

Effect of pH on the antibacterial properties of turmeric

Harshita Ganga¹, Samantha McDonald²

¹William Mason High School, Mason, Ohio

²Duke University, Durham, North Carolina

SUMMARY

Some spices have antimicrobial or antibacterial properties that people have already tested. For example, turmeric is a spice in the ginger family that has been shown to reduce bacterial growth. Turmeric has a wide variety of uses and has even been implemented in alternative medicine as a treatment for cancer, inflammation, osteoarthritis, and other diseases. The chemical properties of turmeric often changes depending on the temperature and pH, which could influence the efficacy of turmeric as an antibiotic. Thus, we tested the antimicrobial effects of turmeric under two different pHs to characterize this effect *in vitro*. While all turmeric treatments decreased bacterial growth, the acidic treatment appeared to have the strongest effect on inhibiting the growth of cultured bacteria. In conclusion, decreasing the pH of a solution of turmeric may increase antibacterial properties, an effect that should be studied further to better understand any potential antibacterial treatments.

INTRODUCTION

Antibiotic resistance is often caused by the overuse of antibiotics. When paired with the high mutation rate that is inherent to bacteria, the overuse of antibiotics can result in multi-drug resistant bacteria. For example, *Salmonella enteritidis* is a multi-drug resistant, pathogenic bacterium that is found in certain foods, and any preservatives that are used to control its growth often have carcinogenic properties (1). Bacteria evolve to be resistant to antibiotics through overuse, which counteracts the 50 to 70 billion dollars that Americans spend to create antibiotics every year (2). One of the methods that we can use to combat antibacterial resistance is household spices. Spices such as cinnamon and cumin have been shown to possess natural antibacterial properties against certain pathogenic bacteria (3). Turmeric, specifically, is a spice derived from the root of the plant *Curcuma longa*, which is a part of the ginger family (4). Turmeric has many uses including as a food condiment, cure for liver ailments, and laxative (5).

Curcumin is the active ingredient of turmeric and is the source of many of the antibacterial properties of turmeric, among other properties (Figure 1) (6). Curcumin is a dimeric derivative of ferulic acid (Figure 2) (6). In 1949, it was shown to have antibacterial properties *in vivo*. It was also shown that it had high efficacy against Gram-positive cocci, spore-forming bacilli, some Gram-negative bacteria, and fungi (5). This behavior occurs as curcumin inhibits quorum sensing

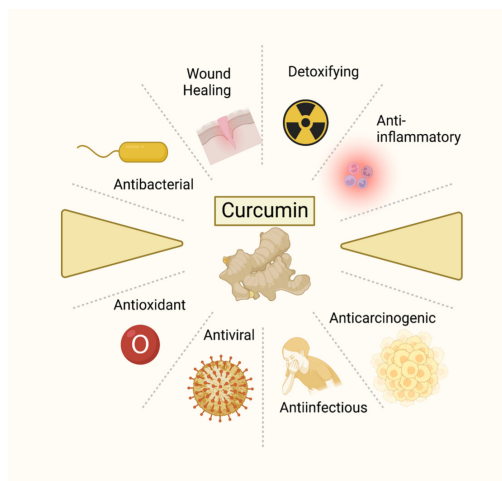


Figure 1: Properties of Curcumin. Curcumin has several intrinsic therapeutic benefits (e.g., antibacterial properties, antioxidant properties) which make it favorable for biomedical applications (6). This figure was created with Biorender.

(the communication system between bacteria) (6). Similarly, curcumin destroys biofilms (bacterial cells enclosed in a polymeric matrix) which limits the population density (6).

In this research study, we explored the effect of pH on the efficacy of the antibacterial properties of turmeric. A previous study found that synthetic antibiotic efficacy was highest at pHs between 6.5 and 7.5 (7). Another study showed that Rifampin is significantly more effective at acidic pHs and Chlorpromazine is significantly more effective at basic pHs (8). Although the effect of pH has been studied on the commonly-used FDA-approved antibiotics, studies looking at this effect on the antibacterial properties of spices have been lacking. We hypothesized that changing the pH of the

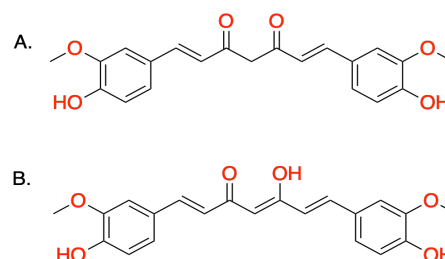


Figure 2: Chemical Structure of Curcumin. Curcumin is a lipophilic polyphenol with a chemical formula of C₂₁H₂₀O₆ and a molecular weight of 368.38 g/mol (5). Structure A shows curcumin in an acidic or neutral environment. Structure B shows curcumin in a basic environment.



Figure 3: Bacteria growth on agar plates two weeks after turmeric treatment. Bacteria were grown in the petri dishes and then treated with turmeric at different pHs (acidic, neutral, and basic). The resulting colonies are shown one week after treatments were added. Colonies were allowed to grow for another week before they were counted. The cloudiness below the colonies is the turmeric treatment. Colonies showed up as little white dots on the petri dishes. In the positive control, there is a lawn of bacteria that is hard to distinguish from the turmeric treatment.

turmeric solution would affect the number of cultured bacteria able to grow on agar plates. We predicted that at extreme pHs, turmeric would be more effective in killing bacteria than at neutral pH, since most bacteria are neutrophiles, which means they optimally grow at a pH near 7 (9). Due to known tautomerization of curcumin (**Figure 2**) in alkaline conditions, we believe that the basic pH will differ from the acidic and neutral pHs.

RESULTS

In this experiment, we exposed bacteria swabbed from a doorknob to turmeric at different pHs. For the acidic, neutral, and basic treatment groups, we prepared solutions of turmeric at pH 2, 7, and 9, respectively. We found that the acidic treatment had the least number of colonies ($M=438$, $SD=31$) (**Figure 3,4**). The average number of colonies in the neutral treatment was the highest ($M = 740$, $SD = 20$) (**Figure 3,4**). Lastly, the average number of colonies in the basic treatment (just turmeric) was intermediate ($M = 556$, $SD = 25$) (**Figure 3,4**). One-way ANOVA indicated that pH had a significant effect on bacterial growth ($F_{2,6} = 5.14$, $p = 0.007$). However, post-hoc F-tests indicated that the tested treatments did not statistically significantly affect bacterial growth (acidic vs. neutral: $F = 0.99 > F_{crit} = 0.05$, $p = 0.50$, basic vs. neutral:

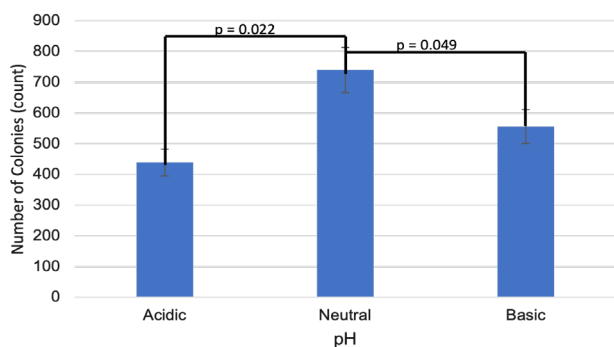


Figure 4. Average bacterial colonies two weeks after treatment. Bar graph showing mean \pm SD colonies of bacteria. Bacterial cells were grown in different pHs and treated with turmeric. The resulting colonies were counted and averaged. Statistical significance was assessed via one-way ANOVA followed by post-hoc F-tests.

$F = 0.068 > F_{crit} = 0.053$, $p = 0.06$). Similarly, there appeared to be no statistically significant difference between the acidic and basic groups ($F = 0.069 > F_{crit} = 0.053$, $p = 0.06$).

DISCUSSION

Through this study, we aimed to find the effect of pH on the efficacy of turmeric as an antibiotic. We hypothesized that extreme pH of turmeric solutions would cause a decrease in bacterial growth. Turmeric's partial inhibition of bacterial growth was stronger when the turmeric was adjusted to acidic pH as the total number of colonies decreased. It is important to note that, while standard deviation shows a significant difference between the results, the ANOVA test did not, indicating that more observing more pHs or trials will give better results. The bacteria grown in a basic environment resulted in more colonies than the acidic environment, yet less than in the neutral environment. Therefore, turmeric may have antibacterial properties which could make it of interest for antibiotic research. The results of this experiment were unexpected, as commercially available measures being taken against bacteria are basic, such as bleach. Therefore, it was surprising that the efficacy of the turmeric treatment was best when acidic. The results were consistent with the hypothesis as the basic and acidic pHs were predicted to perform differently, and the acidic pH showed fewer colonies than the basic pH. Curcumin undergoes keto-enol tautomerization, which could explain the differences of the efficacy of turmeric as an antibiotic at different pHs. At acidic pHs, the diketone form is prevalent, while at basic pHs, the keto-enol form is prevalent (See Figure 2) (11). Immediate future directions include studying other types of pH treatments and repeating this experiment in a sterile environment. We also would like to investigate the efficacy of pH treatments on other types of bacteria. It is also important to study the effects of this research in vivo since this experiment was done in vitro.

This data contains limitations as the experiment was performed in a home, which is a non-sterile environment, and conditions may have varied as the experiment was not performed in strictly controlled conditions. One potential area of future study is to look at the sole effect of pH on bacteria, so that we can compare those results to the ones with turmeric. In addition, the types of bacteria used were not identified. Common bacteria found in doorknobs include *S. aureus*, the *Pseudomonas* species, and *E. coli* (10). Further research could focus on using pH treatments on specific cultures of bacteria or using different spices and pH adjustments to check the effect of changes in pH. There is also the possibility of mutation or contamination. However, there were no observable changes in border or color, leading us to believe that mutations are not likely to be a source of experimental variability. Results may have varied as the turmeric was prepared at home and the turmeric solution wasn't filtered.

Continued research can advance the efforts of previous research in combating the rise of antibiotic resistance or finding an alternative to antibiotics altogether (2). Household spices are often used as treatments when an individual cannot visit a medical professional. This research builds on using turmeric as an alternative to FDA-approved antibiotics (12). Research has shown the importance of finding new, accessible, and abundant antibiotics as bacteria have slowly begun to become resistant to even alcohol sanitizers (a method that has been advertised as killing 99% of bacteria in

a single use) (13). Ultimately, more effective treatments can be beneficial in overcoming antibiotic-resistant bacteria.

MATERIALS AND METHODS

Preparation of Spices:

Turmeric was shipped from India, where it was prepared by drying and grinding roots of *Curcuma longa*. Then, it was stored in stainless steel containers until it was used for the experiment.

Preparation of Luria Bertani (LB) agar plates:

1000 mL of LB agar stock was melted in a microwave. 15 agar plates were divided into the 5 groups and labelled with the group and the plate number. The label was on the bottom of the plate, so that bacteria would be clearly visible. LB agar was poured into each petri dish, so that a layer of LB agar completely covered the bottom of the dish (approximately 12 mL of agar). Then, the LB agar in the petri dishes was left out to solidify overnight (with lids on) at room temperature.

Culturing and subculturing the bacteria:

First, the bacteria were swabbed from a door handle by a dry cotton swab. Then the swab was dipped in 5 mL of LB broth. The mixture of the broth and the bacteria was left to sit for 3 days. Then, the T-streaking method was used to spread three drops of the bacteria culture onto a petri dish. The petri dish was left to sit for a week. Then, a single culture was taken by a cotton swab and mixed into 5 mL of LB broth. This mixture was left to sit for 3 days. Then, three drops of this mixture were added to the experimental group's dishes using an L-shaped spreader. This method involves taking the L-shaped spreader and slowly pushing the bacteria across the plate so that the solution covers the whole plate. The bacteria were grown in a dark room at a temperature of 21 degrees Celsius.

Spice Treatments:

The neutral spice treatment was a saturated solution of turmeric in water. This was prepared by putting an excess of turmeric in water. Excess turmeric was not in the solution. The acidic spice treatment was created by mixing 3 mL of vinegar (5% acidity) and 3 mL of the neutral saturated turmeric solution. The basic spice treatment was created by mixing 3 mL of saturated solution of sodium bicarbonate in water and 3 mL of the neutral saturated turmeric solution. Three drops of the spice treatment were added to the respective petri dishes after the bacteria was spread. Then, pHs were measured using pH paper and recorded. The pH of the acidic solution was 2. The pH of the neutral solution was 7. The pH of the basic solution was 9.

Colony Counting:

To analyze the growth, colonies were counted manually. The bacteria were counted 2 weeks after subculturing and treatment.

Statistical Tests:

ANOVA tests with post-hoc testing were performed in Excel to assess the statistical significance of our findings. We used $\alpha = 0.05$ and $n = 3$.

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