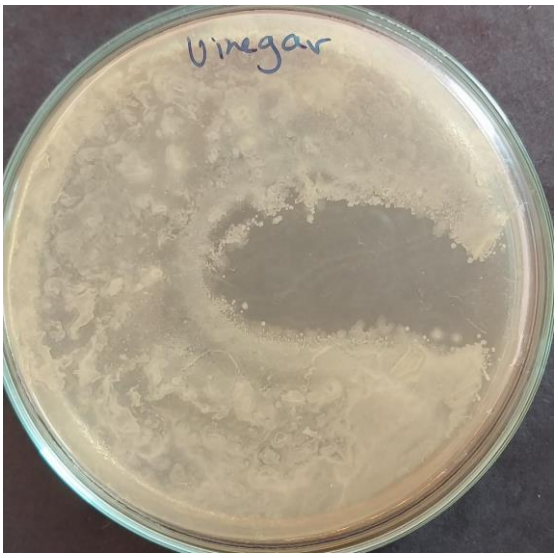

Figure 6: Petri dish containing vinegar

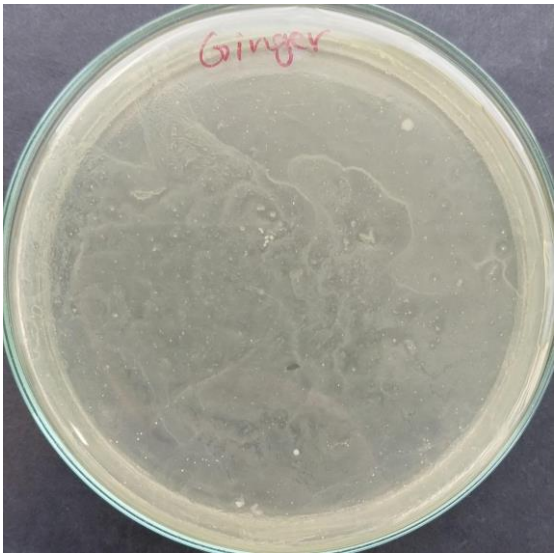

Figure 7: Petri dish containing ginger

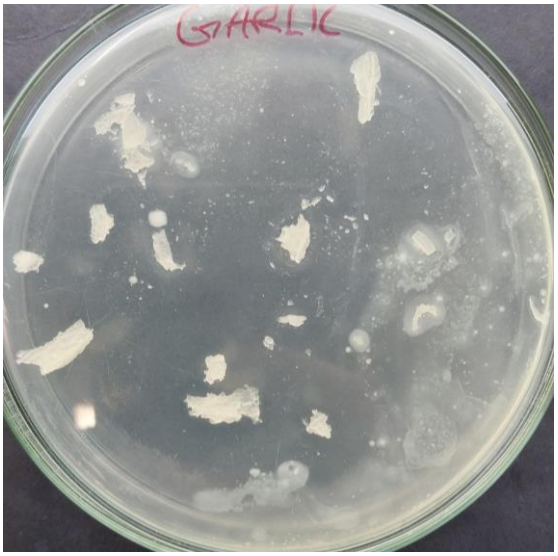

Figure 8: Petri dish containing garlic

After 48 hours, I observed the plates and noticed that the negative control, Triben, hand sanitiser, vinegar, ginger, and garlic plates had colony growth- although varying amounts. The negative control plate had numerous colonies of E.coli growing (see Figure 2). The colonies were small but present nonetheless. The plate containing Fucicort- the positive control (see Figure 3), as well as the plate containing Triben- the antifungal- had a pasty white colour due to the medication applied. There was no observable colony growth on the positive control plate. The plate with Triben applied had many small colonies growing (Figure 4). The plate containing hand sanitiser (Figure 5) had very few colonies growing and the ones present were very small. The plate with apple cider vinegar however had the growth of a biofilm on a large part of the plate (Figure 6). There was however a section in the middle where there were no E.coli colonies present. The petri dish in Figure 7 had ginger applied to it and had visible bacterial colonies growing. They were mostly small however some larger colonies were present as well. Finally, the petri dish with garlic applied (Figure 8) had colony growth, located mostly around the garlic pieces. There were colonies of various sizes.

6. Interpretation

The Fucicort in the positive control stopped the E.coli growth as there were no observable colonies, unlike the negative control, a result that was expected as this medication is an antibacterial available on the market.

The many small colonies growing on the plate containing Triben show that Triben as an antifungal does not affect the growth of E.coli. This allows us to conclude that Triben cannot be used as a replacement for antibacterial medications.

The plate containing hand sanitiser showed that the growth of the E. coli colonies was greatly lessened, therefore hand sanitiser could be an alternative to antibacterial medications. Although it is not natural, it is cheaper and more easily accessible than medications and can be used when there is no alternative.

The petri dish which had apple cider vinegar applied to it shows that apple cider vinegar did not work as an antibacterial substance. Rather, the growth of bacteria seems to have been facilitated. It is interesting that there was an area of no colony growth. This could be due to an experimental error, that the E. coli aliquot was not evenly spread around the plate, or perhaps the area without any bacteria is where there was the most vinegar. This would mean that the vinegar is effective but would need to be used in larger quantities than other remedies. Further experimentation should be carried out to properly analyse this result.

The plate containing ginger had significant colony growth showing ginger did not work as an antibacterial substance. The Shogaols present in ginger should have had antimicrobial properties and therefore stopped the growth. It is possible that the concentration of Shogaols relative to the bacteria was not enough because there are more Shogaols in dried ginger than in fresh ginger and this experiment used fresh ginger.

Finally, in the plate containing garlic, the colony growth was mostly surrounding the garlic pieces, therefore it may be contamination from the garlic. It would be useful to redo the experiment with purchased garlic oil which would be more sterile. The garlic did however seem to inhibit the E. coli colony growth therefore garlic is likely a good alternative for antibacterial medications due to the allicin found in it.

7. Experimental Errors and Limitations

Understanding experimental errors is extremely important in scientific experiments as they impact the reliability of results. In this experiment, I tried to minimise errors to ensure accurate interpretations of my results by being as exact as possible when weighing the medications being applied to the petri dishes and trying to ensure a sterile environment through the use of a Bunsen burner. However, it is still possible that errors occurred. A source of error is likely the contamination of the petri dishes with bacteria other than E. coli that was present in the air, eventually landing on the petri dishes leading to microbes other than E.coli growing. Furthermore, there may have been an error while measuring the amounts of substances which would lead to inaccurate results that cannot be compared. Additionally, there was likely contamination of some of the petri dishes from the antibacterial substances, which could hinder the results.

Every experiment has its limitations, which are important to understand for accurate interpretation of results. This experiment has the limitation that due to the amount of bacteria being applied to each plate not being equal, it is not possible to compare the different substances directly. In order to surpass this, the experiment could be repeated while keeping the volume of E. coli constant. Another limitation is that it was performed on agar dishes to mimic human skin. Agar can be used to represent human skin however it is important to keep in mind the differences between agar and skin due to the natural oils and the fact that agar has no response to stimuli, such as abrasions, that might aggravate the skin. Furthermore, the experiment does not take into account that there are steroids in some of the medications which would affect the results if the experiment were performed on a human. Additionally, this experiment only

considers skin infections, when a large part of bacterial infections are internal. This means that some of the remedies may need to be ingested, which this experiment does not take into account, focusing solely on external problems.

8. Conclusion

To conclude, this experiment showed that certain home remedies have antibacterial effects against E. coli. Hand sanitiser was a good antibacterial and was able to stop the growth of E. coli. Garlic also decreased the growth of bacterial colonies but it is possible there was contamination on the plate making it difficult to draw concrete conclusions. This experiment also showed that Triben, an antifungal, cannot be used in place of an antibacterial when fighting against a bacterial infection.

The future of this experiment would be to test similar home remedies against different types of bacteria that are found in different places in the body. Also, to develop a combination of various different natural remedies that effectively targets numerous bacterial infections. Further experiments should be done to clearly determine if antifungals and antibacterials are interchangeable. Furthermore, the experiment should be repeated for garlic, using garlic oil, for ginger, using dried ginger, and for apple cider vinegar, keeping in mind the necessity of evenly distributing the vinegar as well as the aliquot, and perhaps using a larger quantity of vinegar.

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