RESEARCH PLAN

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Microbiology

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Question:

Are natural substances effective at killing *E. coli* (k12) bacteria?

Hypothesis

If natural substances such as grapefruit extract and raw honey are applied to *E. coli* (k12), they will kill them as effectively as over the counter antiseptics.

Rationale

A growing problem in modern society is the increased resistance of bacteria to anti-biotics and anti-septics. Overuse of prescription and over-the-counter anti-biotics and anti-septics has placed selective pressure on bacterial populations, increasing variants that have resistant mechanisms. It is vital to find alternative, less costly anti-septics for cleaning and treating wounds. Several natural substances are believed to have anti-microbial properties; however, these properties must be demonstrated in controlled experimentation before they can be recommended for general use. For example, Honey is a natural antiseptic and there are several studies on how honey has been used to treat wounds. Applying honey to wounds helps to prevent infections, as it contains antimicrobial agents that kill the bacteria in and around the wound. Many types of bacteria cannot survive in honey so the wound heals, swelling eases, and the tissues can regrow. Grapefruit extract is another natural substance that has probable anti-septic properties. Grapefruit Seed Extract in higher concentrations may be a possible disinfectant choice for many hospitals and clinics throughout the United States and may be safe for individual home use.

Materials

Steens Manuka Honey UMF 24 (MGO 1122)

Grapefruit Seed 4:1 Powdered Extract 32 OZ (2 LBS) 907 G NutriCargo, LLC.
Petridishes

Neosporin First Aid Ointment

Clorox Clean-Up All Purpose Cleaner with Bleach

Nutrient Agar

E. coli K12 slant (pure culture)

Cotton swabs

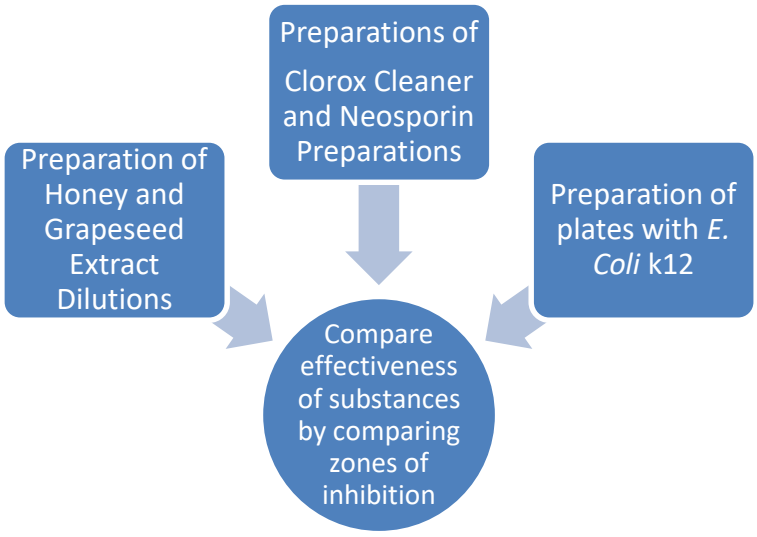
Test tubes for dilution preparation.

Sterile filter paper disks.

Deionized water.

Incubator

Procedures--Overview



Preparation of Honey and Grapeseed Extract Solutions

1. Three dilutions of honey and grapeseed extract solutions were prepared. For the purpose of this experiment, full concentration will be the undiluted honey or extract purchased.
2. A 50% solution of each will be prepared by diluting 10 mL of honey/extract with 10 mL of deionized water.
3. A 25% solution will be prepared by diluting 10 mL of honey/extract with 30 mL of deionized water.
4. A 10% solution will be prepared by diluting 10 mL of honey/extract with 90 mL of deionized water.

Preparation of Clorox All Purpose Cleaner and Neosporin Solutions

5. Three dilutions of Neosporin and Clorox All Purpose cleaner were prepared. For the purpose of this experiment, full concentration will be the undiluted cleaner or ointment purchased.
6. A 50% solution of each will be prepared by diluting 10 mL of cleaner/neosporin with 10 mL of deionized water.
7. A 25% solution will be prepared by diluting 10 mL of cleaner/neosporin with 30 mL of deionized water.
8. A 10% solution will be prepared by diluting 10 mL of cleaner/neosporin with 90 mL of deionized water.

Preparation of Agar Plates with E. Coli k12

9. Plates will be lined up along the edge of a lab table. Each plate will receive approximately 5 mL of sterile nutrient agar that had been melted in a microwave oven.
10. Plates will cool to room temperature and be stacked in a plastic sleeve (upside-down) in a refrigderator until use.
11. Plates will be divided into five sections using a sharpie on the bottom of the petri dish.
12. Using the pure culture provided by the Designated Supervisor of *E. coli* k12, a cotton swab will be used to streak 80 plates with the bacteria using aseptic technique.

Preparation of Zone of Inhibition Experiment

13. Twenty plates will receive 4 sterile disks dipped in the 4 concentrations of honey. The fifth section of the plate will contain a blank sterile disk as a control.
14. Twenty plates will receive 4 sterile disks dipped in the 4 concentrations of grapefruit extract. The fifth section of the plate will contain a blank sterile disk as a control.

15. Twenty plates will receive 4 sterile disks dipped in the 4 concentrations of Neosporin. The fifth section of the plate will contain a blank sterile disk as a control.
16. Twenty plates will receive 4 sterile disks dipped in the 4 concentrations of Clorox All Purpose Cleaner. The fifth section of the plate will contain a blank sterile disk as a control.
17. Plates will be placed in the incubator at 37 degrees C and monitored for 24-48 hours. The radius of the zone of inhibition will be measured around each disk in mm with a ruler.
18. At the conclusion of the experiment all materials such as petri dishes, cotton swabs that may have bacteria exposure will be disinfected in a 10% bleach solution.

Risk/Safety

There is always a risk of broken glassware causing cuts or damage to the eye. Contamination or transfer of bacteria is a concern. Gloves, googles and labcoats will be worn inside the lab. All materials used in the experiment with bacteria will be disinfected with a 10% bleach solution and given to the Qualified Scientist for proper disposal.

Data Analysis

The radius of zone of inhibitions will be averaged and graphed in Excel for each treatment for comparison. This data will be compared to determine if natural substances work as well as purchased ones at killing this bacteria species.

Possible Conclusions

It may be possible to determine if natural substances kill bacteria as effectively as the conventional ointment and cleaner to confirm or reject the hypothesis. Additionally, the data may indicate which concentrations work with similar effectiveness to the conventional antiseptics.

PHBAs

The bacteria E. coli K12 is designated as a BSL1 bacteria as it is non-pathogenic. All experiments will be conducted with a qualified scientist in a BSL1 lab. Precautions such as gloves, googles and a lab coat will be used. Bacterial plates will be disposed of using a 10% bleach solution.

Bibliography

1. Zone of Inhibition Experiments: http://users.bergen.org/donleo/EXPTECH/DISCDIFF/The%20End%20Zone_%20Measuring%20Antimicrobial%20Effectiveness%20with%20Zones%20of%20Inhibition.pdf
2. Natural Antibiotics: **http://www.naturaltherapypages.com.au/article/Natural_Antiseptics**
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5. ISEF Rulebook**: https://member.societyforscience.org/document.doc?id=747**
6. Microbiology Techniques**:** http://www.umsl.edu/~microbes/techniques.html