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|  | **Procedure - 1st experiment**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Oak Gall | Cinnamon Stick | Ginger | Garlic glove | Lavendar Leaves | | Sage Leaves | Large Beaker | Growth Medium (agar) | Laboratory Sterilizer | Dispensable inoculating loops (bacteria wands) | | Two petri dishes | Bleach | Tap water | Lab apron | Lab goggles | | Plastic gloves | Spot plate | Oil pencil | Scale | Pipette | | Distilled Water | Toothpicks (lots) | Bio-hazard bags | Chromotography paper | Hole punch | | Tweezers | Bunsen burner | Autoclave | Metric ruler | Pestle | | Bacillus cereus (for first experiment) | Microwave | Paper towels | E. coli (for second experiment) |  |   **Day 1**  1. Put on lab apron, goggles and plastic gloves.  2. Sterilize all glassware with laboratory sterilizer.  3. Clean tweezers and pipette with distilled water.  4. Swab micro-scale spot plate, pestle, and hole punch with alcohol and allow to air dry.  5. Turn on the autoclave, preheating it for later use.  **If doing Experiment #1:** Set autoclave for 30#C.  **If doing Experiment #2:** Set autoclave for 37#C.  6. Disinfect lab area with 30% bleach solution mixed with tap water.  7. Prepare two agar plates as according to instructions that comes with bacteria. Prior to putting agar in plates, divide each plate into four sections, labeling sections A-H from under side of plates with oil pencil.  8. Label wells of a plastic micro-scale spot plate A, B, C, D, E, F, G, H.  9. Measure 0.5g of the following, grind thoroughly with pestle being sure to cleanse pestle with alcohol and wipe dry between each ingredient, and place in separate wells: Well A - oak gall, B - cinnamon stick, C - ginger stick, D - garlic glove, E - lavender leaves, F - sage leaves.  10. Using pipette and avoiding pipette contact with substances within wells, place 5 mL distilled water in wells A-G. Mix each well thoroughly with a clean toothpick. Discard the used toothpicks.  11. Punch out eight discs of chromatography paper with the hole-punch. Place one disk in wells A-H and allow them to remain there for at least fifteen minutes.  12. **If doing Experiment #1:** Place a bacteria-wand into the test tube containing Bacillus cereus, obtaining some on the end of the wand.  **If doing Experiment #2:** Place a bacteria-wand into the test tube containing Escherichia coli.  13. Establish an even bacterial lawn on the agar, streaking the surface (do this twice, once for each petri dish):  A. Lift the petri dish's lid and streak the bacteria wand back and forth evenly across entire agar-surface of petri dishes. Be sure to streak all the way to the edges.  B. Rotate the petri dish forty-five degrees and streak the agar using the same pattern, at right angles to previous lines.  14. Lift the disc in well A with tweezers and place it in region labeled A, making certain that disc is in full contact with agar.  15. Disinfect tweezers with Bunsen burner, allowing to turn red at least twice. Then allow to cool.  16. Repeat steps 12 & 13 for wells B-H.  17. Incubate the petri dishes in autoclaves for 24 hours.  18. Disinfect the lab area with the 30% bleach solution. Discard the solution in the sink after using it, washing it down with plenty of water.  **Day 2 (and onward)**  1. Put on apron, gloves, and goggles.  2. Disinfect lab area with bleach solution.  3. Get petri dishes from autoclave.  4. Measure the zone of inhibition for each disc with the metric ruler. This is the ring between the paper disk and the bacteria, where no bacteria whatsoever is growing. Observe all changes or results, recording the data.  5. Return the petri dishes to the autoclave for 24 hours.  6. Continue this experiment for as many days as necessary (until all zones have gone), following steps 1-5.  7. Repeating the entire experiment several times is advisable to ascertain results.  **To discard contents of petri dishes:**  1. Place the petri dishes in the autoclave, increasing temperature to 100iC. Leave them overnight.  2. The next day, pour 100% bleach into each petri dish.  3. Allow them to stand until the agar disintegrates.  4. Place petri dish and contents in a bio-hazardous bag and dispose of the bag.  **To discard bacteria:**  1. Add 100% bleach to bacteria test tube until full.  2. Close the cap tightly.  3. Place capped tube in a bio-hazardous bag and dispose of the bag.  **To dry sage leaves and lavender flowers:**  1. Lay out fresh sage leaves (or lavender flowers) on a paper towel so that no leaves are overlapping.  2. Place the paper towel in the microwave on "High" for 30 seconds.  3. Open the microwave. If the paper towel is damp, place the leaves on a new, dry one. Microwave the leaves for another 30 seconds.  4. Repeat Step 3 another four times or longer, until leaves are fully dried.  **Data Collected**:   |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | Zone Of Inhibitios in *mm* - Found In Experiment #1 (Bacillus cereus) | | | | | | | | | | | | |  | **Trial Number** | | | | | | | | | | | | **Herb** | 1 | | 2 | | 3 | | 4 | | | Average | | | DAY | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 3 | 1 | 2 | | Oak Gall | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | .75 | .25 | | Ginger | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | Garlic Cloves | 0 | 0 | 2 | 0 | 2 | 0 | 1 | 0 | 0 | 1.2 | 0 | | Lavender leaves | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | Sage Leaves | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | .25 | 0 | | Cinnamon Stick | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | .25 | 0 | | Control 1 ( water) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | Control 2 (disk only) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | Zone Of Inhibitios in *mm* - Found In Experiment #1 (Bacillus cereus) | | | | | | | | | | | **Trial** | **Day** | Oak | Cinnamon | Ginger | Garlic | Lavendar | Sage | Control  Water | Control  Disk  only | | 1 | 1 | 7 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 2 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 3 | 1 | 4 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 4 | 1 | 4 | 0 | 0 | 8 | 0 | 2 | 0 | 0 | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 6 | 1 | 2 | NOT | 0 | 5 | 0 | 0 | 0 | 0 | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 7 | 1 | 0 | NOT | 0 | 12 | 0 | 2 | 0 | 0 | | 2 | 0 | ------ | 0 | 0 | 0 | 1 | 0 | 0 | | 3 | 0 | ------ | 0 | 0 | 0 | 0 | 0 | 01 | | 8 | 1 | 1 | NOT | 0 | 1 | 0 | 1 | 0 | 0 | | 2 | 0 | ------ | 0 | 0 | 0 | 0 | 0 | 0 | | 9 | 1 | 1 | NOT | 0 | 2 | 0 | 1 | 0 | 0 | | 2 | 0 | ------ | 0 | 0 | 0 | 0 | 0 | 0 | | 10 | 1 | 8 | NOT | 0 | 1 | 0 | NOT | 0 | 0 | | 2 | 6 | ------ | 0 | 1 | 0 | ------ | 0 | 0 | | 3 | 2.5 | ------ | 0 | 1 | 0 | ------ | 0 | 0 | | 4 | 0 | ------ | 0 | 0 | 0 | ------ | 0 | 0 | | Average | 1 | 3 | 0 | 0 | 4.1 | 0 | 0.78 | 0 | 0 | | 2 | 0.6 | 0 | 0 | 0.1 | 0 | 0.1 | 0 | 0 | | 3 | 0.25 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |  | | | | | | | | | |   **Data, Results and Discussion**  This experiment was designed to test the susceptibility of the two major groups of bacteria, gram positive and gram negative, to the herbs being tested for antiseptic properties. Bacteria from both groups were tested in case herbs documented as being antiseptic were in fact only so against one type. One representative was chosen from each group: Bacillus cereus from the gram positive group and Escherichia coli from the gram-negative group. These two groups are distinguished by fundamental differences in the structure of their cell walls. Gram negative bacteria have cell walls that are thinner, with distinct layers, made primarily of lipopolysaccharide. Gram positive bacteria have cell walls consisting mostly of peptidoglycan, polysaccharides, and teichoic acids.  The testing was conducted in the same manner for both bacteria. They were tested for antiseptic properties, measured by a visible zone of inhibition, against oak gall, lavender, garlic, sage leaves, cinnamon, and ginger. There were two controls - one was distilled water and chromatography paper and the other was plain chromatography paper. These controls were included to insure that neither the distilled water nor the chromatography paper introduced lurking variable. Bacillus cereus proved to be far more resistant to the tested herbs than E. coli. Herbs that showed any antiseptic properties were oak gall, garlic, cinnamon, and sage. However, as cinnamon only had a zone of inhibition for the first experiment, after which the procedure was altered in order to control more variables, there is no credible evidence that it is, in fact, antiseptic. The herbs that showed no zones of inhibition whatsoever when tested against Bacillus cereus were ginger, lavender, and both controls. Garlic had the greatest average zones of inhibition, 1.25 mm, on average for Day 1, and only oak had any zone of inhibition after day one.  The results from the Bacillus cereus tests suggests that oak and garlic both have mild antiseptic properties and that sage, cinnamon, and oak warrant further testing. None of these three herbs had reliably large results and consequently had negative values in a 90% confidence interval. A 90% confidence interval gives the range of values between which sample means will fall in 90% of all tests, if the tests were from a normal population (not skewed by lurking variables, etc.). The fact that the lower range of this interval was negative implies that there will be no zone of inhibition in some proportion of the tests, if all tests have a distribution of results similar to the results obtained through this test. However, if there was a larger sample size, it would be far clearer both from experimental and statistical evidence whether or not these herbs were in fact antiseptic. Statistical evidence would include interval values that were not negative. Neither lavender nor ginger showed zones of inhibition. This discredits them as antiseptics, as they did not show any evidence of killing bacteria on surfaces.   |  |  |  | | --- | --- | --- | |  | **90 % Confidence Interval for Herbs with Positive Results**  **Experiment #1 (Bacillus cereus) Day 1** |  | | **Herb** | **Minimum** | **Maximum** | | **Oak** | -0.3766 mm | 1.8766 mm | | **Garlic** | 0.12341 mm | 2.3766 mm | | **Sage** | -0.3383 mm | 0.83834 mm | | **Cinnamon** | -0.3383 mm | 0.83834 mm |   The second test, against Escherichia coli, was then conducted, in order to find out whether or not herbs were effective against only one type of bacteria, both or neither. This test had positive results for garlic, oak, and sage, and negative results for cinnamon, ginger, lavender, distilled water, and chromatography paper. These results were the same, with the exception of cinnamon, as the results from the bacillus cereus. This seems to refute the idea that herbs as antiseptics would be effective or ineffective depending on the bacteria's cell wall.   |  |  |  | | --- | --- | --- | |  | **90 % Confidence Interval for Herbs with Positive Results**  **Experiment #2 (Escherichia coli) Day 1** |  | | Herb | Minimum | Maximum | | Oak | 1.3833 mm | 4.6167 mm | | Garlic | 1.7901 mm | 6.4099 mm | | Sage | 0.26124 mm | 1.2943 mm |   However, there is a wide range in the difference between the width of the zones of inhibition found for oak and garlic against bacillus cereus, versus the zones of inhibition found for these herbs against Escherichia coli. This may indicate that, while whether a bacteria is gram negative or gram positive does not make it wholly unsusceptible to certain kinds of herbs, gram negative bacteria is more susceptible to herbal antiseptics than gram positive bacteria, perhaps due to their different cell wall structure.  This is somewhat refuted by the fact that sage did not show especially convincing statistical evidence to a difference between the zones of inhibition of Escherichia coli and Bacillus cereus. This could change if there were larger sample sizes.   |  | | --- | | **Two Sample T-tests Illustrating the Difference Between Zones of Inhibition found for Herbs tested against Bacillus cereus versus Escherichia coli (for Day 1)**  **Null-Hypothesis:** The means from both samples are from the same population.  **Alternate-Hypothesis:** The mean E. coli zones will be significantly larger than the mean zones from Bacillus cereus. | | **Oak**  t-statistic = -2.2422  p-value = .0223  Reject Null-Hypothesis at the 5% level. There is significant statistical evidence to reject the null hypothesis at the 5% level, with a p-value of 2.23%. It is very unlikely that both sample come from a similar population of zones of inhibition, suggesting that zones of inhibition for oak against E. coli are significantly larger than those against Bacillus cereus. | | **Garlic**  t-statistic = -2.1143  p-value = .0290  The Null-Hypothesis is rejected at the 5% level. There is significant statistical evidence to reject the null hypothesis at the 5% level for garlic, with a p-value of 2.90%. It is very unlikely that both samples - one from Bacillus cereus, one from E. coli - come from the same population of zones of inhibition, suggesting that zones of inhibition for garlic against E. coli are larger than those against Bacillus cereus. | | **Sage**  t-statistic = -1.4123  p-value = .0948  Fail to reject the Null-Hypothesis at the 5% level. There is insufficient statistical evidence that zones of inhibition for sage differ between Bacillus cereus and E. coli, with a p-value of 9.48%. |   Although garlic was clearly the most effective antiseptic (see 90% confidence interval charts), there was some indication that oak gall is capable of lasting a longer period of time, although such differences were not significant and were rare. There was a strong fall off of effectiveness between day one and day two for all effective herbs, with very few retaining a zone of inhibition for longer than one day.  The results of this experiment are as follows: there is compelling evidence to label both oak gall and garlic as antiseptic herbs, but more testing is certainly needed. Sage shows a wealth of possibility as to being antiseptic towards gram negative bacteria, and perhaps experimenting further with sage and gram positive bacteria would show more positive results than my testing yielded. There is an extremely small amount of evidence to suggest cinnamon as an antiseptic; one trial against Bacillus cereus. Although that trial was somewhat discredited by several lurking variables, cinnamon certainly warrants further testing before its' antiseptic properties are decided. There is no evidence whatsoever to support lavender or ginger as being antiseptic. While these herbs may provide valuable immune-boosting uses when ingested, this experiment found no indication at all of any resistance to bacterial growth or antiseptic properties. | |
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