JOURNAL OF
EMERGING INVESTIGATORS

Article

Antibacterial effectiveness of turmeric against gram-positive *Staphylococcus epidermidis*

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SUMMARY

Infections caused by antibiotic resistance are a leading issue faced by the medical field. A potential solution to this involves using common household spices as natural antibacterial agents to reduce infections. One option may be turmeric, as it has high levels of polyphenols that have previously exhibited antibacterial properties. We studied the antibacterial effectiveness of turmeric against gram-positive *Staphylococcus epidermidis* using antibiotic sensitivity disks. The experiment had a negative control, positive control of Penicillin, and experimental group with turmeric. We infused blank antibiotic sensitivity disks with a 5% concentrated solution of turmeric and placed them on agar plates inoculated with bacteria. The zone of inhibition (ZOI) surrounding each disk was measured to determine antibacterial effectiveness. However, there was no measurable ZOI surrounding the turmeric disk so the measurements for all trials were 0 cm, suggesting that turmeric at a 5% concentration is not an effective antibacterial against *S. epidermidis*.

INTRODUCTION

Various strains of bacteria have produced antibiotic resistance to certain antibiotics. Although this can occur naturally, the process is sped up when antibiotics are misused and overused by both humans and animals (1). According to the Centers for Disease Control and Prevention, more than 2.8 million antibiotic-resistant infections occur in the United States each year making it one of the most difficult challenges faced by the medical establishment (2). Rather than creating new antibiotics, an economical and renewable solution is the use of herbs and spices as natural antibacterials. Many spices, like turmeric and cumin, have been used for centuries to fight infections due to their high polyphenolic content (3). Polyphenols are naturally occurring organic compounds that are abundant in plants that work as antioxidants in the human body and protect cells against diseases and foreign cells (4). Since many herbs that possess antimicrobial properties are incorporated into our food, it would be easy to use them to fight against bacteria that negatively affect human health.

This research focused on the antibacterial effectiveness of turmeric against gram-positive *Staphylococcus epidermidis*. By conducting this research, we hoped to have expanded the knowledge of turmeric as a natural antibacterial and its potential implementation in the medical field. Using herbs as a substitute for antibiotics will decrease the number of antibiotic-resistant bacteria, thus making current and future antibiotics more successful. The use of natural antibacterials will also decrease the number of bacterial infections caused by antibiotic resistance.

We hypothesized that turmeric would have an antibacterial effect on *S. epidermidis*. If this hypothesis is correct there will be a measurable zone of inhibition (ZOI) surrounding the turmeric-infused disks. Additionally, presuming that the ZOI measurements of the turmeric disks are less than that of penicillin, it was hypothesized that the antibacterial effectiveness of turmeric would be less than that of penicillin. The effectiveness will be based on the ZOI around the disk.

RESULTS

Table 1 shows the average ZOI measurements for all tested treatments: positive control, negative control, and turmeric. The data for the turmeric plates and positive control is compared in **Figure 1**. No statistical analysis was done as the ZOI measurements for turmeric were all 0 cm.

An unfamiliar growth occurred during Experiment #1, which led to many troubleshooting steps to solve the problem (**Figure 2**). The first change made to the procedure was to add exactly 20uL of the turmeric solution to each diffusion

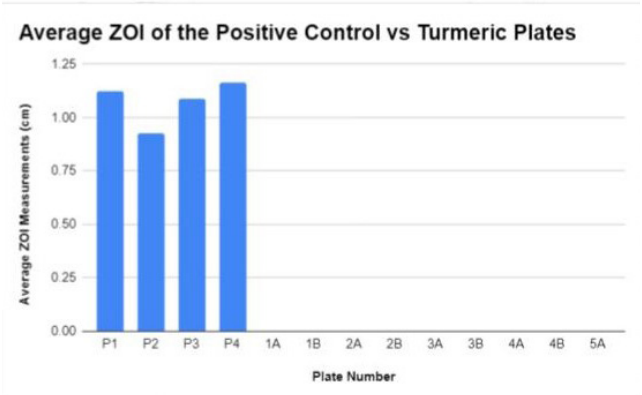

Figure 1. Zone of inhibition measurements. The bars represent the average ZOI measurements for the positive control and turmeric plates corresponding to the plate number listed in the table. Since the ZOI measurements for the turmeric plates are zero there are no representative bars.

Table 1. Zone of inhibition measurements.

Spice	Plate #	Average ZOI (cm)
Positive Control	P1	1.1250
	P2	0.9250
	P3	1.0875
	P4	1.1625
Negative Control	N1	0
	N2	0
	N3	0
	N4	0
5% Concentrated Turmeric	1A	0
	1B	0
	2A	0
	2B	0
	3A	0
	3B	0
	4A	0
	4B	0
	5A	0

NOTE: *The ZOI measurements for the positive control (n = 4), negative c`ontrol (n = 4), and turmeric (n = 9). Measurements were taken after a 96-hour incubation period.*

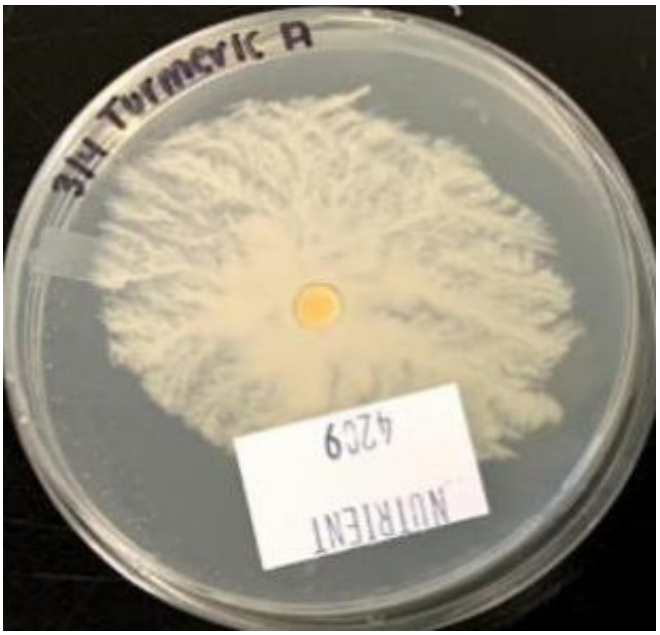

Figure 2. Example of unknown growth. This is the unknown growth that surrounded the turmeric-infused disks after using the procedure in Experiment #1. The procedure was then altered for Experiment #2 after viewing this unexpected result.

disk and allow them to dry, as it was thought that the disks were too saturated and the solution was spreading across the plates. After incubation, the unknown growth was again present.

Aiming to solve the problem, a new *S. epidermidis* broth culture was purchased, and a 5% turmeric solution was made. The solution was sterilized to rule out contaminated turmeric as the issue. The solution was poured into a petri dish and sterilized with ultraviolet light in a biosafety cabinet for 10 minutes. These disks were then added to plates using the same procedure as before with the new bacterial culture. After incubation, the same unknown growth surrounded the turmeric disks.

Next, we assessed if the turmeric was too highly concentrated and inhibiting bacterial growth on the whole plate. The 5% solution was then diluted to a 0.5% and 0.05% concentration and added to bacterial plates the same way as before. Unfortunately, the unknown growth was still present around the disks after incubation. To determine if either the turmeric or blank disks were the issue, the same procedure was used to make a 5%, 0.5%, and 0.05% concentration of McCormick ginger. Ginger was chosen due to its high amount of gingerols which have been used in the past as a natural antibacterial (6). The procedure in Experiment #2 was used to add ginger disks to bacterial plates. After incubation, the same unknown growth surrounded the disks. This led to the conclusion that the blank disks were the underlying issue and caused the unknown growth because both turmeric and ginger exhibited the same results.

To fix this, new blank BBL brand antibiotic sensitivity disks were purchased. We additionally tested McCormick cinnamon and McCormick nutmeg along with turmeric (data not shown). Ten blank disks were infused with a 5% turmeric concentration, six were infused with a 5% cinnamon concentration, and six were infused with a 5% nutmeg concentration. The disks were all added to their respective bacteria plates and incubated for the standard time period, and we observed no unexpected growths (**Figure 3**). The data is not shown for the ginger, cinnamon, and nutmeg.

DISCUSSION

This research was intended to determine the antibacterial effectiveness of turmeric at a 5% concentration against gram-positive *S. epidermidis*. The hypothesis of this study was that turmeric would have an antibacterial effect on the chosen bacteria. The data did not support the hypothesis, as there was no ZOI surrounding the 5% turmeric-infused diffusion disks. Since there was no ZOI, this means that turmeric, at a 5% concentration, did not inhibit the bacterial growth. This was surprising because turmeric has shown anti-inflammatory

Figure 3. Example of a plate from Experiment #2. After using a new procedure in Experiment #2 with turmeric-infused disks, the plates did not exhibit the unknown growth that was present in Experiment #1. There is no zone of inhibition surrounding these disks.

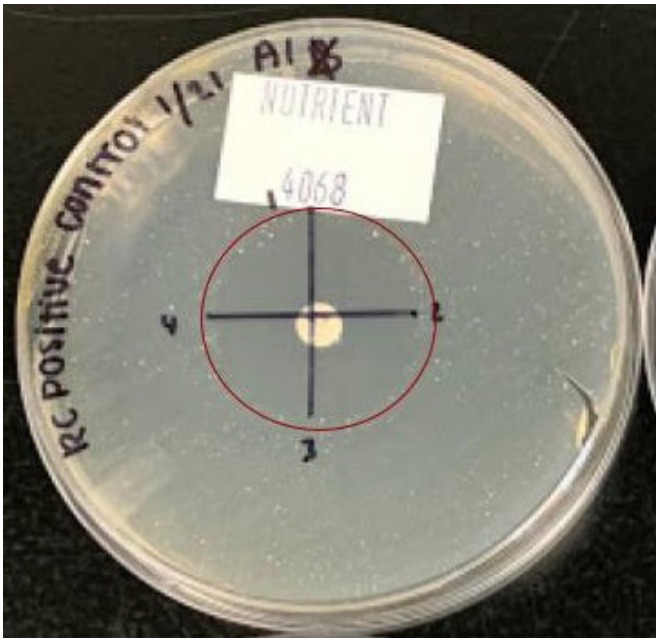

Figure 4. Positive control plate. A penicillin-infused diffusion disk was placed on a plate cultured with *S. epidermidis* and incubated for 96 hours. The area inside the red circle represents the zone of inhibition caused by penicillin.

properties in previous studies (3). Further research is needed to eliminate turmeric as an effective antibacterial agent, including, but not limited to, testing turmeric against other species of bacteria, testing other concentrations of turmeric, infusing turmeric into nutrient agar, or using turmeric in combination with other spices. Additionally, it was hypothesized that the antibacterial effectiveness of turmeric will be less than that of penicillin. This was supported by the data because the ZOI measurements of the penicillin disks were greater than the ZOI measurements of turmeric.

There were many limitations throughout the course of this experiment. The predominant limitation was the COVID-19 pandemic, as classes would be switched to remote for a week or two at a time. This was quite challenging because most of the lab work needed to be done in the school laboratory. The project was also limited in time because there was about a 15-week period to conduct research, with only two to three class periods per week. This limited the project to use only one testing method and one final concentration of turmeric. If there was a longer time period it also would have been possible to test against both gram-positive bacteria and gram-negative bacteria, rather than only gram-positive. Another limitation was the expense, with a larger budget more supplies could have been purchased to conduct more trials. Finally, if the school's laboratory was more advanced with proper safety equipment, like a biosafety cabinet and biohazard disposal method, we would have been able to test turmeric against bacteria like *Escherichia coli*. Although this research did not go as planned, it was a great way to see the scientific process by exhibiting various problem-solving techniques.

MATERIALS AND METHODS

In this study, the effectiveness of turmeric was tested as an antibacterial agent against *S. epidermidis*. A 10% and 5% concentration of turmeric was used as an experimental group along with a blank negative control and positive control of penicillin. These concentrations were chosen based on previous research on the antibacterial effectiveness of common spices (5).

Positive and negative control

To determine an optimal incubation period, the bacterial culture was inoculated on an agar plate, purchased from Carolina Biological Supply Company, using an aseptic technique with a sterile swab dipped in the nutrient broth culture. The agar plate was then incubated at 37° C for 48 hours. Since there were no visible bacterial colonies at this time the incubation period was increased to 96 hours. This was then set as the standard incubation period for the remainder of the experiment. The disk diffusion method was modeled after a previous study that examined the antibacterial activity of various plant extracts (6).To conduct a positive control, four agar plates were inoculated with bacteria and a Biogram penicillin-infused antibiotic sensitivity disk was placed in the center of each plate using sterile forceps (**Figure 4**). The forceps were sterilized in a small equipment sterilization box and a flame to ensure no outside contaminants affected the results. After incubation, the ZOI was measured using a ruler

by sectioning the zone into four quadrants and finding the mean length of each quadrant (**Figure 4**). The ZOI surrounding the disks was recorded to show that *S. epidermidis* growth could be inhibited by penicillin. To conduct a negative control group, the same procedure was done with blank, sterile Biogram antibiotic sensitivity disks.

Experiment #1

First, a 10% turmeric solution was made by mixing 1g of Good & Gather ground turmeric and 10 mL of sterile water. Then, twelve blank sensitivity disks were soaked in the turmeric solution for one minute. Next, using the same procedure as when conducting the positive and negative controls, three agar plates were inoculated with bacteria and sectioned into quadrants, with each quadrant receiving a turmeric-infused disk. These plates were then incubated at 37° C for 96 hours.

Experiment #2

A 5% concentrated turmeric solution was made by mixing 30mL of sterile distilled water and 1.5 g of ground turmeric. Different sterile, blank disks were used in this study and were purchased from the brand BBL. In order to infuse each disk with the same amount of solution, a micropipette was used to add 20 µL of the turmeric solution to each of the nine blank disks. These disks were then allowed to dry for thirty minutes in a sterile petri dish. Next, the agar plates were inoculated with the broth culture and sat for fifteen minutes before the turmeric disks were added. These plates were also incubated at 37°C for 96 hours, and the ZOI was measured on each plate.

ACKNOWLEDGMENTS

Throughout this process, I was in close contact with Dr. Ed Robinson (volunteer for the MSA research class who specializes in veterinary science and biology) for advice on my ever-changing procedure and recurring dilemma.

Received: June 9 , 2021
Accepted: October 31, 2021
Published: January 10, 2022

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