THE ANTIMICROBIAL ACTIVITY OF GREEN TEA (*Camellia sinensis*) ON *Staphylococcus aureus* IN COMBINATION WITH ASCORBIC ACID, ACETIC ACID, AND SODIUM CHLORIDE

Timothy Barnum, Steven Castellano, Annie Chen, Neha Jariwala, Andrew Jung, Kelvin Mei, Christina Sedberry, Margaret To, Heather Tynan, Charles Zou

Advisor: Rachel Sandler
Assistant: Danielle Cusmano

ABSTRACT

Green tea contains polyphenolic catechins that have been demonstrated to effectively inhibit *Staphylococcus aureus* and related bacteria. This study aimed to determine if home-brewed green tea could inhibit *S. aureus* through paper disk diffusion and minimum inhibitory concentration experiments. It was hypothesized that green tea brewed for varying periods of time in solutions with ascorbic acid, acetic acid, and sodium chloride would most significantly inhibit *S. aureus*. The paper disk diffusion test showed that solutions of vinegar and vinegar with green tea produced zones of inhibition. However, the minimum inhibitory concentration test exhibited relatively uniform turbidity between the control and solutions, thus producing inconclusive results. It was determined that green tea did not significantly inhibit *S. aureus* and that vinegar had the greatest impact on bacterial growth.

INTRODUCTION

Due to the increased occurrences of bacterial resistance to synthetic pharmaceutical antibiotics, there has been a shift in medical research towards natural remedies that could be used as effective antimicrobial agents [1]. Green tea, a beverage known for its antibacterial properties, has been proposed as one such natural treatment for bacteria [2, 3, 4]. A particular bacterium of recent concern is *Staphylococcus aureus,* especially its methicillin-resistant varieties that oppose the majority of antibiotics used to treat *S. aureus* infections. According to the U.S. Food and Drug Administration, intoxication by *S. aureus* is the most common form of food-borne illness [5]. It also is known for its ability to quickly spread to new hosts [6]. Therefore, green tea's antibacterial properties could be potentially used to hinder such growth.

Green tea, *Camellia sinensis*, is an Eastern beverage that has been known for its health benefits since 2737 BCE when the Yan Emperor Sheng Nong first used this herb as a relaxing agent and a remedy for headaches [7]. According to folklore, he noticed such medicinal values in green tea when he accidently brewed this beverage after tea leaves fell into his pot of water. Green tea then developed into the most common beverage in China after Emperor Chien-lung noticed the soothing powers of green tea over two thousand years later. As its popularity grew within China, green tea also spread to other nations through international trade. In the 6th century, a Buddhist monk brought green tea to Japan, and by the 16th century, green tea was introduced to Europe by a Portuguese missionary. Since then, other varieties of tea, including oolong and black tea, have been developed, but none have as many medicinal properties as green tea.

Anecdotal accounts of green tea's antimicrobial properties have been reported for centuries, but only within the last 25 years have scientific studies confirmed green tea's effectiveness as an antimicrobial agent [8]. The most active antimicrobial agents of green tea are suspected to be its polyphenols, particularly the catechins. Epigallocatechin gallate (EGCG) constitutes ten percent of the dry mass of green tea, making it the most abundant of the catechins [9]. Although the catechin epicatechin gallate (ECG) has the greatest ability to bind to bacterial cell membranes, it is less influential than EGCG because of its lower abundance [10].

Unlike oolong and black tea, green tea is a particularly potent medical remedy because it contains most of its original phenols without undergoing oxidation [8]. Such components include epicatechin (EC), catechin (C), gallocatechin (GC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), catechin gallate (CG), epicatechin gallate (ECG), theaflavins, caffeine, and various organic acids [11]. The structures of these green tea catechins are shown in Figure 1 below. Green tea catechins contain multiple phenol groups, which are aromatic rings, composed of six carbons in a plane, with one or more hydroxyl groups attached. These hydroxyl groups are integral to the polyphenols' antioxidant properties.

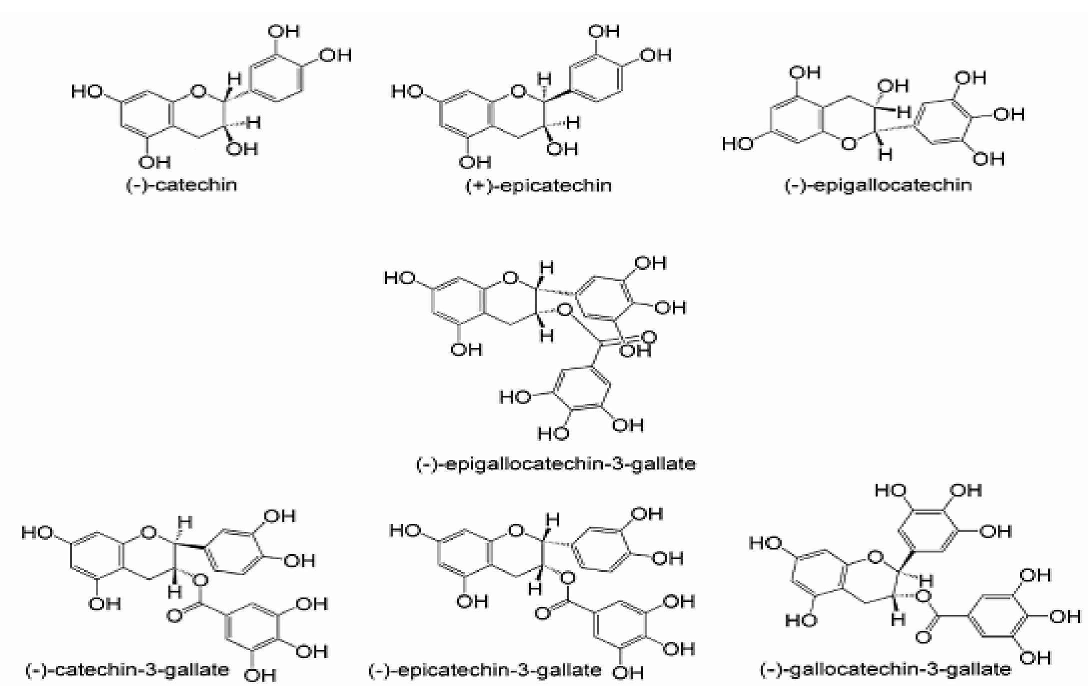

Figure 1. The structures of the primary catechins occurring in *Camellia sinensis* [12]

Green tea's health benefits are considered to be a result of its antioxidant properties, which eliminate free radicals in the body [13]. Free radicals, formed during several biochemical reactions and cellular functions, are molecules or atoms that have one free electron. Because electrons prefer to be in pairs, reactivity of this unstable molecule greatly increases as the molecule seeks to complete an electron pair. During this process, the free radical interacts with cellular components such as DNA, proteins, lipids, and fatty acids, causing mitochondria malfunction, DNA damage, cell membrane damage, and apoptosis [13]. Typically, antioxidants that are naturally occurring within organisms counteract free radicals at a rate that maintains homeostasis. Polyphenol hydroxyl groups donate electrons to satisfy the radical's need for another electron, producing harmless end products, such as water [14].

Since Nong's discovery of green tea's medicinal properties, scientists have continued to discover health benefits associated with green tea. The "Asian Paradox" describes the inconsistency that although people in Asia have one of the highest cigarette consumption rates in the world, they have one of the lowest cardiovascular disease rates per capita [15]. This could be a result of the salubrious effects of green tea. The catechins in green tea are responsible for inhibiting the growth of cancer cells, lowering low density lipoprotein (LDL) levels, and inhibiting the formation of blood clots, both which has been demonstrated to be a major source of heart attacks [11]. Green tea has been shown to increase plasma LDL's resistance to oxidation and to reduce the incidence of atherosclerosis [16]. Antioxidants are also able to inhibit the growth of cancer cells and speed up the body's metabolism for weight loss.

Green tea also seems to be able to alleviate the symptoms for a variety of diseases. Parkinson's disease is believed to be caused by oxidative stress, an imbalance of free radicals, within the central nervous system [17]. Green tea has been associated with cancer chemoprevention. Studies in animal models have shown that EGCG can inhibit carcinogenesis at all stages of cancer progression, due to a combination of antioxidative, antiproliferative, and pro-apoptotic effects [18]. In contrast, these same results were less noticeable in humans because the effective dosage for test animals far exceeds levels usually consumed by humans [17]. Another attribute of EGCG is its inhibition of HIV by interfering with the replication of HIV reverse transcriptase and the binding of the viral envelope [19].

EGCG has been reported to be able to combat bacteria. However, EGCG's mechanism for destroying bacteria is not fully understood, and several different studies report different mechanisms. One such study hypothesizes that EGCG destroys bacteria by binding to the compound peptidoglycan, a carbohydrate component unique to the cell walls in bacteria. The bound EGCG then reduces the structural integrity of the cell wall. This makes the bacteria cells highly susceptible to lysis in the ionic and low osmotic pressure environments in which they would normally thrive [20]. Gram positive bacteria were found to be especially susceptible to lysis after treatment with EGCG compared to Gram negative bacteria [21]. This is because the Gram positive species contain a thick peptidoglycan-based cell wall outside of the cell membrane, while Gram negative species have a significantly thinner cell wall between an inner and outer membrane. As a result, this data supports the argument for an EGCG-peptidoglycan mode of action.

Other studies suggest that several catechins, especially ECG, have the ability to bind to lipid bilayers. The intercalation of these compounds between the phospholipids of the outer and inner bacterial membranes could also be responsible for lysis and cell death. The green tea catechins' greater effect on Gram positive bacteria may be caused by the presence of negatively charged lipid polysaccharides on the outside of Gram negative bacteria. These charged chains prevent certain chemicals from binding to the cell membrane, such as those that recognize the cell in the human immune response [9].

A particular bacterium of recent concern is *Staphylococcus aureus*, known colloquially as "golden staph." *S. aureus* are the spherical Gram-positive bacterial (Figure 2). A *S. aureus* infection can cause a large range of symptoms, including pimples, food poisoning, Toxic Shock Syndrome, pneumonia, and meningitis [22].

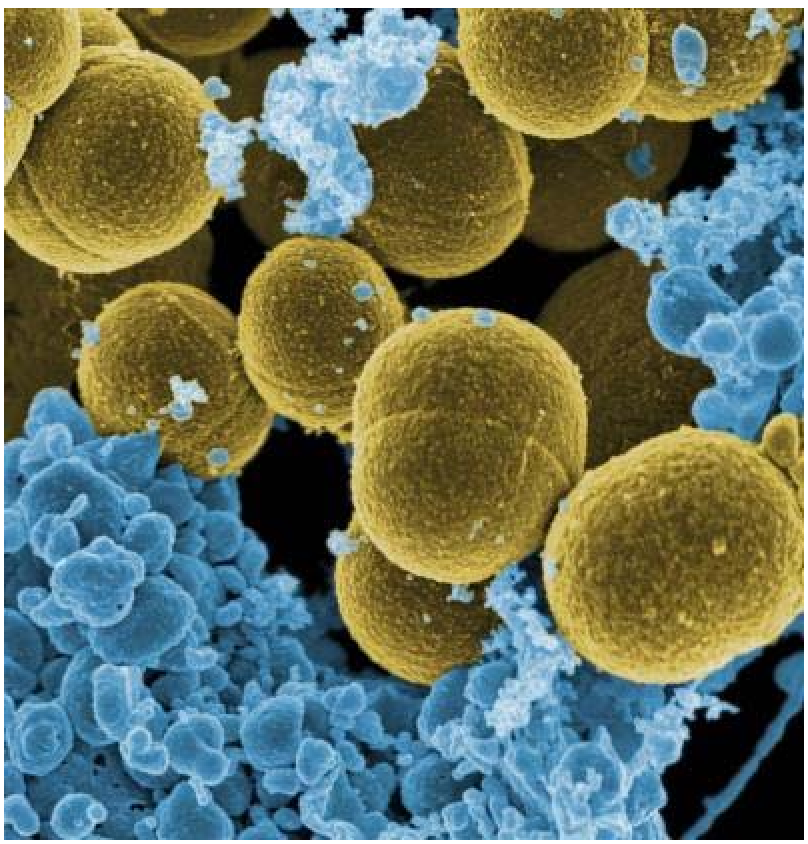

Figure 2. *Staphylococcus aureus* in gold, surrounded by green tea polyphenols in blue [23]

Unusual for a food-borne bacterial pathogen, *S. aureus* has the capacity to grow in the presence of high concentrations of salt [23]. Some strains can grow in NaCl concentrations as high as 15% salt by mass [24]. Halotolerance in *S. aureus* results from its ability to accumulate osmoprotective molecules, such as choline, glycine betaine, and L-proline [22, 25, 26, 27] and to reduce cellular sodium through sodium-hydrogen exchange pumps [28]. Often, *S. aureus* infections occur after forming colonies on the surface of the skin, a high-salt environment [26], which is why *S. aureus* can rapidly spread via direct contact or skin shedding. Epidemic strains of *S. aureus* constitute a high proportion of hospital-acquired systemic infections in Europe, North America and Asia because of this ease of transmission [29].

Many studies have concluded that green tea is an effective antimicrobial agent against *S. aureus*. Toda et al [30] reported in 1989 that tea extracts inhibited and killed a number of bacteria including *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Salmonella typhimurium, Salmonella enteritidis, Shigella flexneri, Shigella dysenteriae, and Vibrio cholerae.* Inhibition of all these bacteria occurred at "cup of tea" concentrations of 3 mg of tea leaves per mL water [31]. The results of these studies have implications for using home brewed green tea as an antibacterial household cleaner, especially against *S. aureus* that typically spreads between surfaces and the skin.

Furthermore, the phenolic compounds in green tea have been effective against methicillin-resistant *Staphylococcus aureus* (MRSA), an antibiotic resistant strain of the *S. aureus* bacteria [32]. According to the Centers for Disease Control and Prevention, the number of incidences of MRSA in the United States increased from 127,000 in 1999 to 278,000 in 2005, and it is suspected that more people died of MRSA than of AIDS worldwide in 2007 [33]. A recent study found that green tea compounds suppressed the growth of both antibiotic susceptible and resistant species after a 6 hour incubation period [34]. The green tea catechins have the ability to reverse β-lactam resistance in MRSA by changing the structure of the bacterial membranes to make β-lactam more penetrable [35]. The catechins can also cripple the halotolerance typically present in MRSA through a similar process [27]. Overall, the antioxidative, bacteriostatic, and enzyme inhibiting properties of catechins [31], along with their low toxicity and high tolerability in humans [36], make them potential candidates as inhibitors of *S. aureus*.

The antimicrobial activity of green tea may be enhanced by the addition of other substances known to be effective against bacteria. Sengun and Karapinar examined the bactericidal activity of ascorbic acid in lemon juice and acetic acid in vinegar against *Salmonella typhimurium* on carrots, and their results showed statistical significance in the immediate effect of lemon juice against *S. typhimurium*. The vinegar also exhibited similar behavior in its antimicrobial effects on cells immediately after contact with the carrots, and maximum effect was seen after a 60-min. treatment dipping carrots in vinegar [37]. Sodium chloride is also known to inhibit the growth of *S. aureus*, particularly in concentrations exceeding ten percent [38].

As a result of green tea polyphenols' effectiveness as antimicrobial agents and their ability to weaken *Staphylococcus aureus*, it was hypothesized that green tea would be significantly more effective than a control at inhibiting the growth of *S. aureus*. It was further hypothesized that the effects of green tea could be enhanced when combined with sodium chloride, ascorbic acid found in lemon juice, and acetic acid found in vinegar.

MATERIALS AND METHODS

Preparation of green tea solutions

Stash™ premium green tea bags were cut open, and each bag's content was massed. To eliminate the variation in the masses of the different green tea bags, 2.25 grams of tea (dry weight) was measured and placed into tea bags. Each tea bag was brewed in 225 mL of de-ionized water. Brewing times for the tea bags were 0.5 minute, 1.0 minute, 5.0 minutes, and 10.0 minutes. A control without any green tea was also included in the study.

In addition to testing the antimicrobial activity of green tea alone, combinations of green tea with lemon juice, household vinegar, and table salt were also tested. The green tea was combined with lemon juice, salt or vinegar for a total of 20 different variations. In three of the four beakers, Stop & Shop™ lemon juice, Stop & Shop™ distilled white vinegar, or table salt was added. The fourth beaker contained only green tea.

Using journal articles as reference, specific concentrations for the lemon juice, vinegar, and sodium chloride solutions were determined [39]. Twenty four percent by volume of vinegar at a concentration of 5% acetic acid was added to the green tea [42]. For the table salt solution, a concentration of 2% m/v was used [27]. Two percent by volume of lemon juice at a concentration of 47% ascorbic acid was added to the green tea [43].

Paper disc diffusion method

Twenty grams of Mueller-Hinton agar powder were dissolved in 500 mL of de-ionized water. The solution was then autoclaved for 20 minutes.

Approximately 40 mL of agar were poured into each 14-cm Petri dish. The plates were then inoculated with a 72-hour culture of *Staphylococcus aureus* (1.14×10^8 CFU per ml). Sterile swabs were used to streak four plates in three directions to ensure complete coverage of the Petri dishes. Paper disks were then soaked into each of the twenty solutions. One disk from each solution was placed on each of the four inoculated plates as well as on one control plate without *S. aureus*. Each solution was assigned a letter (Table 1) which was used to differentiate the disks on the plates (Fig. 3). After incubating the plates for 48 hours, the zones of inhibition were measured in millimeters.

Table 1. Green Tea Combinations in Solutions

Brewing Time (minutes)	Green Tea	Green Tea and Lemon Juice	Green Tea and Vinegar	Green Tea and Table Salt
0.0	A	B	C	D
0.5	E	F	G	H
1.0	I	J	K	L
5.0	M	N	O	P
10.0	Q	R	S	T

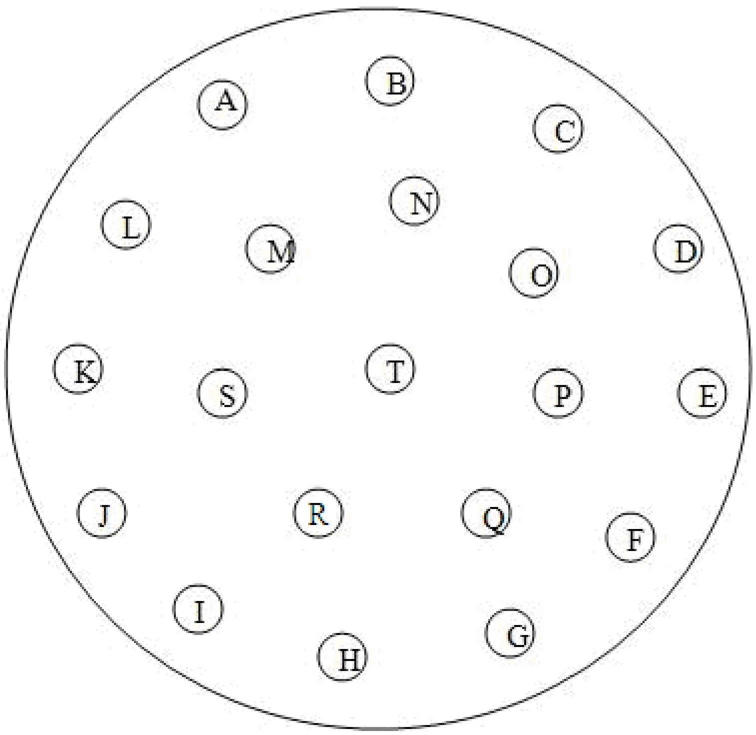

Figure 3. Diagram of Petri Dish Set Up

Minimum Inhibitory Concentration (MIC)

Thirty grams of tryptic soy broth were dissolved in one liter of distilled water. Five mL of broth was added to each of the 60 culture tubes for the MIC test. There were three trials for each of the twenty mixtures for a total of 60 culture tubes. The culture tubes were autoclaved for 20 minutes.

The tea solutions were cooled to room temperature, and 0.1 mL of green tea solution was added to each of the 5 mL of broth in the culture tubes [41]. The 60 culture tubes were inoculated with a loop-full of a 72-hour culture of *Staphylococcus aureus*. The culture tubes were then incubated for 48 hours at 37°C. After the incubation period, the turbidity was visually examined by placing the culture tubes next to each other to determine whether any bacteria had grown.

Data Analysis

The measurements for the solutions on each of the plates were averaged together and analyzed statistically. To assess the effect of the various combinations of green tea with lemon juice, vinegar, and table salt, the one-way ANOVA test was applied. For this study, α was set at 0.05.

RESULTS

Paper Disc Diffusion

The four Petri dishes exhibited similar results for the solutions that produced zones of inhibition. Only five of the twenty solutions produced zones of inhibition, specifically the solutions composed of green tea and household vinegar at the five different brewing times (Figure 2). None of the non-vinegar solutions produced measurable zones of inhibition. The one-way ANOVA statistic test showed that the differences between the diameters of the zones of inhibitions for the five different solutions of vinegar with green tea were not statistically significant. The calculated p-value was 0.712.

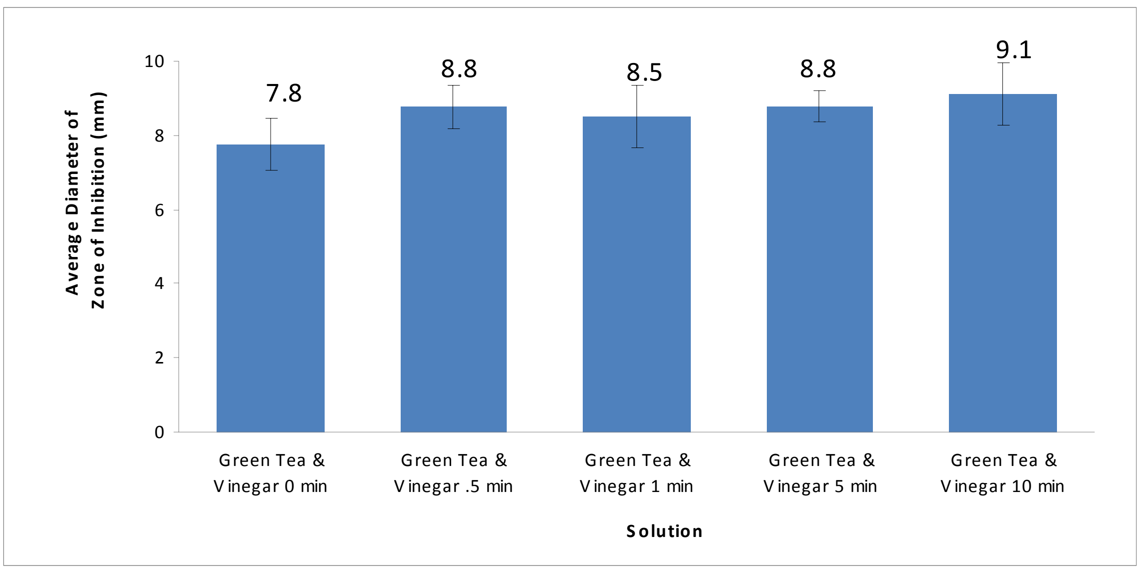

Figure 2: Graph of Average Diameter of Zones of Inhibition vs. Green Tea Solution with Vinegar

Table 2: Results from Statistical Analysis Using the One-Way ANOVA Test

ANOVA

ZoneSize

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.155	4	1.039	.535	.712
Within Groups	29.106	15	1.940		
Total	33.261	19			

Minimum Inhibitory Concentration (MIC)

The results were inconclusive because of insignificant differences in the growth of bacteria. A scale from zero to three was used to evaluate the test tubes for the minimum inhibitory concentration. Zero was indicative of no growth, while three was indicative of significant growth of bacteria. All of the test tubes exhibited bacterial growth and the amount of bacterial growth in each tube appeared relatively uniform in comparison to other tubes, including the control tube containing only bacteria. Because a spectrophotometer was not used, slight variations could not be detected.

DISCUSSION

Because the solutions with vinegar had the largest zones of inhibition, they were determined as the most effective antimicrobial. However, comparing the vinegar and green tea solutions of different brewing times revealed that green tea itself did not alter the zones of inhibition. In fact, all the green tea and vinegar solutions had similar zones of inhibition. Even though there were minute variations, the ANOVA statistics test concluded that the results were statistically insignificant. This suggests that the vinegar itself was inhibiting the growth of the bacteria, not the green tea. Because vinegar is a well-established household cleaner, the results were not surprising [42]. This implication is further supported by the fact that none of the other solutions of green tea and ascorbic acid or sodium chloride produced a zone of inhibition.

In terms of the MIC results, the bacteria were able to proliferate in all test tubes. The test tubes showed similar turbidity, with most of the tubes scoring a one or a two on the scale. Differences in bacterial growth among the test tubes were difficult to determine. However, a spectrophotometer can be used in future experiments to distinguish minute differences in the turbidity, or a sample can be plated to obtain the number of bacteria present. It can be concluded that none of the solutions could significantly inhibit the growth of *S. aureus*.

To improve the results of this study in the future, several changes can be made. For the MIC, the results could have been changed by altering the ratio of broth and bacteria to green tea and by limiting the possible interactions between the active chemicals and the materials in the media. Because the concentrations of polyphenols in the green tea are unknown, this ratio may have been too low to affect bacterial growth in the liquid medium.

The ingredients of the green tea should also be investigated. It is possible that the company who made the green tea could have included some additives into their tea bags or processed the tea leaves slightly more. This may have had a negative effect on the polyphenols. Because polyphenols in green tea are most effective in their monomer state, fermentation, which results from processing the tea leaves, would reduce the polyphenols' effects [19].

The brewing technique used is another area to be examined. Other experiments showed that a concentration of 3 mg of green tea per 1 mL of water was enough to produce a zone of inhibition [24]. However the ratio of 2.25 g of green tea per 225 mL of boiled water used, almost four times greater, did not produce significant results. This can be attributed to the brewing technique. When the tea bag was placed into the flask, it floated on top of the water, instead of in the water as is usual in the household brewing of tea. This continued for the duration of the brewing process. By decreasing the surface area exposure of the green tea leaves to the water, the polyphenol content could have been significantly reduced. Therefore, the polyphenol concentrations in the green tea may not have been as high as those established in previous studies [7], which extracted the polyphenols from the tea leaves using acetone and methanol instead of brewing the tea in water [21, 7]. Also, the extraction using acetone and methanol may extract more polyphenols than water alone if the polyphenols are more soluble in acetone and methanol than water. The results of such experiments show that green tea is highly effective against different strains of bacteria. However, these results may be exaggerated as a result of the polyphenol extraction process, which concentrated and purified the polyphenols. In future experiments, the tea bag should be either submerged into the water or squeezed immediately after to release more of the tea's compounds into the water.

In future experiments, the green tea concentration should be increased, thus increasing the concentration of the polyphenols in the solutions. This may be accomplished by either brewing more teabags or by decreasing the ratio of milliliters of water to grams of tea used, but these methods have to be proven in future studies. Furthermore, the bacterial concentration should also be taken into account. Since *S. aureus* is often found in low concentrations in the human body and on surfaces that transmit the bacterium, the results may have been affected by the sheer number of bacteria. It is possible that the *S. aureus* cultures had a significantly greater amount of bacteria than in a real life situation because there were not enough resources to determine whether or not the bacteria count used in this study was representative of that on an average countertop. Therefore, the polyphenols may have damaged the cell walls of several colonies of bacteria, which would have an effect in real-life situations, and yet, there could still be no visible results.

Even though the green tea did not significantly affect the zones of inhibition, it is not necessarily ineffective as an antimicrobial. There are many improvements and adjustments that can be made in future experiments to truly determine the effectiveness of green tea polyphenols.

Although this study does not support green tea as a household cleaner, it still has potential as an antibacterial. For instance, green tea polyphenols could be used as active ingredients in toothpaste because polyphenols can act as anti-cavity agents by attacking bacteria in the mouth. One such study conducted by Elvin-Lewis and Steelman have reported a significant difference in the number of cavities between children who drank green tea on a regular basis compared to the number of those who did not. Other sources state that the catechins may be useful in mouth hygiene [4].

Another prospective use of green tea for anti-bacterial purposes is in deodorizers. Since other studies have reported of green tea's antimicrobial benefits, green tea can potentially kill odor causing bacteria. However, further studies must be conducted to confirm its effectiveness as an antibacterial. If this is true, it can also be utilized as an organic alternative to artificial air fresheners [24].

Due to time and resource limitations, this study could not give supporting evidence for the effectiveness of green tea as an antimicrobial agent in the household. However, in spite of the inconclusive results obtained from this experiment, the unique properties of green tea may still be applied for other household products. Future research in this area may provide more conclusive results in favor of green tea as a potent antibacterial substance to be incorporated into daily life.

REFERENCES

[1] Almajano M P, Carbo R, López Jiménez J A, Gordon M H. Antioxidant and antimicrobial activities of tea infusions, Food Chemistry 2008; 108 (1): 55-63. Available from: ScienceDirect. Accessed 2008 July 14.

[2] Caturla N, Vera-Samper E, Villalain J, Mateo C R. The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membrances, Free Radical Biology Medicine 2003;34: 648-662. Available from: ScienceDirect. Accessed 2008 July 26.

[3] Hatano T, Tsugawa M, Kusada M, Taniguchi S, Yoshida T, Shiota S, Tsuchiya T. Enhancement of antibacterial effects of epigallocatechin gallate, using ascorbic acid, Phytochemistry 2007; Available from: ScienceDirect. Accessed 2008 July 14.

[4] Sakanaka S, Juneja L R, Taniguchi M. Antimicrobial effects of green tea polyphenols on thermophilic spore-forming bacteria, Journal of Bioscience and Bioengineering 2000; 90 (1): 81-85. Available from: ScienceDirect. Accessed 2008 July 14.

[5] Food and Drug Administration (U.S.) [USFDA]. Bad Bug Book. Staphylococcus aureus. 1992. 5 p. M Walderhaug. Available from: http://www.cfsan.fda.gov/~mow/chap3.html. Accessed 2008 July 23.

[6] Watson J L, Vicario M, Wang A, Moreto M, McKay D M. Immune cell activation and subsequent epithelial dysfunction by Staphylococcus enterotoxin B is attenuated by the green tea polyphenol (−)-epigallocatechin gallate, Cellular Immunology 2005; 237 (1): 7-16. Available from: ScienceDirect. Accessed 2008 July 14.

[7] An B J, Kwak J H, Son J H, Park J M, Lee J Y, Jo C, Byun M W. Biological and anti-microbial activity of irradiated green tea polyphenols, Food Chemistry 2004; 88 (4): 549-555. Available from: ScienceDirect. Accessed 2008 July 14.

[8] Nagle D G, Ferreira D, Zhou Y D. Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives, Phytochemistry 2006; 67 (17): 1849-1855. Available from: ScienceDirect. Accessed 2008 July 26.

[9] Kajiya K, Kumazawa S, Nakayama T. Effects of External Factors on the Interaction of Tea Catechins with Lipid Bilayers, Bioscience, Biotechnology, and Biochemistry 2002; 66: 2330-2335. Available from: JSTOR. Accessed 2008 July 26.

[10] Kajiya K, Kumazawa S, Nakayama T. Steric Effects on the Interaction of Tea Catechins with Lipid Bilayers, Bioscience, Biotechnology, and Biochemistry 2001; 65: 2638-2643. Available from: JSTOR. Accessed 2008 July 26.

[11] Yam T S, Shah S, Hamilton-Miller J M. Microbiological activity of whole and fractionated crude extracts of tea (Camellia sinensis), and of tea components, FEMS Microbiology Letters 1997; 152 (1): 169-174. Available from: ScienceDirect. Accessed 2008 July 14.

[12] Ho M. Green Tea, The Elixir of Life?. In: Mayday [discussion list on the Internet]. 2007 Jan. 18; [cited 2008 July 21]. Available from: http://www.i-sis.org.uk/graphics/Tea-polyphenols.jpg.

[13] Satoh E, Tohyama N, Nishimura M. Comparison of the antioxidant activity of roasted tea with green, oolong, and black teas, International Journal of Food Sciences and Nutrition 2005; 56 (8): 551-559. Available from: PubMed. Accessed 2008 July 26.

[14] Lin S D, Liu E H, Mau J L. Effect of different brewing methods on antioxidant properties of steaming green tea, LWT- Food Science and Technology 2008; 41 (9): 1616-1623. Available from: ScienceDirect. Accessed 2008 July 14.

[15] Sumpio, Bauer E. "Green Tea, the 'Asian Paradox,' and Cardiovascular Disease." Journal of the American College of Surgeons 202.5 (May 2006): 813-25. ScienceDirect. Elsevier. 23 July 2008 <http://www.sciencedirect.com/>.

[16] Kang W S, Lim I H, Yuk D Y, Chung K H, Park J B, Yoo H S, Yun Y P. Antithrombotic activities of green tea catechins and (-)-epigallocatechin gallate, Thrombosis Research 1999; 96 (3): 229-237. Available from: PubMed. Accessed 2008 July 26.

[17] Hara Y. Green Tea: Health Benefits and Applications. CRC Press; 2001. 252 p. Available from: Google Scholar. Accessed 2008 July 26.

[18] Chow H S, Cai Y, Hakim I A, Crowell J A, Shahi F, Brooks C A, Dorr R T, Hara Y, Alberts D S. Pharmacokinetics and Safety of Green Tea Polyphenols after Multiple-Dose Administration of Epigallocatechin Gallate and Polyphenon E in Healthy Individuals, Clinical Cancer Research 2003; 9: 3312-3319. Available from: http://clincancerres.aacrjournals.org/cgi/content/abstract/9/9/3312. Accessed 2008 July 26.

[19] Zaveri N T. Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications, Life Sciences 2006; 78 (18): 2073-2080. Available from: ScienceDirect. Accessed 2008 July 14.

[20] Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechines damage the lipid bilayer, Biochimica et Biophysica Acta (BBA) - Biomembranes 1993; 1147: 132-136. Available from: ScienceDirect. Accessed 2008 July 26.

[21] Mbata T I, Debiao L U, Saikia A. Antibacterial Activity of the Crude Extract of Chinese Green Tea (Camellia sinensis) on Listeria monocytogenes, African Journal of Biotechnology 2008; 7 (10): 1571-1573. Available from: AcademicJournals.org. Accessed 2008 July 17.

[22] Anderson C B, Witter L D. Glutamine and proline accumulation by Staphylococcus aureus with reduction in water activity, Applied and Environmental Microbiology 1982; 43: 1501-1503. Available from: PubMed. Accessed 2008 July 26.

[23] Reedy J. University of Washington News. 2008 Mar. 24; Available from: UWNews.com. Accessed 2008 July 20.

[24] Wang H, Provan G J, Helliwell K. Tea flavonoids: their functions, utilisation and analysis, Trends in Food Science & Technology 2000; 11 (4-5): 152-160. Available from: ScienceDirect. Accessed 2008 July 14.

[25] Amin U S, Lash T D, Wilkinson B J. Proline betaine is a highly effective osmoprotectant for Staphylococcus auerus, Archives of Microbiology 1995; 163: 138-142. Available from: PubMed. Accessed 2008 July 26.

[26] Graham J E, Wilkinson B J. Staphylococcus auereus osmoregulation: roles for choline, glycine betaine, proline, and taurine, Bacteriol 1992; April (174): 2711-2716. Available from: PubMed. Accessed 2008 July 26.

[27] Stapleton P D, Gettert J, Taylor P W. Epicatechin gallate, a component of green tea, reduces halotolerance in Staphylococcus aureus, International Journal of Food Microbiology 2006; 111 (3): 276-279. Available from: ScienceDirect. Accessed 2008 July 14.

[28] Hiramatsu T, Kodama K, Kuroda T, Mizushima T, Tsuchiya T. A putative multisubunit Na+/H+ anitporter from Staphylococcus aureus, Bacteriol 1998; 180: 6642-6648. Available from: PubMed. Accessed 2008 July 26.

[29] Jones E M, Bowker K E, Cooke R, Marshall R J, Reeves D S, MacGowan A P. Salt tolerance of EMRSA-16 and its effect on the sensitivity of screening cultures, Journal of Hospital Infection 1997; 35: 59-62. Available from: ScienceDirect. Accessed 2008 July 26.

[30] Toda M, Okubo S, Hiyoshi R, Shimamura T. The bactericidal activity of tea and coffee, Letters in Applied Microbiology 1989; 8 (4): 123-125. [Abstract] Available from: http://md1.csa.com/partners/viewrecord.php?requester=gs&collection=ENV&recid=2157310. Accessed 2008 July 26.

[31] Scott W J. Water relations of Staphylococcus aureus at 30 C, Australian journal of Biological Sciences 1953; 6 (4): 549-564. Available from: PubMed. Accessed 2008 July 26.

[32] Stapleton P D, Shah S, Hamilton-Miller J M, Hara Y, Nagaoka Y, Kumagai A, Uesato S, Taylor P W. Anti-Staphylococcus aureus activity and oxacillin resistance modulating capacity of 3-O-acyl-catechins, International Journal of Antimicrobial Agents 2004; 24 (4): 374-380. Available from: ScienceDirect. Accessed 2008 July 14.

[33] American Medical Association (USA) [JAMA]. Invasive Methicillin-Resistant Staphylococcus Aureus Infections in the United States. CDC-Information Center; 2007 Oct 17. 9 p.

[34] Tiwari R P, Bharti S K, Kaur H D, Dikshit R P, Hoondal G S. Synergistic antimicrobial activity of tea & antibiotics, Indian Journal of Medical Research 2005; 122: 80-84. Available from: http://icmr.nic.in/ijmr/2005/july/0709.pdf. Accessed 2008 July 26.

[35] Zhao W H, Hu Z Q, Okubo S, Hara Y, Shimamura T. Mechanism of synergy between epigallocatechin gallate and β-lactams against methicillinresistant Staphylococcus aureus, Antimicrobial Agents and Chemotherapy 2001; 45: 1737-1742. Available from: PubMed. Accessed 2008 July 26.

[36] Si W, Gong J, Tsao R, Kalab M, Yang R, Yulong Y. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. Journal of Chromatography A, 2006;1125:204-210. Available from: ScienceDirect. Accessed 2008 July 26.

[37] Sengun I Y, Karapinar M. Elimination of *Yersinia enterocolitica* on carrots (*Daucus carota* L.) by using household sanitizers, Food Control 2005; 16 (10): 845-850. Available from: ScienceDirect. Accessed 2008 July 23.

[38] Parfentjev I A, Catelli A R. Tolerance of Staphylcoccus aureus to sodium chloride, Bacteriol 1964; (88): 1-3. Available from: PubMed. Accessed 2008 July 26.

[39] Rusak G, Komes G, Likić S, Horžić D, Kovač M. Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used, Food Chemistry 2008; 110 (4): 852-858. Available from: ScienceDirect. Accessed 2008 July 14.

[41] Iralu V. Trypanosoma cruzi Cultivation in Trypticase Soy Broth with Hemin and Serum, The Journal of Parasitology 1967; 53 (3): Available from: http://www.jstor.org/. Accessed 2008 June 21.

[42] Squires K. The Essential Cleaning Kit. Real Simple; 2007. Accessed 2008 July 20.

[43] The World's Healthiest Foods Association [WH Foods]. Lemons/Limes n.d.