Effect of Beverages Containing Plant-Derived Polyphenols on Supragingival Plaque Bacterial in Children

BY

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THESIS
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LIST OF ABBREVIATIONS

AAPD  American Academy of Pediatric Dentistry
ANGT  All Natural Green Tea (Snapple®, Plano, TX)
ANRT  All Natural Raspberry Tea (Snapple®, Black and Green Tea, Plano, TX)
BT-ET  English Teatime, RC Bigelow®, Fairfield, CT
BT-CS  Cinnamon Stick, RC Bigelow®, Fairfield, CT
BT-RR  Raspberry Royale, RC Bigelow®, Fairfield, CT
CDM  Chemically Defined Medium
CJ-OS  Ocean Spray® Cranberry Juice Cocktail, Lakeville-Middleboro, MA
CJ-S  Simply ™ Cranberry Cocktail, Apopka, FL
GT  Pure Green Tea 100% Natural, Lipton®, Englewood, NJ
MIC  Minimal Inhibitory Concentration
MBIC  Minimal Biofilm Inhibitory Concentration
O.D.  Optical Density
PBS  Phosphate Buffered Saline
SSB  Sugar Sweetened Beverage
S. mutans  Streptococcus mutans
UT  Pure Leaf™ Unsweetened Tea (Purchase, NY)
SUMMARY

Many widely consumed polyphenol-rich foods or beverages including tea, coffee or wine, have been shown to benefit oral health, with anti-gingivitis and anti-caries properties. 

Objective: To investigate the effect of commercially available teas and bottled beverages containing plant-derived polyphenols on in vitro growth and biofilm formation of supragingival plaque bacteria isolated from children. Methods: Supragingival plaque bacteria obtained from tooth surfaces of children were treated with nine commercially available teas and beverages. The effects of test teas, juices or beverages on in vitro growth and biofilm formation of plaque bacteria were examined. Results: All teas tested inhibited the growth and biofilm formation of the supragingival plaque bacteria. Bottled beverages varied in the inhibition of growth and biofilm formation. Biofilm formed in the presence of plant-derived polyphenols were loosely adherent and easily dislodged from surfaces, indicating weakened biofilm attachment. Conclusions: Commercially available teas and beverages containing plant-derived polyphenols may benefit children’s oral health by suppressing growth and biofilm formation of the supragingival plaque bacteria. The concept of oral diseases prevention using natural foods or beverages may be a novel and practical approach to promote oral health in children.
I. INTRODUCTION

A. Background

Dental caries and its associated consequences are one of the most prevalent health problems in children in the United States (Peterson, 2003). It is a multifactorial disease that involves the presence and interaction of four factors: host defense, dental plaque bacteria, availability of carbohydrate as bacterial energy source, and duration of bacterial challenge that results in dental caries (Wu, 2009). As stated in the AAPD Policy on Dietary Recommendation for Infants, Children, and Adolescents, dietary habits that include prolonged contact of fruit juices or sugar sweetened beverages are significant risk factors for dental caries.

Dental caries result from demineralization of tooth enamel from acid challenge (Tahmassebi et al., 2006; Skinner et al., 2015). Dental plaque biofilm, a complex multispecies microbial community adhere to tooth surfaces, is the prime etiologic agent of dental caries. Plaque bacteria, especially *Streptococcus mutans*, ferment sucrose and other carbohydrates and produces lactic acid from metabolism. The lactic acid in the dental plaque subsequently lowers the pH level in the oral cavity to 5.5, which leads to demineralization of the enamel and dentin. *S. mutans* also produces glycosyltranferases (GTFs) which hydrolyze sucrose to produce the adherent polysaccharide matrix facilitating accumulation and stickiness of the plaque biofilm (Tahmassebi et al., 2006).

Polyphenols are natural-occurring phytochemicals that can be found in many edible plants, and the major classes of them are flavonoids, phenolic acids or alcohols, stilbenes, and lignans, with flavonoids being the most abundant one found in dietary food products (Wu, 2009; Ferrazzano 2009; Jeon et al., 2011). Dietary polyphenols in both edible and
non-edible plants have been shown to benefit health, including their antioxidant, anticancer, and anti-inflammatory properties within the last decade (Pandey and Rizvi, 2009; Gupta et al., 2011). Many widely consumed polyphenol-rich foods or beverages such as tea, coffee or wine, have been shown to benefit oral health, with antigingivitis and anticaries properties (Yoo et al., 2011; Gupta et al., 2011).

Existing research has shown that tea prepared from the plant *Camellia sinensis* inhibited *in vitro* growth and biofilm formation of *Streptococcus mutans* (Ooshima et al., 1994; Xu et al., 2011). Population with habits of drinking tea have decreased caries incidence, lower levels of cariogenic bacteria, and/or healthier plaque scores (Ooshima et al., 1994; Hamilton-Miller, 2001). Short-term rinsing with black tea had also been reported to inhibit regrowth and glycolysis of supragingival plaque bacteria from human when compared to water-rinse group (Wu, 2009). Polyphenols from fruits and berries such as cranberry (*Vaccinium macrocarpon*) or lingonberries have also been shown to have bacteriostatic effect on *Streptococcus mutans* and evident anti-adhesion properties (Kontiokari et al., 2005; Koo et al., 2006; Duarte et al., 2006; Yoo et al., 2011; Riihinen et al., 2014). Many commercially available beverages contain dietary plant-derived polyphenols, which include various cranberry cocktails and flavored teas. But it is unknown whether these drinks affect cariogenic bacteria or provide oral health benefits for its consumers. Children nowadays have access to a wide variety of beverages including juices and tea drinks. With the increase in sugar content in these beverages, it is a major concern that some beverages may contribute to dental caries. The influence of dietary habits towards oral health is indisputable, and understanding how commercially available beverages may affect dental health can serve as a new approach to promote dental health. There are
available studies of soft drinks on enamel erosion (Zero, 1996; Tahmassebi et al., 2006), but how some beverages affect plaque bacterial especially children is less well-understood. The oral health benefit of these beverages is worth investigating. In this study we want to examine if beverages containing theses antimicrobial polyphenols affect the growth and biofilm formation of children’s dental plaque.

B. Purpose of the Study

The purpose of the study is to investigate the effect of selected commercially available teas and beverages containing dietary plant-derived antimicrobial polyphenols on *in vitro* growth and biofilm formation of supragingival plaque isolated from children.

C. Hypotheses

We hypothesize that commercially available teas and bottled beverages that contain dietary plant-derived antimicrobial polyphenols may benefit children’s oral health by inhibiting the growth of children’s supragingival plaque bacteria and biofilm formation.
II. CONCEPTUAL FRAMEWORK AND RELATED LITERATURE

A. Dietary Plant-derived Polyphenols

Polyphenols are natural phytochemicals that can be found in many fruits, plants, and vegetables (Pandey and Rizvi, 2009). Polyphenol-rich plants such as grapes, cocoa beans, tea plant, cranberries, apples, and cherries can contain 200-300 mg polyphenols per 100 grams of weight, and even the food products or beverages manufactured from them contain a significant amount of polyphenols (Pandey and Rizvi, 2009). Polyphenols may contribute to the astringent or bitter taste of the foods, and they have high oxidative stability (Pandey and Rizvi, 2009). Epidemiological studies and meta-analyses have strongly suggest that consuming polyphenol-rich foods offer long term health benefits and protect from cardiovascular diseases, diabetes, and osteoporosis (Graf et al., 2005; Pandey and Rizvi, 2009).

Traditionally, the notion of dental caries prevention has placed much focus on mechanical removal of dental plaque and food debris, but in recent years there has been an increased interest in using natural food product to aid in dental caries prevention in the research field (Gupta et al, 2011). Dietary plant-derived polyphenols, such as catechins, which can be found in tea plant (Camellia Sinensis), American cranberry fruit (Vaccinium macrocarpon), and Cinnamon (Cinnamomun cassia) have been found to possess several health promoting benefits, and its anti-caries and anti-gingivitis properties have gathered attention in the dental research field (Yoo et al., 2011; Gupta et al., 2011).
B. Dental Plaque Biofilm and Dental Caries

Dental Caries is one of the most prevalent chronic diseases, and it affects U.S. children significantly. The etiology of dental caries is multifactorial, but it can be attributed to an interaction between oral bacteria, dietary habits, and host response (Peterson, 2003; Wu, 2009; Xu et al., 2011). Since the introduction of water fluoridation in 1940s, there has been a decline in the caries prevalence; however dental caries still remained a national issue (AAPD Guidelines on Fluoride Therapy).

Dental plaque is a sticky biofilm community that adheres to the surfaces of the teeth. It contains salivary proteins and pellicles, mucopolysaccharide matrix, food debris, and more than 800 species of oral bacteria (Aimutis, 2004). *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque are recognized as the main etiologic oral bacterial species for dental caries (Duarte et al., 2006; Xu et al., 2011). These cariogenic bacteria metabolize available sucrose and carbohydrates in the oral cavity that results in acid production, biofilm formation, and stress tolerance (Koo et al., 2006; Xu et al., 2011). A well-documented virulence factor for *S. mutans* that is implicated in biofilm formation is the presence of the enzyme, glucosyltransferases (GTFs); it produces the water-insoluble sticky glucans from sucrose (table sugar) that mediates the attachment and adherence of the plaque biofilm to tooth surfaces (Schilling and Bowen, 1992; Xu et al., 2011).

Consumption of sugar sweetened beverage (SSBs) has increased significantly over the last twenty years in the United States, and it is shown to cause dental caries (Shenkin et al., 2003; Skinner et al., 2015). A national survey conducted in Australia in 2007 reported that 25% of children age between 2 to 16 drinks SSBs on a daily basis, and research has
shown that consuming 3 or more cups of SSBs per day resulted in higher mean DMFT in the pediatric population (Armfield et al., 2013; Skinner et al., 2015).

The sugar content in the beverages is a main risk factor for dental caries (Tahmassebi et al., 2006). Oral cariogenic bacteria metabolize sugar and produce acid, which lowers oral pH and demineralize enamel and dentin (Tahmassebi et al., 2006). With repeated consumption of SSBs, the saliva loses the ability to neutralize the acid challenge and to protect the dentition (Tahmassebi et al., 2006).

C. Tea and Oral Bacteria

Tea is the world’s most popular beverage; it is estimated that more than two billion cups are consumed everyday (Abd Allah et al., 2011). They are all prepared from an infusion with the processed leaves of the plant *Camillia sinensis*, including green tea, black, and oolong tea. One of the fundamental differences chemically between green tea and black tea is the type of polyphenols each contains; green tea has mainly monomeric polyphenols called catechin, whereas black tea has larger molecules of polyphenols called theaflavins due to the fermentation and oxidation process (Hamilton-Miller, 2001; Yoo et al., 2011). Both catechin and theaflavins are biologically active molecules that may promote dental health. *In vitro* studies have shown that these molecules were a) bactericidal against *Streptococcus mutans*, and b) anti-adhesion against dental plaque, c) inhibition of human salivary amylase, and d) inhibition of glucosyl transferase which limits glucan biosynthesis and adhesion (Hamilton-Miller, 2001).

Studies had been done in the past to delineate a relationship between tea drinking and status of dental health. Elvin-Lewis and Steelman (1986) reported lower DMFT and
plaque score in children who drank one to three cups of tea per day compared to those who only drank one to two cups per week. Rinsing with black tea in Egyptian populations has been shown to reduce *Streptococcus mutans* count in saliva at 60% immediately after rinsing and 99.9% one hour after rinsing with black tea (Abd Allah *et al.*, 2011). Short-term rinsing with black tea had also been reported to inhibit regrowth and glycolysis of supragingival plaque bacteria from human when compared to water-rinse group (Wu, 2009).

D. **Cranberry Juice and Bacteria**

Cranberry juice is prepared from the American Cranberry fruit (*Vaccinium macrocarpon*) and is distinctively abundant in polyphenols, such as flavonoids (Bonifait and Grenier, 2010). Its consumption is widely accepted by the public, and its therapeutic effect in treating urinary tract infection via antibacterial activity against *Eschericia coli* is well established (Kontiokari *et al.*, 2005). The potential benefits of cranberry juice or cranberry extract toward inhibition of *Streptococcus mutans* have been investigated extensively in the past decade. Koo *et al.* (2006) had investigated the effect of Cranberry Juice on biofilm development and discovered that cranberry juice inhibited bacterial adhesion through blocking the binding sites in salivary pellicle and in glucans. Yamanaka *et al.* (2004) had further delineated that the anti-adhesion property of cranberry juice was due to a reduction of hydrophobicity in the cell surface of *Streptococcus mutans*, which led to an inability to attach to hydroxyapatite surfaces.
E. Cinnamon and Bacteria

Cinnamon (*Cinnamomum cassia*) is one of the natural antimicrobials that have gathered attention in the last decade in the medical field for personal care and well beings. The polyphenolic compounds found in Cinnamon had been shown to have protective properties against diabetes and cardiovascular disease (Anderson, 2008). In addition, Gupta *et al.* (2011) studied the antimicrobial effects of Cinnamon oil against a series of common oral bacteria, including *Streptococcus mutans* and *Lactobacillus species*, and reported clinically significant inhibition against the growth of these bacteria when compared to Clove oil and chlorhexidine. Some studies have attributed the antimicrobial properties of cinnamon to its chemical content, which included polyphenols, benzoic acid, benzaldehyde, and eugenol (Ramos-Nino *et al.*, 1996; Ranasinghe *et al.*, 2002; Qin *et al.*, 2012). Zhu *et al.* (2013) had shown that chewing gum containing cinnamic aldehyde promoted short-term germ-kill effect of human salivary bacteria.
III. MATERIALS AND METHODS

A. Materials

1. Test Beverages

A total of 9 teas and bottled beverages were used in this study. They were all purchased from Jewel-Osco grocery store in Chicago, IL, including:

a. Teas

i. Green Tea (Pure Green Tea 100% Natural, 1.8 grams/bag, 7.2 mg/ml, Lipton®, Englewood, NJ) [Abbreviated as “GT”]

ii. Black Tea without flavor (English Teatime, 2.3 grams/bag, 9.2 mg/ml, RC Bigelow®, Fairfield, CT) [Abbreviated as “BT-ET”]

iii. Black Tea with Cinnamon (Cinnamon Stick, 1.8 grams/bag, 7.4 mg/ml, RC Bigelow®, Fairfield, CT) [Abbreviated as “BT-CS”]

iv. Black Tea with Raspberry (Raspberry Royale, 1.8 grams/bag, 7.1 mg/ml, RC Bigelow®, Fairfield, CT) [Abbreviated as “BT-RR”]

b. Bottled Tea Beverages

i. Unsweetened Tea (Pure Leaf™, Purchase, NY) [Abbreviated as “UT”]

ii. All Natural Green Tea (Snapple®, Plano, TX) [Abbreviated as “ANGT”]
iii. All Natural Raspberry Tea (Snapple®, Black and Green Tea, Plano, TX) [Abbreviated as “ANRT”]

c. Cranberry Juice Cocktails

i. Cranberry Juice Cocktail (Ocean Spray®, Made From Concentrate, Lakeville-Middleboro, MA) [Abbreviated as “CJ-OS”]

ii. Cranberry Cocktail (Simply™, Not From Concentrate, Apopka, FL) [Abbreviated as “CJ-S”]

B. Methods

1. Supragingival Plaque Sample Collections

Supragingival plaque samples were obtained from 16 children (less than 14 years old) of University of Illinois at Chicago Pediatric Dental Clinic. No demographic or protected health information were obtained or recorded from the subjects. This study was exempt from IRB review as non-human subject research (Protocol#2014-1216). The samples were collected from the buccal and lingual surfaces of all available teeth using a specialized cotton swab (Huby®-340, Constix swabs, SC-9, Spartanbug, SC, manufacturer: Sanyo co. LTD, Japan). The cotton swab was then kept in a capped 15ml Conical tube (Falcon®, 352097, Corning Science, Reynosa, Tamaulipas, Mexico) for storage, and labeled with an identification number (#1 -#16). All tubes containing plaque samples were stored on ice until laboratory processing.
2. **Preparation of Children’s Supragingival Plaque Samples**

Saline solution (1.75ml, 0.85% NaCl) was added into the storage tube containing the supragingival plaque sample. The tube was vortexed (*Fisher Vortex Genie 2®,* Fisher Scientific, Bohemia, NY) for 30 seconds, followed by ultrasonic dispersion (FS30, Fisher Scientific) at 20% power for 2 minutes. The tubes were let stand on ice for 1 more minute for the content to settle. Clear supernatant was carefully transferred to a glass tube, and the optical density (O.D.) was adjusted to O.D. = 0.2 ± 0.01 at 600 nm by adding needed amount of 0.85% NaCl solution. This suspension was used as an inoculum for further experiments.

Chemically Defined Medium (CDM, 2X) was added to the cell suspension to achieve a 1:20 dilution for the Minimal Inhibition Concentration Assay (MIC); for the Minimal Biofilm Inhibition Concentration Assay (MBIC), an additional 2% sucrose solution was added to the 2X CDM.

3. **Designation of 96-well Microtiter Plates**

For testing all of the selected teas and beverages, four 96-well microtiter plates (*Costar® 3596*, Corning Incorporated, Corning, NY) were used for each supragingival plaque sample.
Figure 1. Microtiter plates designations

Plate-A was used for analyzing the following teas:

1) Lipton® Pure Green Tea 100% Natural  
2) Bigelow® English Teatime, Black Tea  
3) Bigelow® Cinnamon Stick, Black Tea  
4) Bigelow® Raspberry Royale, Black Tea

Plate-B was used for analyzing the following beverages:

1) Pure Leaf ™ Real Brewed Tea, Unsweetened  
2) Snapple® All Natural Green Tea  
3) Snapple® All Natural Raspberry Tea, Green and Black Tea  
4) Ocean Spray® Cranberry Juice Cocktail, From Concentrate  
5) Simply ™ Cranberry Cocktail, Not From Concentrate

4. Preparation of Test Beverages

a. Teas

Test tea bags (listed in III.A.1) were cut open and dried tea leaves were removed. One gram of tea leaves was measured on a standardized scale for use. The tea leaves (1g) were placed in 10ml of electric kettle-boiled and filtered water to achieve final stock concentration of 100 mg/ml (weight/volume). The tea solution was allowed to gradually cool to room temperature over a 30 minutes period, filtered sterilized using a 0.45 µm syringe filter (Millex®-HV 0.45 µm, Merck Millipore ltd, Tullagreen, Carrigtohill, Ireland), and placed in a 15ml conical tube (Falcon®, 352097, Corning Science, Reynosa, Tamaulipas, Mexico). Fresh tea solution was prepared for each batch of plaque sample testing. Tea concentration
was expressed in weight/volume, i.e. mg of tea leaves/ml of water; or as percentage (%)
Equivalent to a cup of brewed tea, i.e. 7mg leaves in a bag/ 250ml of water.

b. Bottled Tea Beverages and Cranberry Juice Cocktails

The bottled tea beverages and cranberry juice cocktails were used at the original concentration.

5. Serial Dilution of Teas and Bottled Beverages

a. Tea (Plate-A)

Double distilled water (100 µl) was added to all 96 wells (Row A to H) in the microtiter plate for use in serial dilution. Tea solution (100 µl of 100mg/ml stock) was added to Row A of the plate. Serial two-fold dilution was performed from Row B to G, and 100 µl of diluted solution from Row G was discarded. Wells in Row H were used as bacterial and plaque controls, ie bacteria and plaque were added to wells containing growth medium CDM but no test teas were added.

b. Bottled Tea Beverages and Cranberry Juice Cocktails (Plate-B)

Double distilled water (100 µl) was added to Row B to H of the 96-well microtiter plate for the use in serial dilution. Selected tea beverages and cranberry juice cocktails (200µl, full strength) were added to Row A. Water was not added to Row A of B-plate to obtain a 50% test concentration of the beverages. Serial two-fold dilution was performed from Row B to Row G as described in previous section, and 100 µl of mixture from Row G was discarded. Wells in Row H were used as
bacterial and plaque controls, ie bacteria and plaque were added to the wells containing CDM but no testing beverages were added.

6. Growth Condition of Plaque Samples

After serial dilution was performed, 100 µl of 2X CDM was added to each well in column 1 to 4 in Plate-A and Plate-B (listed in the designation of plates) to serve as the color control, and 100 µl of supragingival plaque sample was added to column 5 through column 12 in Plate-A and Plate-B to serve as testing groups. The plates were then incubated in an anaerobic chamber (Forma Scientific Anaerobic System, Marietta, OH) with mixed gas (5% H₂, 5% CO₂, 90% N₂, Praxair) at 37°C for 24 hours for growth inhibition (MIC) assay or for 48 hours for biofilm inhibition (MBIC) assay.
Figure 2. Plate A: Test Concentrations of Teas

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Figure 3. Plate-B: Test Concentrations of Bottled Beverages

<p>| | | | | | | | | | | | |</p>
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<td>SnapG or Unsw CDM</td>
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<td>Rasp Plaque</td>
<td>6</td>
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<td>B</td>
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<tr>
<td>D</td>
<td>6.25% bev. dilution</td>
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<td>E</td>
<td>3.1% bev. dilution</td>
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<td>1.6% bev. dilution</td>
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<tr>
<td>G</td>
<td>0.8% bev. dilution</td>
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</tbody>
</table>

7. Effect of Teas and Beverages on *In Vitro* Growth of Supragingival Plaque Bacteria: Minimum Inhibitory Concentrations (MIC) Assay

Minimum Inhibitory Concentration was defined as the lowest concentration where no growth (O.D. ≤ 0.05) of the plaque bacteria was observed. After 24 hours of incubation anaerobically, the microtiter plates designated for MIC determination were read in the spectrophotometer (*Power Wave 200-I, Bio-Tek Instruments®*) at 550nm, and the optical density (O.D.) of the wells were recorded. All plates were also examined visually for
bacterial growth. The Minimal Inhibitory Concentration was recorded based on the O.D. readings, which was defined as ≤ 0.05 in this study. If the MIC could not be determined based on O.D. readings, then visual inspection of the microtiter plates was done to determine MIC.

8. **Effect of Teas and Beverages on In Vitro Biofilm Formation of Supragingival Plaque Bacteria: Minimum Biofilm Inhibitory Concentrations (MBIC) Assay**

Minimum Biofilm Inhibitory Concentration was defined as the lowest concentration where more than 90% of biofilm formation was inhibited. After 48 hours of incubation of plaque bacteria and test agents anaerobically, the non-adherent cells in each of the wells were carefully removed and discarded. The wells were washed twice with 200µl of Phosphate-buffered saline (PBS, 0.05M, pH=6.8), and the adherent biofilms were air dried for 10 minutes. Crystal Violet (0.1%, 100µl) was added to each well for 10 minutes to stain the biofilm at room temperature. The dye was carefully removed, and the stained biofilm in the wells were washed gently three times with PBS. Ethanol (90%, 200µl) was added to the stained biofilm in each well, and the plates were placed on a shaker (Maxi rotator, Lab-line, Melrose Park, IL) for 30 minutes to extract the stain retained by the biofilm. 100µl of the content of each well was then transferred to new microtiter plates, and placed in the spectrophotometer (Power Wave 200-I, Bio-Tek Instruments®) for O.D. reading at 550 nm. The intensity of the stain correlates with the quantity of biofilm formed. The biofilm formation of the treated sample was compared to the control, and percent biofilm formation of the control was calculated. Percent (%) biofilm formation = O.D. of the treated
biofilm / O.D. of the control biofilm. The Minimal Biofilm Inhibitory Concentration was determined as the lowest concentration of test beverages which afforded < 10% biofilm formation of the control.

9. **Statistical Analysis**

Statistical analyses were carried out using IBM SPSS version 22. Descriptive statistics including the mean, median, minimum, and maximum of values were calculated. The MIC and MBIC were compared ($P<0.05$) using the Friedman Test. Pairwise comparisons were performed in a post hoc manner using Wilcoxon Signed Ranks.

The concentrations of the tea in the MIC and MBIC results were converted from mg/ml to percent solution (%) by taking the tea weight per bag (in milligrams) divided by the amount of water used (250 ml) to divide the data in mg/ml. The data of teas in percent solution was then compared with bottled beverages.

In order to perform certain comparisons, missing data were imputed based on the average value or excluded in the statistical analysis when appropriate. There were eight missing data points for Unsweetened Tea (*Pure Leaf™*, Purchase, NY); the MICs for this beverage were filled in with the values that all other subjects had, which was 51. The MBIC analysis excluded this beverage due to the missing data. Missing data was imputed on four subjects for All Natural Green Tea (*Snapple®, Plano, TX*) for MIC and MBIC; MIC was imputed using the average value of the other subjects, which were between 50 and 51. MBIC was imputed as 51 because that was what all the other subjects had shown. Missing data were imputed on 1 subject for the two cranberries cocktails (*Ocean Spray®, Made From Concentrate, Lakeville-Middleboro, MA; Simply ™, Not From Concentrate, Apopka, FL*)
for both MIC and MBIC; the imputation was done by regressing all the other beverages’ MIC or MBIC values on each cranberry cocktail, and using the predicted value as the imputed value.

For the comparison of significance between teas and beverages, the data for teas were converted from mg/ml to percent of teas by computing the concentrations based on a standardized tea preparation protocol.
IV. RESULTS

A. Effect of Teas and Beverages on *In Vitro* Growth of Supragingival Plaque Bacteria from Children: Minimum Inhibitory Concentrations (MIC)

<table>
<thead>
<tr>
<th>Drinks</th>
<th>Total Subjects</th>
<th>Median MIC (%*)</th>
<th>Minimum MIC (%*)</th>
<th>Maximum MIC (%*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT</td>
<td>16</td>
<td>11.0 [0.8 mg/ml]</td>
<td>5.5 [0.4 mg/ml]</td>
<td>42.6 [3.1 mg/ml]</td>
</tr>
<tr>
<td>BT-ET</td>
<td>16</td>
<td>34.1 [3.1 mg/ml]</td>
<td>4.4 [0.4 mg/ml]</td>
<td>68.8 [6.3 mg/ml]</td>
</tr>
<tr>
<td>BT-CS</td>
<td>16</td>
<td>31.8 [2.4 mg/ml]</td>
<td>5.4 [0.4 mg/ml]</td>
<td>84.5 [6.3 mg/ml]</td>
</tr>
<tr>
<td>BT-RR</td>
<td>16</td>
<td>88.3 [6.3 mg/ml]</td>
<td>11.3 [0.8 mg/ml]</td>
<td>353.1 [25 mg/ml]</td>
</tr>
<tr>
<td>ANRT</td>
<td>16</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>UT</td>
<td>8</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>ANGT</td>
<td>12</td>
<td>&gt; 50</td>
<td>50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>CJ-OS</td>
<td>15</td>
<td>50</td>
<td>3.1</td>
<td>50</td>
</tr>
<tr>
<td>CJ-S</td>
<td>15</td>
<td>50</td>
<td>3.1</td>
<td>50</td>
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</table>

*Percentage concentration of Teas and Beverage was expressed. For teas, % value represents the percent concentration of a cup of brewed tea (weight per bag/250ml H₂O). MIC (mg/ml) was divided by weight of tea leaves per bag in 250ml H₂O. >50 represents no inhibition of growth observed. For teas, the tea in mg/ml was also shown. GT: Pure Green Tea 100% Natural, Lipton®, Englewood, NJ| BT-ET: English Teatime, RC Bigelow®, Fairfield, CT| BT-CS: Cinnamon Stick, RC Bigelow®, Fairfield, CT| BT-RR: Raspberry Royale, RC Bigelow®, Fairfield, CT| ANRT: All Natural Raspberry Tea (Snapple®, Black and Green Tea, Plano, TX)| UT: Unsweetened Tea (Pure Leaf™, Purchase, NY)| ANGT: All Natural Green Tea (Snapple®, Plano, TX)| CJ-OS: Cranberry Juice Cocktail (Ocean Spray®, Made From Concentrate, Lakeville-Middleboro, MA)| CJ-S: Cranberry Cocktail (Simply™, Not From Concentrate, Apopka, FL)

Minimum Inhibitory Concentration was defined as the lowest concentration where no growth (O.D. ≤ 0.05) of the plaque bacteria was observed. The lower the MIC, the more effective the inhibition. All nine drinks tested differed significantly in MIC (Friedman ANOVA, *P*<0.001). As shown in Table 1, all of the teas tested inhibited growth of plaque bacteria with MICs ranging from 0.8 mg/ml (11% of a cup of brewed tea) to 6.3 mg/ml (88.3% of a cup of brewed tea).
TABLE II.  PAIRWISE COMPARISONS OF THE MICS OF TEAS AND BOTTLED BEVERAGES

<table>
<thead>
<tr>
<th></th>
<th>ANRT</th>
<th>UT</th>
<th>ANGT</th>
<th>BT-ET</th>
<th>BT-CS</th>
<th>CJ-S</th>
<th>CJ-OS</th>
<th>GT</th>
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<tbody>
<tr>
<td>BT-RR</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>0.007</td>
<td>0.004</td>
<td>0.001</td>
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<tr>
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<td></td>
<td></td>
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<td>UT</td>
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<td></td>
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<tr>
<td>BT-ET</td>
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<td></td>
</tr>
<tr>
<td>BT-CS</td>
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<tr>
<td>CJ-S</td>
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<tr>
<td>CJ-OS</td>
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</tr>
</tbody>
</table>

*NS: Not significant
GT: Pure Green Tea 100% Natural, Lipton®, Englewood, NJ
BT-ET: English Teatime, RC Bigelow®, Fairfield, CT
BT-CS: Cinnamon Stick, RC Bigelow®, Fairfield, CT
BT-RR: Raspberry Royale, RC Bigelow®, Fairfield, CT
ANRT: All Natural Raspberry Tea (Snapple®, Black and Green Tea, Plano, TX)
UT: Unsweetened Tea (Pure Leaf™, Purchase, NY)
ANGT: All Natural Green Tea (Snapple®, Plano, TX)
CJ-OS: Cranberry Juice Cocktail (Ocean Spray®, Made From Concentrate, Lakeville-Middleboro, MA)
CJ-S: Cranberry Cocktail (Simply™, Not From Concentrate, Apopka, FL)

Overall, GT had the lowest MIC compared to all other teas and beverages but was only marginally lower than BT-ET and BT-CS. The two cranberry cocktails tested, CJ-OS and CJ-S, had the next lowest MIC following BT-ET and BT-CS but were not significantly different from them or from each other. ANGT had a marginally lower MIC than ANRT, UT, and BT-RR, with BT-RR having the highest MIC (P < 0.0014, Bonferroni Correction). Overall, the effect of teas and bottled beverages on children's supragingival plaque in terms of strength of inhibition of growth from strongest to weakest was GT > BT-CS and BT-ET ≈ CJ-OS and CJ-S > ANGT > ANRT and UT > BT-RR.
### TABLE III. GROWTH OF PLAQUE BACTERIA AT 1:1 DILUTION OF BOTTLED BEVERAGES

<table>
<thead>
<tr>
<th>Drinks</th>
<th>Total Subjects</th>
<th>Mean Growth (%)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Minimum Growth (%)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Maximum Growth (%)&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>ANRT</td>
<td>16</td>
<td>28.3</td>
<td>7.9</td>
<td>75.5</td>
</tr>
<tr>
<td>UT</td>
<td>8</td>
<td>45.2</td>
<td>2.6</td>
<td>4.9</td>
</tr>
<tr>
<td>ANGT</td>
<td>12</td>
<td>37.6</td>
<td>2.4</td>
<td>110.5</td>
</tr>
<tr>
<td>CJ-OS</td>
<td>15</td>
<td>2.6</td>
<td>0.0</td>
<td>7.6</td>
</tr>
<tr>
<td>CJ-S</td>
<td>15</td>
<td>4.9</td>
<td>0.0</td>
<td>59.5</td>
</tr>
</tbody>
</table>

*Percent of Growth

ANRT: All Natural Raspberry Tea (*Snapple®*, Black and Green Tea, Plano, TX)
UT: Unsweetened Tea (*Pure Leaf™*, Purchase, NY)
ANGT: All Natural Green Tea (*Snapple®*, Plano, TX)
CJ-OS: Cranberry Juice Cocktail (*Ocean Spray®*, Made From Concentrate, Lakeville-Middleboro, MA)
CJ-S: Cranberry Cocktail (*Simply™*, Not From Concentrate, Apopka, FL)

The five bottled beverages were further analyzed for growth at the dilution level of 50% to describe the differences between the bottled beverages. The percentage of growth compared to control at 1:1 dilution (50%) of the bottled beverages showed a range from 2.6% to 45.2% of control (Table III). Both brands of cranberry cocktails showed the lowest mean growth overall, suggesting highest growth inhibition of the plaque bacteria among all the bottled beverages tested at the original concentration, and this was consistent with the findings from MIC comparison.
B. Effect of Teas and Bottled Beverages on *In Vitro* Biofilm Formation of Supragingival Plaque Bacteria from Children: Minimum Biofilm Inhibitory Concentrations (MBIC)

**TABLE IV. EFFECT OF TEAS AND BOTTLED BEVERAGES ON *IN VITRO* BIOFILM FORMATION OF CHILDREN’S SUPRAGINGIVAL PLAQUE BACTERIA: MINIMUM BIOFILM INHIBITORY CONCENTRATIONS (MBIC)**

<table>
<thead>
<tr>
<th>Drinks</th>
<th>Total Subjects</th>
<th>Median MBIC (%*)</th>
<th>Minimum MBIC (%*)</th>
<th>Maximum MBIC (%*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT</td>
<td>16</td>
<td>22.0 [1.6 mg/ml]</td>
<td>5.5 [0.4 mg/ml]</td>
<td>343.4 [25.0 mg/ml]</td>
</tr>
<tr>
<td>BT-ET</td>
<td>16</td>
<td>8.8 [0.8 mg/ml]</td>
<td>4.4 [0.4 mg/ml]</td>
<td>68.8 [6.3 mg/ml]</td>
</tr>
<tr>
<td>BT-CS</td>
<td>16</td>
<td>21.6 [1.6 mg/ml]</td>
<td>10.8 [0.8 mg/ml]</td>
<td>41.9 [3.1 mg/ml]</td>
</tr>
<tr>
<td>BT-RR</td>
<td>16</td>
<td>22.6 [1.6 mg/ml]</td>
<td>5.65 [0.4 mg/ml]</td>
<td>353.11 [25.0 mg/ml]</td>
</tr>
<tr>
<td>ANRT</td>
<td>16</td>
<td>9.4</td>
<td>1.6</td>
<td>25.0</td>
</tr>
<tr>
<td>UT</td>
<td>8</td>
<td>4.7</td>
<td>1.6</td>
<td>12.5</td>
</tr>
<tr>
<td>ANGT</td>
<td>12</td>
<td>&gt; 50.0</td>
<td>&gt; 50.0</td>
<td>&gt; 50.0</td>
</tr>
<tr>
<td>CJ-OS</td>
<td>15</td>
<td>&gt; 50.0</td>
<td>1.6</td>
<td>&gt; 50.0</td>
</tr>
<tr>
<td>CJ-S</td>
<td>15</td>
<td>50</td>
<td>1.6</td>
<td>&gt; 50.0</td>
</tr>
</tbody>
</table>

*Percentage of Beverage; >50 represents no inhibition of biofilm observed. For teas, the tea in mg/ml was also shown.

GT: Pure Green Tea 100% Natural, Lipton®, Englewood, NJ
BT-ET: English Teatime, RC Bigelow®, Fairfield, CT
BT-CS: Cinnamon Stick, RC Bigelow®, Fairfield, CT
BT-RR: Raspberry Royale, RC Bigelow®, Fairfield, CT
ANRT: All Natural Raspberry Tea (*Snapple*®, Black and Green Tea, Plano, TX)
UT: Unsweetened Tea (*Pure Leaf™*, Purchase, NY)
ANGT: All Natural Green Tea (*Snapple*®, Plano, TX)
CJ-OS: Cranberry Juice Cocktail (*Ocean Spray*®, Made From Concentrate, Lakeville-Middleboro, MA)
CJ-S: Cranberry Cocktail (*Simply™*, Not From Concentrate, Apopka, FL)

Minimum Biofilm Inhibitory Concentration was defined as the lowest concentration where more than 90% of biofilm was inhibited. The MBICs of all teas and bottled beverages except UT differed significantly based on Friedman’s Test (*P*<0.001). UT was excluded because data were not available for half of the subjects. As shown in Table 4, all of the teas and beverages except ANGT and CJ-OS inhibited biofilm. UT showed the lowest MBIC when compared to other teas, showing promise for providing the strongest inhibition against biofilm formation, but needing further study.
Overall, ANRT had the lowest MBIC among all the teas and bottled beverages; it was significantly lower than the cranberry cocktails, ANGT, and GT. BT-ET was significantly lower than ANGT and BT-RR. BT-CS was significantly lower only than ANGT but higher than ANRT. Both BT-RR and GT did not inhibit the biofilm formation any differently from all teas and beverages except being weaker when compared to ANRT. Both cranberry cocktails had a lower MBIC than ANGT. ANGT had the highest MBIC overall but was not significantly different from BT-RR and GT, and possibly CJ-OS. Overall, the effect of teas and bottled beverages on children’s supragingival plaque in terms of strength of inhibition of biofilm was highest in ANRT and BT-ET and lowest in ANGT.
TABLE VI.  BIOFILM FORMATION OF PLAQUE BACTERIA AT 1:1 DILUTION OF BOTTLED BEVERAGES

<table>
<thead>
<tr>
<th>Drinks</th>
<th>Total Subjects</th>
<th>Mean Biofilm (%)*</th>
<th>Minimum Biofilm (%)*</th>
<th>Maximum Biofilm (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANRT</td>
<td>16</td>
<td>0.9</td>
<td>0.0</td>
<td>10.0</td>
</tr>
<tr>
<td>UT</td>
<td>8</td>
<td>2.0</td>
<td>0.0</td>
<td>4.6</td>
</tr>
<tr>
<td>ANGT</td>
<td>12</td>
<td>118.9</td>
<td>26.6</td>
<td>201.6</td>
</tr>
<tr>
<td>CJ-OS</td>
<td>15</td>
<td>42.5</td>
<td>0.0</td>
<td>195.9</td>
</tr>
<tr>
<td>CJ-S</td>
<td>15</td>
<td>27.7</td>
<td>0.0</td>
<td>253.9</td>
</tr>
</tbody>
</table>

*Percent of Biofilm Formation
ANRT: All Natural Raspberry Tea (Snapple®, Black and Green Tea, Plano, TX)
UT: Unsweetened Tea (Pure Leaf™, Purchase, NY)
ANGT: All Natural Green Tea (Snapple®, Plano, TX)
CJ-OS: Cranberry Juice Cocktail (Ocean Spray®, Made From Concentrate, Lakeville-Middleboro, MA)
CJ-S: Cranberry Cocktail (Simply™, Not From Concentrate, Apopka, FL)

The five bottled beverages were further analyzed for biofilm formation at the dilution level of 50% to describe the differences between the bottled beverages. As shown in Table VI, ANRT and UT showed the lowest mean biofilm formation (0.9% and 2.0%, respectively), suggested highest inhibition against biofilm formation, and this was consistent with the MBIC findings. No inhibition of biofilm at 1:1 dilution was observed in ANGT. CJ-S at 1:1 dilution showed less biofilm formation (27.7%) when compared to CJ-OS, suggesting perhaps stronger inhibition against biofilm formation.
V. DISCUSSION

Dental caries is one of the most prevalent chronic diseases, and it affects U.S. children significantly. Since the introduction of water fluoridation in 1940s, there has been a decline in the caries prevalence; however dental caries still remains a national issue (Guideline on Fluoride Therapy; Brunelle and Carlos, 2015). Since plaque and dental caries are closely associated, mechanical removal of dental plaque and food debris has been emphasized for caries prevention. In recent years, effort has been made to investigate foods and diet that are anti-cariogenic and beneficial for oral health (Ferrazzano et al., 2009). Dietary plant-derived polyphenols, which can be found in many plants such as the tea plant (Camellia sinensis) and American cranberry fruit (Vaccinium macrocarpon), have been reported to possess in vitro antimicrobial activity and health promoting benefits (Graf et al., 2005; Pandey and Rizvi, 2009). Its potential for anti-caries and anti-gingivitis properties has been suggested and attracted attention in preventive dentistry research (Gupta et al., 2011; Yoo et al., 2015). These components have been widely used in the food industry and are commonly consumed in the forms of beverages, such as teas, tea beverages, or cranberry juices. In this study, we examined the effects of various commercially available teas and beverages containing dietary plant-derived antimicrobial polyphenols on the growth and biofilm formation of supragingival plaque in children, and have found selected teas inhibited plaque growth and biofilm formation of children’s plaque bacteria.

Based on existing literature, populations that drink tea have been shown to have decreased caries rate and healthier plaque scores (Ooshima et al., 1994; Hamilton-Miller,
In vitro studies have shown that tea extracts inhibited growth and biofilm formation of cariogenic bacteria, *S. mutans* (Ferrazzano et al., 2009; Naderi et al., 2011; Xu et al., 2011; Araghizadeh et al., 2013). However, not much is known in the effect on children’s plaque. In the current study, we discovered that green tea and black tea are both effective at inhibiting the *in vitro* growth and biofilm of supragingival plaque bacteria in children. At concentrations tested, Green Tea (Pure Green Tea 100% Natural, *Lipton®, Englewood, NJ*) was the strongest at inhibiting growth of plaque bacteria, whereas black tea was best at inhibiting biofilm formation among teas. This confirmed previous studies by Xu *et al.* (2011) showing inhibiting effects of tea polyphenols on specific virulence factor of *S. mutans*, namely biofilm formation and acid production.

Previous studies showed that cinnamon was antibacterial (Pelczar et al., 1988; Gupta et al., 2011). Therefore, we anticipated that flavored tea such as Black Tea with Cinnamon flavor may have synergistic inhibitory effect on plaque bacteria. Our study showed that the effect of Black Tea with Cinnamon (Cinnamon Stick, *RC Bigelow®, Fairfield, CT*) was still comparable to Black Tea without flavor (English Teatime, *RC Bigelow®, Fairfield, CT*), indicating that adding cinnamon flavor to black tea did not appear to negatively affect the antibacterial properties of tea. However, we did not observe any enhanced effect above black tea, which may be due to the unknown quantities of each component presented in Black Tea with Cinnamon (Cinnamon Stick, *RC Bigelow®, Fairfield, CT*).

On the contrary, it was interesting to note that Black tea with Raspberry flavor (Raspberry Royale, *RC Bigelow®, Fairfield, CT*) was the weakest in inhibiting the growth of children’s plaque bacteria and the second weakest in inhibiting biofilm formation when
compared to all teas and bottled beverages tested. Comparing to Black Tea without flavor (English Teatime, RC Bigelow®, Fairfield, CT), the result suggested that adding raspberry flavor to the tea weakened the protective properties of black tea. Perhaps during the manufacturing process of this specific raspberry flavor tea, the content of tea was reduced in each tea bag as raspberry fruits or flavoring agents were added. With reduced tea content, Black tea with Raspberry flavor (Raspberry Royale, RC Bigelow®, Fairfield, CT) became the weakest in inhibiting the growth of plaque bacteria among all the teas and beverages tested, or perhaps there was something plaque promoting in this particular product.

Koo et al (2006) reported that twice daily topical applications with cranberry juice for 1 minute was effective in reducing biofilm formation of S. mutans in vitro. They further concluded that cranberry juice was more effective at preventing initial biofilm formation than preventing subsequent accumulation of biofilm. In our study, supragingival plaques were isolated from children and used in the laboratory, which represents a complex bacterial community than a single bacterial species, ie, S. mutans. Our results simulate the reality better when compared to previous studies utilizing only one test bacteria species, and we were able to show that cranberry cocktails inhibited biofilm formation of supragingival plaque bacteria from children.

It is noteworthy that in based on descriptive data, Simply ™ Cranberry Cocktail (Not From Concentrate, Apopka, FL) seemed to perform better in both inhibiting of the growth of plaque bacteria and inhibiting of biofilm formation when compared to Ocean Spray® Cranberry Juice Cocktail (Made From Concentrate, Lakeville-Middleboro, MA). One disclosed difference between the two products was that while they both contained 27% of
juice, *Ocean Spray®* Cranberry Juice Cocktail was made from concentrate yet *Simply™* Cranberry Cocktail was not. Despite our results showed that these two cranberry cocktails didn’t differ from each other significantly, whether the difference in juice content was the reason for better performance is worth further investigation. Nonetheless, because the ingredients and proportion of each ingredient in their juice preparation were not disclosed in details, it was difficult to interpret our data in terms of actual accountability of the effect observed. But overall, both cranberry cocktails showed notable inhibition against biofilm formation at 1:1 dilution.

The inhibition of growth and biofilm formation of the bottled teas, namely Unsweetened Tea (*Pure Leaf™*, Purchase, NY), All Natural Green Tea (*Snapple®*, Plano, TX), and All Natural Raspberry Tea (*Snapple®*, Plano, TX) seemed to vary in widely. For example, All Natural Raspberry Tea (*Snapple®*, Plano, TX) showed the strongest inhibition against biofilm formation among all teas and bottled beverages tested, and its effect was not significantly different from Black Tea without flavor (English Teatime, *RC Bigelow®*, Fairfield, CT), which was known to have strong inhibitory effect towards biofilm. In contrast, All Natural Green Tea (*Snapple®*, Plano, TX) was the weakest in inhibiting biofilm formation and second weakest in inhibition growth of the plaque bacteria. Perhaps this may be attributed to the widely varying composition of ingredients in these beverages, in regards to the proportion of tea, water, preservative, or other additives etc.

In this study, we noticed variations in the effect of teas and beverages among different children’s plaque samples obtained, suggesting the complexity and heterogeneity in the composition of human plaque samples. Individual plaque samples may react differently to each tested drinks. These differences may be attributed to the variations in
dietary habits, last meal consumed before plaque sampling, or oral hygiene status in different individuals. For instance, a subject who already has a habit of drinking tea may have selected for plaque bacteria that were less adherent to start with, and hence the effects of teas may not be as obvious.

In our study, the inhibition observed for tea preparation from loose tea leaves from the tea bags on the growth of the plaque bacteria and biofilm formation was significant; however these were the results of laboratory experiments and one should not extrapolate \textit{in vitro} results to \textit{in vivo} situation. Nonetheless, with the support of the data presented in the current study, rinsing studies using the teas or bottled beverages may provide further insights into the anti-cariogenic potential of these beverages. This study can be easily reproduced to accommodate a larger investigation in order to improve the study design and clinical applicability. One of the limitations for the current study was the uneven sample size for Unsweetened Tea, and therefore we could not conclude our findings in this beverage. However it’s certainly worth further investigation to examine the effect of this beverage as the limited data showed promising results.

Children’s frequent consumption of sugar sweetened beverages is one of the risk factors for dental caries. Investigating the anti-cariogenic potential of natural foods or commercially available beverages containing dietary plant-derived polyphenols can help the consumers, especially children, make better decisions in their diet to promote oral health. The awareness of healthy diet should not only emphasize on general health but also promote oral health, which is intricately associated with overall well-beings. With the discoveries made in this study, dietary plant-derived polyphenols present in commercially
available teas and beverages can be a novel and practical approach to dental caries prevention in children.
VI. CONCLUSIONS

The following conclusions can be drawn from this study:

1. Selected commercially marketed teas/beverages tested in this study were able to suppress growth and biofilm formation of children's supragingival plaque bacteria.

2. Biofilms formed by plaque bacteria in the presence of selected beverages were significantly less adherent and easily dislodged by rinsing. This suggests that polyphenols may have preferential inhibitory activity against GTF enzyme responsible for biofilm integrity and adherent properties.

3. The concept of oral diseases prevention using natural foods or beverages in the diet may be a novel, practical, and acceptable approach to promote oral health in children.
Notice of Determination of Human Subject Research

December 12, 2014

*20141216-86863-1*

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RE: Protocol # 2014-1216
In Vitro Analysis of the Effect of Natural Anti-microbial Compounds on Pediatric Dental Plaque

Sponsor: None

Dear Dr. Huang:

The UIC Office for the Protection of Research Subjects received your “Determination of Whether an Activity Represents Human Subjects Research” application, and has determined that this activity DOES NOT meet the definition of human subject research as defined by 45 CFR 46.102(f).

You may conduct your activity without further submission to the IRB.

If this activity is used in conjunction with any other research involving human subjects or if it is modified in any way, it must be re-reviewed by OPRS staff.

Cc: Christine Wu, Pediatric Dentistry, M/C 850

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CITED LITERATURES


Yoo, S., Murata R., Duarte S.: Antimicrobial traits of tea- and cranberry-derived polyphenols against *Streptococcus mutans.* *Caries Research.* 45:327-335, 2015.


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RESEARCH
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PROFESSIONAL MEMBERSHIPS:
American Academy of Pediatric Dentistry - June 2013
American Dental Association - June 2013
Illinois State Dental Society - March 2015
Illinois Society of Pediatric Dentistry - March 2015
Chicago Dental Society - March 2015