Comparison of Antimicrobial Activity in Home-brewed and Store-Bought Kombucha Using Escherichia coli
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Introduction or Overview
The drink Kombucha is a sugar fermented tea that is said to help with digestion, ridding the body of toxins, and boost immune systems. Though it is supported through previous experiments the health effects of kombucha, there is still a gap in understanding if there are better health benefits between store-bought or homebrewed kombucha. This study aimed to close that knowledge gap by observing the antimicrobial activity of home-brewed and store-bought kombucha. This experiment will be performed on *Escherichia coli* using two store-bought lemon ginger kombucha, Health ADE and Brew Dr., as well as unflavored homebrewed kombucha.

Research Hypothesis/Objectives
The aim of this project was to test the effects on immune system health by comparing home-brewed and store-bought kombucha through looking at the antimicrobial properties against a lower intestine bacterium, *Escherichia coli*.

Methodology or Approach

**Reviving Freeze-Dried Strain**
Cells were obtained from American Type Culture Collection (ATCC) strain 25922. About 1 mL of LB media was added aseptically with a Pasteur pipette to the freeze-dried *Escherichia coli* and mixed until uniform. The material was left to rehydrate for fifteen minutes. A purity check was performed. The plate and test tube were incubated for 24 hours at 37ºC. Subsequent subcultures were made. Cells were added in 200 μL aliquots along with 200 μL of glycerol and stored in a -80ºC freezer until needed.

**Kombucha Fermentation**
Tea was brewed using 2 cups of water, 6 tea bags, and 1 cup cane sugar. The final brew was diluted to a gallon. The Fermentaholics Organic SCOBY that was purchased through Amazon was added and the jar was covered with a filter to keep out unwanted organisms. The jar was kept in 20ºC to ferment for 7-21 days or until an optimal pH of 3.5 was achieved. This was measured every other day.

**Antimicrobial Experiments**
Bacteria were removed from the freezer and thawed. Upon thawing, the density was measured using a Vernier Go Direct Spectro Vis Plus spectrophotometer that was blanked with deionized water. If the density reading was too high, LB broth media was added and measured again until optimal density was achieved. Optimal density was found to be 1.105 g/mL or measured at an absorbance of 0.03. Bacteria were grown on Mueller-Hinton plates using 200 μL medial culture to create a lawn growth. Cellulosic absorbent disks were made using a sterilized hole punch and soaked in varying flavors of 45 μL of kombucha. After soaking, two disks were equally spaced on each inoculated plate using sterile forceps. These plates were incubated upside down for 24 hours at 37ºC. The zones of inhibition were measured and recorded. A series of 32 measurements on sixteen plates were taken per sample. The store-bought samples were stored in a refrigerator for a period of a month in between sampling times. All samples of home-brewed and store-bought kombucha were plated and measured simultaneously. Statistical software Jamovi was used to run and analyze the analytical tests, One-Way ANOVA and Tukeys Hov.

**Major Outcomes, Results and Conclusion**
There was no statistical difference found between antimicrobial activity of home-brewed or store bought kombucha.