Research Article

Antimicrobial efficacy of white tea mouthwash against bacteria in early childhood caries

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ABSTRACT

Aim: The aim of this study is to evaluate the effectiveness of green tea mouth rinse against oral bacteria found in early childhood caries affecting children. **Materials and Methods:** In this study, antibiotic susceptibility testing was done comparing different concentrations of white tea with vancomycin against pure isolates of *Streptococcus mutans* and *Lactobacillus acidophilus* following incubation for 24 h and measuring the zone of inhibition in millimeters. **Results:** From this study, it is evident that white tea acts as an effective mouth rinse in creating a barrier for the growth of oral *S. mutans* and *L. acidophilus*. The results are statistically significant. **Conclusion:** This study is done to evaluate the use of white tea as a prevention modality against bacterial diseases in the oral cavity and its use as an adjunct to other mouth rinses and oral hygiene methods usually in children with motor disabilities.

KEY WORDS: Antimicrobial, Early childhood caries, Polyphenols, White tea

INTRODUCTION

The human body has been estimated to harbor around 90% of microorganisms that essentially make up the resident flora of the host.[1] The oral cavity, being the entrance to various microbes, is colonized by a broad range, and therefore, diseases of the oral cavity are the fourth most expensive diseases to treat.[2] This calls upon the need for measures to prevent oral diseases before they settle in and at an early age. Many children have inadequate general and oral health due to active and uncontrolled dental caries.[3] Dental caries in children below 6 years is called early childhood caries (ECC), and its severe debilitating form is called severe-ECC. It is a preventable, localized infectious, multifactorial disease resulting from the interaction among host, diet, and microflora on the tooth surface over a period of time, resulting in localized demineralization of hard tissues.[4] ECC is associated with a marked reduction in the value of life and overall health.[5] Mutans are mainly responsible for the initial phase of the caries lesion, especially in the enamel (initiation), whereas *Lactobacillus* is more involved with the progression of caries.[6]

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There have been various antibacterial agents which have been introduced in science and in everyday life to prevent the irreversible disease that is dental caries. Proper toothbrushing and flossing, on a daily basis, becomes the key principle in prevention followed by the usage of mouthwash, chlorhexidine (CHX) being the "gold standard." The concept of mouthwashes or mouth rinses was initially propagated by ancient Romans and Egyptians.[4] As early as 40–90 A.D, Pedanius Dioscorides, a Greek physician suggested a concoction of olive juice, pomegranate fillings, wine, and gum myrrh to treat halitosis, while Hippocrates is known to have advocated mouth rinsing with a mixture of alum, salt, and vinegar in the ancient times.[7] They are used for their multiple properties such as analgesic, antimicrobial, anticariogenic, and anti-inflammatory activities.

The American Dental Association (ADA) endorses that mouth rinses should be effective at modifying the microbiota by selectively eliminating pathogens without negatively affecting the normal commensals of oral cavity. Evidence illustrates that the long-term twice-daily use of antiplaque and anti-gingivitis

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mouth rinses, namely, 0.12% CHX gluconate and essential oils with methyl salicylate which are approved by the Council on Dental Therapeutics of the ADA, does not have any negative effect on the oral microbial flora.[8] CHX works by impairing the bacterial cell wall and interferes with osmosis.[9] Studies have shown a number a side effect with CHX usage such as altered taste sensation, inflammation of the parotid, discoloration of teeth, mucosal irritation, and etcetera.

Studies have also established that the use of mouth rinses in children provided a significant decrease in the decayed extracted filled surface index.[9] However, strong, astringent mouthwashes cannot be used by children due to the tingling after taste which is not preferred, and therefore, there is a need for a fainter, alcohol-free mouthwash for general population with allergies, children, and children with motor disabilities.[10]

There have been increasing studies with the usage of green tea mouthwash with its favorable taste and close to CHX antimicrobial activity. Tea is the second most widely consumed drink in the world due to it is cultural popularity and health benefits. Results from epidemiological studies as well as laboratory experiments suggest that consumption of tea confers protection against the development of chronic diseases such as cardiovascular disease and cancer.[11] It essentially has a reputation for its antioxidant and anticancer properties due to the high levels of polyphenols. The composition of tea and inadvertently the number of polyphenols is affected by the fermentation process into not fermented (green and white tea), partially fermented (red and oolong tea), and completely fermented (black tea). During fermentation of fresh tea leaves, some catechins are oxidized or condensed to larger polyphenolic molecules (dimer or polymer) such as theaflavins (3–6%) and thearubigins (12–18%).[12] The effectiveness of the antioxidant property in tea depends on the purity of tea and the content of epigallocatechin gallate (EGCG). White tea has a high content of EGCG as it is the least fermented followed by oolong tea and green tea. Catechins, the polyphenols in tea, proanthocyanidins, and hydrolyzable tannins show antimicrobial activity. The tolerance of bacteria to polyphenols depends on the bacterial species and the polyphenol structure.[13]

The objective of this study was to estimate the antimicrobial efficacy of white tea when formulated as a mouthwash toward oral bacteria found in ECC.

MATERIALS AND METHODS

The antibacterial efficacy was evaluated using antimicrobial sensitivity testing.

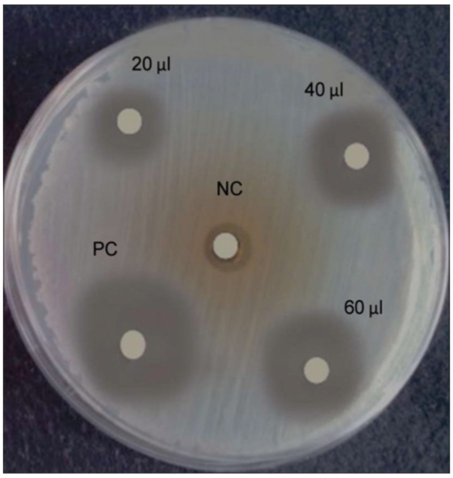

Figure 1: Zone of inhibition (mm) of *Streptococcus mutans*

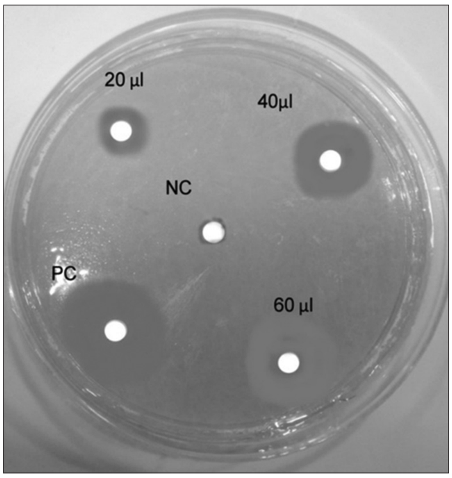

Figure 2: Zone of inhibition (mm) of *Lactobacillus acidophilus*

Mouthwash Preparation

The mouthwash was prepared by dissolving 250 mg of white tea powder in 25 ml of distilled water. About 25 ml of white tea extract was added to the 25 ml of the mixture containing 2% of sodium chloride, 2% sodium benzoate, and 2% sodium bicarbonate and mixed with amaranth solution (0.092%) which was then filtered and then made up to 100 ml with distilled water. The solution was transferred to 100 ml plastic bottles for antimicrobial testing. Three concentrations were obtained, 20 µl, 40 µl, and 60 µl.

Bacterial Strains and Media

Streptococcus mutans and *Lactobacillus acidophilus* pure isolates were used in this study which was obtained from HiMedia Laboratories. All samples were cultured and subcultured again for purity on blood agar plates. Colony morphology and Gram staining were carried out to confirm the identity of working strains.

Antimicrobial Susceptibility Testing

About 15 ml of sterile Muller-Hilton agar (HiMedia) in Petri dish was seeded with 1.0 ml of standard broth culture of

Table 1: Antibacterial activity of white tea mouthwash

Concentration (μg/ml)	Zone of inhibition (mm)	
WT mouthwash	*Streptococcus mutans*	*Lactobacillus acidophilus*
20 (μl)	5.6±0.01*	6.05±0.54*
40 (μl)	7.1±0.24*	8.01±0.61*
60 (μl)	10.1±0.89**	12.3±0.74**
Positive control	14.8±1.2***	16.1±1.0***
Negative control	NI	NI

NI: No inhibition. *P<0.05, **P<0.01, and ***P<0.001 as compared with negative control

Table 2: Comparing the antimicrobial efficacy of 20 μl against 40 μl white tea mouthwash

Concentration (μg/ml)	Zone of inhibition (mm)	
WT mouthwash	*Streptococcus mutans*	*Lactobacillus acidophilus*
20 (μl)	5.6±0.01*	6.05±0.54*
40 (μl)	7.1±0.24*	8.01±0.61*

*P<0.01

Table 3: Comparing the antimicrobial efficacy of 40 μl against 60 μl white tea mouthwash

Concentration (μg/ml)	Zone of inhibition (mm)	
WT mouthwash	*Streptococcus mutans*	*Lactobacillus acidophilus*
40 (μl)	7.1±0.24*	8.01±0.61*
60 (μl)	10.1±0.89**	12.3±0.74**

*P<0.05, **P<0.01

Table 4: Comparing the antimicrobial efficacy of 60 μl white tea mouthwash against positive control vancomycin (10 μg)

Concentration (μg/ml)	Zone of inhibition (mm)	
WT mouthwash	*Streptococcus mutans*	*Lactobacillus acidophilus*
60 (μl)	10.1±0.89**	12.3±0.4**
Positive control	14.8±1.2***	16.1±1.0***
	*P<0.001	

NI means no inhibition zone. Each value is expressed as mean±SD (n=3). *P<0.05, **P<0.01, and ***P<0.001 as compared with negative control

the bacteria (1.0 × 107 colony-forming unit/ml) and spread gently to ensure uniform distribution of microorganisms and then allowed to solidify on a flat surface. Five wells were made in the plate (about 0.5 mm diameter) using a sterile cork borer. About 20, 40, and 60 μl concentrations of white tea mouthwash were transferred into the wells using micropipette and then impregnated in sterilized 6 mm blank discs. The discs which had been impregnated with mouthwash using sterile forceps were applied on the inoculated Mueller-Hinton agar once it has completely dried. The discs were pressed gently to ensure uniform contact with agar surface. Then, the plates were inverted and incubated for 24 h at 37°C.

About 10 μg concentration of vancomycin was taken as a standard reference and distilled water-loaded discs were used as negative controls for mouthwash, respectively. All impregnated discs, before the application on bacteria, were allowed to stand for 1 h for pre-diffusion to the extract to occur and were incubated at 37°C for 24 h. After incubation for 24 h, the zone of inhibition was calculated by measuring the diameter of zones of growth inhibition using colony counter. The diameter of inhibition zone either around the treated discs or around the control discs was measured for the antibacterial activity assessment. If present, their diameters were measured to the nearest whole millimeter with a ruler. All tests were carried out 3 times to ensure the reliability, and the average of the three replicates for each volume of mouthwash and antibiotic was calculated.

RESULTS

The results were obtained by measuring the zone of inhibition of *S. mutans* [Figure 1] and *L. acidophilus* [Figure 2] against positive control – vancomycin, negative control – distilled water, and three different concentrations of white tea mouthwash [Table 1].

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Statistical significance was determined by one-way analysis of variance and *post hoc* least significant difference test. P < 0.05 was considered statistically significant.

DISCUSSION

The purpose of the study was to evaluate and compare the antimicrobial efficacy of white tea mouth rinse to that of vancomycin on *S. mutans* and *L. acidophilus* which is an arch criminal in ECC. The present study was carried out under *in vitro* conditions.

According to the results of the study, white tea was found to be a very effective antibacterial mouth rinse against *S. mutans* and *L. acidophilus*. There have been various studies that have stated the antibacterial efficacy of green tea mouthwash, which proved to be effective due to the presence of catechins. Like the other types of tea, white tea too comes from *Camellia sinensis* plant. However, the leaves are picked and harvested before the leaves open fully when the buds are still covered with fine white hair and, hence, the name "white tea." White tea is a very rare and expensive connoisseur's tea that is only produced in China, mainly in Fukien Province. Once harvested, white tea is not oxidized or rolled, but simply withered and dried by steaming. White tea undergoes the least amount of processing and, hence, has the maximum number of polyphenols, which are not oxidized or destroyed during processing. White tea is full of potent antioxidants and may be even more beneficial than green tea.[14]

Tea is generally known to inhibit a wide range of microbes, namely, *Vibrio cholerae*, *Salmonella typhi*, *Campylobacter jejuni*, *Campylobacter coli*, *Helicobacter pylori*, *Shigella*, *Salmonella*, *Clostridium Pseudomonas*, *Candida*, *Mycoplasma*, and *Cryptococcus*.[15] Subinhibitory concentrations of EGCG and ECG can suppress the expression of bacterial virulence factors and reverse the resistance of the opportunistic pathogen *Staphylococcus aureus* to β-lactam antibiotics.[16]

The results from the study state that with increasing concentrations of the mouthwash, there is a similar increase in the zone of inhibition, thus an increase in the antibacterial efficacy. When the concentration is increased from 20 µl to 40 µl, [Table 2] the zone of inhibition increases by 2% in *S. mutans, Lactobacillus* equally. *P* value is significant with this comparison being <0.05%, whereas the increase to 60 µl similarly boosts up to 3% increase in antibacterial efficacy with the *P* value being <0.01% [Table 3]. Thus, it can be well expected that there would be 4% or more increase by further doubling the concentration of the white tea mouthwash with its significance being <0.001% on comparison of the antibacterial efficacy of white tea mouth rinse to that of 10 µg vancomycin, we found that it was a significant competitor and by increasing the concentrations of the mouth rinse it could have similar effects as vancomycin [Table 4]. However, due to the better acceptance due to its taste, white tea mouth rinse may rise up to be used commercially among children and adults alike.

Further studies are required to test the effects of white tea mouth rinse in real subjects, an *in vivo* study.

CONCLUSION

From the results of our study, it can be concluded that white tea mouth rinse could be a very effective and child-friendly mouth rinse as there is an increasing demand for the betterment of oral hygiene maintenance methods in children with special needs. However, further studies are required to evaluate any potential adverse effects with long term, everyday use of this mouth rinse.

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