Extraction Methods of Cilantro and its Effects on Antimicrobial Activity

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Abstract

Cilantro contains Dodecanol which has been found effective against salmonella. The objective of this project was to find out if the extractions methods of cilantro affected the effectiveness of the Dodecanol against Salmonella. We tested this by diluting fresh, boiled and cilantro extract. Using disc diffusion, we were able to look for areas of death to see the effectiveness. Unfortunately, there was little to no cell death and a different type of bacteria formed in 2 of the plates.

Introduction

It has been established that certain compounds within cilantro, such as (2E)-Dodecanol (c(12)) have the capacity to minimize bacterial concentrations of Salmonella (Kubo, et al., 2004). Our study aimed to evaluate how different extraction methods of cilantro affected the number of colonies of Salmonella. Our preparation methods included boiling cilantro, cilantro extract and fresh cilantro done with a dilution series (1:1, 1:2, 1:3). Our research aimed to answer whether different extraction methods of cilantro affected the effectivity of Dodecanol and therefore effected number of countable Salmonella colonies. Our hypothesis was that using fresh cilantro would have the greatest effectivity in minimizing colonies of Salmonella.

Methods

Materials & Location:

The experiment was conducted in the biology lab at Whatcom Community College using cilantro and cilantro liquid extract obtained from a local grocery store. The solvent for all extracts used was water. The boiled extract was heated in a beaker on a hot plate. A mortar and pestle was used for grinding the cilantro. A vortex was used on the dilutions as well as a centrifuge when it was needed.

Disk diffusion plating was prepared on TSA plates using sterile swabs for inoculation of Salmonella culture and sterile discs were used to place the extracts onto the plates, plates were then placed in an incubator.

For molecular analysis, bacterial lysis was done, a thermocycler was used to do PCR, and gel electrophoresis was performed which was stained and visualized.

Procedure

To prepare the fresh cilantro extract 3 dilutions were made through a serial dilution. 10g of cilantro was measured and was ground using a mortar and pestle. 10^{-1} dilution: 1 mL of the slurry was added into 9 mL of sterile water and vortexed for 30 seconds. 10^{-2} dilution: 1 mL of the 10^{-1} dilution was added into 9 mL sterile water and vortexed for 30 seconds. 10^{-3} dilution: 1 mL of the 10^{-2} dilution was added into 9 mL of sterile water and vortexed for 30 seconds.

To prepare the boiled cilantro extract 10g of cilantro was measured and boiled for 5 minutes using 100 mL of DH2O in a beaker that was heated on a hot plate. This was then ground using a mortar and pestle. A serial dilution was made; 10 mL of the slurry was added into a test tube and vortexed for 30 seconds to create the 10^{-1} dilution. 1 mL of the slurry was added into 9 mL of sterile water and vortexed for 30 seconds to create the 10^{-2} dilution then 1 mL of this dilution was added into 9 mL of sterile water and vortexed for 30 seconds to create the 10^{-3} dilution.

To prepare the cilantro liquid extract dilutions 1 mL of the liquid extract was added in 1 mL of sterile water for a 1:1 and 1 mL of the liquid extract was added into 2 mL of sterile water for the second dilution of 1:2, as well as a pure droplet of the extract was tested.

Dodecanol Molecule

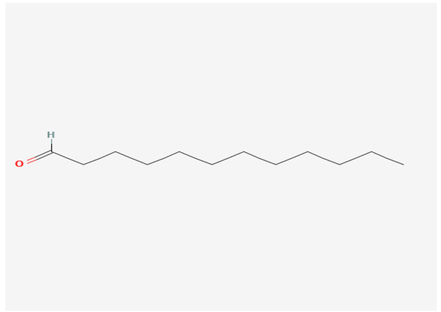
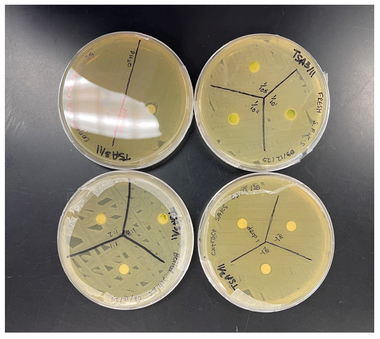
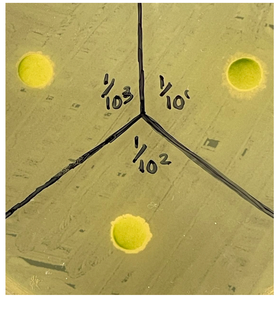


Image of Dodecanol molecule.
PubChem.https://pubchem.ncbi.nlm.nih.gov/compound/8194#section=2
D-Structure

Plates with Salmonella and Cilantro dilutions



This image show one of the trials, we were expecting to see a circle of cell death around the discs which was not present.



After incubation, discs showed growth of seemingly different bacteria.

To prepare the disk diffusion plates 5 plates were inoculated with Salmonella with a sterile swab while using an ethanol candle to maintain aseptic technique. Sterile filter paper discs were then dipped in each dilution and placed on their corresponding locations on the plates. An ethanol disc and sterile water disc were used as a control. Parafilm was then added to the plates and incubated at 37º for 48 hours

After incubation, bacterial lysis was performed on the colonies on the surrounding areas of the disks of the cilantro liquid extract due to it having changed appearance in preparation for PCR. A gel was run for 40 minutes to analyze the PCR products. The gel was then stained with EtBr and visualized using a UV light box. Then the cleaned PCR products were sent to be sequenced.

Discussion

In this project, we tested whether cilantro could kill Salmonella, based on research showing that a compound in cilantro called Dodecanol has antimicrobial properties. However, our results did not support the original hypothesis. None of the three methods we used—fresh cilantro paste, boiled cilantro, and cilantro extract—were able to kill the Salmonella on the agar plates after 48 hours of incubation. This was unexpected because other research has suggested that cilantro can help reduce harmful bacteria.

There are a few reasons why our experiment may not have worked. One major factor could be that our cilantro was too diluted, meaning there wasn't enough of the active compound, Dodecanol, to kill the bacteria. Another possible reason is that we had too much Salmonella on the plates for the cilantro to make a difference. Also, the way we did the experiment—such as mixing the cilantro with water, boiling it, or using an alcohol-based extract—might have affected how strong the Dodecanol was. Compared to other studies that used stronger antimicrobial mediums like electrolyzed water (Jung, et al., 2022) or higher concentrations, our method might have been too mild to see an effect.

Even though our experiment didn't show the results we hoped for, it still gave us useful information. It showed us that just using cilantro in the ways we tried might not be enough to kill bacteria like Salmonella. For future research, it might be helpful to try stronger cilantro concentrations or different ways of applying it. One idea is to apply the cilantro dilution before incubation—right after adding the Salmonella—and letting it sit for 48 hours before incubating. This could give the Dodecanol more time to work before the bacteria start growing into colonies. If that method worked, it would support the idea that the timing and concentration of the treatment are really important.

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References

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