Journal of
Emerging Investigators

Disk Diffusion Tests Show Ginger to be Ineffective as an Antibacterial Agent

Kruti Patel[1], Kathleen Mungai[1], Yaa Konama Pokuaa[1], Katelyn St. Louis[1], Jahnvi Patel[1], Kathryn Mansour[1], Keroline Santos[1], Kimberly A. Gonzalez[2]

[1]Lowell High School, Lowell, MA

[2]Middlesex Community College, Bedford, MA

Summary

Before the discovery of antibiotics, alternative medicines were used to treat infectious diseases. Such treatments included ginger, *Zingiber officinale*, yet investigation into ginger's effectiveness has produced mixed results. Some studies have shown ginger to be an effective against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, while other studies have shown it is ineffective. This study continues the exploration of ginger as an antibacterial agent. Ginger powder and ginger root extract were tested to see if inhibition of growth of *E. coli*, *S. aureus*, and *B. subtilis* would occur. Various preparations and concentrations of ginger were tested by disk diffusion tests. Our results revealed that neither ginger preparation had an observable antibacterial effect on growth of *E. coli*, *S. aureus*, and *B. subtilis* at any of the tested concentrations. This is important because many have believed that ginger is an effective antibacterial agent, but according to this study, this does not appear to be the case. These results can guide people to seek treatment with appropriate antibiotics if they have an infection due to *E. coli*, *S. aureus*, and/or *B. subtilis*, as ginger does not appear to inhibit the growth of these organisms. Future studies need to be done to validate these results with other tests, and determine if ginger may enhance the effectiveness of antibiotics.

Received: January 20, 2017; **Accepted:** August 7, 2017; **Published:** October 30, 2017

Introduction

Before the discovery of antibiotics, aromatic herbs and medicinal plants were the primary treatment for infectious diseases (1). Even with the discovery of antibiotics, many people still turn to these herbs and plants for relief because they are thought to contain a broad-spectrum of antibiotic activity against pathogens. With antibiotic resistant infections on the rise, the exploration of these foods as antimicrobials is becoming increasingly necessary.

Ginger (*Zingiber officinale*) is an ayurvedic, which in the classical Hindu system of medicine, is thought to be a "whole body medicine", used to treat a variety of ailments including sore throats, nausea, fever, and infectious diseases (7). Studies examining the effects of ginger on inhibition of microbial growth have produced mixed results (1,3). Ginger has been shown to be an effective antimicrobial agent against *Aspergillus niger*, *Saccharomyces cerevisiae*, *Mycoderma sp.*, *Lactobacillus acidophilus*, and *Bacillus cereus* (1), yet ginger appears to be ineffective against organisms in the Enterobacter and Klebsiella genera (3). It is possible that differences in success of ginger as an antimicrobial could be attributed to differences in cell wall permeability of the organisms being tested. In successful studies, the antimicrobial activity of ginger has been attributed to the presence of the biomolecules gingerol, paradol, shogaol and zingeroneoleoresin (4). Biomolecules extracted from ginger exhibit antioxidant activity against the growth of pathogens, as well as having the potential to lyse cells, and reduce protein synthesis, resulting in the death of pathogens (9). In patients, these active biomolecule substances also help to lessen the body's inflammatory responses as a result of infection. In relation to our study, ginger root and ginger powder both contain biomolecules, such as gingerols, which are anti-inflammatory compounds. The process of turning ginger root into ground ginger involves a reduction in the amount of gingerols, but it increases the amount of shogaols, another antimicrobial biomolecule.

Since there are conflicting reports about the effectiveness of ginger, we aimed to continue the examination of ginger root and ginger powder as alternative antibacterial agents, hypothesizing that ginger is in an effective antibacterial agent, with there being no observed difference between the two forms. In this study, ginger root was used to explore its potential antimicrobial activity, in its purest form. Ginger powder was also used to see if ground, dehydrated ginger, would show different results when compared to the ginger root. Testing of each preparation was done using the disc diffusion method on three different concentrations of whole ginger root extract, as well as three different

concentrations of ginger powder preparations, against the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. *E. coli* is a gram-negative bacteria, *S. aureus* and *B. subtilis* are gram-positive bacteria. These organisms were chosen because they are organisms present both in the environment, as well on and in the body. Our result show that neither the ginger root nor ginger powder seemed to have any demonstrable antimicrobial activity against *E. coli*, *S. aureus*, or *B. subtilis*, thus rejecting our hypothesis. This may be attributed to the fact that the biomolecule concentrations are not high enough in these preparations to be effective at preventing bacterial growth, however, this warrants further investigation. The results of this study provide further support to conclusions that ginger on its own may not be an effective antibacterial agent.

Results

To determine whether or not whole ginger root extract and/or ginger powder were effective antimicrobial agents, disc diffusion tests using various concentrations of whole ginger root extract (10%, 50%, and 100%), as well as ginger powder (10%, 25%, and 100%), were performed on *E. coli*, *S. aureus*, and *B. subtilis*. Positive (antibiotic discs) and negative (blank discs) control tests were also performed. Susceptibility patterns were determined by measuring the zone of inhibition (ZOI) of bacterial growth, in millimeters. These values were the compared to the National Committee for Clinical Laboratory Standards (NCCLS) antimicrobial sensitivity values for each organism (5). **Tables 1-6** show the results of each test. For the antibiotics tested, all organisms showed sensitivity to the antibiotics, and all organisms show no ZOI for the blank paper disc.

Table 1 shows streptomycin, chloramphenicol, kanamycin, and tetracycline were used as positive controls with *E.coli*. *E.coli* was sensitive to three out of four antibiotics, with the fourth antibiotic being interpreted as intermediate susceptibility. *E.coli* showed no zone of inhibition to any of the ginger root extract preparations. In **Table 2**, *E.coli* was tested against the same antibiotics and ginger powder preparations. The results were similar to the ginger root extract, in that *E. coli* was sensitive or intermediate to the antibiotics, but there was no observed ZOI for the various ginger power concentration. **Figure 1** shows *E. coli* being tested in each of the aforementioned conditions.

Table 3 shows *S. aureus* tested against tetracycline and erythromycin. These two antibiotics were chosen because *S. aureus* is generally sensitive to these antibiotics. The results show *S. aureus* was sensitive to the antibiotics but resistant to the ginger root extract. In **Table 4**, *S. aureus* was being tested against tetracycline, erythromycin, and the ginger powder preparations. *S.*

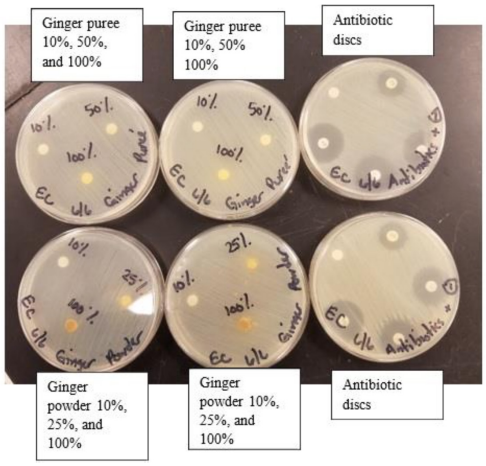

Figure 1: Zones of Inhibition Plates for *E. coli*. *E. coli* was sensitive to the tested antibiotics but not to the blank disk or the various ginger preparations.

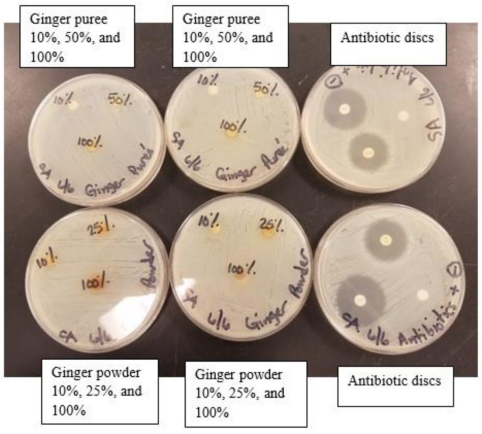

Figure 2: Zones of Inhibition Plates for *S. aureus*. *S. aureus* was sensitive to the tested antibiotics but not to the blank disk or the various ginger preparations.

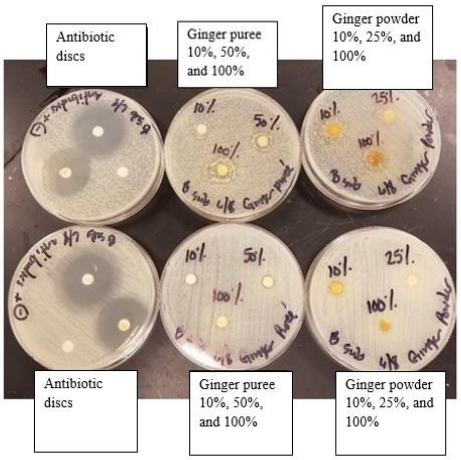

Figure 3: Zones of Inhibition Plates for *B. subtilis*. *B. subtilis* was sensitive to the tested antibiotics but not to the blank disk or the various ginger preparations.

E. coli Ginger Root Extract	Trial 1 ZOI (mm)	Trial 2 ZOI (mm)	Average ZOI (mm)	S.D.	Interpretation
Blank disc	6	6	6	0	Resistant
Streptomycin	16	23	20	4	Sensitive
Chloramphenicol	20	26	23	3	Sensitive
Kanamycin	20	28	24	4	Sensitive
Tetracycline	15	20	18	3	Intermediate
10% root extract	6	6	6	0	Resistant
50% root extract	6	6	6	0	Resistant
100% root extract	6	6	6	0	Resistant

Table 1: Zone of inhibition (ZOI) and standard deviation for *E. coli* and ginger root extract.

S. aureus Ginger Root Extract	Trial 1 ZOI (mm)	Trial 2 ZOI (mm)	Average ZOI (mm)	S.D.	Interpretation
Blank disc	6	6	6	0	Resistant
Tetracycline	25	26	26	1	Sensitive
Erythromycin	30	30	30	0	Sensitive
10% root extract	6	6	6	0	Resistant
50% root extract	6	6	6	0	Resistant
100% root extract	6	6	6	0	Resistant

Table 3: Zone of inhibition (ZOI) and standard deviation for *S. aureus* and ginger root extract.

B. subtilis Ginger Root Extract	Trial 1 ZOI (mm)	Trial 2 ZOI (mm)	Average ZOI (mm)	S.D.	Interpretation
Blank disc	6	6	6	0	Resistant
Tetracycline	34	35	35	1	Sensitive
Erythromycin	38	45	42	4	Sensitive
10% root extract	6	6	6	0	Resistant
50% root extract	6	6	6	0	Resistant
100% root extract	6	6	6	0	Resistant

Table 5: Zone of inhibition (ZOI) and standard deviation for *B. subtilis* and ginger root extract.

E. coli Ginger Powder	Trial 1 ZOI (mm)	Trial 2 ZOI (mm)	Average ZOI (mm)	S.D.	Interpretation
Blank disc	6	6	6	0	Resistant
Streptomycin	23	27	25	2	Sensitive
Chloramphenicol	28	28	28	0	Sensitive
Kanamycin	28	28	28	0	Sensitive
Tetracycline	20	21	21	1	Intermediate
10% powder concentration	6	6	6	0	Resistant
25% powder concentration	6	6	6	0	Resistant
100% powder concentration	6	6	6	0	Resistant

Table 2: Zone of inhibition (ZOI) and standard deviation for *E. coli* and ginger powder preparation

S. aureus Ginger Powder	Trial 1 ZOI (mm)	Trial 2 ZOI (mm)	Average ZOI (mm)	S.D.	Interpretation
Blank disc	6	6	6	0	Resistant
Tetracycline	30	33	32	2	Sensitive
Erythromycin	32	35	34	2	Sensitive
10% powder concentration	6	6	6	0	Resistant
25% powder concentration	6	6	6	0	Resistant
100% powder concentration	6	6	6	0	Resistant

Table 4: Zone of inhibition (ZOI) and standard deviation for *S. aureus* and ginger powder preparation.

B. subtilis Ginger Powder	Trial 1 ZOI (mm)	Trial 2 ZOI (mm)	Average ZOI (mm)	S.D.	Interpretation
Blank disc	6	6	6	0	Resistant
Tetracycline	31	35	33	2	Sensitive
Erythromycin	40	41	41	1	Sensitive
10% powder concentration	6	6	6	0	Resistant
25% powder concentration	6	6	6	0	Resistant
100% powder concentration	6	6	6	0	Resistant

Table 6: Zone of inhibition (ZOI) and standard deviation for *B. subtilis* and ginger powder preparation.

aureus was sensitive to the antibiotics and resistant to each ginger powder concentration. ZOI results can be seen in **Figure 2**. **Table 5** shows *B. subtilis* tested against tetracycline and erythromycin as positive controls, due to its sensitivity to these drugs. *B. subtilis* was also resistant to the ginger root extract concentrations. Finally, in **Table 6**, tetracycline and erythromycin were again tested against *B. subtilis*, along with various ginger powder preparations. The microorganism was resistant to the ginger powder concentrations, and the ZOI results can be seen in **Figure 3**. Looking at the whole picture, there appeared to be no antimicrobial activity against any of the microorganisms tested, at any ginger preparation or concentration used.

Discussion

In this study, we hypothesized that ginger was an effective antibacterial agent against *E. coli*, *S. aureus*, and *B. subtilis*. We used disc diffusion assays with various concentrations of both raw ginger extract and ginger powder preparations. The results of testing showed no inhibition of bacterial growth of any of the species tested using the raw ginger extract or the ginger powder, therefore rejecting our hypothesis. This experiment supports a study by Azu and Onyeagba (6), which also showed that raw ginger extract was ineffective in inhibiting the growth of *E. coli*, *B. subtilis*, and *Salmonella typhi*. However, the Azu and Onyeagba study showed that ethanol and cold water extraction of ginger had an inhibitory effect on the growth of the organisms. We did not attempt extraction of specific biomolecules for this study, and as such, this may explain the negative results we obtained. However, in terms of a limitation, this may be insignificant when considering that in treating infections with ayurvedic herbs and plants, medicinal preparations are often accomplished by frying, soaking in water, steaming, or grinding and mixing the therapeutics in a form that can then be taken into the body (7). Alcohol extraction of active ingredients is not necessarily something that would take place in the home use of these alternative medicines, so therefore, our methodology of using pureed ginger root and dehydrated ginger powder may support a scenario that is more likely to happen in home treatment of infectious diseases with ginger. It should be noted, however, that ginger could still potentially be used with antibiotics to inhibit bacterial growth, and future studies could examine if our ginger preparations, taken along with antibiotics, would inhibit the growth of bacteria. This could be done by mixing ginger in the agar and seeing if the antibiotic discs inhibit the growth of the microorganism. A study by Sundar *et al*. showed that extracts of ginger and cloves

enhanced the antibiotic activity of the aminoglycoside antibiotics in a similar testing fashion (8).

The major limitation of our study is sample size. The study size was N=2, so additional rounds of testing are needed to validate these initial results and make a significant conclusion. Another limitation of our study is that it does not test ginger extracts in the presence of ethanol, to see if ginger shows an antimicrobial effect in other studies because of the presence of ethanol. It is also possible our findings resulted from some contamination in the ginger. While precautions were taken to ensure that contamination did not occur, it is possible that the ginger could have been contaminated after being sterilized, most likely due to inappropriate handling.

In spite of the limitations or possible sources of error within this study, our results show that neither ginger root nor ginger powder are effective antibacterial agents against the organisms *E. coli*, *S. aureus*, and *B. subtilis*. This information can be used to steer people away from using solely ginger as an antimicrobial therapeutic.

Methods

Ginger Root Preparation

Ginger root was peeled and blended in a NutriBullet® blender in order to extract the fluid from the root. The ginger puree was filtered through a 20µm filter paper into a plastic container, effectively collecting the ginger juice. The ginger root fluid was then vacuum filtered through 0.45-µm filter paper in order to remove potential microbial contaminants. From the filtered ginger solution, 10%, 50%, and 100% ginger extract solutions were made. For the 10% solution, 1 mL of sterilized ginger root filtrate was added to 9 mL of sterilized, distilled water (dH_2O) in a sterile test tube. For the 50% solution, 5 mL of sterilized ginger root filtrate was added to 5 mL of sterilized, dH_2O in a sterile test tube. Finally, for the 100% solution, 10 mL of pure, sterilized ginger root filtrate was added to a sterilized test tube.

Ginger Powder Preparation

For a 10% powdered ginger solution, 1.0 g of powdered ginger (McCormick gourmet organic ground ginger powder) was added to 9 mL of sterilized, dH_2O. For a 25% powdered ginger solution, 0.5 g of powdered ginger was added to 1.5 mL of sterilized, dH_2O. A 25% powdered ginger solution was chosen over a 50% powdered solution due to the viscosity of the powder-dH_2O mixture. For a 100% ginger powder test, a moistened, sterile paper discs was placed directly into the ginger powder solution. This disc was then placed directly onto the inoculated nutrient agar plate.

Following preparation of the ginger solutions, 0.5 McFarland standards of *E. coli*, *S. aureus*, and *B. subtilis* were made. This was accomplished by adding 5 mL of sterile, dH_2O to three separate test tubes, inoculating one organism per test tube to visually match a known 0.5 McFarland latex standard. From the McFarland standard solutions containing each organism, bacteria were spread onto nutrient agar plates using X, Y, and Z quadrants to create a confluent lawn of growth. Next, paper discs containing antibiotics, sterile blank paper discs, and sterile, dry paper discs dipped in the ginger filtrate solutions (10%, 50%, 100%) or the ginger powder solution (10%, 25%, 100%), with the exception being that the 100% powder preparation required the disc to me moistened with sterile dH_2O. The discs were added to their respective plates containing each organism. Antibiotics chosen were based on NCCLS susceptibility ranges for the organisms being tested, as different microorganisms have different sensitivity patterns (5). *S. aureus* and *B. subtilis* were tested with tetracycline and erythromycin; *E. coli* was tested with streptomycin, chloramphenicol, kanamycin, and tetracycline. Chloramphenicol, kanamycin, and tetracycline have a concentration of 30 µg/mL, Streptomycin has a concentration of 10 µg/mL, and erythromycin has a concentration of 15 µg/mL. All plates were then incubated at 35°C for 24 hours. After 24 hours, the zones of inhibition were measured using a standard metric ruler, to determine susceptibility. Each trial was duplicated.

References

1. Sasidharan I and Menon AN. “Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (*Zingiber officinale Roscoe*)”. *Int J Curr Pharm Res*. 2.4 (2010): 40-3.
2. Islam K, Rowsni AA, Khan MM, Md Kabir S. “Antimicrobial activity of ginger (*Zingiber officinale*) extracts against food-borne pathogenic bacteria.” *IJSET* 3.3 (2014): 867-71.
3. Karuppiah P, Shyamkumar R. Antibacterial effect of Allium Sativum cloves and Zingiber Officinale rhizomes against multiple-drug resistant clinical pathogens. *Asian Pac J Trop Biomed* 2.8 (2012): 597–601.
4. Rahmani AH, Shabrmi FM, Aly SM. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. *Int J Physiol Pathophysiol Pharmacol.* 6.2 (2014): 125–36.
5. [CLSI] Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 2014. Twenty-Fourth Informational Supplement.
6. Azu N and Onyeagba R. Antimicrobial properties of extracts of Allium cepa (Onions) and *Zingiber officinale* (Ginger) on *Escherichia coli*, *Salmonella typhi* and

Bacillus subtilis. *Int J Trop Med*. 3.2 (2007):1–10.
7. Jaiswal Y, Liang Z, and Zhao Z. Botanical drugs in ayurveda and traditional Chinese medicine. J Ethnopharmacol. 194 (2016): 245–59.
8. Sundar S, Jayasree T, Ditya D, Sanjana J, Sowmya M, Jahnavi S. Antimicrobial activity of aminoglycoside antibiotics combined with clove and ginger. *World J Pharm Pharm Sci.* 4.6 (2015): 1515-24.
9. Pedtapti A. Antimicrobial properties of vegetables and spices – an overview. *Biotech Articles* (2015). http://www.biotecharticles.com/Agriculture-Article/Antimicrobial-Properties-of-Vegetables-and-Spices-An-Overview-3442.html. Accessed 23 October 2017.