THE INHIBITORY EFFECTS OF GREEN TEA (*CAMELLIA SINENSIS*) ON THE GROWTH AND PROLIFERATION OF ORAL BACTERIA

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ABSTRACT

Camellia sinensis, commonly known as green tea, has been shown to possess antimicrobial properties and to lower the risk of cardiovascular disease and periodontal diseases. This study investigates the effects of brewing green tea at varying concentrations and durations on its antimicrobial activity against common oral bacteria, such as *Streptococcus mutans, Porphyromonas gingivalis,* and *Staphylococcus epidermis*. Gram stain tests revealed that our bacteria cultures had a mixture of Gram-positive and Gram-negative bacteria. A paper disk diffusion test revealed that increasing the concentration of green tea and decreasing brewing time increased the zones of inhibition; the tea brewed at a concentration of 80 mg/mL for 20 minutes had the greatest antibacterial effect. In the mouthwash paper disk diffusion test, a new bottle of Scope® was found to be most effective against common oral bacteria, while Listerine® was found to have little effect. The minimum inhibitory concentration test implied a positive correlation between the concentration of green tea and bacterial growth. Tests indicated that Scope® had a considerable effect against bacterial growth, green tea had minimal effect, and water had no effect; however, these results were inconclusive due to small sample size. As confirmed by the study, green tea does have antibacterial properties, but further investigations are required to make a definitive conclusion.

INTRODUCTION

Green tea is a beverage made from the evergreen plant *Camellia sinensis* and has been enjoyed for thousands of years. Generally, *Camellia sinensis* leaves are added to hot water and allowed to brew for several minutes. The resulting beverage lowers the risk for cardiovascular disease and periodontal diseases, and it also has antibacterial and antimicrobial properties[1]. However, many of its health benefits are not well known since studies on green tea have been conducted only fairly recently.

Green tea has long been believed to be beneficial to one's health and has a long history of widespread consumption. Evidence shows that green tea was consumed as early as the third century AD, yet multiple stories suggest it was brewed much earlier. One legend says that in 2737 B.C. an herbalist named Shen Nung was boiling water to drink while resting under a tree. A breeze caused green tea leaves to fall into his steaming water. When he drank the resulting liquid, Shen Nung was pleasantly surprised by the stimulating taste, and the tradition of tea consumption began. Since the third century, green tea has been used for medicinal purposes, such as depression, stomach problems, and anxiety. The cultivation of green tea rapidly increased during the Tang Dynasty[2], and Lu Yu wrote a famous book called *Tea Classic*, which

discussed the production, consumption, and culture of green tea[3]. Around 1211, a Buddhist name Eisai wrote *Kissa Yohjoh Ki*, the first book discussing the health benefits of green tea on the "five vital organs[4]." During the Ming Dynasty in China, green tea became a common drink of the Chinese populace and helped to prevent scurvy in Chinese seamen due to its vitamin C concentration. Today, China and Japan are the world's leading producers of green tea[2], the second most popular drink in the world after water[3]. Publicized studies on the health benefits of green tea have only been available since the 1990's. However, green tea's popularity in the West can be attributed to the growing interest in its potential health benefits[2].

Green tea's numerous health benefits are the result of the large percentage of polyphenols found within the tea, even though the polyphenol content varies due to environmental factors like rainfall and season. The major polyphenols are the catechins: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), epigallate (EG), and catechin (C). EGCG, the most active component of green tea, provides most of its properties because it contains multiple chemically reactive hydroxyl groups (Fig. 1). In one study where green tea leaves were kept in the mouth for several minutes, high concentrations of catechins remained in the mouth after rinsing. These results suggest that tea leaves are a dependable natural source of catechins[8]. Green tea also contains a myriad of other compounds including gallic acid, quercetin, kaempferol, myricetin, caffeic acid, and chlorogenic acid[1].

In addition to green tea, black and oolong tea are also common beverages derived from *Camellia sinensis* that contain polyphenols. However, black tea is fully fermented, while oolong tea is partially fermented. The fermentation process oxidizes many of polyphenols catalyzed by polyphenol oxidase, degrading EGCG and reducing tea's antibacterial potency. Unlike black and oolong tea, green tea is unfermented, thus containing the highest concentrations of polyphenols and most likely possessing the greatest antibacterial effect[1].

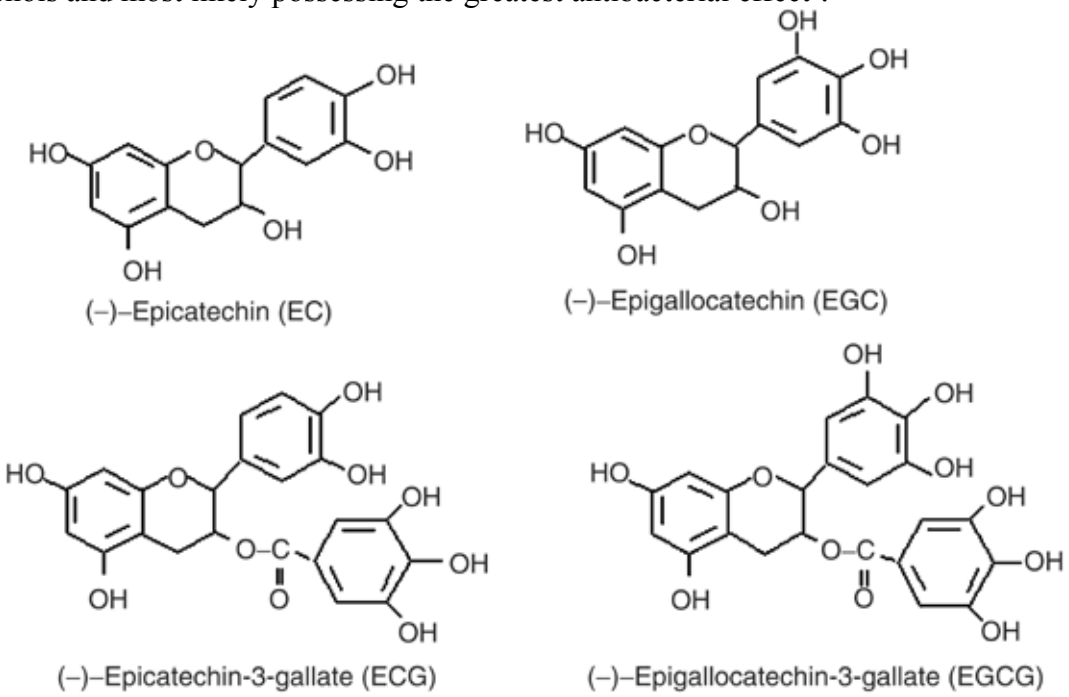

Fig. 1. Chemical structures of major catechins[1]. The most common polyphenols in green tea.

Many conditions, including high cholesterol, diabetes, liver and kidney complications, aging and related degenerative diseases, can be traced back to free radicals, which are extremely reactive in the body. The polyphenols in green tea, mainly EGCG, display anti-oxidative properties by acting as free radical scavengers. The flavonoids can react with free radicals and effectively eliminate possible negative health effects. The presence of three hydroxyl moieties at 3', 4', and 5' on the B ring in the EGCG are primarily responsible for this inhibitory activity[5].

Previous studies have found that green tea polyphenols can decrease LDL cholesterol levels, thereby increasing the ratio of good cholesterol (HDL) to bad cholesterol (LDL)[1]. In addition, EGCG has been found to lower plasma cholesterol and triglyceride levels, consequently reducing the risk of cardiovascular disease and inhibiting the growth of abnormal blood clots[6].

Several studies testing green tea's anticarcinogenic effects have concluded that green tea prevents and inhibits several forms of cancer, including biliary tract, bladder, breast, colon, esophageal, prostate, and skin cancers. Cancer is the uncontrolled growth and division of cells, which interferes with normal body processes. Cancer cells rely on inflammation to promote tumor angiogenesis. The polyphenols reduce this form of inflammation, effectively inhibiting cancer progression[7]. In addition, the polyphenols in green tea prevent the growth of blood vessels, slowing down metastasis by inhibiting the distribution of nutrients to the cancer cells[6]. Green tea also slows the release of tumor necrosis kappa-B function, which is critical for tumor growth (Fig. 2).

Furthermore, EGCG and other polyphenols found in green tea promote cell cycle arrest and induce apoptosis in cancerous cells. The polyphenols, which contain strong nucleophilic centers, react with the electrophilic carcinogenic species in order to prevent tumorigenesis. In addition to activating killer caspases, this reaction changes the expression of cell cycle proteins, specifically the Bax/Bc12 function. Moreover, polyphenols can arrest tumor growth internally because EGCG regulates signal transduction pathways involved in cell proliferation, transformation, and metastasis. These polyphenols affect the cancer cells without harming normal body cells, making green tea viable for cancer research. Although the anticarcinogenic effects of green tea have been widely observed, much research has yet to be done in order to determine specific mechanisms that inhibit cancer growth[7].

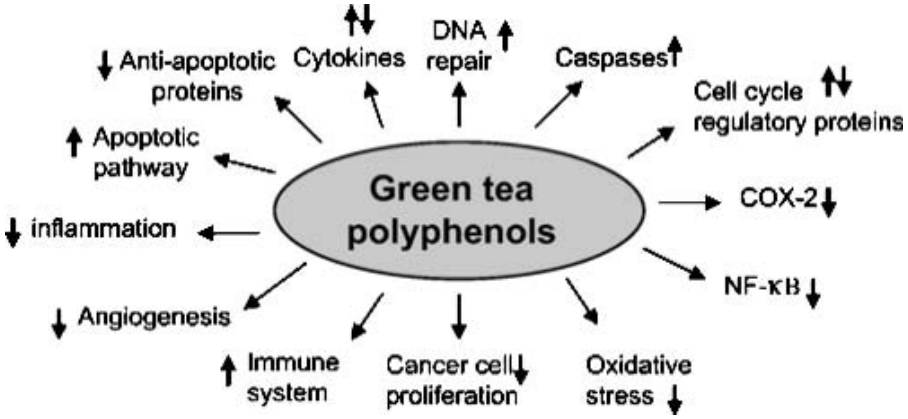

Fig. 2: **Anticarcinogenic property of green tea[1].** Various effects of green tea polyphenols in the body that either directly or indirectly inhibit cancer.

In addition to the health benefits of the polyphenols, these compounds also exhibit powerful antimicrobial and antiviral properties. For instance, green tea actively strengthens the immune system by preventing the binding of HIV to human T-cells. Evidence shows that the EGCG inhibits the infection of human CD4 lymphocytes by HIV. However, further research is required to fully understand green tea's antiviral effects[1].

Many studies have shown EGCG to be the most effective antibacterial polyphenol at typical or slightly lower concentrations than found in regular brewed green tea[8]. The exact mechanisms of EGCG's antibacterial activity are unknown, but it is believed that EGCG disrupts the cell membrane and prevents DNA supercoiling, ultimately leading to the destruction of the bacterial cell. *In vitro* experiments suggest that EGCG affects fungal pathogens, Gram-positive bacteria, and Gram-negative bacteria, but Gram-positive bacteria are particularly vulnerable to the polyphenols. The difference between Gram-positive and Gram-negative bacteria lies in the cell wall. The Gram-positive cell wall has several layers of peptidoglycans that are joined together to form a thick, rigid wall, whereas the Gram-negative cell wall has an additional membrane covering the thinner wall of peptidoglycans. This outer membrane contains lipopolysaccharides and lipoproteins, which are vital to the bacteria's survival under enormous bacterial pressure (Fig. 3)[9].

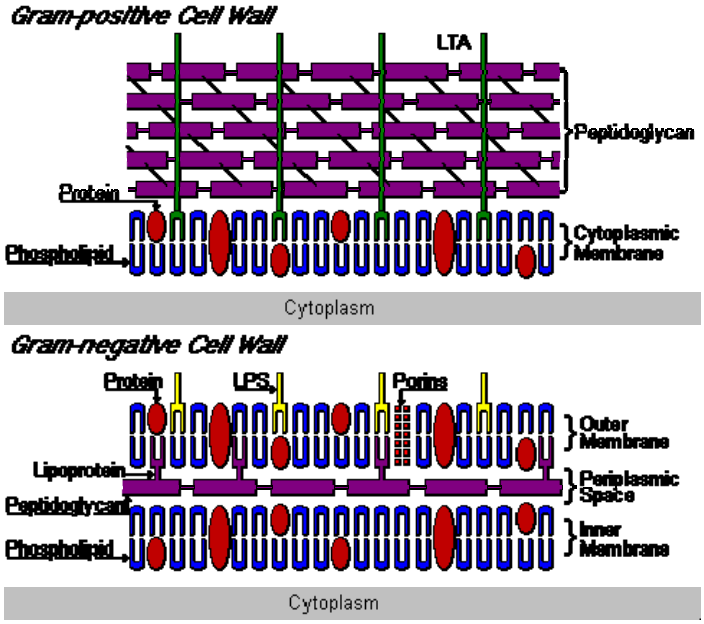

Fig. 3: Bacterial Cell Wall. The differences between Gram-positive and Gram-negative cell walls on a molecular level[10].

Not only do catechins inhibit the growth of both Gram-positive and Gram-negative bacteria, but they increase the number of beneficial bacteria, such as lactobacilli and bifidobacteria. Previous successful studies have discovered that polyphenols found in tea have been able to inhibit the growth of and/or kill the following pathogenic bacteria: *Staphylococcus aureus, Salmonella typhi, Salmonella typhimurium, Salmonella enteridis, Shigella flexnieri, Shigella dysenteriae, Streptococcus sobrinus, Lactobacillus rhammosus, Actinomyces viscosus, Listeria monocytogenes, Streptococcus salivarius, Streptococcus mitis* and *Vibrio chlorae*[11]. EGCG also inhibits oral bacteria such as *Streptococcus mutans, Porphyromonas gingivalis,* and

Staphylococcus epidermidis[12]. These bacteria exhibit high virulence and specialized methods of infestation. However, many of these species can be killed or disrupted by the polyphenols, and green tea extracts have now been added to facemasks, mouthwashes and antiseptic creams for this reason[13]. Tea has such effective antibacterial properties that a tea ointment was successfully used as a topical medicine for impetigo[8].

Streptococcus mutans are Gram-positive bacteria and grow in chains and rods. These bacteria are primarily responsible for oral health issues, such as tooth decay and plaque[14]. They degrade carbohydrates, such as lactose and sucrose, by producing lactic acid[15], which erodes tooth enamel. *S. mutans* build up on teeth by metabolizing sucrose into a sticky dextran-based sugar that allows the adherence and accumulation of plaque. The plaque collects in the mouth at dips, fissures, and indentations. Moreover, *S. mutans* are able to create long-term infections in the human mouth due to their tolerance to acidity[16], as shown by several studies. Hamilton-Miller found that a mixture of catechins extracted from green tea caused "very substantial inhibition of adherence of *S. mutans* to saliva-coated hydroxyapatite[17]." Yoshio *et al* also found that extracts of green tea leaves suppressed the growth of *S. mutans*[18]. Muroi and Kubo found that the combination of green tea flavor compounds was bactericidal against *S. mutans*, as well[19].

Streptococcus sobrinus is another species of oral bacteria with better adhesion capabilities than *Streptococcus mutans*. However, *S. sobrinus* are not as commonly found in fissures as *S. mutans*[20]. Furthermore, *S. sobrinus* have been proven to be cariogenic. Therefore, humans with large quantities of *S. sobrinus* also tend to have a build-up of plaque and numerous dental caries, as compared to those individuals with no caries. These bacteria are sometimes considered more cariogenic than *S. mutans*[13]. Because S*treptococcus mutans* and *S. sobrinus* have similar shapes and cause dental caries, it can sometimes be difficult to differentiate between the two[21].

Staphylococcus epidermidis, bacteria that commonly grow on human skin and mucus membranes, are also Gram-positive[14]. These white, facultatively anaerobic bacteria are a major concern in many hospitals because of their abundance and ability to cause an infection[22]. Contained in a polysaccharide intercellular adhesion capsule (PIA), these bacteria can adhere to blood and extracellular matrix and congregate with other bacteria, forming biofilms that are associated to infections. In addition, they are enduring organisms that display a wide range of resistance and tolerance to many antibiotics including ciprofloxacin, vancomycin, and other glycopeptide drugs[22].

Porphyromonas gingivalis are very potent and virulent bacteria that also inhabit the human mouth. They often grow in black colonies due to the iron in the hemin. *P. gingivalis* invades cells and disables proper immune responses with the destruction of the cellular matrix. Furthermore, *P. gingivalis* disables leukocytes, rearranges the cytoskeleton in infected target cells, and degrades the IgG antibodies. *P. gingivalis* uses well adapted fimbriae to bind and adhere to saliva-coated apatites and oral cells in the human mouth. In addition, this virulent bacterium has been found to produce multiple enzymes that damage and degrade host proteins both *in vitro* and *in vivo*. These Gram-negative bacteria are a major contributor to periodontal disease, which affects over 49 million people in the United States as of 1995[15].

Green tea is notably beneficial to dental health because it prevents the adhesion of *Streptococcus mutans, Porphyromonas gingivalis,* and *Streptococcus sobrinus*. The process of plaque formation begins when bacteria adheres to the surface of the tooth and creates a glycocalyx film[18]. This film contains a glucan that mediates the adherence to the tooth. These glucans are formed by glucosyltransferases (GTF) from sucrose, and many studies have focused on GTF's for the prevention or treatment of oral diseases[23].

Many such experiments have shown that catechins in green tea inhibit the growth of *S. mutans* and *S. sobrinus* with a minimum inhibitory concentration between 50 and 1000 μg/ml, and 250-500 μg/mL for *P. gingivalis*. These concentrations all fall within the range of concentration found in a typical cup of tea[13]. A cup of green tea, which contains about 50 to 100 mg of polyphenols in which 60% is ECG and EGCG, can inhibit glucan synthesis and/or bacterial adherence[24]. These anti-plaque effects of green tea have been demonstrated by experimentation on human subjects. A 0.2% tea solution, 0.25% catechin mouthwash, and tablets containing tea polyphenols all resulted in a considerable reduction of the plaque index. In one study, after drinking one cup of green tea every day for 250 days, Japanese schoolchildren had fewer dental caries. Morse, Kehres, and Tsuji similarly found that after drinking a cup of green tea every day for 28 days, men and women both had greatly reduced numbers of dental bacteria. Green tea inhibits the growth and adherence of bacteria such as *Prevotella* and *Porphyromonas gingivalis*, which are involved in periodontal disease. The halitosis that is associated with this disease was reduced by the catechins of green tea when they deodorized the methyl mercaptan[13].

The polyphenols EGCG and EGC are very active in inhibiting the growth of *Streptococcus mutans*[13]. The minimum inhibitory concentration was in the range 250 to 1000 μg/ml, which is higher than that of antibiotics. A cup of green tea contains 50 to 100 mg of polyphenols, a concentration higher than the minimum inhibitory concentration. In addition, the tea polyphenols greatly reduced the colony forming units (cfu) of *S. mutans* after only five to ten minutes of exposure. It is known that tea inhibits formation of dextran and levan from the sucrose of the cariogenic bacteria such as *S. mutans*[5].

Since the literature supports the theory that catechins possess antibacterial properties, green tea was predicted to have an inhibitory effect on the bacteria sample taken from the mouth. This hypothesis was examined by using paper disk diffusion, determining minimum inhibitory concentration, and comparing the effects of green tea to those of commercial mouthwashes in an oral rinsing study[25].

MATERIALS AND METHODS

Preparation of the Oral Culture

To prepare the oral culture, two human mouths were swabbed and the samples streaked onto tryptic soy agar plates with 5% sheep's blood. The culture was incubated at 37°C for 72 hours. After incubation tryptic soy broth was inoculated with a loop of the culture. The number of colony forming units was estimated through serial dilutions.

Paper Disk Diffusion

The antimicrobial properties of green tea were tested using paper disk diffusion. Tryptic soy agar plates were inoculated by spreading a lawn of the oral culture across each plate. The green tea was brewed at 90ºC with varying concentrations of crushed dried tea leaves and varying brewing times, resulting in fifteen different concentration-time combinations (Table 1). Small paper disks were infused with the green tea by soaking in the green tea for approximately 5 minutes. The disks were then placed onto the inoculated Petri dishes. Fifteen control disks were also prepared by infusing each with a different green tea combination and then placed on a plate that was not inoculated. The plates were incubated at 37°C for 48 hours. After incubation the zones of inhibition were measured using a ruler.

Table 1: Green Tea Concentrations and Brewing Time Combinations

Brewing Time (min)	Concentration (mg/mL)
60	0
	20
	40
	60
	80
40	0
	20
	40
	60
	80
20	0
	20
	40
	60
	80

The paper disk diffusion was repeated with eight different types of mouthwash in order to compare the antibacterial properties of commercial mouth cleansers to those of green tea. Paper disks were soaked for 5 minutes in the following brands of mouthwash: Act® Restoring™, Oasis® Moisturizing Mouthwash, Tom's of Maine® Natural Cleansing Mouthwash, Scope® Original Mint Mouthwash, Listerine® Antiseptic, Crest® Pro-Health™ and Cēpacol® Antibacterial Mouthwash with Ceepryn®. Each bottle was opened in March 2009. In addition, paper disks were soaked for 5 with minutes Scope® Original Mint Mouthwash, which was opened on the day of the test. The paper disks were placed on tryptic soy agar plate inoculated with a lawn of the oral culture. The plates were then incubated at 37°C for 48 hours and then a ruler was used to measure the zones of inhibition.

Minimum Inhibitory Concentration

In order to determine the lowest concentration of green tea that can inhibit the growth of bacteria commonly found in the human mouth, a minimum inhibitory concentration test was performed. Pearl Green Tea® was brewed at 90°C for 20 minutes at concentrations of 0, 1.5,

2.5, 3.5, 4.5, 5.5, 6.5, and 7.5 mg/mL. The green tea and the tryptic soy broth were prepared at double the required concentrations. Test tubes were prepared by combining 4.9 mL of tryptic soy broth, 5 mL of green tea and 0.1 mL of the bacterial culture. For each concentration of green tea, a control was created by adding 0.1 ml standard concentration tryptic soy broth instead of the bacterial culture. The tubes were incubated at 37°C for 48 hours. After incubation, 0.1 mL of each sample was removed, spread on a tryptic soy agar plate and incubated at 37°C for 48 hours. The plates were visually analyzed for signs of bacterial growth.

Oral Rinsing Study

An oral rinsing study was conducted on seven test subjects to compare the effectiveness of green tea as an oral antiseptic to commercial mouthwash. Salada® green tea was prepared according to the package directions. 237 mL (one cup) of filtered water were brought to a rolling boil in a microwave and then allowed to cool for one minute. One tea bag was allowed to steep for three minutes. The tea was cooled to room temperature. Seven tryptic soy agar plates were prepared. Each plate was divided into four quadrants, one for each time the subject's mouth was swabbed. Seven subjects were asked to rinse with 30 mL of either green tea, Scope® Original Mint Mouthwash or a control of boiled cooled and filtered water. Three subjects rinsed with tea, two rinsed with Scope® Original Mint Mouthwash, and two rinsed with boiled cooled filtered water; the rinsing took place for 30 seconds. The mouth of each participant was swabbed with a sterile swab before rinsing, immediately after rinsing, 30 minutes after rinsing and 45 minutes after rinsing. The swab was brushed in a consistent manner between trials: from left to right along the inside of the bottom row teeth. Swabs were streaked onto each plate according to the time interval.

RESULTS

Paper Disk Diffusion

In this experiment, the concentration of the green tea infused into the paper disks was proportional to the zones of inhibition around the disks (Fig. 4). The results showed that the most effective concentration was 80 mg/mL with an average zone of inhibition of 0.13 cm and a standard deviation of 0.064 cm. However, for the 40 minute trials, the 60 mg/mL disks were more effective than the 80 mg/mL disks (0.10 cm on average compared to 0.073 cm). Comparing all the trials at an 80 mg/mL concentration to all the trials at a 60 mg/mL concentration using a two-sample T-test at a significance level of 0.05, a p-value of 0.0016 was calculated, indicating that 80 mg/mL concentration produced zones of inhibitions that were significantly larger than those created by the 60 mg/mL concentration. It was also found that at a concentration of 20 mg/mL, green tea proved ineffective in preventing the growth of bacteria with all three trials exhibiting no zones of inhibition. There was increased bacterial growth with no zones of inhibition around the disk infused with water and on the ends where the tea did not reach.

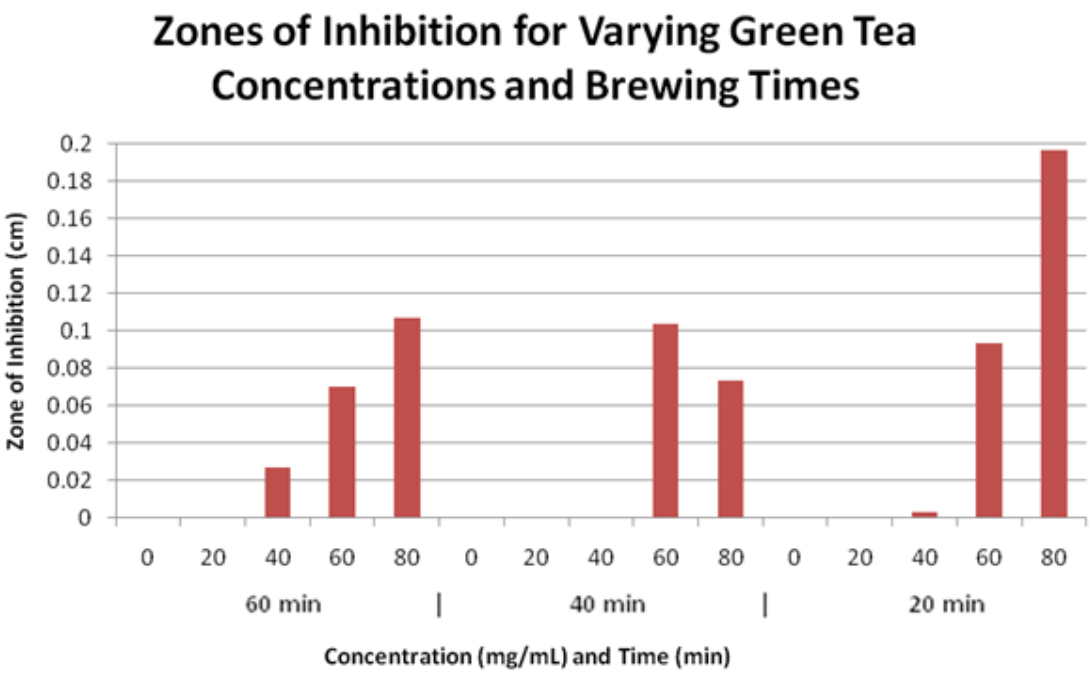

Fig. 4: The Antimicrobial Effects of Green Tea. The average zones of inhibition observed in bacterial cultures exposed to paper disks soaked in green tea brewed in varying ways.

Mouthwash Diffusion

The mouthwashes varied drastically in inhibiting capability. For example, the newly opened bottle of Scope® mouthwash was found to be incredibly effective, with an average zone of inhibition of 0.57 cm and a standard deviation of 0.11 cm. In contrast, Listerine® and Tom's® mouthwash were much less effective, with zones of inhibition of 0.14 cm and 0 cm respectively (Fig. 5). In addition, a two-sample T-test was run to compare the zones of inhibition of the brand-new Scope® against the Scope® that was open since last year. At a significance level of 0.05, a p-value of 0.042 indicates that there is a statistically significant difference between the zones of inhibition created by the old and new Scope® mouthwashes.

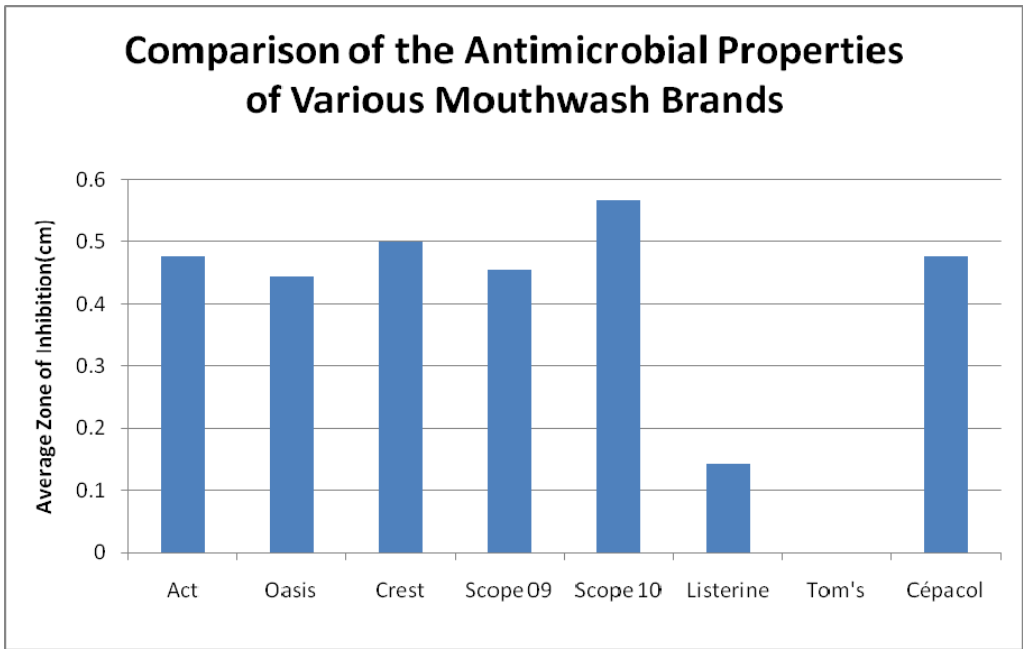

Fig. 5: Antimicrobial Properties of Various Mouthwashes. Average zones of inhibition created by eight different mouthwash brands against oral bacteria.

Minimum Inhibitory Concentration

A trend was observed of more bacteria growing on the plates with higher concentrations of green tea. There was a positive correlation between green tea concentration and bacterial growth. The control groups without bacteria showed no bacterial growth.

Fig. 6: Minimum Inhibitory Concentration Plates with 0 mg/mL of Green Tea. Bacteria were added to the left dish, and neither plate had any visible growth.

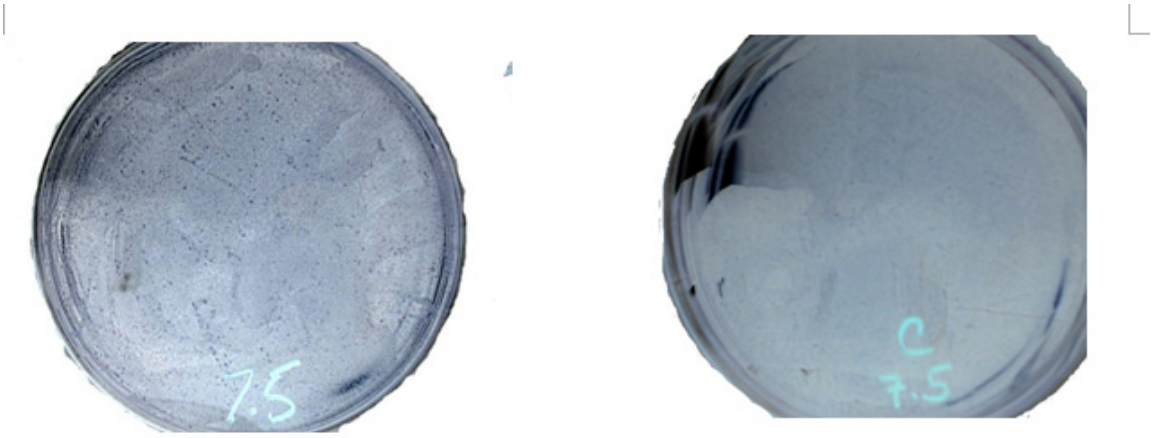

Fig. 7: Minimum Inhibitory Concentration Plates with 7.5 mg/mL of Green Tea. Bacteria only grew on the left Petri dish, and the control had no growth.

Oral Rinsing Test

The cultures grown from the subjects' oral bacteria after rinsing with Scope® and green tea exhibited significantly less bacterial growth than the cultures grown from the bacteria before rinsing. The control group of water showed little change throughout the experiment. In addition, while the tea and Scope® trials did show immediate results, the bacterial swabs taken at 30 minutes and 45 minutes after the rinsing showed considerable growth.

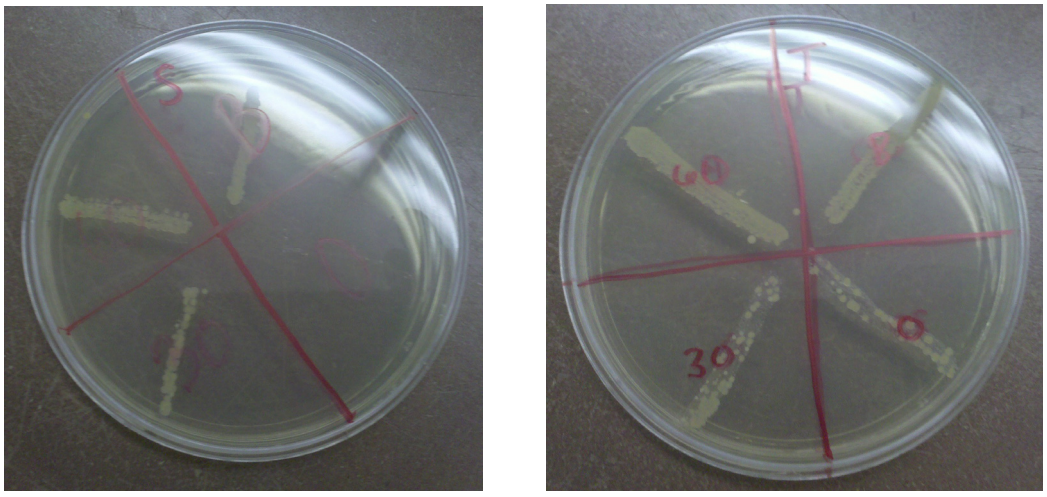

Fig. 8: Oral Rinsing Test Results. The Petri dish on the left shows the results of the Scope® trial, and the Petri dish on the right shows the results of the green tea trial.

Gram Stain

While the bacteria examined were mostly Gram-positive, closer examination using an oil immersion microscope revealed small but distinctive circular Gram-negative bacteria. On most of the minimum inhibitory concentration Petri dishes, the cultures were Gram-positive.

On the 1.5 mg/mL of green tea dish, however, some of the cultures were Gram-negative. The larger colonies along the periphery of the paper disk diffusion dishes and around the control disks were Gram-positive, while the smaller colonies closer to the paper disks were Gram-negative.

Serial Dilution

Through a serial dilution, the concentration of oral bacteria in the original culture was determined to be 1.35×10^8 cfu.

DISCUSSION

After performing Gram stains on the minimum inhibitory concentration and paper disk diffusion cultures, both Gram-positive and Gram-negative bacteria were found in each of the experiments, but more Gram-positive bacteria were detected by the stains. Some of the Gram-negative bacteria that were noticed could have resulted from human error while Gram staining. These results show that the green tea was effective in inhibiting some of the bacteria in the mouth, as supported by previous literature. However, several circular Gram-negative cocci colonies could be located after close examination with an oil immersion microscope.

The results of the paper disk diffusion experiment supported the hypothesis that increasing concentration of green tea would increase inhibition of bacterial growth. The paper disk with the highest concentration did create the largest zone of inhibition. Since the average concentration in a cup of green tea, 20 mg/mL, was ineffective in killing the cultures, the results implied that one cup of green tea is not enough to kill the microbes in the mouth. The results showed that more concentrated tea is necessary to exhibit green tea's antibacterial activity. Since the paper disks infused with water showed no zones of inhibition, it can be inferred that the zones produced in the high concentrations were due to the antimicrobial effects of green tea. The tea that brewed for 20 minutes was the most effective in killing the microbes. The tea that was brewed for 40 minutes and the tea that was brewed for 60 minutes were equally effective in killing the cultures, but were not as efficient as the tea brewed for 20 minutes. One explanation for these results could be that the longer the green tea leaves were infused in the hot water, the greater the breakdown of the antibacterial polyphenolic compounds, rendering the tea less effective.

The microbes used in the experiment were unknown, which could have created some error. In previous studies, green tea inhibited the growth of Gram-positive microbes, *S. mutans* and *S. sobrinus*[17]. Because the cultures used in the present study could have been Gram-positive and/or Gram-negative, the type of microbes that the green tea killed could not be conclusively determined. Furthermore, the microbes that actually grew on the dishes were unknown. Gram stains were performed; large colonies that were found only along the outer edge of the Petri dishes and near the control (but not near the disks) and the small colonies closer to the disks were stained. The large colonies were Gram-positive, and the small ones were Gram-negative. The results support the hypothesis that green tea can act as an antimicrobial agent in the mouth, particularly against Gram-positive bacteria.

Another source of error could be due to inconsistencies in heating the tea. When brewing the green tea at 90°C, the temperature could not be maintained constantly because of the shortage of hot plates and the uneven heat distribution on the hot plate. Therefore, the teas most likely varied in temperature rather than staying steadily at 90°C.

Inconsistencies in the methods used to prepare the disks were also present. When the soaked paper disks were ready to be placed onto the Petri dishes, only some of the disks became very saturated with tea. As a result, the tea spread out around the disk on the Petri dish. The disks that acted in this manner could have killed more cultures than the ones that did not. Also, when transferring the paper disks, some were accidentally dropped onto the table. To correct this error, new paper disks were soaked in the proper concentrations; however, the time the disks were soaked were different and could have skewed the results. Finally, the zones of inhibition measurements should have been rounded to the nearest tenth of a millimeter with the ruler, rather than just the nearest millimeter, to increase accuracy. Although this is not a source of error, this could be considered a restriction that limited the results. In the future, a microscope with a measuring scale could possibly be used.

Because of these potential sources of error, further experiments should be conducted by trying to maintain the proper temperature. In addition, more concentrated green tea extract could be used. Perhaps the extract could have more catechins, even for an average cup of green tea. Moreover, since the experiment was only performed with one brand of green tea leaves, perhaps performing the same procedures with various brands of green tea could offer an even better understanding of green tea and its antimicrobial effects.

According to the paper disk diffusion, the most effective brand of mouthwash was Scope® Original Mint Mouthwash because it produced the largest zone of inhibition. However, unlike the other bottles, this bottle of Scope® was opened the day of the experiment. The other bottles were opened in March 2009. After analyzing only the bottles of brands opened in March 2009, Crest® Pro-Health™ proved to be the most effective mouthwash, outcompeting the 2009 Scope® Original Mint Mouthwash. The results showed that Listerine® Antiseptic was an ineffective mouthwash even though it is a popular brand. The active ingredients in Listerine® include menthol, thymol, methyl salicylate, and eucalyptol. Ethanol serves to dissolve these ingredients into the solution[26]. The concentration of ethanol is not toxic to microbes, but it is the cause of the burning sensation in the mouth. These ingredients, while containing some antiseptic properties, are not enough to kill the cultures at rates equivalent to competing brands of mouthwash. Compared to the eight mouthwashes tested, Listerine® Antiseptic was the second to least effective.

Scope®, on the other hand, contains cetylpyridinium, chloride, and domiphen bromide, all of which serve as its primary antimicrobial ingredients. Alcohol also makes up a significant portion of the product because it prevents germs from growing in the bottle after it is opened[27]. Similarly, Crest® Pro-Health™ mouthwash contains cetylpyridinium chloride as its active ingredient[28]. The main difference between Crest® and Scope® is that Crest® lacks alcohol. These results lead to the conclusion that alcohol does not serve as a strong antiseptic in the concentrations found in mouthwashes. The Scope® opened this year had a greater zone of inhibition than the one opened last year. The ingredients in the mouthwash probably became less

effective and lost its antiseptic properties, preventing the mouthwash from creating larger zones of inhibition.

The least effective mouthwash was Tom's of Maine® mouthwash. The active ingredient in this brand is sodium fluoride, an ingredient that serves to prevent tooth decay but lacks antiseptic properties[29]. Thus, having a negligible zone of inhibition was expected and observed.

The microbes used in this experiment were mouth flora, but their exact identities remain unknown. In future experiments, focus should be directed to specific types of microbes, such as *Streptococcus mutans*, the primary cause of dental caries, or *Streptococcus sobrinus*, also a cause of plaque and caries. Other sources of error include the inaccuracy of the measurements of the zones of inhibition. Measurements could only be taken to the nearest millimeter, so the variation from brand to brand in the 0.4 to 0.5 cm range could be a simple estimating error. Further studies should implement more accurate measurements of length. In addition, fresh bottles of mouthwash should be used.

In another part of the experiment, the minimum inhibitory concentration test, the results showed increased concentrations of green tea actually promoting the growth of the cultures. The control had the least bacterial growth when treated with water, but the most bacteria grew on the plates with the highest concentration of tea, 7.5 mg/mL. When the cultures from these plates were Gram stained, both Gram-positive and Gram-negative bacteria were found. In past trials, green tea had been found to be effective against both types, particularly Gram-positive bacteria[30]. However, the presence of both Gram-positive and Gram-negative microbes indicates that the tea was ineffective at killing either type of bacteria.

The control groups used were agar plates with the same concentrations of green tea as used in the experimental groups but with no bacteria. These groups displayed little to no growth, indicating that the cultures grown on the experimental dishes was not due to contamination directly from the green tea. One of the controls, the 2.5 mg/mL dish, did exhibit three distinct colonies of bacteria. If all the controls showed this type of bacteria growth, it could be assumed that the source of contamination was from the green tea. However, since the 2.5 mg/mL dish was the only control with cultures, this contamination must have been from an outside source, such as the air.

Certain steps in the procedure caused the green tea to be ineffective as an antimicrobial agent. Perhaps, in order for the green tea to be effective, a longer brewing time was required at a higher temperature or a shorter brewing time at a lower temperature. Another error could have occurred while the tea was being made. Although the tea was filtered, stray tea leaves may have fallen into the solution. Perhaps the tea leaves provided a source of nutrition to aid bacterial growth, thereby explaining why the bacteria proliferated with increasing green tea concentration.

Under the conditions specific to this procedure, green tea not only failed to inhibit bacterial growth, but also promoted it. Under different conditions, green tea's antimicrobial effectiveness may differ. Varying brewing times and temperatures may also lead to better results, as the conditions used in this experiment - 20 minutes at 90°C - may not have been the optimal brewing conditions for green tea. Twenty minutes was chosen as the brewing time because past studies have shown that tea brewed for 20 minutes was effective in killing

Streptococcus mutans[31]. 90°C was chosen as the temperature of the green tea because bringing the tea to a full boil could have caused the tea to lose some of its antiseptic properties in the steam, and evaporation would consequently increase the concentration of catechins per milliliter of liquid. Another group also used 90°C successfully for the same experiment the year prior[32]. Therefore, it was decided that 90°C would be a suitable temperature for the green tea. Even though literature was used to determine the brewing conditions for green tea, better methods for brewing the green tea most likely exist and should be tested.

Another possible explanation is that green tea catechins have been shown to increase the levels of beneficial bacteria[13]. The microbes found in the experimental dishes may actually be beneficial to the body, which would explain why the higher concentrations of green tea promoted its growth.

Within the minimum inhibitory concentration test, there were several possible sources of error. At many times, the green tea, bacterial samples, and plated Petri dishes were exposed to possible contamination. The samples were open to the air during the brewing, inoculation, and plating processes. Air contamination could have occurred as a result and caused some extraneous bacterial growth. The actual bacterial culture used to create the samples was cloudy, indicating that a large number of microbes were already dead, the culture was contaminated, or the tea was very concentrated. During the plating process, ethanol was used to sterilize the glass spreading rods. Incorrect sterilization of the glassware or improper handling of the Petri dishes may have introduced contamination. Perhaps, not enough culture was put on the plate, so it was too dilute. If the procedure is changed so that there is a greater concentration of microbes added to the plates, it would probably yield better results.

In addition to possible contamination, errors within the procedure may have altered the results. A hot plate was used to brew the green tea, which made maintaining a steady temperature difficult. The temperature fluctuated from 78°C to above 100°C. Boiling of the green tea was another problem, as it may have caused a change in the chemical composition of the tea catechins. The brewing tea was removed from the hot plate if boiling was noticed, and it is possible that the actual brewing times were slightly off from the preferred 20 minutes. The pipetting process also introduced several sources of error. In the final step, before transferring the samples to Petri dishes, homogenizing the mixtures with a vortex was difficult. The vortex was unable to lift debris at the bottom of some of the test tubes and homogenize the debris with the rest of the mixture. Therefore, a pipette was necessary to mix the solution thoroughly. A pipette tip was dropped into the 3.5 mg/mL sample during inoculation. Although the forceps used to remove the pipette from the sample was sterilized, there is still always a chance that the forceps contaminated the sample. However, the sample did not seem contaminated; therefore, the extra bacterial growth was most likely due to a greater initial concentration of culture.

Further studies should use a much larger sample size in order to better identify trends. Flame sterilization should be used at all times when possible. Flame sterilization, as opposed to ethanol, is a more effective way of sterilizing equipment and could reduce the amount of contamination. A more consistent heating method that can maintain a constant brewing temperature is also needed. Other possible experiments could target specific microbes to determine which strains of bacteria are most susceptible to green tea.

In another portion of the experiment, the effectiveness of mouthwash and green tea on killing oral bacteria was tested. Scope® was expected to be more effective in destroying bacteria than green tea because of the mouthwash's antiseptic properties. Although some of the results indicated that both Scope® and green tea killed bacteria, the results were inconclusive because there were not enough trials for each of the rinses. Of the seven subjects, two rinsed with Scope®, two rinsed with water, and three rinsed with green tea. As expected, there were no differences in bacteria growth before and after the two subjects rinsed their mouths with water, indicating that any bacteria that were killed had been killed by the solution in the rinse and not by the actual act of rinsing.

In the Scope® mouthwash and green tea trials, the results were often contradictory. For instance, in one of the Scope® trials, there was a distinct decrease in the growth of bacteria immediately after rinsing, and then a continuous increase in growth as time went on. In the other trial for Scope®, the distinct decrease in bacterial growth immediately after rinsing, as well as an increase in bacteria growth after thirty minutes, was still evident. However, the streak placed on the agar after forty-five minutes had minimal bacterial growth. The cause of the disappearance of bacteria in the last streak is most likely due to an error in the swabbing, but this cannot be proven since this result could not be compared with other results because there was only one other trial. If more results had been collected, a common trend or correlation between rinsing with Scope® and the growth of bacteria may have been found.

Some discrepancies were found in the results for the trials that used a green tea rinse. Although one trial did show a distinct decrease in bacteria in the test swab immediately after rinsing, the agar plates for the other two green tea trials did not indicate any decrease in bacteria immediately after rinsing. Therefore, the green tea probably does not kill more than a negligible amount of bacteria in the mouth, for there were two trials that showed no decrease in bacteria in the time before and after rinsing. However, more trials are needed to reach a stronger conclusion.

On the other hand, the two trials that showed no decrease could have been due to ineffective rinsing of the mouth. Differences in the way subjects rinsed their mouths could have caused some of the differences in bacteria growth on the agar plates. Because plates were labeled by trial and not by name for anonymity reasons, it is not possible to link the plate's results directly to the rinsing method.

In order to improve this experiment, more trials of each of the rinses must be run. Using a larger number of test subjects may lead to more of a general consensus regarding the actual effect the rinse has on bacteria in the mouth, since it would be easier to detect a trend. The results would also be more substantial if the rinsing method between the subjects was kept as uniform as possible. For instance, a rule can be made that all subjects rinse their mouths by swishing the mouthwash or tea from left to right. The experiment would also be improved if a greater concentration of tea is used. Based on the results, the concentration of tea used in this experiment did not kill many bacteria or inhibit growth for very long. Using tea of a greater concentration could increase the antiseptic effect and kill more microbes. Another way to improve the experiment would be to identify the cultures as harmful or beneficial bacteria. The

microbes that grew from the mouth swabs could have been beneficial, which would explain why the green tea had no effect on its growth.

The objective of this experiment was to determine whether or not green tea is effective in killing oral bacteria. Based on the results, green tea is effective in killing microbes, but its efficiency is dependent upon certain conditions. Most of the results from the experiments showed that the average concentration of green tea in a single cup was not enough to kill a significant amount of bacteria. The paper disk diffusion experiment found that the zones of inhibition increased as concentrations of the tea increased. The concentration of an average cup of tea – 20 mg/mL – produced a negligible zone of inhibition, indicating that drinking a cup of tea will not have much of an effect on microbes in the mouth. The mouth swab test also showed that a "normal" cup of tea does not kill many bacteria. After subjects rinsed their mouths with tea made by following the instructions on the box, there was barely a decrease in the amount of oral bacteria before and after the rinsing. Moreover, the mouthwash was far more efficient than green tea at killing microbes. The results from the mouth swabbing showed a distinct decrease of bacteria immediately after rinsing while tea exhibited barely any decrease. Furthermore, the zones of inhibition of the paper disks soaked with mouthwash were much larger than the zones created by the tea soaked paper disks. In the future, this lab can be further expanded upon by finding green tea's effects on the most common types of bacteria in the mouth. Specific strains of bacteria could be isolated to determine which bacteria are most susceptible to green tea. The effects green tea has on beneficial bacteria could also be determined. By modifying the limitations of this study and others similar to it, future projects should be able to discover interesting and applicable information about green tea and its antimicrobial properties.

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