THE SPICES OF LIFE: TESTING THE ANTIMICROBIAL EFFECTS OF GARLIC (ALLIUM SATIVUM), CINNAMON (CINNAMOMUM ZEYLANICUM), AND CLOVE (SYZYGIUM AROMATICUM) AGAINST STREPTOCOCCUS MUTANS

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ABSTRACT

It is obvious that spices have been used to enhance the flavor of food for centuries, but what other uses do they possess? This question was posed in the hopes of discovering antimicrobial properties in various household spices. Garlic (Allium sativum), cinnamon (Cinnamomum zeylanicum), and clove (Syzygium aromaticum) were chosen to examine their effects on the bacteria Streptococcus mutans. To obtain an extract from the spices, an aqueous extraction method combined with filtration was used. The extracted compounds were applied to the bacteria through the paper disk diffusion method. The same disk diffusion method was then used to examine commercial mouthwashes and synergistic effects of the spices on the S. mutans. After incubation, the amount of bacteria revealed no inhibition from the spices. There was, however, inhibition when the bacteria were exposed to the mouthwashes. Overall, the results were inconclusive, but it is likely that the method of extraction used for the spices prevented inhibition from being observed.

INTRODUCTION

Background, Properties, and Chemical Composition of Spices: Garlic, Cinnamon, Clove

Spices are the flavor of life. All throughout the world, cultures and countries utilize a variety of spices for a plethora of reasons. These purposes may include enhancing flavor and preserving perishable food. Spices are defined as any dried, fragrant, or aromatic vegetable or plant substance that contributes flavor in a whole, broken, or ground form. Spices include all parts of herbaceous plants except the leaf, which is considered an herb. Spice use is a common feature in the cultures of all countries.

While spices are heavily used for seasoning and increasing palatability, they have also expressed antimicrobial properties. Spices found in a garden may be used to effectively kill harmful, sometimes even deadly, microbes. Some studies have concluded that these spices may be very valuable because bacteria develop resistance to conventional antibiotics. Consequently, effective methods of utilizing the antimicrobial activities in spices would be advantageous.
Phytochemicals, or essential oils, are the main antimicrobial agents in spices. Most of these oils seem to derive their antimicrobial properties from a benzene functional group. The presence of sulfur may also be an important part in the antimicrobial mechanisms of some spices. Nevertheless, it seems that the antimicrobial effects of spices are determined by the integrity, mass, and target of the spice. The overall temperature is also believed to play a role in the spice’s effectiveness, as at different temperatures spices seem to show changes in antimicrobial activities. These properties vary among different spices. This study explores the specific antimicrobial effects of garlic, cinnamon, and clove.

*Allium sativum*, known to most as garlic, or the “stinking rose,” is widely recognized for having an array of medical benefits, with antiviral, anti-fungal, and antibacterial properties. Garlic is made of portions known as cloves, which may be separated for cooking and eating. Within these cloves, there are quite a few sulfur-containing chemicals, such as allicin, alliin, and ajoene (Fig. 1). Allicin produces the odor that is characteristic of the garlic bulb. It is produced when the plant tissue is broken, allowing enzymes like alliinase to react with alliin to form allicin. Allicin then acts to protect the plant from further damage. However, the enzymes responsible for converting alliin to allicin usually denature in humans due to the low pH of the stomach. Allicin itself also usually begins to break down at temperatures over 40° C. Along with its protective abilities, allicin is believed to be the chemical responsible for garlic's antimicrobial effects.

![Figure 1: The sulfur-containing compound of garlic](image)

Not only do essential oils protect the garlic plant, but they also demonstrate antioxidant and anticancer effects in humans. Studies have found garlic to be effective in blocking the formation of nitrosamines. Nitrosamines are carcinogenic compounds that are either taken in by the body or formed from nitrite and other compounds. Many sulfur-containing compounds from garlic convert the nitrite needed for nitrosamines into nitrosothiols, thus limiting the production of these carcinogenic compounds.

Various studies have shown that garlic is also effective against many gram-negative and gram-positive bacteria, such as *Escherichia coli*, *Salmonella*, *Staphylococcus*, and *Streptococcus* species. Many of these bacteria do not develop resistance to allicin, despite being resistant to
antibiotics. One study demonstrated the potential for garlic to act as a meat preservative. Garlic extract was shown to kill about 75% of the *E. coli* and non-pathogenic *Salmonella* on chicken meat as well as a majority of the contaminating bacteria on the meat after 10 minutes of exposure. The study also showed that the garlic could limit the growth of all the bacteria tested. It has also been observed that allicin is capable of killing methicillin-resistant *Staphylococcus aureus* (MRSA). In a study conducted by Culter and Wilson, it was found that a more stable extract of allicin inhibits all strains of MRSA tested at a concentration of 256 µg/mL. Garlic was also shown to be effective in combating *Helicobacter pylori*, which has been linked to stomach cancer. Using 5 mg/mL of an aqueous garlic extract, researchers were able to inhibit the growth of 90% of their *H. pylori* populations. These studies have shown that garlic possesses some antimicrobial properties.

Another spice, cinnamon or *Cinnamomum zeylanicum*, found in the inner bark of Cinnamomum trees, is commonly used in cooking for its aroma, flavor, and taste. Historically, cinnamon has been used by the Egyptians for embalming, most likely due to its antimicrobial properties. Eugenol and cinnamaldehyde (Fig. 2) are the two major chemical components in cinnamon that are responsible for its health benefits. Eugenol, a phenol compound, inhibits mold and adds flavor and aroma to bakery items. It also contains antiviral properties in vitro. Additionally, eugenol and cinnamaldehyde inhibit *H. pylori* growth at a low pH, showing their efficacy in eliminating the bacteria present in the human stomach. The electronegative cinnamaldehyde also inhibits amino acid decarboxylase. Cinnamaldehyde interferes with electron transfers and reactions with nitrogen-containing compounds, resulting in impeded growth of microorganisms.

Tests have shown that cinnamon oils have strong antimicrobial effects against most bacteria and fungi, even more so than cinnamon extract. An extract is obtained when plant parts are cold pressed and soaked in liquid, such as alcohol, in order to amplify a certain quality of the plant. An oil is obtained when a plant’s essence is distilled off, leaving behind a concentrated liquid. Gupta *et al.* showed that cinnamon oil was effective against all 10 bacteria tested, including *Staphylococcus aureus*, *Listeria monocytogenes*, and *E. coli* while cinnamon extract was only effective against most of the food-borne microbes. In another study which examined the antibacterial effects of various plant essential oils, cinnamon oil showed maximum activity.
against the gram positive bacteria *B. subtilis* and *K. pneumoniae* and the gram negative bacteria *P. aeruginosa* and *E. coli*.

Like garlic and cinnamon, clove (*Syzygium aromaticum*) has been proven to be effective against many different types of bacteria. Cloves are the dried immature flower buds of a tropical tree of the Myrtaceae family. These trees are native to Indonesia but also cultivated in other tropical locations. Cloves have been used for centuries as natural medicine against illnesses such as diarrhea, hernia, ringworm, and nausea and have been shown to be effective in reducing toothache. Recent experiments conducted by Japanese researchers have also revealed that cloves, like many other spices, contain antioxidants that can help prevent cancer. Additionally, another study demonstrated that clove essential oil displayed the greatest inhibition of a radical known as DPPH, or diphenylpicrylhydrazyl when compared to the essential oils of oregano, thyme, rosemary, and sage.

Cloves also have a strong inhibitory effect against microbes and are able to kill species of bacteria and fungi such as *S. aureus*, *L. monocytogenes*, and *Aspergillus*. This property is a result of the spice’s main antimicrobial chemical ingredient: eugenol, the phenol compound also found in cinnamon (Fig. 2). Eugenol makes up the majority of clove bud oil, at 60-90%.

A study performed by Barbosa et al. compared the essential oils of clove, lemongrass, basil, oregano, ginger, marjoram, and thyme through an agar dilution method and a minimum inhibitory concentration test. The oils were tested against two Gram-positive bacteria, *S. aureus* and *L. monocytogenes*, and two Gram-negative bacteria, *E. coli* and *S. enteritidis*. The clove essential oil had the highest antimicrobial activity out of all the spices tested. Leuschner and Ielsch tested ground clove, fresh garlic, and red dried chili in broth at two temperatures against *L. monocytogenes*. Once again, the clove’s antimicrobial properties proved to be the most effective at killing the bacteria.

Although many different herbs and spices have been tested for their antimicrobial properties, fewer investigations of the synergistic effect of such substances have been conducted. Synergy, the combination of compounds to create more profound microbial action, may also be an important factor in using spices to kill bacteria. Combinations of extracts can also lead to additive or antagonistic effects. The additive effect is equal to the individual effects, whereas the antagonistic effect is less potent than the individual effects. Synergism often results from components of one spice aiding the other, improving the efficacy. For example, in one study, researchers combined various amounts of cilantro, dill, coriander, and eucalyptus essential oils and tested them on several bacteria. Cilantro and eucalyptus had additive effects on all gram-positive bacteria and also had a synergistic effect on the gram-negative bacteria, *Y. enterocolitica*. Synergy serves as another option to amplify the antimicrobial properties of spices.

**Oral Antiseptics and *Streptococcus mutans***
Ever since the validation of the germ theory, antimicrobial properties have proven desirable in the fight against pathogens. Products upon products have hit the market promising to disinfect and sterilize. But while succeeding in eliminating bacterial growth, these products have introduced synthetic chemicals into the natural world. The purpose of this experiment was to step away from the artificial and explore the extent to which natural substances such as garlic, cinnamon, and clove provide antimicrobial effects. The ability of these household spices to inhibit the growth of bacteria and the possibility of using this potential to create a natural, yet effective, mouthwash was observed.

Commercial mouthwashes are popular means of maintaining dental hygiene. They are antiseptic solutions that contain ingredients such as fluoride, hydrogen peroxide, and chlorhexidine at very low concentrations. Fluoride prevents dental caries by making teeth less susceptible to acid deterioration caused by bacteria and sugars, while hydrogen peroxide is added to whiten teeth. One of the strongest antimicrobials, chlorhexidine, is a cationic solution that attaches to anionic surfaces like bacterial cell walls. This results in the prevention of plaque formation and bacterial metabolism. Chlorhexidine also has a prolonged effect, resulting in continuous protection from harmful micro-organisms. Listerine and Scope are two of the leading brands of mouthwashes, and therefore, have been incorporated in this experiment.

It is important to maintain dental hygiene as the mouth serves as an environment that is conducive to the growth of many types of bacteria, includes Streptococcus mutans. S. mutans is a coccus-shaped, non-motile bacterium found in virtually every person’s mouth. Its thick cell wall distinguishes it as gram positive. This strain of bacteria is considered harmful to humans due to the fact that it causes tooth decay. They congregate in large numbers of Streptococci plaques on the surface of a tooth. This particular species is the most predominant of all bacteria growing on the teeth, tongue, cheeks, and in the saliva of humans. It is mostly found residing in the crevices and pits of human teeth.

S. mutans uses sucrose from food particles left in the mouth after a meal. It generates a sticky polysaccharide that enables the bacteria to stick to one another, resulting in plaque. The bacteria release acids in the presence of food, resulting in the degradation of tooth enamel. This can eventually result in dental decay and cavities. To obtain food particles that are attached to tissues in the mouth, S. mutans secretes enzymes that metabolize the tissues into small pieces. It is then possible for the tissues to be consumed by the bacteria. The S. mutans consumes high molecular weight dextrans and other glucans only from sucrose. The sucrose is needed for polysaccharide production. The polysaccharides are then stored for later use as energy reserves. These energy reserves are tapped into during the production of lactic acid, a compound that can aid in the deterioration of tooth enamel.

The purpose of this study was to determine the antimicrobial effects of garlic, cinnamon, and clove on S. mutans at various concentrations. Two separate methods, a paper disk diffusion and a minimum inhibitory concentration (MIC) test, were used to determine the inhibitory effects of the spices. Entire spices, rather than select essential oils, were also combined to test for...
synergy. This avoided antagonistic effects due to the lack of crucial minor components or essential oils in the plant\textsuperscript{25}. The same paper disk diffusion method was also used to test the antimicrobial properties of various mouthwashes as a basis of comparison. Based on previous research\textsuperscript{9,12,32}, it was hypothesized that antimicrobial effectiveness would decrease in the order of garlic aqueous extract, cinnamon aqueous extract, and clove aqueous extract. When used together, it was hypothesized that the synergistic effects of garlic and cinnamon extracts would be more effective at killing \textit{S. mutans} than either of the other combinations\textsuperscript{25,26,33}.

MATERIALS AND METHODS

Spice Preparation

McCormick \textsuperscript{®} ground garlic and cloves and ShopRite \textsuperscript{®} ground cinnamon were prepared by mixing 20 g of each spice with 100 mL of distilled water. Clove and garlic were boiled for 15 minutes. Cinnamon was allowed to stand for 20 minutes. The garlic and cinnamon were then filtered, while clove was centrifuged to remove the ground spice particles from the mixture.

Paper Disk Diffusion

Two trials of paper disk diffusion were used. For the first trial, tryptic soy agar plates were prepared with a lawn of the \textit{S. mutans} culture. Paper disks were placed on the lawn and various amounts of the spice extracts were pipetted onto paper disks (Fig. 3). Then, water was added until the water and spice extract totaled 10 µL on each disk. Control disks were set up with only water. A control plate with no inoculation was also set up in the same manner. An effect would be observed upon the formation of zones of inhibition. A zone of inhibition results when a circular field around the paper disk forms, indicating the prevention of bacterial growth. These zones were measured with a ruler. No statistical analysis of error was done but more than one trial was performed.

For the second trial, agar plates were again prepared with a lawn of \textit{S. mutans}. This time, spice extracts were added first onto the disks, and allowed to dry before being placed onto the inoculated lawn. The spices were added in the amounts 0 - 60 µL, with water added in decreasing order so that each disk had a total of 60 µL of liquid (Fig. 4). To determine the synergistic effects of the spices, the extracts were applied to the disks in pairs. They were applied in three combinations, using 30 µL of each extract. Additionally, some disks were completely soaked in the individual extracts for 30 minutes. Control plates were also set up in the same manner with no inoculation.
Minimum Inhibitory Concentration

1.5 μL of tryptic soy broth were pipetted into each well of a 24-well microtiter plate. Garlic, cinnamon, and clove extracts, as well as water were added to the wells in the same quantities as in trial 1 of the paper disk diffusion (Fig. 3). Then 10 μL of *S. mutans* culture were added to all the non-control wells. The bottom row served as the control with only 10μL tryptic soy broth.
Mouthwash Experiment

As a comparative experiment, separate agar dishes were prepared with a lawn of *S. mutans*. Disks were placed on the plates, each with 10 µL of one of the various mouthwashes: Oasis (Tri Hydra Technology)®, ACT Bubblegum Blowout®, Scope Outlast®, Listerine Antiseptic®, Listerine Zero®, Crest Pro-Health®, Care One Antiseptic®, and Cepacol Antibacterial Rinse®.

RESULTS:

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Figure 5: Disk Diffusion Tests. A. Garlic 0-10 µL. B. Garlic 0-60 µL. C. Cinnamon 0-10 µL. D. Cinnamon 0-60 µL. E. Clove 0-60 µL. F. Clove 0-60 µL. G. Soaking and Synergy Extracts I. H. Soaking and Synergy Extracts II.

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Figure 6: MIC Tests. A. Garlic MIC plate. B. Cinnamon MIC plate. C. Clove MIC plate.
In this experiment, the extracts of garlic, cinnamon, and clove were unsuccessful at inhibiting the growth of *S. mutans*. In trial 1, in which concentrations varied from 0-10 µL, all three extracts failed to produce zones of inhibition in the disk diffusion tests (Fig. 5 A, C, and E). Those same concentrations also showed no activity for the MIC. All inoculated wells showed turbidity, indicating a failure to eliminate *S. mutans* growth (Fig. 6). In trial 2, in which concentrations varied from 0 – 60 µL, the extracts similarly failed to produce zones of inhibition in the disk diffusion test (Fig. 5 B, D, and F). There was, however, a contamination in the garlic extract as seen in the control plate of that spice (Fig. 7). There should have been no growth in the control plate; however, cloudy white growth was visible. On the bottom left part of the disk, there appeared to be a fungal contamination as opposed to a bacterial contamination. Furthermore, the method of soaking the disk in the extract did not prevent bacterial growth. In the test for synergy, the combinations of these spices also failed to prevent growth (Fig. 5 G and H).

Mouthwash Experiment Results:

![Figure 7: Garlic Control Contamination.]


The disk diffusion of the mouthwashes showed varied antimicrobial effects of the different products (Table 1, Fig. 8). Cepacol ® (I) proved to be the most effective, showing zone of inhibition of diameter 2.550 cm. Crest ® (G) followed next with a zone measuring 2.325 cm, followed in order by ACT ® (B) (1.700 cm), Scope ® (C) (1.625 cm), and Oasis ® (A) (1.525 cm). Listerine ® (D), Listerine ® (E), and CareOne ® (H) all failed to prevent *S. mutans* growth.

[3-9]
DISCUSSION

The results revealed that the garlic, cinnamon, and clove extracts had no antibacterial properties against *S. mutans* since there were no zones of inhibition. It can be concluded that the spices did not kill any bacteria. The mouthwashes, however, produced zones of inhibition, demonstrating that the bacteria culture was viable.

To equally compare the inhibitory properties of the spices and mouthwashes, the paper disk diffusion method was used to test each. Although none of the tested spices inhibited *S. mutans*, a few brands of mouthwash effectively killed the bacteria. Of the eight mouthwashes tested, Cepacol® Antibacterial Mouthwash with Cypreen was the most successful. Both types of Listerine did not eradicate any bacteria. Possible explanations were analyzed for this behavior. On the Cepacol Mouthwash label, it quotes “Cepacol Antibacterial kills the germs that cause bad breath” whereas, the Listerine mouthwash bottles read “helps reduce plaque and gingivitis.” Limsong *et al.* observed that Listerine does not kill *S. mutans*, but instead prevents it from adhering to teeth. The testing methods used did not rinse away bacteria, which possibly presented misleading results.

Since the mouthwash demonstrated that the bacteria could be inhibited, it is probable that the extraction method used on the spices in this study was not effective. Other experiments have noted the use of ethanol or vacuum filtration in the derivation of extract. Nzeako and his team obtained significant results used boiling techniques similar to the ones in this experiment. Therefore, other reasons for the failure of the experiment have been considered. The extract, for instance, could have been boiled for too long and burned, thus destroying any enzymes responsible for antimicrobial properties. If ethanol was used instead of water, it is possible that boiling would not even be necessary. It is also possible that *S. mutans* is immune to the antimicrobial properties of the spice extracts. This is highly unlikely, however, because Delaquis.

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<th>Mouthwash</th>
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<td>B. ACT Bubble Gum Blowout</td>
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<td>C. Scope Outlast</td>
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<td>D. Listerine Antiseptic</td>
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<td>E. Listerine Zero</td>
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<td>G. Crest Pro-Health</td>
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<td>H. CareOne Antiseptic</td>
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<tr>
<td>I. Cepacol Antibacterial Rinse</td>
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et al. have found these spices to be effective against *S. mutans*\(^{26}\). As a result, antimicrobial activity should have been observed from the extracts used in the experiment.

Additionally, it is possible that the state of the original spice was the reason for the lack of observed antimicrobial activity. The spices were store-bought and in a powdered form. The industrial method of transforming the spice into a powdered form may have removed the extract from the spices. This would occur if the spices were processed using heating methods that denatured the enzymes in the spices. Also, in the ground state of the spice, the extract may have evaporated because it was not contained by the spice’s natural structure. It is also possible that more than 100 mL of water should have been used in extraction. In previous studies with whole garlic, only 50 mL was used because the whole garlic still contained some natural water\(^ {35}\). The powdered form used in this experiment was already dried, so it is possible that more water should have been used to make up for the previous drying of the spice. Previous studies showed essential oils to be more effective than extract\(^ {14}\). Therefore, if the essential oils were isolated instead of the extract, antimicrobial effects may have been observed. Also, spices could potentially require the same rinsing that mouthwashes such as Listerine may need, in order to remove bacteria from the surface. Further testing would have to be done to verify these possibilities.

Trial 2 of the experiment provided further evidence of potential errors that may have been responsible for failure. For instance, it may have been possible that filter disks did not provide effective diffusion of the extract to the bacteria. The second trial dismissed this notion since brown rings were seen around the clove disks indicating that the clove extract must have diffused out. Still, bacteria were present in these rings, so the extract showed no antimicrobial effects. In addition, during the first trial, the bacteria may have been swabbed on too thickly, preventing any significant antimicrobial activity from being observed. Consequently, in the second experiment, a light film of the bacteria was swabbed. Yet again, no antimicrobial activity was observed. After the first trial, it was assumed that not enough of the extract was used so in the second trial, some disks were soaked in the extract, but still no antimicrobial effects were observed. Soaking provides a concentrated amount of extract, so if a low concentration of extract was the problem, this technique should have resulted in inhibition.

While conducting the study, the team committed several human errors that may have also contributed to the inconclusive results obtained. Since the experiment revolved around the use of bacteria, it was imperative that all tools and materials were sterilized. Poor sterilization of tools may have led to the contamination of the agar plates. Additionally, the paper disks were dried in the second trial in open air, potentially allowing airborne bacteria, other than *S. mutans*, to contaminate them. When the plates were prepared, errors were made in the pipetting of the spice extracts. Some of the cinnamon extracts could have been contaminated with minute amounts of garlic since the same water was used; however, the wells with garlic-contaminated water were cleared with a pipette and components were added again. Other pipette errors involved clogged pipettes and pipette contamination. Human errors like these may have contributed to the lack of conclusive results in the experiment.
The bulk of the errors in the experiment may have also occurred while preparing the spices for the bacteria. Though the initial method of extraction included boiling and filtering the spices, the method was unsuccessful. By boiling the garlic, allicin may have been partially degraded. The boiled solution, however, still emitted a garlicky scent, indicating that some allicin was present. During the boiling process, some clove boiled over and burned. Clove and cinnamon are also woodier substances than garlic. They subsequently absorbed the water, which left less water to form a solution. The mixtures of clove and cinnamon were clumpy and viscous, respectively. As a result of this complication, non-uniform methods of extraction had to be employed to obtain the extract. The cinnamon had to be re-mixed, then its components were extracted through filtration without boiling, and the clove was centrifuged to form a supernatant. Another issue with the filtration method was that the spices, especially clove and cinnamon, did not filter completely and yielded lower concentrations.

Originally, the team purposely chose an extraction method suited for home-use rather than lab-use. Because water, stoves, and filters are common in the household, the method of extraction incorporated such items to emulate how spices could naturally and easily be used as a mouthwash on a daily basis. Through this method, the natural, organic, and unprocessed antimicrobial properties of cinnamon, clove, and garlic could be harnessed. The results of this study, however, indicate that the method of extraction failed to isolate the components necessary for antimicrobial activity. It can be concluded that commercially ground spices cannot be simply cooked or boiled for use as a mouthwash, but require additional processing or more advanced techniques. Based on this study, this home-use method is ineffective.

As a result of the unsuccessful home-use method, future experiments can be performed with a different procedure. Prior research about the individual spice or herb and its unique extraction method would lead to optimal results. Utilizing the whole, fresh spice is ideal. In the home-kitchen environment, the entire spice is consumed; therefore, this preparation method would be most economical. However, when extracting the antimicrobial components of the spices and herbs, water should not be used as the solvent. During the experiment, water was ineffective in extracting the spices’ antibacterial ingredients. A vacuum centrifuge may be a better filtration method. Although this cannot be performed in the home-kitchen environment, this preparation method may be a more effective way of extracting the needed antimicrobial components.

Even though extraction of spices at home for their properties may not be viable, antimicrobial mouthwashes composed of spices should not be abandoned. Since experimental evidence demonstrates that spices do have the ability to kill some types of bacteria, including S. mutans, they still have the potential to be effective components of mouthwashes. Spices, however, may need to be extracted in a lab using more advanced techniques and equipment. Consequently, spice infused mouthwashes may only be produced commercially. Although commercial production will result in slight loss of the natural and chemical-free benefits of the spice mouthwash, it may be the only viable option based on these results.
Overall, the experiment revealed that spices cannot be used as an effective mouthwash if prepared at home, using the procedure from this study. The commercial mouthwashes demonstrated antimicrobial activity against the *S. mutans* while the spices had no effect. Nonetheless, spices extracted in a lab can still present an opportunity for natural mouthwashes. In comparison to chemical mouthwashes, which often have labels warning against the dangers of accidental ingestion of the product, the risk-free spice mouthwash will most likely attract many consumers. Spice mouthwashes could possibly succeed in the near future with an upgraded procedure and ingredients.
REFERENCES


[3-15]


