Examining the Efficacy of Toothpastes to Prevent Biofilm Formation by Three Bacterial Species Associated with Tooth and Gum Disease

William Roshto, Biology Human Medicine and Dawn Simms, M.S., Assistant Professor of Biology

FRANCISCAN MISSIONARIES OF OUR LADY UNIVERSITY

Abstract

Dental plaque is a leading cause of cavities (AKA dental caries) and gum disease. This plaque consists of a biofilm formed by bacteria that live in the mouth and metabolize sugars from the foods we eat. By-products of these metabolic processes include lactic acid which degrades tooth enamel. This study sought to examine and compare the efficacy of several fluoridated and herbal toothpastes against three plaque forming bacterial species (i.e., *Streptococcus mutans*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) known to cause dental caries. Antimicrobial susceptibility tests were performed via the disc diffusion and microdilution methods, which allowed for measuring inhibition of microbial growth. For determining the concentration of each toothpaste needed to completely inhibit growth of these primary plaque colonizers, the minimum inhibitory concentration (MIC) for each toothpaste was identified. Data were analyzed using one-way ANOVA and Tukey's multiple comparison tests (significant at $p < 0.05$). It was hypothesized that herbal toothpastes would show antimicrobial properties comparable to those of fluoridated toothpastes. Preliminary data showed the highest levels of inhibition occurred in toothpastes containing sodium fluoride and sodium bicarbonate.

Introduction

Tooth and gum disease are the most common diseases in the world, and they are caused by dental plaque that attaches to teeth and gums. Plaque is a biofilm formed by bacteria of the oral microbiome which contains 50 to 200 bacterial species in humans, and some of these species are more likely than others to cause the formation of plaque and dental caries[1]. For example, *Streptococcus mutans* (*S. mutans*) has long been recognized as the primary source of tooth decay due to its ability to accumulate into a biofilm and metabolize sugars[2]. Moreover, *S. mutans* can contribute to chronic oral infections due to its tolerance to acidity[3]. Bacteria belonging to the genus *Staphylococcus* form biofilms with the aid of polysaccharide intercellular adhesins. Some strains of *Staphylococcus aureus* (*S. aureus*) have developed resistance to beta-lactam antibiotics such as penicillin, amoxicillin, ampicillin, methicillin, oxacillin, and cephalosporins[4]. *Staphylococcus epidermidis* (*S. epidermidis*) is known to show resistance to many antibiotics, such as ciprofloxacin and vancomycin[5]. Biofilm formation is further aided by a sticky, dextran-based sugar produced as a byproduct of the metabolization of sucrose. Metabolism of lactose and glucose produces lactic acid which dissolves the thin (1-2 mm), porous layer of protective tooth enamel[6]. For centuries, toothpastes have been used to fight tooth decay; however, toothpastes are only effective at preventing bacterial growth if they include a therapeutic agent, such as fluoride, as an active ingredient[7]. Dozens of clinical studies show fluoride effectively prevents the formation of dental caries by preventing bacteria from making the enzymes needed to metabolize sugar[6,7]. However, with fluoride comes the risk of dental fluorosis, a condition that causes undesirable changes in tooth enamel, especially in children age 8 and younger[8]. Rare cases of dental fluorosis can cause severe damage to tooth enamel. For this reason, many people have switched from toothpastes containing fluoride to those containing natural, herbal ingredients such as eucalyptus oil, plant extracts, and essential oils[6]. Recent studies have shown these herbal ingredients to be effective at preventing bacterial growth when incorporated into toothpastes[9].

Methods

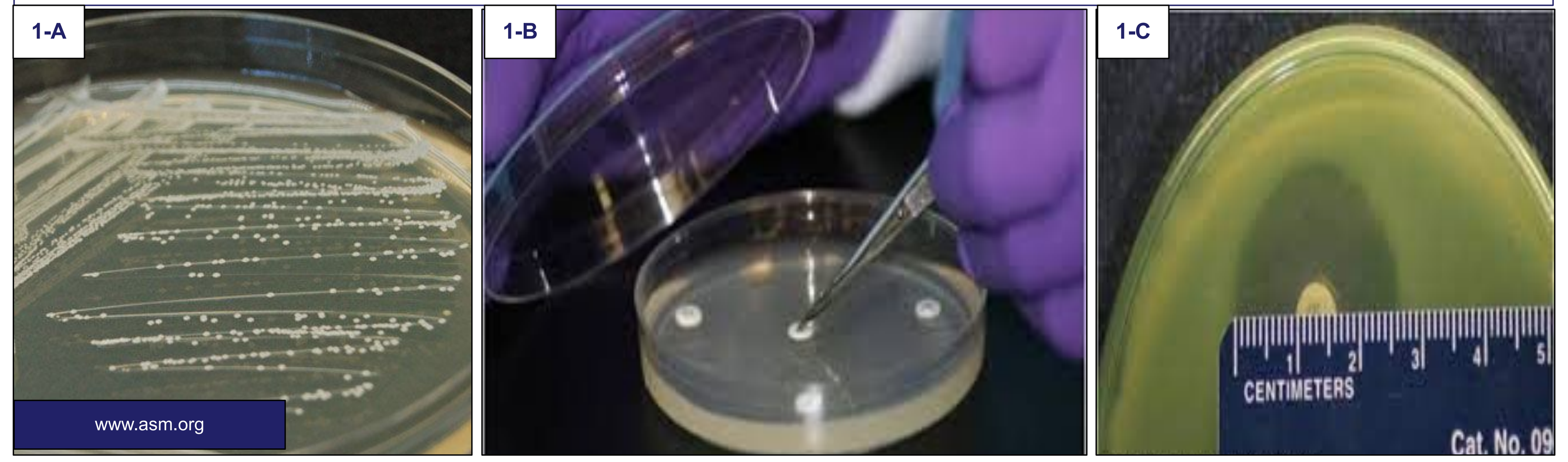
The hypothesis that herbal toothpastes show antimicrobial properties significantly similar to fluoridated toothpastes was tested by directly comparing the effects of five brands of toothpaste (Arm & Hammer Advance White™, Crest Complete® 3D White, and Colgate® Total Whitening, Colgate® Herbal Original and Dr Bronner's All-One Cinnamon) against three species of bacteria commonly associated with tooth decay and gum disease (i.e., *S. mutans*, *S. aureus*, and *S. epidermidis*). The ingredients for each toothpaste were compiled and classified by type (e.g., antimicrobials, abrasives, detergents, etc.; see Table 1).

Table 1: Type and Classification of Toothpaste Ingredients

Toothpaste	Active Ingredient(s)	Abrasives	Sweeteners	Humectants	Whiteners	Surfactants	pH Balancing	Thickeners	Dyes
Arm & Hammer Advance White™	Sodium Fluoride	Hydrated Silica, Sodium Bicarbonate	Sodium Saccharin	PEG-8, PEG/PPG-116/66 Copolymer	Sodium Bicarbonate, Sodium Carbonate Peroxide	Sodium Lauryl Sulfate	Sodium Bicarbonate	Tetrasodium Pyrophosphate	None
Crest Complete® 3D White	Sodium Fluoride	Hydrated Silica	Sodium Saccharin	Sorbitol	Disodium Pyrophosphate	Sodium Lauryl Sulfate, Polysorbate 80	Sodium Hydroxide	Xanthan Gum, Carbomer, Cellulose Gum	Blue 1, Yellow 5
Colgate® Total Whitening	Calcium Peroxide	Hydrated Silica, Sodium Bicarbonate	Sodium Saccharin	Glycerin, PEG-12	Sodium Bicarbonate, Calcium Peroxide	Sodium Lauryl Sulfate	Sodium Hydroxide	Cellulose Gum, Carrageenan	Titanium Dioxide
Colgate® Herbal Original	Sodium Fluoride, Commiphora Myrrha Resin Extract, Mentha Piperita Oil, Chamomilla Recutita Flower Extract, Eucalyptus Globulus Leaf Oil	Hydrated Silica	Sodium Saccharin, Salvia Officinalis Oil	Glycerin, PEG-12, Sorbitol	Limonene	Sodium Lauryl Sulfate	Limonene	Cellulose Gum	CI 74260, CI 77891
Dr Bronner's All-One Cinnamon	Aloe Barbadensis Leaf Juice, Potassium Cocoate Cinnamomum Cassia Oil, Mentha Arvensis Crystals	Hydrated Silica, Sodium Bicarbonate	Stevia Rebaudiana Leaf / Stem Extract	Glycerin	Sodium Bicarbonate	Cocos Nucifera Flour	Citric Acid, Cocos Nucifera Oil	Xanthan Gum	None

Tryptic soy broth (TSB) was used to grow *S. aureus* and *S. epidermidis*. TSB + 5% glucose was used for cultivating *S. mutans*, which requires glucose for proper cell division[10]. Bacteria were suspended in 10 ml of broth and incubated at 37°C for 24 hrs. To ensure cultures were pure, *S. aureus* and *S. epidermidis* were streaked for isolation on nutrient agar (Fig. 1-A), and *S. mutans* was isolated on blood agar. Minimum inhibitory concentration (MIC) of each toothpaste was determined via ten-fold serial dilutions, followed by plate counts to quantify colony-forming units per milliliter (CFU/mL). Based on MIC results, 10^{-3} (1%), 10^{-2} (10%), and $5x10^{-1}$ (50%) were chosen for disc diffusion tests (Fig. 1-B). Broth cultures were spread onto sterile Muller-Hinton (MH) agar plates (5 per dilution, n = 15). Sterile filter paper discs were placed onto the surface of each spread plate. Controls were inoculated with sterile water. Test discs were each inoculated with one of the 15 toothpaste dilutions. MH plates were incubated at 37°C for 24hrs, and the presence and size of inhibition zones were recorded (Fig. 1-C).

Figure 1: Culturing, Isolation, and Testing for Inhibition



Disc diffusion tests were five-fold, such that average inhibition zones could be calculated and used for statistical analyses. Average zones of inhibition were analyzed using one-way ANOVA and Tukey's multiple comparison tests (significant at $p < 0.05$).

Results

One of the toothpastes previously thought to contain fluoride (Colgate® Total Whitening) instead contained calcium peroxide as its active ingredient (see Table 1). Additionally, one herbal toothpaste (Colgate® Herbal Original) contained fluoride as one of its active ingredients. MIC was 10^{-3} (1%) for each toothpaste against *S. aureus* and *S. epidermidis*. *S. mutans* showed growth at 1% (mean = 1.3 X 10^6 CFU/mL, n = 5), but no growth at 10%. It was rationalized that due to how toothpaste is used, the 50% concentration would be included in the study, so all three concentrations (1%, 10%, and 50%) were used for disc diffusion testing.

Figure 2: Inhibitory Effects of Toothpastes against Biofilm-forming Bacteria

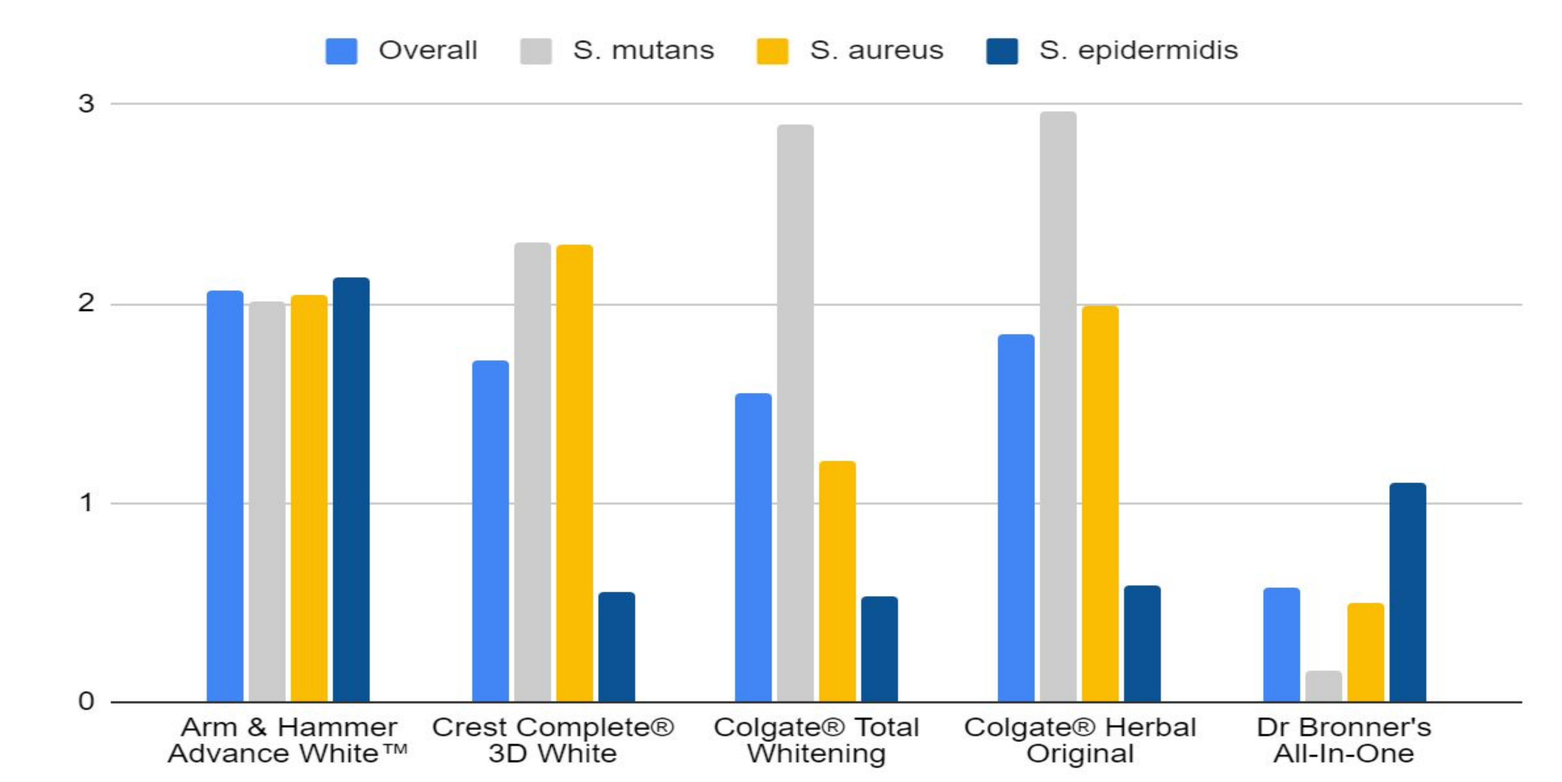


Figure 2 shows mean inhibition zone size (in mm) for each toothpaste against each bacteria. Arm & Hammer Advance White™ exhibited antibacterial activity toward all three bacterial species tested (mean = 2.07 mm, n = 15). Dr. Bronner's All-In-One exhibited the lowest antibacterial activity among all five toothpastes, with inhibition zones between 0.16 mm and 1.1 mm (mean = 0.58 mm, n = 15). Table 2 shows the results of one-way ANOVA tests (significant at $p < 0.05$, shown in light blue). Arm & Hammer Advance White™, Crest Complete® 3D White, and Colgate® Herbal Original all showed significantly more antimicrobial activity than Dr Bronner's All-In-One, as did fluoridated vs. non-fluoridated toothpastes, overall.

Table 2: Results of One-Way ANOVA Significance Testing Among Toothpastes

	Arm & Hammer vs. Crest 3D	Arm & Hammer vs. Colgate Total	Arm & Hammer vs. Colgate Herbal	Arm & Hammer vs. Dr. Bronner's	Crest 3D vs. Colgate Total	Crest 3D vs. Colgate Herbal	Crest 3D vs. Dr. Bronner's	Colgate Total vs. Colgate Herbal	Colgate Total vs. Dr. Bronner's	Colgate Herbal vs. Dr. Bronner's	Fluoridated vs. Non-fluoridated
Overall	p = 0.53	p = 0.37	p = 0.72	p = 0.00 (Arm & Hammer)	p = 0.80	p = 0.85	p = 0.02 (Crest 3D)	p = 0.67	p = 0.06 (marginal, Colgate Total)	p = 0.02 (Colgate Herbal)	p = 0.04 (fluoridated)
Streptococcus mutans	p = 0.80	p = 0.53	p = 0.50	p = 0.00 (Arm & Hammer)	p = 0.71	p = 0.68	p = 0.04 (Crest 3D)	p = 0.97	p = 0.03 (Colgate Total)	p = 0.03 (Colgate Herbal)	p = 0.30
Staphylococcus aureus	p = 0.79	p = 0.33	p = 0.95	p = 0.02 (Arm & Hammer)	p = 0.26	p = 0.77	p = 0.03 (Crest 3D)	p = 0.43	p = 0.28	p = 0.07 (marginal, Colgate Herbal)	p = 0.04 (fluoridated)
Staphylococcus epidermidis	p = 0.00 (Arm & Hammer)	p = 0.00 (Arm & Hammer)	p = 0.01 (Arm & Hammer)	p = 0.01 (Arm & Hammer)	p = 0.95	p = 0.94	p = 0.28	p = 0.89	p = 0.19	p = 0.34	p = 0.42

Discussion & Conclusions

The hypothesis that herbal toothpastes show antimicrobial properties significantly similar to fluoridated toothpastes was rejected in this study. While antimicrobial properties were observed for herbal plant-based ingredients, sodium fluoride appeared to be the superior active ingredient. This was most pronounced with Arm & Hammer Advance White™ Toothpaste (p = 0.00), which showed consistent efficacy against all three bacterial species. Previous studies demonstrated the benefits of herbal toothpastes; however, direct comparisons between herbal and fluoridated toothpastes are largely missing from the scientific literature[9]. Indeed, the current study included only one truly herbal, non-fluoridated brand (i.e., Dr. Bronner's All-In-One Cinnamon Toothpaste) which performed significantly poorly as compared to the other pastes (Table 2: p = 0.00 - 0.02). While fluoride is a proven therapeutic toothpaste ingredient[7], it is important to remember that ingredients alone do not necessarily dictate how effective a toothpaste will be at preventing tooth decay and gum disease. For example, results of MIC tests performed in this study showed that the amount and concentration of the toothpaste used may impact its efficacy. Additionally, it is imperative that one brushes for the correct amount of time and with the appropriate frequency[11]. Significant synergistic effects may exist for toothpastes and mouthwashes, which warrants future investigation, as do comparisons between *in vitro* and *in vivo* tests and specimen.

References

1. Valm, A. 2019. The Structure of Dental Plaque Microbial Communities in the Transition from Health to Dental Caries and Periodontal Disease. Journal of Molecular Biology. Retrieved from doi: 10.1016/j.jmb.2019.05.016.
2. Alaluusua, S. and O. Renkonen. 1983. *Streptococcus mutans* establishment and dental caries experience in children from 2 to 4 years old. European Journal of Oral Sciences. Retrieved from; https://doi.org/10.1111/j.1600-0722.1983.tb00845.x.
3. Hamilton, I. R. and N. D. Buckley. 2007. Adaptation by *Streptococcus mutans* to acid tolerance. John Wiley & Sons. Available from: http://www3.interscience.wiley.com/journal/119994459/abstract.
4. David, M. Z. and R. S. Daum 2010. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clinical Microbiology Reviews 23:616-687.
5. Koch, J. A., Taylor, M., Pust, A., Cappellini, J., Jonathan, B., Mandell, D. M., Neel, B., Shah, K., Brothers, M. and K. L. Urish. 2020. *Staphylococcus epidermidis* Biofilms Have a High Tolerance to Antibiotics in Periprosthetic Joint Infection. Life. 10: 253.
6. Ali, Y., Abdulhaq, A., Eldin, N., and S. Alam. 2015. Efficacy of Various Toothpastes Used in Kingdom of Saudi Arabia against Dental Caries Causing *Streptococcus mutans*. International Research Journal of Natural and Applied Sciences. 2: 42-57.
7. Cury, J.A. and M.A. Tenuta. 2014. Evidence-based recommendation on toothpaste use. Oral Health. Retrieved from: https://doi.org/10.1590/S1806-83242014.50000001
8. Center for Disease Control and Prevention. 2019. Community Water Fluoridation: Fluorosis. Retrieved from https://www.cdc.gov/fluoridation/faqs/dental_fluorosis/index.htm
9. Peck, M. T. 2011. An in-vitro analysis of the antimicrobial efficacy of herbal toothpastes on selected primary plaque colonizers. International Journal of Clinical Dental Science. Retrieved from: https://repository.uwc.ac.za/handle/10566/928.
10. Kovacs, C. J., Faustoferri, R. C., Bischer, A. P., and R. G. Quivey Jr.. 2019. Streptococcus mutans requires mature rhamnose-glucose polysaccharides for proper pathophysiology, morphogenesis and cellular division. Molecular Microbiology. 112: 944-959. Retrieved from: https://doi.org/10.1111/mmi.14330.
11. Gallagher, A., Sowinski, J., Bowman, J., Barrett, K., Lowe, S., Patel, K., Bosma, M.L., and J.E. Creeth. 2009. The Effect of Brushing Time and Dentrifice on Dental Plaque Removal *in vivo*. The Journal of Dental Hygiene. 83: 111-116. Retrieved from: https://jdh.adha.org/content/jdenthyg/83/3/111.full.pdf.

Acknowledgements

Thanks go out to Jodi Wilder for early work on this project. The instructor of record was Dr. Debanjana Bhattacharya. Funding was provided by the Fran U Biology Department and the Fran U Sr. Edana Corcoran Professorship.