INHIBITORY EFFECTS OF *Camellia sinensis* (GREEN TEA) ON *Streptococcus mutans*

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ABSTRACT

Green tea (*Camellia sinensis*) contains polyphenolic catechins reported to exhibit significant antimicrobial properties. This study tested green tea at a higher concentration against *Streptococcus mutans*, a common cariogenic bacterium. An *in vitro* study using paper disk diffusion determined that green tea at higher concentrations brewed at 90ºC proved most efficient in the inhibition of bacterial growth. A minimum inhibitory concentration (MIC) test was also conducted to determine pharmacologically effective concentrations of green tea. Although there were signs of contamination, plates inoculated with green tea clearly contained less growth than the water control. A mouth swabbing study was performed with green tea, Listerine®, and water, where only the mouths rinsed with Listerine® showed slightly less bacterial growth. Finally, several brands of mouthwashes were tested for their comparative efficacies against *S. mutans*. A wide range of potencies was observed, with mouthwashes containing cetylpyridinium chloride as most effective. Green tea, at a concentration of 40 mg/mL, brewed at 90° C at 5, 20, and 40 minutes, was determined to be moderately effective against *S. mutans.* Further studies are required to conclusively determine the specific conditions for optimal inhibition and thus, practical use.

INTRODUCTION

In recent years, the American public has become increasingly interested in natural products. From 2002 to 2007, the consumption of organic goods doubled in the United States. The availability of these products has also increased to meet the needs of a more health-conscious population. In 2006, more than 2,000 new organic items were made available for purchase in grocery stores throughout America[1]. In response to this movement and as a result of the growing problem of antibiotic resistant bacteria, studies are now being conducted regarding the antimicrobial effects of compounds found in natural foods, such as green tea[2].

Green tea, *Camellia sinensis,* is a widely consumed beverage that is especially popular in the Far East. Green and black teas are infusions of the leaves of the same evergreen shrub. Black tea, however, undergoes fermentation, while green tea does not[3]. There are also other variations of tea, including white and oolong tea. Oolong tea is an intermediate between green tea and black tea because it undergoes more fermentation than the former but less than the latter. White tea is made from buds and young leaves; in contrast, green, black, and oolong teas are made from more mature leaves[4]. Other herbal teas, such as peppermint, chamomile, and jasmine tea cannot be attributed with the same properties as green tea, as they are not made from the leaves of *Camellia sinensis*[5].

Green tea originated in China, and later spread to India, Japan, Europe, Russia, and eventually to the New World in the 17th century. According to legend, green tea was first discovered by Chinese Emperor Shen Nung when tea leaves accidentally blew into his pot of boiling water. Throughout the 3rd century AD, this refreshing drink was used primarily for medicinal purposes. Then, during the Tang Dynasty, also known as, the "golden age" of tea, people began to consume the drink for both enjoyment and health restoration. The consumption of tea became common not only among the wealthy, but throughout the entire population. Eventually, tea spread throughout the world, largely through trade. Today, tea is one of the most common beverages in the world, second only to water[6].

Green tea has long been valued throughout the world for its therapeutic properties[6]. It is considered mildly refreshing and produces an overall feeling of contentment. In addition, green tea has been shown to strengthen capillaries, facilitate weight loss, and even inhibit the growth of implanted malignant cells [3, 7]. The aforementioned benefits of green tea are often attributed to its antioxidant properties. Antioxidants remove free radicals, which are unstable molecules or atoms with one unpaired electron. The molecule or atom favors having paired electrons and becomes highly reactive, creating an imbalance in the body. Antioxidants donate electrons to free radicals to prevent chemical instability[8]. The medicinal properties of green tea have largely been credited to its catechins, flavonoids, and other chemical constituents.

The chemical composition of green tea varies with climate, season, horticultural practices, and leaf age. Green tea contains a multifarious grouping of antioxidants, vitamins, and minerals, including ascorbic acid (vitamin C) and water-soluble B vitamins. These chemical compounds are quickly released in a cup of tea. A cup of green tea also provides a small amount of potassium, manganese, magnesium, and fluoride[9]. Green tea does not undergo fermentation and thus retains its polyphenols. A phenol is a benzene group with a hydroxyl group attached; the term polyphenol is used when multiple phenols are bonded together. The polyphenols, which create tea's bitter taste, are credited with antimicrobial properties[10]. Specific antioxidant polyphenols, called catechins, play the most active role in green tea's inhibition of bacterial growth. Examples of several significant catechins include: (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC), and (-)-gallocatechin-3-gallate (GCG) (Figure 1) [11, 12].

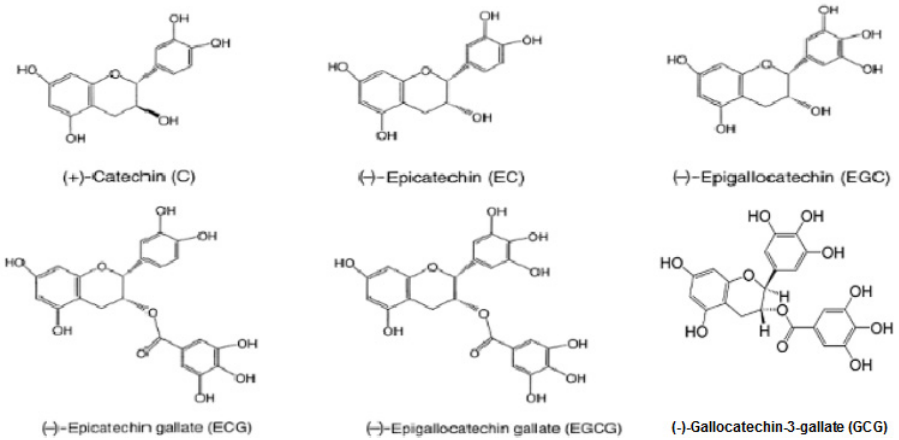

Figure 1. The chemical structure of tea's catechins.

EGCG accounts for 50% of the catechin mass found in green tea. Of the over 8,000 scientific literary works that cite the chemistry, bioactivity, production, and potential health benefits of green tea, about half relate to EGCG. A vast majority of *in vitro* studies conducted demonstrate that EGCG in high concentrations inhibits disease-causing molecular targets and cellular processes. *In vivo* studies conducted on animals confirm EGCG's prevention of tumor growth and cardiovascular disease[13]. EGCG is also hypothesized to inhibit tumorigenesis by preventing the release of tumor necrosis factor-alpha, a catechin believed to stimulate the growth of malignant cells. Scientists believe EGCG can boost metabolism and consequently facilitate weight loss. In an experiment conducted at the University of Chicago, rats lost up to 21% of their body weight. Catechins are believed to have played an active role in the study, inhibiting leptin receptors from helping to create appetite. EGCG has also been reported to inhibit lipid peroxidation, an oxidative process involved in several pathologic conditions, including atherosclerosis, which is the buildup of fatty materials in the arteries[7].

While many of green tea's health benefits are ascribed to EGCG, the overall medicinal potency of the beverage relies on all of the compounds. ECG is the second most abundant catechin found in green tea, comprising 10-20% of the total catechin mass. The two compounds behave in fairly similar ways, suggesting that ECG's effectiveness rivals that of EGCG. According to a study, ECG proved to be cytotoxic to carcinoma HSC-2 cells and induced apoptosis only in the carcinoma cells, indicating that ECG may play a large role in inhibiting tumorigenesis[14]. In another experiment, researchers tested ECG's protection of skin cells from ultraviolet rays, and the study concluded that ECG does indeed prevent skin photoaging[15]. Evidently, both EGCG and ECG are potent compounds in green tea that contribute to its medicinal efficacy.

Several studies have demonstrated the effectiveness of green tea as an antimicrobial. Consumption of green tea has been shown to prevent or reduce gastrointestinal infections, including those caused by *Helicobacter pylori*[2]. The growth of other bacteria, *Campylobacter jejuni* and *Campylobacter coli*, is also lessened by green tea. These bacteria are the principal causes of enteric infections[16]. Previous research has demonstrated the antagonistic power of ECG and EGCG on human immunodeficiency virus (HIV) reverse transcriptase. At 10 to 20 ng/mL concentrations, the presence of these components resulted in 50% inhibition of reverse transcriptase[17]. EGCG also helps restrain tuberculosis by down-regulation of the tryptophan-aspartate containing coat protein, which plays an important part in the maturation of tuberculosis[18]. Green tea polyphenols exhibit inhibitory actions towards thermophilic spore-forming bacteria, as well. In the case of *Bacillus stearothermophilus*, the heat resistance of the strain is lessened, and the bacteria are eradicated[19].

More than 600 oral bacteria exist in the human body; some of these are beneficial and even indispensable to human well-being, while others are considered disease-causing agents[20]. The bacterium present on the surface of teeth and gums assist in food digestion and defend against any detrimental bacteria[21]. For instance, the K-12 strain of *Streptococcus salivarius* fights other *Streptococcus* bacteria that are responsible for causing strep throat[22]. Another disease-causing bacterium is *Porphyromonas gingivalis*. *P. gingivalis* is a gram-negative anaerobe associated with periodontal disease. It attaches to the tooth enamel and replaces the gram-positive bacteria found in that region, resulting in gum inflammation[23]. Additional examples of bacteria that contribute to forms of dental decay include *Actinobacillus*

actinomycetemcomitans, Bacteroides forsythus, Treponema denticola, and *Streptococcus mutans*[24].

Streptococcus mutans is a bacterium responsible for the formation of dental caries, commonly known as cavities[19]. Examined under a microscope, this coccus-shaped bacterium appears as a chain of spheres. *S. mutans* is an immobile facultative anaerobe, preferring an environment without oxygen. The most favorable temperature to culture this microbe is 37°C, which is approximately the human oral temperature[25, 26].

S. mutans is a gram-positive bacterium that contains peptidoglycan, a polymer that reinforces the cell wall[27, 28]. Compared to gram-negative strains, gram-positive bacteria lack an outer membrane, have thick peptidoglycan layers, and exhibit a lower lipid and lipoprotein levels (Figure 2)[29]. When stained, gram-positive bacteria absorb the crystal violet stain, resulting in a purple coloration. (Figure 3)[30]. Examples of gram-positive bacteria include *Streptococcus pneumoniae*, and *Staphylococcus aureus*[27]. In contrast, gram-negative bacteria absorb the safranin counterstain, which causes the bacteria to appear pink (Figure 4)[31]. Gram-negative bacteria include *Neisseria meningitides*, *Neisseria gonorrhoeae*, and *Escherichia coli*[27].

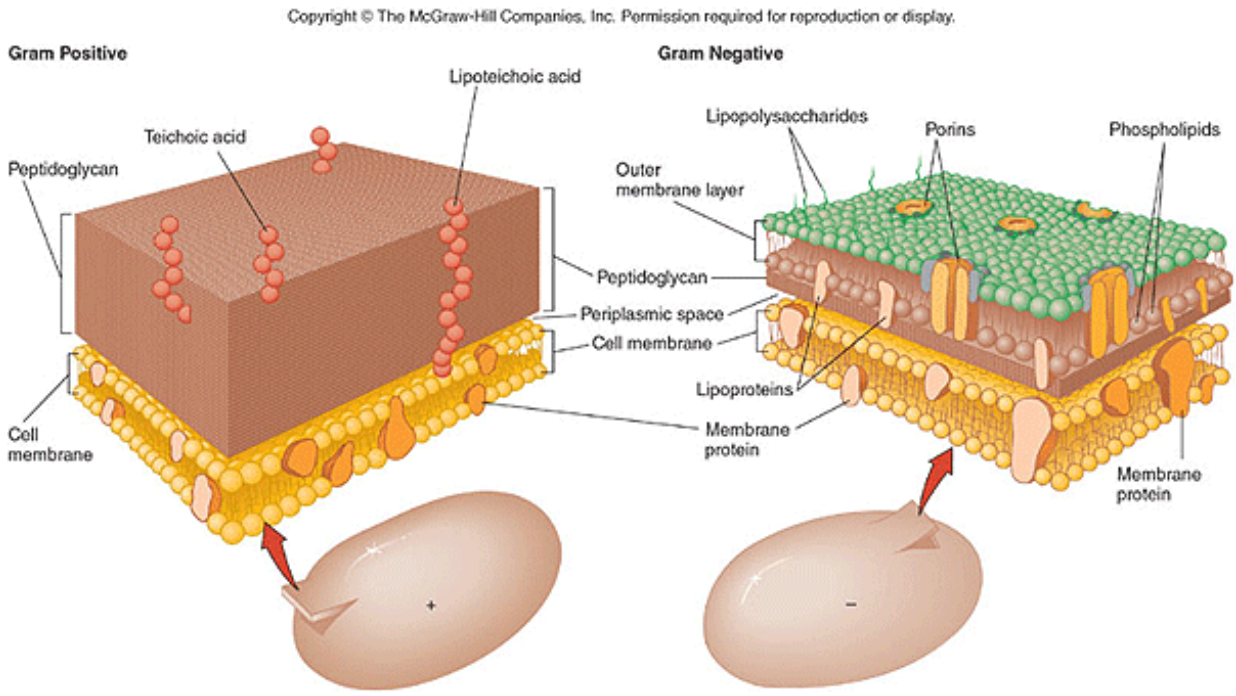

Figure 2. Differences between the cell walls of gram-positive and gram-negative bacteria

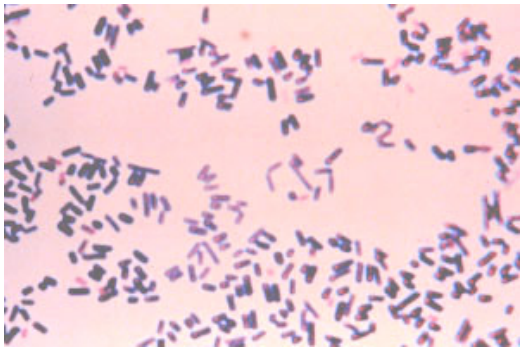

Figure 3. Stain of gram-positive bacteria

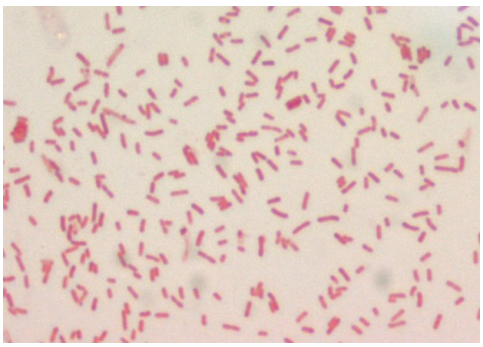

Figure 4. Stain of gram-negative bacteria

The formation of dental caries begins when *S. mutans* adheres to the surface of the tooth enamel. Adhesion results from the fermentation of dietary carbohydrates (primarily sucrose), which initiates the production of dextran. This water-insoluble substance contributes to the formation of plaque on the tooth surface, creating an optimal environment for other cariogenic bacteria[32]. As tooth enamel erodes and bacteria produce lactic acid the pH on the tooth surface decreases to less than 5.0 and a cavity is created[33].

Although *S. mutans* is frequently associated with poor dental health, W. J. Loesche *et al.* have discovered that the presence of this bacterium does not always indicate dental decay. Under certain circumstances, the consumption of dietary carbohydrates may not lead to the exacerbation of dental health. They observed that dental decay did not increase in individuals who consumed half a pound of sucrose per meal; however, when individuals consumed less sucrose more frequently, an increase in dental decay was observed. Based on these results, Loesche *et al.* believe that frequent consumption of dietary carbohydrates allows *S. mutans* to produce more lactic acid from anaerobic respiration. As a result, salivary buffers that normally neutralize acid become overwhelmed and tooth enamel erodes, ultimately causing dental decay[32].

The catechin compounds in green tea may inhibit the bacteria's capacity to adhere to, and ultimately grow in, an oral cavity[34]. In *S. mutans*, two different groups of glucosyltransferases cooperatively synthesize an adherent and water-insoluble glucan responsible for bacterial adherence to the tooth enamel. EGCG and ECG are believed to bind to GTases and irreversibly inactivate them, ultimately preventing the formation of dental caries[19, 34].

In this experiment, the effects of green tea constituents on the inhibition of *S. mutans* were studied. The green tea was hypothesized to decrease the quantity of *S. mutans* in the mouth and thereby act as a natural mouthwash.

MATERIALS AND METHODS

Paper Disk Diffusion

Tryptic Soy Agar was prepared as per instructions and autoclaved. The agar was then cooled and poured into plastic Petri dishes to solidify. Pearl Green Tea® distributed by Walong Marketing Incorporated was crushed. 20 mg/mL of tea were brewed at 60°C, 70°C, 80°C, and 90°C. 40 mg/mL of tea were also brewed at 90°C. Distilled water was heated to each of the temperatures to serve as a control. Tea was removed after five, twenty, and forty minutes, and

25 µL of each of the time-temperature-concentration combinations were micropipetted onto paper disks. After the disks dried, another 25 µL of the same liquid were added. Once a total of 50 µL of either tea or water had been added to the disks, the discs were transferred using sterilized forceps to agar plates, which were inoculated with *Streptococcus mutans* bacteria. The agar plates were marked using the system shown in Table 1. The finished plates were then incubated for 48 hours.

Table 1. Time-Temperature-Concentration Combinations

Brewing Concentration (mg/mL)		**20**				**40**
Brewing Temperature (°C)		**60**	**70**	**80**	**90**	**90**
Brewing Time (minutes)	**5**	A1	B1	C1	D1	E1
	20	A2	B2	C2	D2	E2
	40	A3	B3	C3	D3	E3

Minimum Inhibitory Concentration

A Tryptic Soy nutrient broth was prepared using half of the suggested quantity of water and was autoclaved. To determine the average amount of green tea in a standard tea bag, five Nature's Promise® green tea bags were massed. The average mass of one tea bag was determined to be 1.29 grams. In a standard cup of tea, 5.45 grams of leaves were used to prepare a liter of tea. Five different concentrations of tea were used: 2.73 mg/mL (half of a standard cup of tea), 5.45 mg/mL (a standard cup of tea), 10.90 mg/mL (twice a standard cup of tea), 21.80 mg/mL (four times a standard cup of tea), and 32.7 mg/mL (six times a standard cup of tea). These concentrations of tea were diluted further when added to the nutrient broth and the stock culture solution. The actual concentrations of tea in the solutions from least to greatest were 1.310 mg/mL, 2.616 mg/mL, 5.23 mg/mL, 10.464 mg/mL, and 15.696 mg/mL, and the concentrations were labeled A, B, C, D, and E, respectively. Water was used as a control.

Distilled water was heated to 90°C. The tea preparations were brewed in their respective beakers for ten minutes and filtered into test tubes. 2.5 mL of nutrient broth, 2.4 mL of each tea, and 0.1 mL of stock culture solution (containing the *S. mutans*) were mixed in each test tube. The test tubes were vortexed and then placed into the incubator for 48 hours. Afterwards, 200 µL of liquid were extracted from each test tube and spread into agar plates. The plates were incubated for another 48 hours.

Mouth Swabbing Study

An *in vivo* study was conducted using eleven subjects. Blood agar plates were divided into four quadrants. Each subject swabbed his or her teeth with a sterile swab and densely streaked the swab onto one of the four labeled quadrants on his or her agar plate. The subjects rinsed their mouths for thirty seconds with 20 mL of either Cool Mint Listerine®, water, or green tea. The green tea was prepared from Nature's Promise® green tea bags brewed at twice the suggested concentration. The subjects' teeth were swabbed immediately after rinsing, an hour after rinsing, and two hours after rinsing. The samples were spread in their corresponding quadrants immediately after swabbing, and the plates were incubated for 48 hours. After results were observed, a second study was conducted using blood agar plates divided in halves. Cultures were collected only before rinsing and immediately after rinsing. From the mouth-swabbing test, colonies of bacteria were isolated and spread onto agar plates for further observation.

Bacterial samples were taken from the plates of subjects who were tested with Listerine®, water, and green tea. The samples were placed on slides, and a gram-stain was performed. The slides were then placed under an oil-immersion microscope for observation.

Mouthwash

Blood agar plates were inoculated with *S. mutans.* Paper disks were cut out of filter paper and soaked in various mouthwashes for fifteen minutes. The mouthwashes were Crest Pro-Health®, Listerine®, Cool Mint Listerine®, Tom's Natural Cleansing Mouthwash®, Scope Mouthwash®, Cepacol Antibacterial Mouthwash®, Act-Restoring Anticavity Fluoride Mouthwash®, Original Listerine®, and Oasis Moisturizing Mouthwash®. Nine disks were evenly spaced on the plates and incubated for forty-eight hours.

RESULTS

Paper Disk Diffusion

The average zone of inhibition for E2 was 1.1 cm, while the average zone of inhibition for E3 was 1.3 cm. E2 was soaked with 40 mg/mL of tea brewed at 90ºC for 20 minutes. E3 was 40 mg/mL of tea brewed at 90ºC for 40 minutes. E1, 40 mg/mL of tea brewed at 90ºC for 40 minutes, yielded a visible zone of inhibition only on one of the agar plates and was, therefore, not included in the graph and statistical analysis. All other preparations of tea failed to yield visible zones of inhibition.

A one-tailed two-sample t-test was run at an alpha level of 0.05 to determine if the zone of inhibition produced by E3 was significantly larger than the zone of inhibition produced by E2. A p-value of 0.1607 was generated, indicating that the zone of inhibition produced by E3 was not significantly larger than that produced by E2.

A second one-tailed t-test was run at an alpha level of 0.05 to determine if the zone of inhibition produced by E2 was significantly greater than zero. With a standard error of 0.1, a p-value of 0.0061 was generated. The p-value was less than the alpha of 0.05, which means that the zone of inhibition produced by E2 was significantly greater than zero.

This one-tailed test was repeated for E3. With a standard error of 0.2646 a p-value of 0.0222 was generated. This was greater than the alpha of 0.05, which indicated that the zone of inhibition produced by E3 was significantly greater than zero.

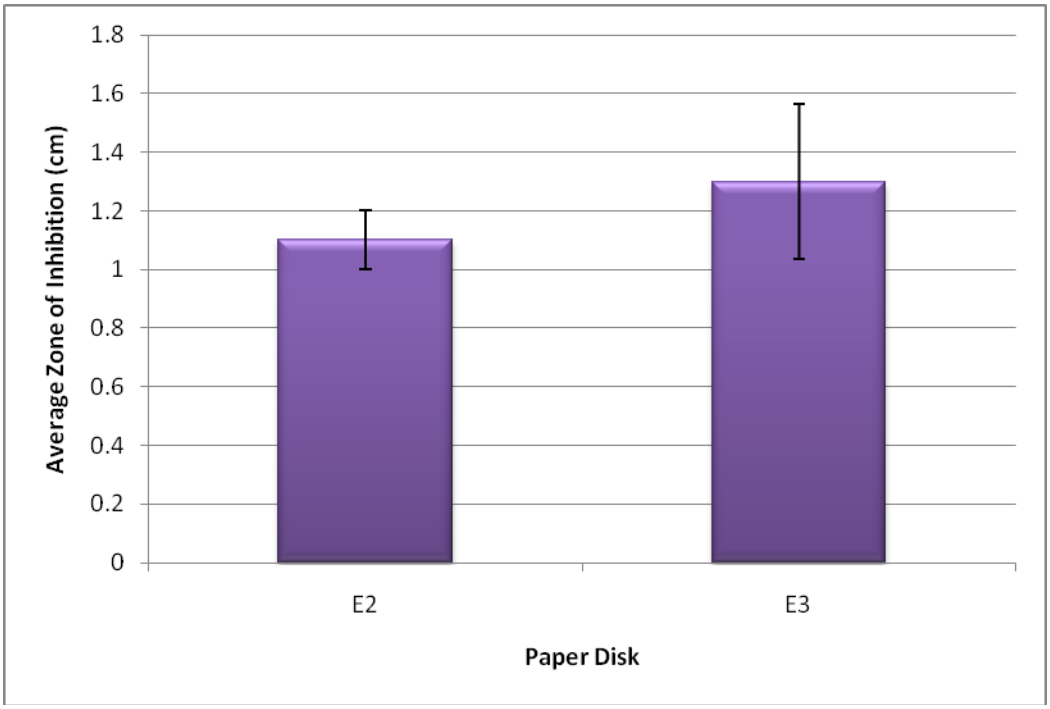

Figure 5. Average Zones of Inhibition for Paper Discs. The error bars represent the standard deviations, which were 0.1 and 0.2646 for E2 and E4 respectively. The sample size for both E2 and E3 was three.

Minimum Inhibitory Concentration

The individual preparations of green tea were all relatively effective, with slight differences between them. Green tea was significantly more effective in preventing bacterial growth than was the water control. However, the highest concentration of tea had the most bacterial growth while the lowest concentration of tea had the lowest bacterial growth.

Mouth Swabbing

The *in vivo* study did not yield conclusive results. Listerine® appeared to have decreased the number of small bacterial colonies immediately after rinsing but had no effect on the number of colonies one hour and two hours after rinsing. Also, green tea and water seemed to have little or no effect on the number of bacterial colonies. After conducting a gram stain, the bacteria appeared violet under the microscope.

Mouthwash

Of the nine mouthwashes, Scope®, Crest®, and Oasis® had the largest zones of inhibition; Tom's Mouthwash® and all Listerine® mouthwashes had no zone of inhibition.

DISCUSSION

In the paper disk diffusion test, two distinct zones (around E2 and E3) of inhibition developed. E2 contained 40 mg/mL of tea brewed at 90ºC for 20 minutes, while E3 contained 40 mg/mL of tea brewed at 90ºC for 40 minutes. According to similar studies, approximately 1039.9 μg/mL of EGCG and 97.9 μg/mL of ECG should have been present in E2[35]. Approximately 1071.4 μg/mL of EGCG and 98.3 μg/mL of ECG should have been present in E3[35]. EGCG and ECG are the main components of tea polyphenols and were brewed in a manner meant to extract maximum quantities of the catechins[19]. The E2 and E3 disks contained preparations of tea with higher concentrations of EGCG and ECG which were successful in inhibiting bacterial growth, while other tea preparations with lower catechin concentrations were not. The statistical analysis demonstrated that these zones were significantly larger than zero, meaning that the tea effectively inhibited bacteria growth. The results support the hypothesis that that *Camellia sinensis* did inhibit the growth of *Streptococcus mutans*.

The results may have been affected by a number of errors. For instance, an uneven and thinner lawn of bacteria could have produced less visible zones of inhibition around the other paper disks. Perhaps smaller zones of inhibition existed but went unnoticed, leading to the false conclusion that various preparations of tea did not interfere at all with the bacteria's growth. Using a different nutrient agar or a higher CFU (colony-forming units) concentration may have formed a denser lawn.

To better understand the ability of green tea to inhibit *S. mutans*, future studies should modify the procedure used. The temperature of the tea could be modified to find the optimal conditions for the catechins to inhibit bacterial growth. The optimal conditions could be determined by testing a variety of brewing temperature and concentration combinations. Further research could examine the higher concentrations of green tea and narrow in on the exact time needed for the tea to be just as effective.

According to the minimum inhibitory concentration test, all test tubes showed reduced bacterial growth. In comparison, the water control showed large amounts of bacterial growth, demonstrating that green tea can serve as an inhibitory agent against *S. mutans*. The two test tubes of the lowest concentrations showed the least bacterial growth. Therefore, contamination of the tea leaves and glassware used for the other test tube samples may have interfered with the results obtained. There was also no control of only tea and nutrient broth, which would have indicated contamination in the green tea leaves.

The unexpected bacterial growth led to the conclusion that the tea concentrations were inconsistent; the inconsistency could have been the result of various brewing times. Although brewing time was targeted at 10 minutes, the timings were estimated, which may have altered the concentrations of tea compounds. The teas were brewed simultaneously, and filtering each tea sample simultaneously was not possible. Another option is brewing and pouring the tea samples in a time-staggered fashion, which would allow fewer individuals to perform the same task.

In future experiments, the pouring of the tea should be done more carefully so that solids do not pass through the filter paper; another option would be the use of finer filter paper. The C tea (5.23 mg/mL), despite having half the concentration of D tea (10.464 mg/ml), appeared

darker. This may have occurred due to the filtering process used to strain the leaves and solid particles out of the tea. When the C tea was poured into the filter paper, some leaves and large powdery leaf fragments went into the paper with the tea. This did not occur with any other tea. The filter paper was expected to filter out all the solid particles, but some may have entered the tea and darkened it, increasing the concentration of solids. The D tea was found to be the exception to the pattern of lower tea concentrations inhibiting the most growth.

In order to gather more conclusive data, better aseptic techniques should be employed. The glass spreader may still have been hot when used, thereby killing the bacteria. By touching an agar plate not in use, the glass spreader can be cooled down without killing the bacteria. Moreover, the experiment was conducted in an open-air environment where there is always a risk of airborne contamination of the tea or equipment.

The results obtained from the first set of mouth swabs were inconclusive. This could be because of faulty methodology or ineffective products. Among the subjects, the swabbing was inconsistent. They swabbed for different lengths of time and may have spent the bulk of the time swabbing different regions of the mouth (which may have different bacteria associated with them). Subjects should have received more detailed instruction on how to swab to ensure consistency. Also, if the swabbing was done by only one person, the procedure could have been more consistent. The streaks made by the swabs in the blood agar were very dense in their respective sections of the plate. Therefore, distinguishing colonies effectively was difficult because the streaks merged into each other. Our results also did not show that the growth of bacteria was time dependent. The amount of bacteria from one and two-hours later was the same as the amount immediately after the rinse.

The experiment with the second set of mouth swabs was conducted as a follow-up to the first set. Subjects were instructed to swab only before and immediately after the rinse because no changes in bacteria were observed with the passage of time. Also, lengthier, more spaced-out streaks were used for the second set of swabs so that colonies could be more easily distinguished, and thus the effects of the liquids better observed.

These effects revealed that the products used did not work as predicted. Listerine® claims to kill 99.9% of germs in the mouth when used as instructed. However, qualitative analysis of the blood agar plates swabbed by those who rinsed with Listerine® reveals that a much smaller percentage of bacteria was killed. Before the rinsing, the bacteria growth from the swab contained both small and large colonies. After the rinsing, only the large colonies remained. Further studies can be conducted to determine both the identity of the bacteria that survived and the cause of its survival. Also, the green tea and water produced a small decrease in the number of bacterial colonies. For green tea to have a significant result, however, the method used in the study (two bags per cup) is insufficient for killing oral bacteria. The methods used to brew the more concentrated green tea in the paper disk diffusion were shown to be more effective in inhibiting the growth of bacteria. Therefore, those methods, though impractical in an average household (brewing at 90ºC for at least 20 minutes), could make for a more effective mouthwash. The use of freshly crushed whole-leaf teas and longer brewing times may also make the tea more effective.

The green tea may not have been effective in the *in vivo* test because methodology may not have been optimal. A Listerine® bottle gives exact figures such as volume of liquid and duration for rinsing, whereas the effective methods for using green tea as a mouthwash are unknown. In addition, the mouthwash is at a fixed concentration, while the concentration of green tea depends on its preparation. These conditions may not have been the most ideal for the catechins to inhibit bacterial growth. For future studies, any factors such as concentration, volume, and rinsing time may have to be modified to greater values in order to inhibit *S. mutans*.

Finally, the bacteria collected from the subjects' mouths were not all *S. mutans*. The mouth is home to many bacteria other than *S. mutans*: over 600 different types of bacteria are estimated to live in the mouth[36]. Future studies could run further tests on the bacteria deposited on the agar plates to better determine its identity. A bigger sample size would have also helped this experiment. More people would have provided more results, and with a greater number of plates to analyze, more reliable conclusions could be drawn.

A paper disk diffusion test was conducted to test the effectiveness of different mouthwashes on the inhibition of *S. mutans.* This was done because it provided quantitative data (zone of inhibition). Also, it ensured that *S. mutans* was definitely the bacteria being tested while the mouth swabbing test could have involved any bacteria. The resulting bacterial growth provided unexpected results. The brand of Listerine® used in the mouth swabbing study did not inhibit the growth of *S. mutans* in two of three plates even though it appeared slightly effective in the mouth swabbing study. The mouth swabbing study conducted previously may have produced better results by using some of the more effective mouthwashes. Each of the three most effective mouthwashes, Scope®, Crest®, and Oasis®, contained cetylpyridinium chloride as an ingredient. This compound was absent from the mouthwashes that did not show any degree of inhibition against *S. mutans.*

Cetylpyridinium Chloride (CPC) is a quaternary ammonium compound known to inhibit bacterial growth based on electrostatic interactions. Bacterial cell walls favor the binding of positively charged cetyl molecules allowing CPC to penetrate the cell wall. Bacterial metabolism is thus disrupted, ultimately leading to cell death[37]. There is evidence that CPC is capable of interaction with *S. mutans* biofilms and can be used for dental plaque control. CPC is able to integrate itself into the bilipid membrane and change its permeability, allowing leaks or defects of cellular components[38]. In further research, examining the effects of a mouthwash containing CPC on bacterial growth may be beneficial. Mouthwashes that contain CPC should be used rather than Listerine®, as the CPC compounds have been shown to work.

The paper disks used in this experiment were hand cut from filter paper; consequently, they were imperfect in size, and accuracy in quantitative measurements of respective zones of inhibition was sacrificed. As an alternative, small premade paper disks should be used. Four paper disks were placed to soak in each mouthwash for 15 minutes although there may have been some slight inconsistencies in time based upon the order they were added to soak. More people may be required to standardize the time, or the disks may be soaked in a time staggered manner (instead of simultaneously).

The paper disks were applied to the plate while still wet. This may account for the discrepancy involving Listerine® on plate 1. On this plate, Listerine® was placed next to Crest®,

a mouthwash that showed significant inhibition. There was no bacterial growth between the two disks. Handling of the plates may have caused the mouthwash to run, accounting for the lack of bacteria between the two disks and Listerine's® apparent effectiveness on this plate only. Paper disks should be allowed to dry to a moist state before application rather than being applied directly from the beaker.

CONCLUSION

The paper disk diffusion test confirmed the hypothesis that green tea inhibits *S. mutans*. However, the tea only demonstrated this property at a higher concentration, so future research should concentrate on teas of higher concentrations. Inconclusive results came out of the MIC test, for lower concentrations of tea showed increased ability to inhibit bacteria. However, the plates with tea showed less growth than those with water, confirming the ability of green tea to inhibit *S. mutans* under certain conditions. Success on a petri dish did not translate to success in subjects' mouths. The *in vivo* test yielded inconclusive results; the plates with tea and water showed little change in bacterial growth before and after swabbing. Uncertainty concerning proper preparation of green tea and the small sample size used may have contributed to the inconclusive results. Future investigation should revise conditions for mouth swabbing and employ a larger sample size. The mouthwash test, performed as a follow-up, revealed that Listerine® had little ability to inhibit bacterial growth, while other mouthwashes worked better (and thus should replace Listerine® in future studies).

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