JOURNAL OF
EMERGING INVESTIGATORS

Article

Antimicrobial properties of common household spices on microbes cultured from two kitchen locations

Kingsley Carroll[1]*, Trinity Coleman[2]*, Yousuf Yousuf[3]*, Choah Kim[4], & Michael Kelberman[5]

[1] Commonwealth Charter Academy, Harrisburg, Pennsylvania
[2] Classical Conversations, Easton, Massachusetts
[3] Palm Tree School, Fairfax, Virginia
[4] Harvard University, Cambridge, Massachusetts
[5] Emory University, Atlanta, Georgia
*Authors contributed equally

SUMMARY

The number of bacterial infections in humans is rising, and a major contributor is foodborne illnesses, which affect a large portion of the population and result in many hospitalizations and deaths. Common household cleaners are an effective strategy to combat foodborne illness, but they are often costly and contain harmful chemicals. Thus, we sought to test the antimicrobial effectiveness of readily available, low cost, and relatively harmless spices (clove, nutmeg, astragalus, cinnamon, turmeric, and garlic) on microbes cultured from refrigerator handles and cutting boards. We hypothesized that cutting boards would have microbes that were more resistant to spices given the properties that make them conducive to microbial growth, whereas the microbes cultured from refrigerator handles would be more prone to the antimicrobial effects of the spices. We found that clove, garlic, and nutmeg were able to completely prevent the growth of microbes from cutting boards. Clove and garlic also prevented microbial growth from refrigerator handle samples, but nutmeg only reduced the growth of these microbes over a week. Similarly, turmeric and cinnamon only reduced the growth of microbes over a week from both culture samples. Our results demonstrate long-lasting, antimicrobial effects of multiple spices that support their use as alternatives to common household cleaners.

INTRODUCTION

Bacterial infections are rising, which places a significant burden on the economy and healthcare sector (1). More specifically, foodborne illnesses affect a large portion of the population annually, resulting in an estimated 70,000 hospitalizations and 1,600 deaths in 2011 (2, 3). Of the total number of foodborne illnesses, approximately 39% were a result of bacteria (2, 4). Evidence also suggests that kitchens contain some of the highest amounts of bacteria in a household (5). To minimize the risk of foodborne illnesses, proper sanitization of kitchen surfaces is essential. However, common household cleaners can be expensive and toxic, which has led consumers to seek alternative, ecofriendly, and low-cost cleaning products (6–8).

One promising avenue to pursue as an alternative cleaning solution is the use of spices. Studies have previously demonstrated antioxidant and anti-inflammatory effects of spices in cells, rodents, and humans, in addition to lowering the risk of some diseases (9). However, evidence also suggests that their use could be expanded past medicinal properties, such as use as antimicrobials. One report tested whether 11 spices could inhibit the growth of *Escherichia coli* (*E. coli*) bacteria (10). 5 of the 11 spices were effective in inhibiting bacterial growth: clove, cinnamon, garlic, oregano, sage, and thyme (10). These results support the antibacterial properties of some spices against specific bacteria. In addition, household spices are natural and easily obtainable; thus, they warrant further study on whether they are effective antibacterial agents, especially in household areas such as the kitchen.

In the present study, we chose to assess three previously tested (garlic, clove, and cinnamon) and three new (nutmeg, turmeric, and astragalus) spices for their potential antimicrobial properties. Although garlic, clove, and cinnamon have previously been shown to be effective at inhibiting the growth of *E. coli*, the kitchen contains a diverse population of microbes on which these spices have yet to be tested. We also chose to test new spices, nutmeg, turmeric, and astragalus, which were not tested by the previous researchers, but have been shown to contain some antibacterial properties. For example, curcumin, which is the primary compound in turmeric, can kill both gram-negative and gram-positive bacteria (11). Meanwhile, evidence suggests that nutmeg might selectively target pathogenic bacterial strains of *E. coli* and *Streptococcus* bacteria (12,13). Astragalus has also been shown to have antibacterial properties (14).

We chose to swab and culture microbes from two different locations in the kitchen: a cutting board and refrigerator handle. We reasoned that different areas of the kitchen may contain different populations and amounts of microbes with variable sensitivities to spices. For example, a cutting board is cleaned after almost every use and may contain grooves after repeatedly being used to prepare food. Through this process, more resistant microbes could emerge through selection. Meanwhile, the refrigerator handle typically is a less often cleaned, highly touched surface, which could suggest that microbes here would be less exposed to common household cleaners and may be easier to kill. In addition to microbial resistance, the overall amount of microbes may be influenced by location. The National Sanitation Foundation (NSF) reported that bacteria thrive in damp environments, such as a cutting board when it is left to dry after cleaning (5). Meanwhile, bacteria grow less efficiently on cool, smooth surfaces, such as a fridge handle

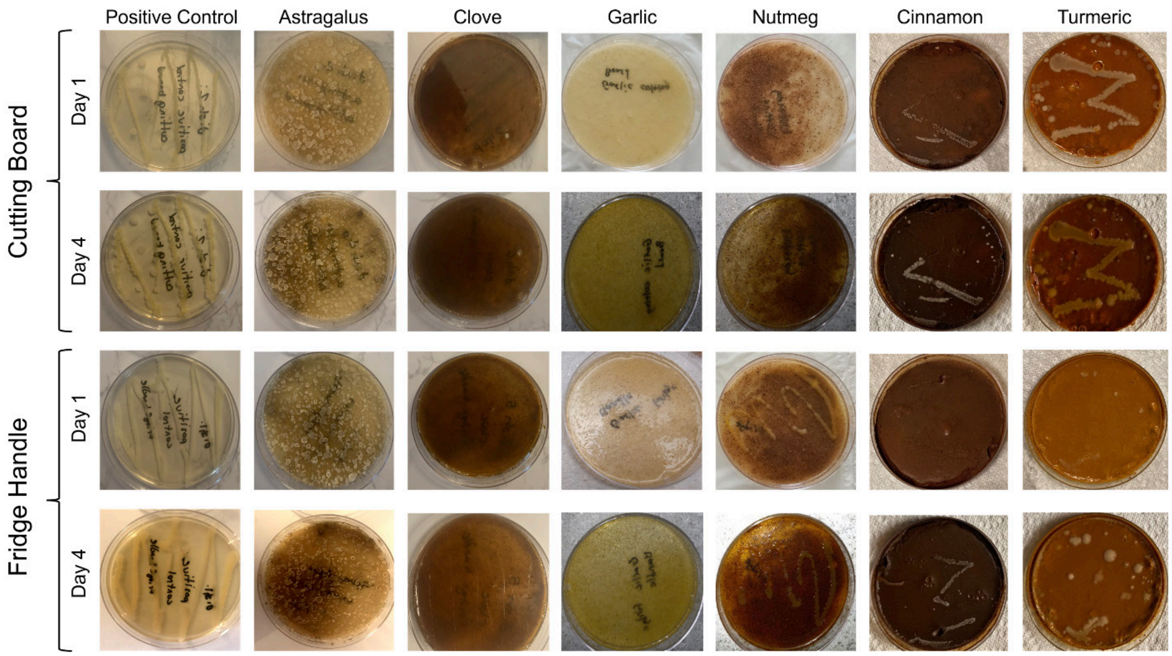

Figure 1: Representative images of subculture plates with and without spices on days one and seven. Microbes were cultured from samples obtained from the refrigerator handle and a single colony was chosen for subculturing. This subcultured microbe was then spread onto a positive control plate or a plate containing approximately ¼ teaspoon of a spice. Nothing was spread onto the negative control plate (image not shown). Microbe growth was recorded on four days over the course of a week (days one, three, five, and seven; days three and five not shown).

(5). We therefore hypothesized that each household spice would be effective at inhibiting microbial growth from cultures of refrigerator handles but that they might be less effective on samples from cutting boards. We found that cinnamon and turmeric reduced microbial growth from both surfaces, while clove and garlic completely prevented growth of microbes from both locations. Meanwhile, nutmeg completely prevented microbial growth from cutting board cultures but only reduced growth from refrigerator handle cultures, thus demonstrating location-dependent anti-microbial effects. Our results warrant further study into the antimicrobial properties of household spices and their utility as household cleaners.

RESULTS

Our objective was to determine the effectiveness of spices as alternative household cleaners. To achieve this, we quantified microbial growth on agar plates infused with spices that were then inoculated with a subcultured microbe from the refrigerator handle and cutting board. We successfully isolated microbes from our chosen swabbing locations, as demonstrated by visible growth on positive control plates **(Figure 1)**. However, we observed some differences in the amount of area covered by microbes when comparing the three positive control plates for both the refrigerator handle and cutting board **(Figure 2)**. This variation precluded comparisons between different spices, and we ultimately separated analysis into three pairwise comparisons with their respective control plates. There was also evidence of microbial growth over time on positive control plates, as the percent area occupied by microbes increased over the four-day monitoring period **(Figure 2)**. Interestingly, we observed less microbial growth across all positive control refrigerator handle plates compared to positive control cutting board plates **(Figure 2)**. For example, the cutting board control 2 showed approximately 53% coverage on day 1 compared to the refrigerator handle control 2, which showed approximately 38% coverage on day 1. We did not observe any microbial growth on the negative control plates (data not shown).

We observed mixed efficacy of the tested spices to prevent and/or slow microbial growth from swabs of the cutting board and fridge handle **(Figure 1)**. In order to compare microbial growth from control plates with those that contained the spices, we quantified the percent area of the plate covered by microbes from the cutting board and refrigerator handle swabs every other day over the course of a week. Garlic and clove were able to completely prevent the growth of microbes over the course of four days for samples from the refrigerator handle and cutting board **(Figure 2)**. Interestingly, nutmeg completely inhibited the growth of microbes from the cutting board but not the refrigerator handle **(Figure 2)**. However, it appeared as though nutmeg was only able to reduce the growth rate of microbes from the refrigerator handle over the course of four days compared to control plate 1 **(Figure 2A)**. Cinnamon and turmeric both appeared to reduce the rate of microbial growth over the four-day observation period compared to the control plate for both the refrigerator handle and cutting board cultures **(Figure 2)**. We were unable to quantify microbial growth on plates containing astragalus due to the rough texture apparent on the plates. However, we did not observe growth of microbes on these plates.

DISCUSSION

In this work, we sought to build upon previous literature on the effectiveness of household spices to inhibit microbial

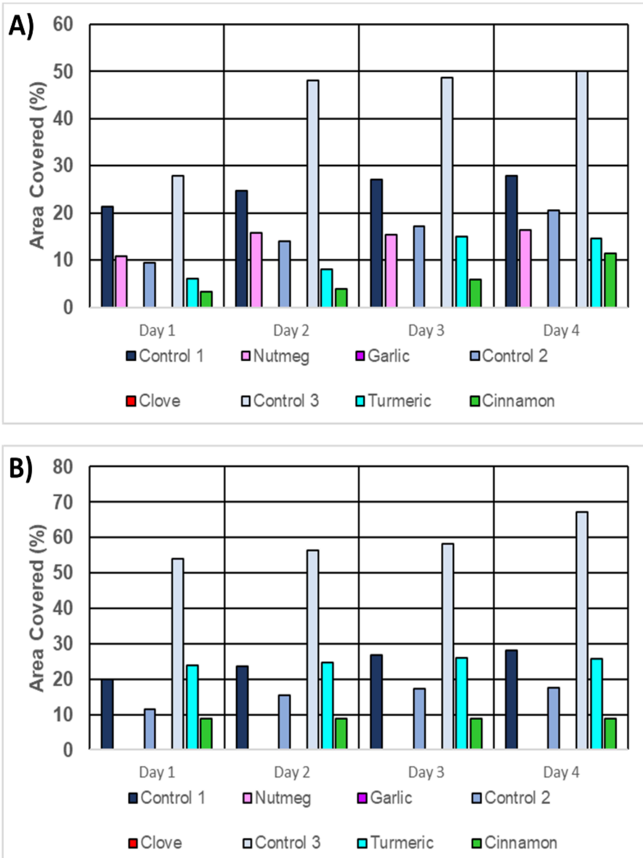

Figure 2: Microbial growth from refrigerator handle cultures. Microbes were cultured from samples obtained from the refrigerator handle (A) or cutting board (B), isolated and subcultured, and spread onto plates with and without spices. Control replicate one was run alongside nutmeg and garlic plates, control replicate two was run alongside clove and astragalus (data not shown), and control replicate three was run alongside turmeric and cinnamon. Microbial growth was monitored over the course of a week and subsequently quantified as a percent of the total plate occupied.

growth. We specifically referenced a study that demonstrated antibacterial properties of various household spices (10). We built on these previous studies in a number of ways. First, we chose to culture microbes from a cutting board and refrigerator handle, instead of using *E. coli*, which we expected would isolate microbes with different susceptibilities to spices. We decided to subculture, as we hypothesized that we would be unable to determine whether spices were effective against a subset of microbes present in a community sample. Thus, subculturing provided a more uniform microbial sample to test within each pairwise comparison. We theorized that growth of microbes cultured from the refrigerator handle might be more vulnerable to spices than cultures from the cutting board, given its suboptimal growing conditions and less frequent cleaning which may lend the microbes less resistance. Second, we tested antimicrobial properties of three spices that had previously been tested against *E. coli* (clove, cinnamon, and garlic), and three spices not previously tested (astragalus, nutmeg, and turmeric). We chose these additional spices based on prior evidence of antibacterial or medicinal properties. Finally, we monitored microbial growth for four days over the course of a week to ascertain the long-term antimicrobial properties of these spices.

In line with previously demonstrated work, clove and garlic were able to completely prevent the growth of microbes from both cultures over the entire monitoring period. This finding suggests both these spices have short-term antimicrobial properties that last over the course of a week. Interestingly, cinnamon did not prevent microbial growth but was able to slow it over the monitoring period when compared to our positive control **(Figure 1).** Unfortunately, we were unable to quantitatively describe the effects of astragalus on microbial growth. When pouring the plates, it appeared as though the hot liquid agar reacted with the astragalus and led to a rough texture that made quantification impossible. However, there was a lack of visual evidence of microbial growth throughout the monitoring period. Interestingly, nutmeg completely prevented the growth of microbes from the cutting board culture but only slowed growth of microbes from the refrigerator handle. We also observed what appeared to be 'bubble' growths on both of the turmeric plates, which was reported in a previous study when testing basil (10). These were hypothesized to be fungal in nature, rather than bacterial, and hence brought about our caution in labeling our results as bacteria-specific. In line with this observation, the NSF reported that there was yeast and mold on the refrigerator handle in 23% of households, while there was yeast and mold on the cutting board in 14% of households (5). Curcumin, a compound in turmeric, has antifungal properties and thus supports the notion that these colonies may have been fungal rather than bacterial, given the lack of growth over the monitoring period (15). Overall, our results support selective vulnerability of cutting board cultured microbes only in response to nutmeg. Although the refrigerator handle plates with cinnamon and turmeric showed less growth than the cutting board plates, the positive control plate also showed less growth. Thus, we cannot rule out the possibility that there were different amounts of starting microbes from each subculture. Finally, due to limitations in resources, we were unable to run duplicates or triplicates. These replicates would be necessary to know whether any reduction in microbial growth met statistical significance, though we note that we saw 10-40% reductions in the amount of microbial growth over the observation period.

There are a number of potential reasons why some spices were able to inhibit microbial growth completely whereas others could not. One possibility is the different compounds in each spice that exert antimicrobial properties. Examples of this include curcumin, found in turmeric, and eugenol, found in clove, nutmeg, cinnamon, and basil (15, 16). Curcumin has been shown to suppress microbial proliferation, gene expression, and enzyme function. In contrast, the free hydroxyl group of eugenol containing compounds is thought to inhibit the ability of microbes to transport ions and ATP, and interferes with normal membrane permeability (15, 16). Follow up studies that isolate active compounds of spices and/or test them in combination could be a useful strategy to produce a natural cleaning product that has utility against microbes. Another possible reason for the differing effectiveness of spices was that we were unable to determine the type of microbe being cultured. Thus, each spice might have different potencies based on which microbe it is being used against. This is compounded by the fact that the frequency of cleaning and type of cleaning product that each person uses to clean their kitchens may differ, which could affect the microbes present for culturing.

Future studies should expand on the work we presented here. Identifying which microbes are inhibited by specific spices would be useful for determining their utility as household cleaners. Another useful follow-up study would be determining the dose-response curve of microbial growth inhibition for each spice in order to be cost effective. Further comparison in terms of cost and effectiveness at preventing microbial growth with common household cleaners would aid in determining the feasibility of spices as a substitute for this purpose. Our study design was particularly useful for determining whether these spices can prevent microbial growth. Adding spices to plates with microbes already present would be a better determinant of their ability to kill microbes by examining whether overall area occupied decreases following their application and may therefore be a better comparison for use as cleaners. Overall, all household spices tested in our study were able to at least slow the growth of microbes cultured from two kitchen locations, with some being able to completely inhibit microbial growth over a number of days. Ultimately, we have added to a growing body of literature on the antimicrobial nature of spices by applying them to a real-world context. Caution must also be taken, given that some spices can be toxic under certain circumstances, and these potential limitations should be explored in greater detail (17). Nonetheless, we anticipate that this research will spur future work and promote the use of spices as natural alternatives for household cleaners.

MATERIALS AND METHODS

Preparation of Herbs and Spices

We used the following spices in powder form: garlic, nutmeg, cinnamon, turmeric, clove, and astragalus. We did not alter spices that were already ground into a fine powder. Whole spices were ground into a fine powder and stored in cabinets until ready to use. Astragalus (Starwest Botanicals), nutmeg (Simply Organic), cinnamon (McCormick Organic), turmeric (Simply Natures), and clove (McCormick Organic) were purchased from Amazon, while garlic (Simply Organic) was purchased at Safeway. The following spices were run together: nutmeg and garlic, clove and astragalus, and turmeric and cinnamon.

Swabbing Locations and Procedure

Sterile swabs were submerged in a vial containing 5 ml of sterile water and used to swab either the refrigerator handle or cutting board from three different households. The swabs were placed into 15 ml Falcon tubes containing 5 ml of tryptic soy broth (VWR). Culture tubes were left to incubate at room temperature for approximately 48 hrs.

Agar Plate Preparation and Microbial Spreading

Tryptic soy agar was melted in the microwave in increments of 30 seconds over the course of approximately 2 minutes, swirling after each increment. Once in a liquid form, petri dishes were filled approximately halfway with the liquid agar and allowed to solidify at room temperature. The end of a bacterial spreader was dipped into a culture tube containing swabs from the refrigerator handle or cutting board. Microbes were spread onto agar plates using the T method, which is similar to the quadrant method (18). Nothing was spread on to the negative control plates. Plates were left to incubate at room temperature for three to four days.

Separately, in newly labelled plates, approximately ¼ tsp of each spice was added to along with 15 ml of melted, liquid tryptic soy agar. Positive and negative control plates had no spices mixed into the tryptic soy agar. These plates were then allowed to solidify at room temperature and set aside until use.

Subculturing and Microbial Spreading

A single, well-isolated colony from the fridge handle and cutting board agar plates were selected for subculturing with a sterile colony picker. Chosen colonies were then submerged in new Falcon tubes containing 5 ml of tryptic broth and incubated at room temperature for approximately 48 hours. After 48 hours, each culture was diluted by adding three drops of the culture into 3 ml of tryptic broth. Bacterial spreaders were submerged into tubes containing the diluted subculture from the refrigerator handle or cutting board and spread onto the respective plates. Positive control plates without spices allowed visualization of the natural growth of microbes over the four-day period. Nothing was spread onto the negative control plates to ensure that plates were not contaminated upon arrival or during the experimental process.

Quantification of Microbial Growth

Images of plates were taken every other day (days one, three, five, and seven) over the course of one week beginning 48 hours after streaking. Images were imported into ImageJ for analysis. Microbe colonies were manually outlined and the area occupied by microbes was quantified as a percentage of the total plate area. This was repeated for an entire week to ascertain the ability of each household spice to prevent or delay microbial growth. Data was entered and graphed in Microsoft Excel.

ACKNOWLEDGEMENTS

The authors would like to thank Michael Mazzola for his guidance during the planning stages of this study and for his support during the Journal of Emerging Investigators Mini-PhD program.

Received: October 4, 2021
Accepted: February 12, 2022
Published: May 12, 2022

REFERENCES

1. Ventola, C. L. “The Antibiotic Resistance Crisis: Part 1: Causes and Threats.” *P T*, vol. 40, no. 4, 2015, pp. 277-283.
2. Scallan, E., *et al*. “Foodborne Illness Acquired in the United States--Unspecified Agents.” *Emerg Infect Dis*, vol. 17, no. 1, 2011, pp. 16-22, doi:10.3201/eid1701.091101p2.
3. “Food Safety: Foodborne Germs and Illnesses.” Centers for Disease Control and Prevention. www.cdc.gov/foodsafety/foodborne-germs.html. Accessed August 21 2021.
4. Lawrence, D. T., *et al*. “Food Poisoning.” *Emerg Med Clin*

North Am, vol. 25, no. 2, 2007, pp. 357-373; abstract ix, doi:10.1016/j.emc.2007.02.014.
5. "2011 NSF International Household Germ Study." National Sanitation Foundation. www.nsf.org/knowledge-library/2011-nsf-international-household-germ-study-exectutive-summary. Accessed August 21 2021.
6. McKenzie, L. B., *et al*. "Household Cleaning Product-Related Injuries Treated in Us Emergency Departments in 1990-2006." *Pediatrics*, vol. 126, no. 3, 2010, pp. 509-516, doi:10.1542/peds.2009-3392.
7. "Table R-1. All Consumer Units: Annual Detailed Expenditure Means, Standard Errors, Coefficients of Variation, and Weekly (D) or Quarterly (I) Percents Reporting, Consumer Expenditure Survey, 2019." U.S. Bureau of Labor Statistics.
8. "Statista-Survey Cleaning Products 2018." Statista. www.statista.com/forecasts/1018986/importance-of-ecofriendly-seals-on-cleaning-products-in-the-us. Accessed September 22 2021.
9. Jiang, T. A. "Health Benefits of Culinary Herbs and Spices." *J AOAC Int*, vol. 102, no. 2, 2019, pp. 395-411, doi:10.5740/jaoacint.18-0418.
10. Gehad, Y., and M. Springel. "Characterization of Antibacterial Properties of Common Spices." *Journal of Emerging Investigators*, vol. 3, 2020.
11. Dogra, N., *et al*. "Polydiacetylene Nanovesicles as Carriers of Natural Phenylpropanoids for Creating Antimicrobial Food-Contact Surfaces." *J Agric Food Chem*, vol. 63, no. 9, 2015, pp. 2557-2565, doi:10.1021/jf505442w.
12. Narasimhan, B., and A. S. Dhake. "Antibacterial Principles from Myristica Fragrans Seeds." *J Med Food*, vol. 9, no. 3, 2006, pp. 395-399, doi:10.1089/jmf.2006.9.395.
13. Takikawa, A., *et al*. "Antimicrobial Activity of Nutmeg against Escherichia Coli O157." *J Biosci Bioeng*, vol. 94, no. 4, 2002, pp. 315-320, doi:10.1263/jbb.94.315.
14. Kanaan, H., *et al*. "Screening for Antibacterial and Antibiofilm Activities in Astragalus Angulosus." *J Intercult Ethnopharmacol*, vol. 6, no. 1, 2017, pp. 50-57, doi:10.5455/jice.20161018101720.
15. Moghadamtousi, S. Z., *et al*. "A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin." *Biomed Res Int*, vol. 2014, 2014, p. 186864, doi:10.1155/2014/186864.
16. Marchese, A., *et al*. "Antimicrobial Activity of Eugenol and Essential Oils Containing Eugenol: A Mechanistic Viewpoint." *Crit Rev Microbiol*, vol. 43, no. 6, 2017, pp. 668-689, doi:10.1080/1040841X.2017.1295225.
17. Ehrenpreis, J. E., *et al*. "Nutmeg Poisonings: A Retrospective Review of 10 Years Experience from the Illinois Poison Center, 2001-2011." *J Med Toxicol*, vol. 10, no. 2, 2014, pp. 148-151, doi:10.1007/s13181-013-0379-7.
18. Sanders, E. R., "Aseptic Laboratory Techniques: Plating Methods." *J Vis Exp*, no. 63, 2012, p. e3064, doi:10.3791/3064.