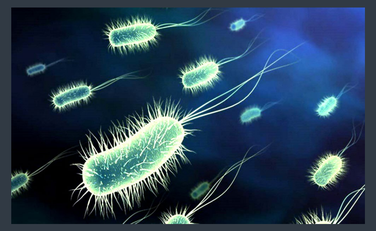

Investigation of Antimicrobial Properties of the Natural Product Curcumin

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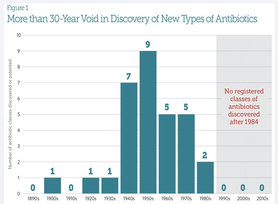
Abstract

Antibiotics has become an essential part of modern day medicine due to its ability to fight the bacterial infection by either killing or limiting the growth of the bacteria.
During our modern era, the increased usage of antibiotics has decreased its benefits thus the resistance to most antibiotics have exponentially increased. Therefore, the research and discovery of new antibiotics have become a crucial demand to resolve this upcoming battle.
This experiment tests liquid and powder forms of curcumin, commonly found in turmeric--often used in southeast Asia cuisine. By using serial dilutions, antibacterial properties that may be found in curcumin was tested against *Salmonella* Typhimurium. This compound was believed to have antibiotic properties due to the common use among herbal medicine practitioners in India.
The results for curcumin in powder have shown to be inconclusive. Interestingly, as the concentration of powder mixture decrease, the absorbance of the *Salmonella* increased. Comparatively, liquid curcumin showed antibiotic ability.
Curcumin could be further tested to explore if the liquid compound can show improvement in other dosage to prove it is a solid candidate to become a compound of a new antibiotic.

Figure 1
More than 30-Year Void in Discovery of New Types of Antibiotics



Source: Adapted from Lynn L. Silver, "Challenges of Antibacterial Discovery," Clinical Microbiology Review (2011)
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Why Do We Need New Classes of Antibiotics?

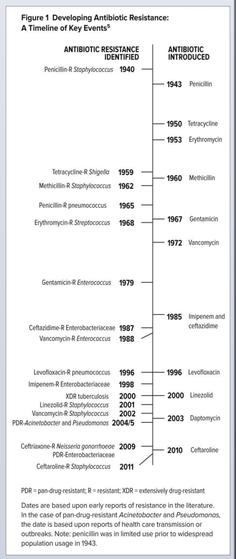
Antibiotics can be categorized based on similarities in their chemical structures (i.e. antibiotic classes). Resistance to one antibiotic often leads to resistance to multiple antibiotics within the same class.

Introduction

It has become such a routine in our modern society when we become sick to take antibiotics and in a few days the infection is gone. During this routine, many of us have never second guessed what would happen if these antibiotics were to suddenly stop working. A reason for this could be something called antibacterial resistance. Antibacterial resistance occurs when the bacteria that antibiotics are targeted to kill, become resistance to the antibiotic, causing the bacteria to survive and multiply. Antibacterial resistance is a rising problem worldwide, and one that has very little attention. Models and studies indicate deaths from antibacterial resistant strains of common diseases will outpace deaths from cancer in 2050.

A common strain of bacteria that is becoming more and more resistant to antibiotics is *Salmonella*. A certain strain of *Salmonella* called *Salmonella* Typhimurium is most common in the United States. The basic symptom of this is gastroenteritis, and lasts up to a few days. This bacteria is commonly transferred between animal feces, and ultimately ends up in livestock. So, consuming undercooked poultry increases the risk of contracting *S.* Typhimurium. Antibiotics are not required for *S.* Typhimurium but in severe cases, they may be necessary.
In an attempt to combat this growing issue, our team chose to test the properties of Curcumin. Curcumin is bright yellow chemical that is derived from the *Curcuma longa* plants. Curcumin is a component of turmeric which is most widely used for its anti-inflammatory properties due to its antioxidant mechanisms. Curcumin shares similar properties to turmeric and has been used to treat inflammation disorders, diabetes, and different kinds of arthritis.
Studies performed on bacteria and fungi show curcumin as a promising antibacterial, especially in the work done by Yu Wang et al. In different experiments, his team was able to prove curcumin worked as an antimicrobial and antifungal using different concentrations of Curcumin in different cups. Another study performed by Emanuele Marini et al. demonstrated Curcumin was a viable synergistic compound when paired with traditional antibiotics that increased inhibitory effects of antibiotics against a resistant strain of Tuberculosis. These two works alone provide substantial evidence to the antimicrobial properties Curcumin possesses.
Based on our preliminary research and studies performed by other researchers, we believe Curcumin presents a viable candidate as an antibiotic against *Salmonella* Typhimurium. As antibiotic strains of all types of bacteria surface around the world, the need for the drugs to fight them grows more intense. This is a race against time and nature to find drugs that will fight off the next possible pandemic.

Figure 1 Developing Antibiotic Resistance: A Timeline of Key Events[5]



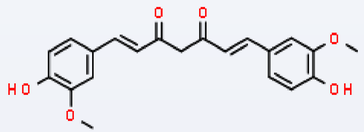
PDR = pan-drug-resistant; R = resistant; XDR = extensively drug-resistant

Dates are based upon early reports of resistance in the literature. In the case of pan-drug-resistant *Acinetobacter* and *Pseudomonas*, the date is based upon reports of health care transmission or outbreaks. Note: penicillin was in limited use prior to widespread population usage in 1943.

Developing antibiotic resistance: a timeline of key events:
This chart highlights some major developments in antibiotic medications and the acquisition of resistance in bacteria. (Ventola 2015)

Hypothesis

It is hypothesized that Curcumin extract will exhibit an antimicrobial property on *Salmonella* Typhimurium in a dose dependent manner when tested at 37 ℃ for 48 hours.



Chemical Structure of Curcumin (C21H20O6)
Picture Provided by (ChemSpider.com)

Turmeric: The parent source of Curcumin

Objectives

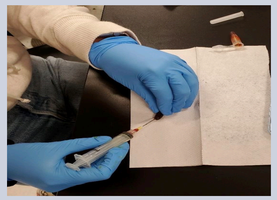
Our objectives were to:

- To test a prospective compound to prove its antibiotic properties will eliminate or stop growth of *Salmonella* Typhimurium.
- Predict and form hypotheses about the behavior of the compounds.
- Perform an experiment that will identify 'hits' and will identify the compound as a possible antibiotic.

Methods

The Curcumin was plated in two 96-well plates, the first containing the powdered form of Curcumin that was then tested at different concentrations. (mg/ml) (0.7, 1.5, 2.9, 5.9, 11.7, 23.5, 47.0, and 93.9). The second form of Curcumin tested was in capsule form that was then serially diluted in a (1:10) dilution. The concentrations for the pill form was the following: (80 mg/ml & 0.8 mg/ml). The concentrations were taken at random within the parameters of 0-100 mg/ml in which the 2% ethanol solution that was used as the negative control was soluble in. 2% ethanol was the negative control for all plates including powdered and capsule form. This was concluded to be the preferred ethanol concentration after testing a separate plate in which different concentrations of ethanol were tested. The positive control for all plates was Ampicillin due to the known antibiotic properties of it. 90 µL of *Salmonella* and 10 µL of compound, negative, or positive control were pipetted in each well. The plates were incubated for 48 hours before being read for absorbance.

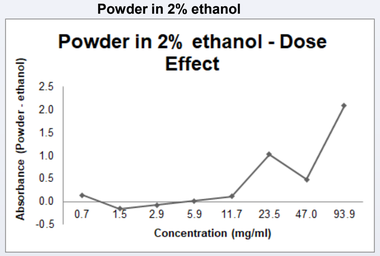
Curcumin came in a tablet, pictured below on the right. Therefore, before testing began, Curcumin had to be extracted from its 'pill' form using a syringe. The reagents used were provided by the University of Colorado at Boulder.



Results

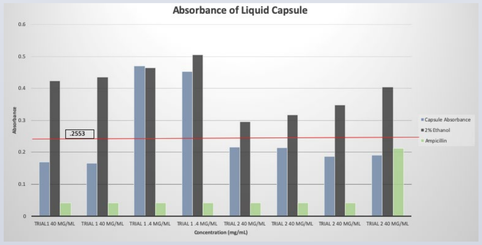
After testing liquid and powder curcumin, we found that liquid curcumin in 2% ethanol showed statistical hits. 'Hits' are identified by comparing the mean absorbance of the compound to the mean absorbance of the negative control (2% ethanol). After calculating the standard deviation of the negative control, anything outside of the range of two standard deviations from the mean is considered a hit. Two standard deviations below the mean meant that there was a low absorbance rate of *Salmonella* in the well, indicating the compound killed the *Salmonella*. This is the type of 'hit' we are looking for in a potential antibiotic.

Powder in 2% ethanol



Curcumin Powder (Serial Dilution) Absorbances: The graph above shows the differences in absorbance of the various concentrations of powder curcumin in 2% ethanol. The powder mean absorbance came out to be 0.9723 while the mean of negative control, 2% ethanol, is 0.5176. This data set included the extreme outliers. If excluding the outliers, the mean of the powder will decrease to 0.5771 which is closer to the negative control. No absorbance in the data set came out to be outside of the 2 standard deviation range from the mean of 2% ethanol, so we could not conclude that there was a hit with the power and 2% ethanol solution.
The graph shows what was the opposite of what was expected--for curcumin to kill the *Salmonella*. As powder concentration increased, the absorbance increased which infers that *Salmonella* increased by feeding on the compound or that curcumin powder itself absorbed the light calculating the absorbance.

. **Capsule in 2% ethanol**



Curcumin Capsule at 4 mg/ml & 40 mg/ml Absorbances:
The graph above shows the average absorbance of 2 different trials. For Trial 1 we tested 2 of the same concentrations of 80 mg/mL of pure liquid curcumin. Then we diluted the 80 mg/mL into a 1:10 dilution (.8 mg/mL) and tested 2 of the same concentrations of it. The graph shows that the diluted concentration of the capsule was growing at an almost identical rate of the negative control, 2% ethanol. Based on these results, we determined the diluted capsule could not qualify as a hit because it had little effect on the *Salmonella*. The results from the 80 mg/mL concentration looked promising because the number was considerably smaller than the negative control average. We ran the same concentration again for the second trial. The red line indicates 2 standard deviations below the mean. Anything below this line is considered a hit because it caused the *Salmonella* to die. We can conclude that the highest concentration of the liquid capsule, 40 mg/mL, killed *Salmonella*. Since it is below the red line, it is a hit.

Effect of concentration on *Salmonella*:
Powder curcumin showed opposite results from what was expected, a positive correlation. The absorbance increased as the concentration increased. With the liquid curcumin, the results were as expected. It showed that at higher concentrations of curcumin, more *Salmonella* was killed. Diluted curcumin from the capsule caused *Salmonella* to grow at almost the same rate as the negative control.

:

Conclusions

Our tests of Curcumin powder and liquid capsules as potential antibiotics resulted in moderately good results. The Curcumin powder tested did not kill adequate amounts of *Salmonella*. The average absorbance value of the liquid extracted from the capsules was just shy of our two standard deviation mark, and killed a large number of *Salmonella* Typhimurium in our tests. It should be noted that all 40mg/mL results included in the ethanol solutions were below the required two standard deviations from the mean, but when the diluted Curcumin trials are included the average value is 0.003 above the two standard deviation mark. We can conclude that the pure liquid Curcumin should be investigated as a potential antibiotic, but the powder form should not.
Early work with the powdered form of Curcumin resulted in very high absorbance values, which normally indicate *Salmonella* growth, but were in fact the result of poor laboratory solution preparation. When those high values were removed, absorbance averages were closer to normal. The very high absorbance values were approximately fifteen standard deviations above the mean. Those particular trials were noted to have clumps of the curcumin powder in the wells due to exceeding the solubility of ethanol. Work with the Curcumin liquid capsules was difficult because precise measurements of the viscous liquid were hard to obtain.
Future experiments should focus on proper laboratory solution preparations to verify the results of the powder and liquid tests. The major issue we encountered was proper Curcumin solution preparation when using the powdered form. While we attempted to test the same concentration of liquid and powder, we quickly found out that we could not do this because the solubility of the powder was much lower in ethanol, DMSO, and water. This was the main obstacle to our work with the powder, and the reason we believe our trials with that substance went so poorly.
Precise use of pipettes should also be a focus for future experiments and would verify liquid capsules as a possible antibiotic. Other tests might look at less viscous forms of liquid Curcumin to see if accurate and precise pipetting will give the same results.
Long term experiments should look at analogues of Curcumin as possible antibiotics as well, following the same or similar procedures as performed in this experiment. Research and experimentation of analogues could reveal an antibiotic based on Curcumin that works just as well if not better than currently available drugs. Other tests and research might go further and test Curcumin against *Salmonella* living in cells. This would test whether or not Curcumin can actually target *Salmonella* in something analogous to its normal environment in a host body. Further experimentation would include clinical trials using varying concentrations of Curcumin to test the efficacy in first mice, and possibly humans if the compound or an analogue of Curcumin shows promise as an antibiotic.

Future Directions

Data from Drug Discovery Lab will be transferred to the Detweiler lab. Future research could include:

1. Retesting the capsule form of curcumin as the results came back as a hit, meaning it was less than 2 standard deviations from the mean.
2. Using DMSO as the solvent to see if concentration can be increased as curcumin is more soluble in DMSO.
3. Letting the liquid capsule curcumin be centrifuged with ethanol in an extraction in order to remove any excess materials such as glycerin and gelatin.

Acknowledgements

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References

Wang, Yu, et al. "Study on the Antibiotic Activity of Microcapsule Curcumin against Foodborne Pathogens." *International Journal of Food Microbiology*, Elsevier, 8 Sept. 2009, www.sciencedirect.com/science/article/pii/S0168160509004668.

Marini, Emanuela, et al. "Curcumin, an Antibiotic Resistance Breaker against a Multiresistant Clinical Isolate of Mycobacterium Abscessus." *Wiley Online Library*, John Wiley & Sons, Ltd, 29 Nov. 2017, onlinelibrary.wiley.com/doi/full/10.1002/ptr.5994.

Prasad, S. (1970, January 1). *Turmeric, the Golden Spice.* Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK92752/.

Kali, A., Bhuvaneshwar, D., Charles, P. M. V., & Seetha, K. S. *Antibacterial synergy of curcumin with antibiotics against biofilm producing clinical bacterial isolates.* Retrieved December 1, 2019, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4910474/. (2016, June).

A Scientific Roadmap for Antibiotic Discovery.). Retrieved December 1, 2019, from https://www.pewtrusts.org/en/res search-and-analysis/reports/2016/05/a-scientific-roadmap-for-antibiotic-discovery. (2016, May 11)