IB Biology- Standard Level

Internal Assessment

*A study of the effect of Lavender, Wild green, Wild orange, and Geranium Essential Oil on non-pathogenic bacterial growth*

**Background**

As a woman, I use various different oils on my skin so this lab could show me which oils are cleaner for my skin and overall healthier. As I did research I was surprised that there are oils that enhance bacteria growth while at the same time there are oils that destroy bacteria. As Costa Rica is home to thousands of plant species, there are hundreds of different types of oils made from these plants.

**Aim**

The aim of the biology lab experiment is to test essential oils, oils that are derived and distilled from plants with a concentration of 40% oil and 60% plant based, and their effect on bacteria derived from the skin, in specific the hand area.

**Research Question**

Do essential oils enhance and aid bacterial growth on the hands or do they destroy it?

**Prediction**

Essential oils contain a wide variety of secondary metabolites that are capable of inhibiting or slowing the growth of non-pathogenic bacteria.

**Safety and Ethical**

Through every procedure gloves were worn alongside masks over the mouth. This prevents both the contamination of the agar plates and the people committing the experiment. Also the surface in which the experiment took place was cleaned with alcohol in order to sterilize it.

**METHOD**

**Preliminary Experiment**

**Method:**

1. Agar plates should be placed in an incubator at approximately 98 degrees Fahrenheit to make sure there is no initial bacteria on it but at our school we do not have an incubator but as we I live in a tropical country the temperature does not vary drastically. Instead agar plates were placed in refrigerator to prevent any extra bacterial growth.
2. Prepare 5 agar plates with one cotton swab of bacteria from counter. Make sure hands are washed before and after the fingerprint is placed. We are using bacteria from finger as safe store bought bacteria is very hard to find.
3. Label 5 beakers with their concentration using tape and permanent marker.
4. Prepare the five different concentrations of essential oils in their beakers. The first concentration will have 0% oil therefore its the controlled, the second 25%, the third 50%, the fourth 75%, and the last one will be 100% oil. To dilute each use olive oil.
5. Place 50 grams of each oil concentration on a agar plate

Then tape the agar plate close and place in incubator for approximately 24 hours.

**Results:**

Results were collected by visually counting how many colonies of bacteria were present after concentration of essential oil was added. Since this was the preliminary it was not necessary to use a quadrant to measure squares filled with bacteria.

|  |  |
| --- | --- |
| Concentration (percentage) | Size (how many colonies of bacteria) |
| 0 | 76 |
| 25 | 53 |
| 50 | 24 |
| 75 | 9 |
| 100 | 10 |

**EXPERIMENT**

**Equipment**

* 100g of Lavender, Wild green, Wild orange, and Geranium Essential Oils
* 400g olive oil
* 25 Petri dishes
* bacteria extracted from finger
* Autoclave
* 100g Tryptone Soy Agar
* Spatula
* Newspaper
* 5 Pipettes
* Sterile swab
* 5 x 100 ml beakers
* Scale
* 1000ml beaker
* Hot stove
* Refrigerator
* Plastic quadrant film (super imposed grid)

**To clean and sterilize petri dishes**

It is very important to sterilize petri dishes in order to secure that there is no bacteria growth apart from the bacteria we add. If extra bacteria grows it will contaminate my results.

1. Place petri dishes on top of each other
2. Wrap petri dishes in newspaper and tape it up with masking tape.
3. Place dishes in autoclave and turn on to medium heart. Leave for 15 minutes or until it lets out steam
4. Take off lid of autoclave and let steam out.

Safety: Gloves or hot mats should be used when extracting the petri dishes from autoclave and petri dishes should be made from heat resistance glass.

**To prepare agar plates**

1. Fill 1000ml beaker with 300 ml of distilled water
2. Heat water until boiling, then add 30 grams of Tryptone Soy Agar and stir with sterilized glass rod.
3. Once agar is dissolved in water turn off heat and let cool down for 5 minutes.
4. Measure 25 ml of substance and pour into petri dish. Wrap petri dish with plastic wrap and put into refrigerator.
5. Repeat 4 times.

Safety: To avoid contamination of agar plates always wear gloves and a mask. Make sure surface used is cleaned with alcohol and all equipment is sterilized.

**Testing Essential Oils**

1. Place the five agar plates that will be used in an incubator at approximately 98 degrees Fahrenheit to make sure there is no initial bacteria on it.
2. Prepare 5 agar plates with one cotton swab of bacteria from hand. The reason bacteria was extracted from fingers was because the school doesn’t have stock bacteria and also because I wanted to specifically test for the effects on skin bacteria as you apply the oils to your skin. Make sure hands are washed before and after the fingerprint is placed. We are using bacteria from finger as safe store bought bacteria is very hard to find.
3. Leave bacteria on petri dishes for 12 hours and then measure the squares filled with bacteria using a plastic quadrant film. Write down results.
4. Label 5 beakers with their essential oil using tape and permanent marker.
5. Prepare the five different essential oils in their beakers. Each oil will be diluted with olive oil in order to make the concentration 40% olive oil and 60% plant based oil which is the common concentration used in soaps.
6. Place 4 ml of olive oil and 6 ml of plant based oil in beaker and mix with a sterilized glass rod. Repeat 4 times for each oil. You will also have one controlled oil which will consist of 10 ml olive oil.
7. Place 5 drops of each oil concentration on the corresponding agar plate with the use of a pipette.
8. Then tape the agar plate close and leave for approximately 24 hours.
9. After 24 hours’ open agar plates and use quadrant to measure squares filled with bacteria. Write down results.

Repeat all steps 4 times in order to have 5 trials.

**Controlled Variables**

* The same volume of agar mixture (20g) was put into every petri dish and left in refrigerator for 24 hours.
* The size of the petri dishes was the same for each trial therefore the surface the bacteria could cover

**Assumptions**

* The temperature is the same everyday when the trials are conducted (23 Celsius).
* The distilled water contains the same impurities.
* All materials are sterilized and contain close to no bacteria.
* No bacteria from the surface worked on or from the breath of the one doing the experiment will contaminate the agar gel.

**Observations**

* The agar jelly was a clear color before bacteria was added. Once bacteria contaminated the agar jelly the color turned yellowish.
* The amount of bacteria that contaminated the agar jelly changed for every petri dish therefore it was necessary to use a plastic quadrant film and compare the results before and after the essential oil was added.
* Once the essential oils were added the agar jelly turned a light yellow brownish color.
* Right away it was obvious that Wild Orange had the biggest effect on the bacteria as it resulted in the least bacterial cover among the agar jelly.

**DATA**

**Trial #1**

The first trial I sterilized the petri dishes in the autoclave letting it steam for approximately 20 minutes. Then we only opened the petri dishes to put in the agar. After approximately one day all 4 of the petri dishes were utterly contaminated (100% coverture with bacteria) as you can see in the photo. The light yellow color is all the bacteria that grew. As they were contaminated I could not use them for my experiment meaning the first trial was a fail.

**Trial #2**

For each trial I recorded Qualitative Results of bacterial growth without the essential oil, Qualitative Results of bacterial growth with essential oil, and the quantitative results. This trial was repeated 4 more times.

Qualitative Results of bacterial growth without essential oil

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Wild Orange | Wild Green | Lavender  | Geranium |
| Color | Clear with a hint of yellow | Clear with a hint of yellow | Clear with a hint of yellow | Clear with a hint of yellow |
| Coverture (estimates) | 5/8 of dish | 2/8 of dish  | 7/8 of dish | 6/8th of dish |

Qualitative Results of bacterial growth with essential oil:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Wild Orange | Wild Green | Lavender  | Geranium |
| Color | Clear with a hint of yellow | Dark yellow color | Very pale white | Clear with a hint of yellow |
| Coverture (estimate) | 6/8 of dish | 4/8 of dish  | 7/8 of dish | 7/8 of dish |

Quantitative Results:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Wild Orange | Wild Green | Lavender  | Geranium |
| Number of grids with bacteria before oil | 26.5 | 15 | 34 | 31.5 |
| Number of grids with bacteria after oil | 24 | 13 | 33 | 31 |

In every trial the bacteria after exposed to the essential oil decreased showing that they are effective in destroying bacteria.

**ANALYSIS**

**Percentage difference**

In order to see if there was a significant difference between the bacteria destroyed/grown with soap versus the bacteria grown/destroyed with essential oils the percentage difference of each trial and oil was calculated. We use this test to see which essential oil is most effective towards fighting bacteria.

****

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Trial 6 |
| Wild orange | 9.9009% | 22.9508% | 18.0062% | 21.3422% | 16.0348% |
| Wild Green | 8.0000%  | 11.7647% | 13.3498% | 9.9390% | 9.5382% |
| Lavender  | 2.9850% | 0.0000% | 4.8726% | 12.0834% | 1.2332% |
| Geranium  | 1.6001% | 21.2766% | 12.028% | 3.1212% | 0.0833% |

**T- test**

In order to statistically test whether the essential oils destroyed the pathogenic bacteria more efficiently then olive oil, a t-test was carried out to investigate whether there is a significant difference between the destruction of bacteria.

****

t-test formula:

Degrees of freedom= 8

Tcalc= 0.64256

Critical value of T= 1.860

I carried out the T-test with wild orange and olive oil in order to test if there was a significant difference between the bacteria destroyed. The T-test resulted in a t calculation of 0.643 which is less than the critical value of t which was 1.860. This suggests that there is not a significant difference. This means that even though there are more bacteria being destroyed by the essential oil, wild orange, than olive oil, the difference is not big enough to be seen as significant.

**Evaluation of Strengths**

One of the strongest points of this lab was that I was able to carry out all 5 trials on the same day. This allowed me to control more variables like the same temperature during each trial and same environment. By being able to control more variables in this experiment it allows for less contamination of the agar plates and therefore more accurate results.

 Another strength was the fact that Costa Rica produces some of the most popular essential oils used in soaps. The easy access to these oils allowed for me to truly test whether these oils destroyed or reproduced the non-pathogenic bacteria.

**Evaluation of Weaknesses with suggested improvements**

The essential oils were retrieved from the brand “Doterra”. This company claims that these oils when added to soap, are made up exactly of 30% olive oil and 70% solely plant derived oils. In the experiment I tried to make it as real as possible therefore creating the concentrations 30 to 70. If this is not true it could be considered a weakness as this experiment would not simulate the oil used in soap. As not all oils that are used in soaps have this same concentration, it is not possible to say that all soaps destroy bacteria. Also since this is experiment was conducted in a tropical country, the humidity and bacteria in the air could have a negative affect on my results. As well, since this is a school lab report, it was conducted in a school lab while other students were conducting their own IAs. This could had lead to bacterial contamination from other students while they were walking by or working next to me.

One huge improvement for this experiment would be to conduct it in a lab that was completely empty. This would allow for everything to be sterilized and avoid any contamination from other people’s non-pathogenic bacteria or the bacteria on the materials and countertop. Also another improvement for the experiment would to carry it out all on the same day and time therefore the temperature and time would become controlled variables. This would lead to a less of a chance of contamination of changes in the preparation of the agar plates.

To further narrow my scope of this experiment I could have tested different brands of essential oils. This would test which brands are more useful and which ones are lying to their consumers about bacteria destruction. This would lead to a more anthropological view on this lab experiment.

**Conclusion**

In conclusion, this lab experiment and its results supported my hypothesis that essential oils such as wild orange, wild green, lavender, and geranium can fight and eventually destroy the growth of the non-pathogenic bacteria. I also found that the most effective essential oil tested to destroy bacteria was wild orange. This is proven by calculating the percentage difference of the bacteria on the petri dish before and after the oil was added. The highest percentage difference for the wild orange occurred in trial 3 where close to 22% of bacteria was destroyed after only 24 hours. The runner up was Germanium who destroyed nearly 21% of the bacteria in trial 3. All this data is seen and compared in the percentage difference graph showing the effectiveness of these oils on fighting bacteria. Now that we know that these essential oils do destroy bacteria I carried out the t-test to see if essential oils destroyed the pathogenic bacteria more efficiently then olive oil. The t-test that there is not a significant difference

Overall this lab concluded that essential oils are effective when fighting bacteria growth and therefore I as a woman will continue using these soaps on my skin as I know I result in cleaner skin after the use thanks to this experiment.

**Works Cited**

What is an Essential Oil? (n.d.). Retrieved November 29, 2016, from https://doterra.com/US/en/what-is-an-essential-oil

Nazzaro, F., Fratianni, F., Martino, L. D., Coppola, R., & Feo, V. D. (2013). Effect of Essential Oils on Pathogenic Bacteria. Retrieved November 12, 2016, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3873673/

Vidaver, A. K., & Lambrech, P. A. (2004). Bacteria as Plant Pathogens. Retrieved November 29, 2016, from http://www.apsnet.org/edcenter/intropp/pathogengroups/pages/bacteria.aspx