A COMPARISON OF COCONUT AND SESAME OILS WITH CHLORHEXIDINE AND LISTERINE AS ANTIMICROBIAL AND ANTI-PLAQUE AGENTS

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MASTER'S THESIS

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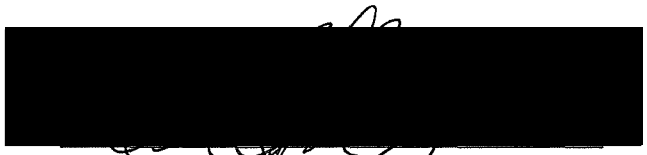

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ABSTRACT

A COMPARISON OF COCONUT AND SESAME OILS WITH CHLORHEXIDINE AND LISTERINE AS ANTIMICROBIAL AND ANTI-PLAQUE AGENTS

ERIN S BAILEY, M.S., COMPREHENSIVE DENTISTRY, 2019

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Introduction: Displeasure and dissatisfaction with the side effect profiles of conventional medical therapies has led to the exploration of diverse approaches to prevent or treat common diseases. Oil-pulling, in complementary and alternative medicine, is a procedure that involves swishing oil in the mouth for oral and systemic health benefits. While it has been used extensively as a traditional Indian folk remedy, sufficient research and evidence is lacking to demonstrate the effects of oil pulling on oral health.

Objectives: The aim of this study is to compare the antimicrobial and anti-plaque effects of edible oils with Chlorhexidine and Listerine.

Methods: This in vitro study was carried out in two different phases: (1) Antibacterial activity of coconut and sesame oil was assessed and compared to the antibacterial activity of Chlorhexidine and Listerine using the Kirby Bauer Disk susceptibility test. (2) Anti-plaque activity was assessed by comparing the microorganism reduction efficacies of the oils to those of Chlorhexidine and Listerine.

Results: Listerine, Coconut Oil, and sesame oil did not show any antimicrobial effect on *Streptococcus mutans* using the Kirby Bauer Disk susceptibility test. Chlorhexidine and Listerine had 100% antiplaque effect against viable organisms in biofilms, thus there were no numbers to

compare against the edible oil and PBS data. Log reduction measure of efficacies of PBS (control), Coconut oil, and Sesame Oil were compared. The p-values indicated that there was no statistical differences in median concentrations of colony forming units at a significance level of .05.

Conclusions: This study's results do not support the claim that edible oils have antimicrobial and anti-plaque properties comparable to Chlorhexidine and Listerine

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LIST OF ABBREVIATIONS

WHO- World Health Organization

CHG – Chlorhexidine Gluconate

ADA – American Dental Association

CAM – Complementary and Alternative Medicine

BHI – Brain Heart Infusion

DFR – Drip Flow Reactor

PBS – Phosphate Buffered Saline

CHAPTER I: REVIEW OF THE LITERATURE

Dental caries is an infectious, transmissible disease which results in the destruction of tooth structure and can ultimately result in tooth loss [1]. According to a systematic analysis conducted by the World Health Organization (WHO), it was reported that untreated caries in permanent teeth was the most prevalent condition, affecting 2.5 billion people worldwide [2]. Therefore, it is imperative that effective oral hygiene practices are employed to help combat this pandemic public health crisis.

Dental caries initiation and progression occurs when there is an intersection between three components: acidogenic bacteria found in dental plaque, fermentable carbohydrates derived from the diet, and susceptible tooth structure. *Streptococcus Mutans* and *Lactobacilli Acidophilus* are the primary bacteria found within plaque that metabolize ingested carbohydrates, producing acid which dissolves natural tooth structure. Disruption of any step in this process, by mechanical or chemical means, will prevent the progression of disease and may allow for remineralization activity which can reverse the effects of disease [3].

While the primary mechanisms for controlling bacterial plaque are mechanical in nature, chemical intervention through the use of antimicrobial mouthwashes can be used to supplement the caries prevention effect. Oral rinses have the ability to access and penetrate areas not easily accessible by a toothbrush and floss. Chlorhexidine gluconate was accepted as one of the first chemotherapeutic agents to be effective against supra-gingival dental plaque and gingivitis [4].

Chlorhexidine gluconate (CHG) is an antimicrobial agent with bacteriostatic and bactericidal effects on microorganisms such as *Streptococcus mutans* [5]. CHG can bind to

salivary mucins which ultimately reduces pellicle formation and inhibits colonization of plaque bacteria [6]. Among topical oral rinses, it is considered the most effective anti-plaque and anti-gingivitis agent [7]. Although chlorhexidine gluconate is highly effective, it has several undesirable side effects that make it less appealing to patients, including dental stain, altered taste sensation, irritation and dryness of the mouth, desquamative lesions, and ulceration of the gingival mucosa [8].

Listerine is an essential oil-containing mouthrinse that carries the American Dental Association (ADA) Seal of approval as an anti-plaque and gingivitis agent. It is composed of eucalyptol, menthol, methyl salicylate, and thymol. These essential oils have the ability to alter bacterial enzyme activity, cause protein denaturation, and have also been shown to penetrate the dental plaque biofilm [6]. In contrast to Chlorhexidine, Listerine does not require a prescription and is commercially available. In comparison to CHG, Listerine has side effects that make it less appealing to some patients. Food and Drug Administration reports of Listerine side effects have included but are not limited to complaints of application site burn, ageusia, oral mucosal exfoliation, and stomatitis [9].

Displeasure and dissatisfaction with the side effect profiles of conventional medical therapies has led to the exploration of diverse approaches to prevent or treat common diseases. These modalities collectively known as Complementary and Alternative Medicine (CAM), do not always fall under the norms of Western medicine. Relating to the field of dentistry, some CAM supporters have beliefs which are not evidence-based, including opposition to community water fluoridation [10]. These individuals are more likely to hold beliefs which are philosophically congruent with Complementary and Alternative Medicine practices. Philosophical congruence has been identified as the most influential reason for healthcare

recipients to pursue CAM measures [10]. These alternatives are more compatible with the patient's values, worldviews, and spiritual philosophies regarding the nature of their health. CAM strategies emphasize holistic balance and the mind-body connection, and prioritize a patient's autonomy and locus of control [10, 11]. CAM practices have become increasingly popular in the United States. In 2007, $33.9 billion was spent on CAM therapies in the United States and an estimated 38% of American adults reported using some form of CAM [10].

Oil pulling is a traditional Indian folk remedy that involves swishing oil in the mouth for prolonged periods of time with purported oral and systemic health benefits. Referred to as Kavala Gandoosha and Kavala Graha in Ayurvedic texts, it is claimed to alleviate or cure over 30 systemic ailments to include caries, halitosis, gingivitis, dry throat, and cracked lips. In the 1990s, Dr. Karach popularized the concept of oil pulling in Russia using edible oils such as sesame oil, coconut oil, and sunflower oil [11]. Sesame oil contains lignans which have antioxidant properties and potentiate Vitamin E action, and polysaturated fatty acids which can reduce free radical injury to the oral tissues [12]. Coconut oil contains lauric acid which has antimicrobial and anti-inflammatory properties [13]. While the exact mechanism of action remains unknown, it has been hypothesized that the action of oil pulling reduces bacteria, toxins, and pus from the interstitium. However, it is scientifically impossible for toxins to be removed from deeper tissues since the oral mucosa is not a semi-permeable membrane. Another possible theory involves a saponification process by which oil is broken down by bicarbonates in the saliva and forms a soap that can cleanse microorganisms or plaque materials. Although the exact mechanism of action remains unknown, both sesame and coconut oil have shown effective antimicrobial activity against *S. Mutans* in in vitro and randomized, controlled, triple-blind studies [12, 13, 14].

Traditional medicine involves knowledge, skills, and practices based upon theories, experiences, and beliefs inherent to different cultures [15]. While there are some studies that show the effect of edible oils on systemic health, sufficient research and evidence is lacking to demonstrate the effects of oil pulling on oral health. To date, safety and efficacy standards have not been met to award any of these products the ADA Seal of Acceptance [16]. The lack of scientific evidence on edible oils and oil pulling has prevented them from being incorporated into modern dental care. The purpose of this study is to compare the antimicrobial and anti-plaque effects of edible oils with chlorhexidine and Listerine to assess the validity of using edible oils as a natural alternative for patients who prefer Complementary and Alternative Medicine choices.

CHAPTER II: MATERIALS AND METHODS

Materials

Kirby Bauer Test

Sterile saline

2 ml tubes

0.5 McFarland standard

Mueller-Hinton agar plates supplemented w/ 5% blood sheep, 100mm

Caliper

Filter paper disks

Antibiotic disk dispenser

Vortex

Blood agar

18-24 hour old pure culture of Streptococci Mutans

Coconut oil

Sesame oil

.12% Chlorhexidine

Sterile swabs

Inoculating loop or needle

Bunsen burner

Alcohol pads

35 °C to 37°C non-CO2 incubator

Drip Flow Biofilm Reactor

6 chamber Drip Flow Reactor

Hydroxyapatite Coupons

Silicone tubing

Norprene tubing

Bacterial air vents

Teflon thread seal tape

Carboy lids

Teflon, metal or rubber spatulas

Culture tubes and culture tube closures

Petri dish 100mm x 15mm

Stainless Steel hemostat clamps

Glass Beakers

Peristaltic pump and four pump heads

Clamp stand and clamp

Environmental shaker

250 ml Erlenmeyer flask

Homogenizer

Homogenizer probe

Sterile Dilution Buffer

Conical bottom sterile disposable centrifugal tubes

CO2 gas generating cart

Brain Heart Infusion Broth + 2% sucrose

Brain Heart Infusion Agar + 2% sucrose

Methods

Kirby Bauer Disk Susceptibilty Test Protocol:

1. S. mutans was cultured in blood agar for 24 hours. Turbidity of the suspension was adjusted to 0.5 McFarland standard.
2. A sterile swab was used to apply the bacterial suspension on the MHA plate.
3. Chlorhexidine, Coconut oil, Sesame oil, Original Listerine, and Distilled water impregnanted disks were placed on the plates using the antibiotic disc dispenser.
4. Plates were placed into an incubator at 35°C for 16-18 hours. Following incubation, zones of inhibition were measured w/ calipers.
5. Susceptibility of the isolates were compared against one another by the non-parametric Kruskal-Wallis test.

Figure 1. Displays a Muehller-Hinton agar plate with impregnated disks. Disk 4 shows what it looks like when there is a zone of inhibition.

Biofilm growth using a Drip Flow Biofilm Reactor:

1. Prepared inoculum culture. Thawed frozen stock culture of S. mutans was added to Brain Heart Infusion broth supplemented with 2% sucrose. Cultures were incubated overnight in an anaerobic chamber with CO2 gas generating cartridges. Viable cell density of the culture were determined by serially diluting and drop plating onto solid BHI agar + 2% sucrose. The plates were incubated overnight in an anaerobic chamber with CO2 gas generating cartridges.
2. Drip flow reactor was prepared and sterilized.

3. Batch phase – reactor was laid flat on a bench top. 15ml of BHI broth + 2% sucrose was added to each of the six chambers. 3ml of S. mutans inoculum was added into each channel, the cover was secured, and incubation occured for 6 hours.
4. Continuous flow phase – after 6-hour batch phase, legs were attached to the DFR adjusted so that the reactor slopes 10°downward. Continuous flow media was pumped into each chamber at approximately 0.5ml/minute for an additional 48 h.

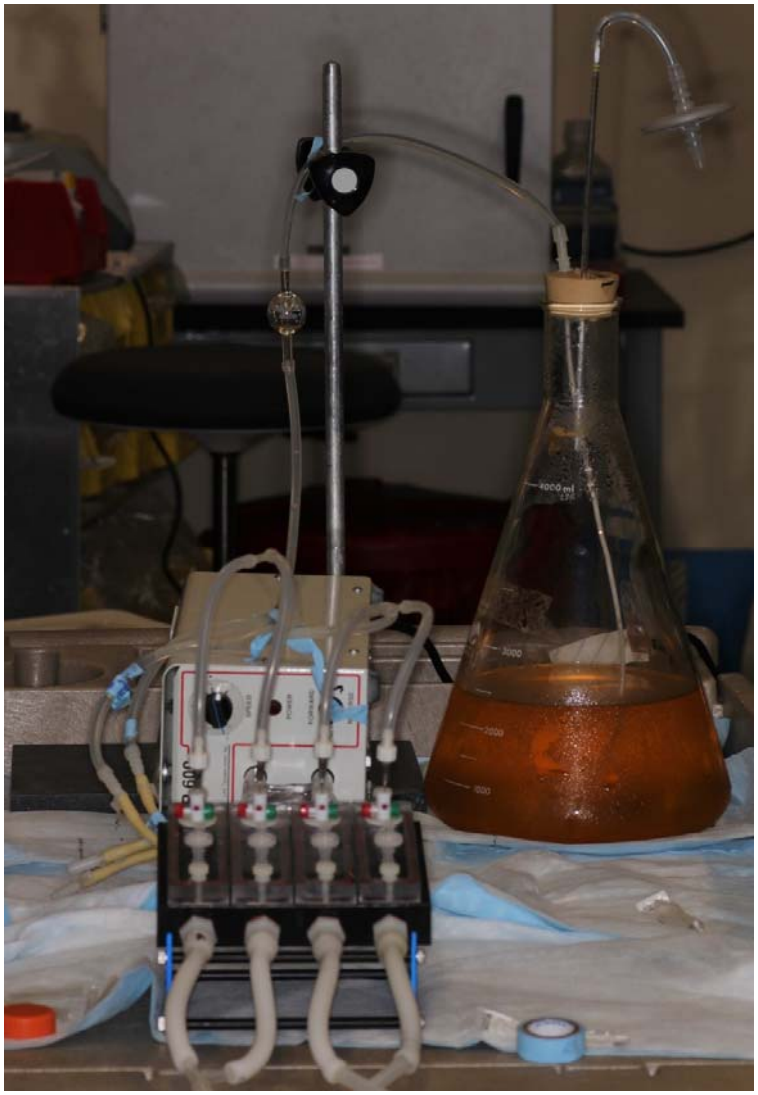

Figure 2. Displays Drip Flow Reactor set up. The flask contains Brain Heart Infusion Broth, the white box is the peristaltic pump which pulls the medium from the flask, into the DFR which consists of 4 parallel channels, each holding one standard glass microscope slide sized coupon.

5. Biofilm sample collection – Each coupon was removed from the reactors and rinsed by sterile buffered dilution. All coupons were placed in a separate sterile glass beaker. Then 10 ml of 0.12% CHG was pipetted into 5 out of the 6 beakers. After 3 minutes the bacteria were scraped into a beaker containing sterile dilution buffer. Scraping was repeated 3-4 times. The coupon was then rinsed with of sterile dilution buffered water. Rinsing was repeated 5 times. The scraped biofilm samples were disaggregated, homogenized, serially diluted, drop plated on BHI + 2% sucrose agar, and incubated for 17-24 hours at 35°C.
6. Colony forming units were counted, the log density per coupon was determined, and the log densities were converted into log reduction measure of efficacy.

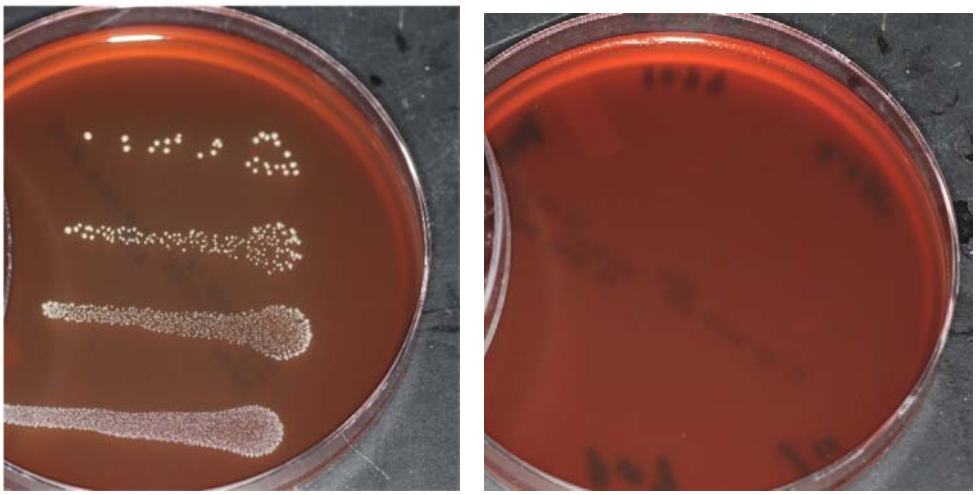

Figure 3. Blood agar plates with viable bacteria obtained from the glass coupons. The left plate represents what viable microorganisms look like when they are able to grow.

7. The steps were repeated, replacing chlorhexidine with Original Listerine, Virgin Coconut Oil, Sesame Oil, and Phosphate Buffered Saline (PBS).
8. At the end Log reduction measure of efficacies of Sesame oil and Coconut Oil were compared with those of Listerine and Chlorhexidine.

CHAPTER III: RESULTS

The outcome variable in the antimicrobial comparison was the zone of inhibition measured in mm. No zones of inhibition were observed for any of the variables except for Chlorhexidine. Chlorhexidine mean zone of inhibition of 21mm was comparable to the mean literature value.

Agents	Avg Zone of Inhibition (mm)
Coconut Oil	*ND
Sesame Oil	*ND
Chlorhexidine	21 mm
Listerine	*ND
Distilled Water	*ND

Figure 4. Average zones of inhibition for each variable. *ND = not determined.

The anti-plaque effect of the edible oils, sesame and coconut were each compared to PBS (a control) and also between the two. A sample of size 8 was obtained for each of the above oils. Descriptively, the means and standard deviations were respectively, 39.88×10^6 cfu/ml (4.22×10^6 cfu/ml), 35.88×10^6 cfu/ml (9.73×10^6 cfu/ml), and 26.38×10^6cfu/ml (18.17×10^6cfu/ml for PBS, sesame, and coconut oils. In addition to the above numerical descriptive statistics, histograms together with a box plot are provided to check for distributional assumptions. Visual inspection shows that the data are not normally distributed. As a consequence, the nonparametric, Mann-Whitney U test was used to compare the median effects of these oils. The p-value between the control and sesame oil was .422, between control and coconut oil was .130, and between sesame oil and coconut oil was .279, in each case, indicating that there are no differences in the median concentrations of colony forming units at a significance level of .05.

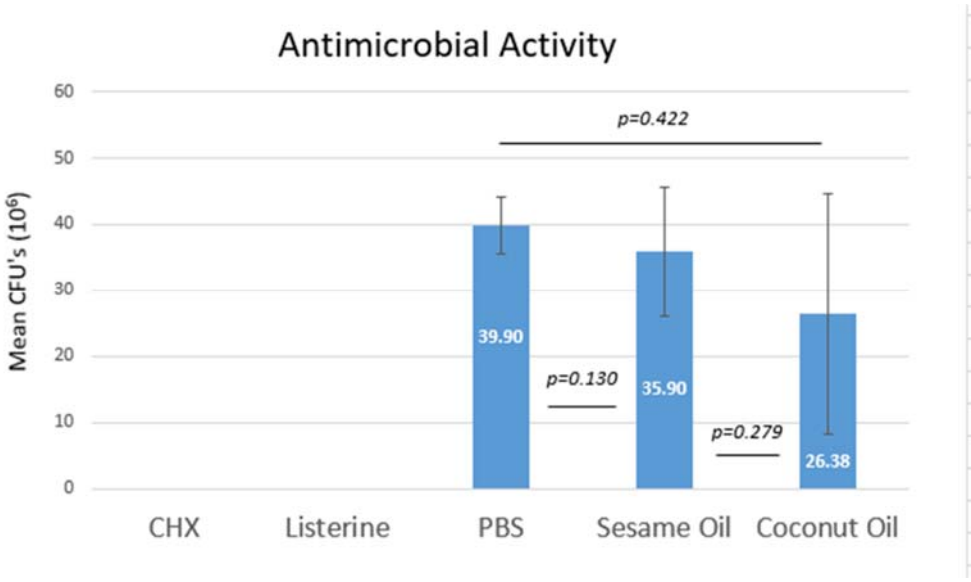

Figure 5. Mean cfu's and p-values for the variables.

CHAPTER IV: DISCUSSION

Antimicrobial effects were not observed with Coconut or Sesame oils. The first explanation as to why they were not observed is that the Kirby Bauer Disk susceptibility test adequately. Oil does not dissolve in water, so the ability of the oil to diffuse through the disk into the agar was compromised which could explain why no zones of inhibition were observed. Better results may have been obtained had the appropriate solvent been used. Another reason that effects were not observed using the oils could be due to their fatty acid content. Studies that have looked at the antimicrobial effect of coconut and sesame oils against different strains of bacteria found that the antimicrobial features come from the fatty acid content [22].

No anti-plaque properties were observed with the edible oils. Most of the studies supporting the antiplaque properties of the edible oils involved an in vivo component where the participants actually perform oil pulling. It has been hypothesized that the ability of the oils to inhibit plaque adhesion comes from an emulsification and saponification process. Emulsification

is the breakdown of fat globules into tiny droplets which results in increased surface area of the fats. Saponification is the conversion of fats or oil into soap and alcohol. The hypothesis is that swishing results in an agitation of the oil that leads to its emulsification which has a two part effect. It alters the adhesion of the bacteria on the tooth surface and allows for the alkali in the saliva to interact with the smaller fat globules forming a soap like substance which also results in reduced plaque adhesion [23].

CHAPTER V: CONCLUSIONS

This study's results do not support the claim that edible oils have antimicrobial and anti-plaque properties comparable to Chlorhexidine and Listerine. In the future, studies evaluating the effects of the oils with different derivative concentrations and their underlying mechanisms should be conducted to support the claims that these oils can be natural adjuvants for oral hygiene.

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