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# Anti-bacterial effect of white tea extract on *Streptococcus mutans* and *Streptococcus salivarius*

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## Abstract

Due to the increasing frequency of drug-resistant strains among different types of microorganisms, the finding of antimicrobial and antifungal compounds of natural substances, which certainly has fewer side effects, has long been of interest to researchers. Therefore, the aim of this investigation was to study the effect of white tea extract on reducing *Streptococcus mutans* and *Streptococcus salivarius*. White tea was prepared in powder form and then its hydroalcoholic extract of it was prepared. The antimicrobial effects of extracts on *S. mutans* and *S. salivarius* were performed using well diffusion and broth microdilution methods. All experiments were performed in triplet replications. Antibacterial susceptibility testing revealed that white tea extract at concentration of 500 µg/ml had a remarkable antibacterial activity against *S. mutans*, and *S. salivarius* with a zone of inhibition, 25±1 mm and 30±1, respectively. The minimum inhibitory concentration (MIC) values of the both tested strains were estimated 31.25 µg/ml. Moreover, the minimum bactericidal concentration (MBC) values of white tea extract were estimated 62.5 µg/ml against both streptococci. Considering the inhibitory power of the white tea extract on the gram-positive bacteria of *S .salivarius* and *mutans*, it can be concluded that this plant can be used in various industries, including the pharmaceutical and sanitary industry and it can also improve oral health as *S. mutans* and *salvarius* are the most important bacteria causing decays.

**Keywords:** Camellia Sinensis, Streptococcus mutans, Tea, Microbial Sensitivity Tests

## 1. Introduction

After water, tea (*Camellia sinensis* (L.) Kuntze) is the most consumed beverage among the people of the world. Tea contains green tea polyphenols Ambe Phytoextracts, and is one of the most traditional and widespread beverages in China and Japan, derived from the young branches of Camellia sinensis. Cathechins and Flavins in tea are also considered active ingredients in microbiology [1]. In 2005, Iran's share of world tea consumption was 3.3 percent, while it accounted for only 1.4 percent of world production [2]. A class of polyphenols called catechins stands out among their bioactive ingredients, of which epigallocatechin gallate is the most common and has high antioxidant properties [3]. Other phenolic compounds for instance caffeine, gallic, proanthocyanidols, chlorogenic or cinnamic acids, quercetin, and theophylline; and minerals as fluorine, manganese or chromium are also found in teas. Studies mostly focused on their antioxidant potential and their effect on the prevention and treatment of degenerative diseases. Various studies have

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investigated on the effect of teas in controlling cardiovascular diseases, increasing bone density, protecting against neurodegenerative diseases and improvement of type 2 diabetes [3]. White tea is known to be the least processed form of tea and have a series of powerful bioactivities, such as antioxidant, anti-inflammatory, anti-mutagenic, and anti-cancer activities [4]. It is reported that ingesting white tea for a long time has a protective role for tissues from acute oxidative stress [5].

Black tea is advisable as anticariogenic food because plaque pH values are not below essential value [6]. Compared with black and green teas, just few studies have been worked on the antimicrobial activity of white tea. White tea is different from others regarding the processing methods. This kind of tea is extracted from young leaves, even before the buds are completely bloomed [7].

Both white and green tea are well-known to contain catechins like epigallocatechin gallate (EGCG), epicatechin gallate, epicatechin in large volumes, and epigallocatechin, Isolated green tea catechins could be suppressor of elastase and collagenase. In comparison noticeably higher anti elastase and anti-collagenase activities are exhibited in white tea. Early investigations have studied that EGCG exhibited strong collagenases inhibitory activity that decrease the organic matrix during erosion. Based on the study results, green and white tea consumption enhanced microhardness of dentine, which is showing functional remineralization. This could be a result of the tea action on the collagen network, which may result in collagen stabilized and left intrafibrillar spaces of collagen network open for remineralization [8]. White tea mouthrinse powerfully inhibits formation of plaque although not as comparable to mouthrinses which contain chlorhexidine. Hence, for those who prefer herbal products, white tea mouthrinse is an appropriate option. As the processing temperature is low in comparison with processing temperature of other kind of teas, white tea retains its nutrients. The components of white tea are alanine, threonine, histidine, aspartic acid, glutamic acid, and amino acids. Polyphenols, which is a natural antioxidant is found in the important ingredients in white tea [9].

Some studies have shown that there is a relation between dental caries and salivary level of *S. mutans*, a gram-positive species [10]. While some studies have implied that *Lactobacillus salivarius* (Gram-positive anaerobic bacteria) WB21 can be helpful for controlling dental caries, and other oral diseases [11]. In this study we are trying to investigate the effect of white tea extract on the reduction of *S. mutans* and *salivarius* bacteria according to dental caries. Tea is extremely popular and usually people use it daily, if we can prove that one type of it is useful to improve oral health, hygiene or immune system against cancer and to control caries that would be valuable information. It can also suggest a healthy lifestyle .This study directly measures the effectiveness of tea extracts on microorganisms that defects oral health and uses global standards and guidelines.

## 2. Methods and Materials

*2.1 Bacterial strains*

This present *in vitro* study was conducted on two strains of Gram-positive cocci including *S. mutans* (ATCC 35668), and *S. salivarius* (ATCC 19258). Bacterial strains were taken from Pastor Technolog Institute (Tehran, Iran). The strain was obtained from the strain stock through being cultured overnight in aerobic conditions at 37 °C on a triptic soy agar (TSA) plate containing fibrous sheep blood 5% (Merck, Germany).

*2.2 Characterization of extract*

Initially, 500 grams of white tea in the Department of Agriculture in the University of Guilan was turned into powder by an electric mill and kept in a glass jar. First, the equipment, including glassware such as Beaker, is sterilized in autoclave (Omran, Type H2A) for 15 minutes at 121 °C and under at least 15 psi of pressure. After sterilization, 250 g of white tea powder was weighed with a scale and poured into the beaker, and the beaker was transported to 1000 ml of distilled water. Then, it was placed on a heater stirrer (IKA, RH basic 2) and used. Next, stirred it from the magnet (IKA, RH basic 2) until the final volume reached 500 ml after a few hours. After the extract was cooled, it was filtered by filtration or sterile gas, then the solution was placed under the laminar flow hood (Wieteg, WLC-V1500) by UV light for 24 hours to be sterilized and free of bacteria. Finally, it was kept in a container in the refrigerator, away from light.

*2.3 Antimicrobial susceptibility testing*

Antibacterial property of the white tea extract was assessed method on Muller-Hinton agar (MHA) via well diffusion (Merck, Germany), enriched with 5% sheep blood [12]. Briefly, a saline suspension of bacterial colonies was made to prepare the bacterial suspension, and adjusted to the tube according to 0.5 McFarland standard (1.5 × $10^8$ colony forming units (CFU)/ml), then inoculated on the MHA surface via sterile swab. For extract antimicrobial activity test, 5 mm wells were punched into the plates. Lastly, 10 µL of extract (500 µg/ml) was added into the wells. Prepared plates were incubated at 37 °C for 24 hours on a rotary shaker at 160 rpm, then the growth inhibition clear zone was evaluated. Furthermore, susceptibility to antibiotic was determined toward Gentamicin (10 µg), and Tetracycline (30 µg) antibiotic discs (MAST, UK) as the positive control, and distilled water as negative control. All tests were done in triplicate. The minimum inhibitory concentration (MIC) of white tea extract was obtained via standard broth microdilution according to the guidelines of clinical and laboratory standards institute (CLSI) ($30^{th}$ edition) [13]. Mueller Hinton broth (Merck, Germany) containing 5% sheep blood was used to determine the MICs for all bacterial strains. Strains were cultured in 96-well microplates. To evaluate the inhibitory effects of the antibacterial agents on bacterial growth, each well was supplemented with a range of concentrations (500 µg/ml to 31.25 µg/ml) of the active agents. The final inoculum concentration was approximately 5 x $10^5$ CFU/ml in each well. Following at 37 °C and 16-18 hours of incubation, the wells were checked for any microbial growth, and the MIC was characterized as the minimum concentration that did not produce visual growth. The minimum bactericidal concentration (MBC) was illustrated as the minimum concentrations resulting microorganisms 99.9% mortality rate in the primary inoculums by seeding on MHA contain 5% sheep blood [14].

*2.4 Statistical analysis*

SPSS™ software version 21.0 (IBM Crop., USA) was used for analysis. The outcomes are provided as relative frequency. The values arereported as mean ± standard deviation (continuous variable) or group percentage (categorical variable).

## 3. Results

Antibacterial susceptibility testing revealed that white tea extract at concentration of 500 µg/ml had a remarkable antibacterial activity against *S. mutans*, and *S. salivarius* with a zone of inhibition, 25±1 mm and 30±1, respectively. The comprehensive results of antibacterial activity of white tea extract illustrated in Table 1.

The MIC and MBC activity of white tea extract were determined by broth microdilution technique. The MIC values of the both tested strains were estimated 31.25 µg/ml. Moreover, the MBC values of white tea extract were estimated 62.5 µg/ml against both streptococci.

Table 1. Antibacterial activity of white tea extract by well diffusion method

| **Strain** | **Extract (500 µg/ml)** | **Positive control** | | **Negative control** |
|---|---|---|---|---|
| | | Gentamicin | Tetracycline | Distilled water |
| *S. salivarius* | 30±1 mm | 45 mm | 30 mm | 0 |
| *S. mutans* | 25±1 mm | 35 mm | 50 mm | 0 |

## 4. Discussion

Complexity of mechanisms that create antibiotic resistance has led to the problem of bacterial resistance to antibiotics that have always plagued medical systems in recent decades. Therefore, finding new antimicrobial compounds with minimal side effects is a topic that has always been on the minds of researchers. In this study we assessed white tea extract's effect on *S. mutans* and *S. salivarius* which had an antibacterial result on them by forming zone of inhibition, more effectively on *S. salivarius* than *S. mutans*.

In a study, the antibacterial effects of polyphenols on several species of pathogenic bacteria, including *Escherichia coli* were evaluated. The MIC for *E. coli* was several times more abundant than other bacteria, such as *S. aureus* (1519±949 and 192±91 µg/mL, respectively) and even more than *Salmonella* (795 ± 590 g µg/mL) [15].

In contrast, there are conflicting reports about the antimicrobial activity of tea extract against pathogenic bacteria. According to Hara and Ishigami *Salmonella typhimurium* and *Campylobacter jejuni* are resistant to tea extract, while other researchers have reported *S. typhimurium* susceptibility to aqueous extract of tea. This conflict can be due to different method used in the study and species [16].

In a study of the effect of a kind of tea called oolong on *S. mutans*, by analyzing extract of the tea, researchers found a fraction of it (OTF10) a polyphenolic compound with an inhibitory effect on the enzyme glucosyltransferase 1, bacterial enzyme necessary to form dextran plaque, and as a result, tooth decay. Interestingly, catechins and other small tea polyphenols did not have such an effect, but black tea thioflavin did. This inhibitory effect, which prevents bacteria from adhering to dental plaque and tooth decay, was greater in OTF10 than in oolong tea extract this different conclusion may be because of different type of tea that was used [17]. Herbal polyphenols are thought to exert their inhibitory effects on bacterial growth by producing hydrogen peroxide [18]. At the same time, bactericidal cathechins have been shown to damage two layers of membrane fat [19]. Although polyphenols are potent antioxidants, they may act as peroxidants under certain conditions [20]. It is possible that tea polyphenols exert their inhibitory effect on bacterial growth in this way.

In another study, the effect of white tea aqueous extract on serum levels of antioxidant enzymes in arsenic-exposed rats was investigated. The results showed that consumption of white tea by reducing the activity of antioxidant enzymes and strengthening the antioxidant defense system reduces the oxidative stress caused by arsenic [20].

In one study, the antimicrobial effect of green tea extract on *S. mutans* and *Enterococcus faecalis* was investigated. Sodium hypochlorite and chlorhexidine were used to determine the susceptibility and resistance of bacteria to green tea. Results showed that in *S. mutans*, the diameter of the green tea growth inhibition zone was not significantly different from chlorhexidine (P = 0.305). Since the results of Ranjbar et al.'s investigation were inconsistent with the results of the present study, it is possible that this may be due to different methods of studies [21].

By studying the results of research on the antimicrobial properties of white tea extract, it is understood that some factors such as; climatic conditions, plant habitat cultivation, plant cultivation and maintenance conditions, drying method, extraction method and concentration used in studies can affect the amount of antimicrobial effects and other effects related to plant extracts. Also, increasing the concentration increases the lethality of the extracts. Considering the results and inhibitory power of white tea extract on the Gram-positive bacteria of *S. salivarius* and *S. mutans*, it can be concluded that this plant has the potential to be used in various industries, including pharmaceutical and health industries, due to its antibacterial properties.

### Authors' contributions

All authors have cooperated in conception and design, or analysis and interpretation of the data for this study; drafting the article or revising it for important content and they approved the final version.

### Conflict of interests

The Authors declare that there is no conflict of interest.

### Ethical declarations

Study did not include any human participants and was ethically approved by National Research Ethics Committee and the code given was IR.GUMS.REC.1396.1238.

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## References


1. Granato D, Prado-Silva Ld, Alvarenga VO, Zielinski AAF, Bataglion GA, Morais DRd, et al. Characterization of binary and ternary mixtures of green, white and black tea extracts by electrospray ionization mass spectrometry and modeling of their in vitro antibacterial activity. LWT - Food Sci Technol. 2016; 65:414-20.
2. Ghoochani M, Shekoohiyan S, Yunesian M, Nazmara S, Mahvi AH. Determination of aluminum and zinc in infusion tea cultivated in north of Iran. J Environ Health Sci Eng. 2015; 13:49.
3. Pastoriza S, Mesías M, Cabrera C, Rufián-Henares JA. Healthy properties of green and white teas: an update. Food Funct. 2017; 8(8):2650-62.
4. Dai W, Xie D, Lu M, Li P, Lv H, Yang C, et al. Characterization of white tea metabolome: Comparison against green and black tea by a nontargeted metabolomics approach. Food Res Int. 2017; 96:40-5.
5. Espinosa Ruiz C, Cabrera L, López-Jiménez J, Zamora S, Pérez-Llamas F. Effects of long-term ingestion of white tea on oxidation produced by aging and acute oxidative damage in rats. J Physiol Biochem. 2018; 74(1):171-7.
6. Gul P, Akgul N, Seven N. Anticariogenic potential of white cheese, xylitol chewing gum, and black tea. Eur J Dent. 2018; 12(2):199-203.
7. Siddiqui MW, Sharangi AB, Singh JP, Thakur PK, Ayala-Zavala JF, Singh A, et al. Antimicrobial Properties of Teas and Their Extracts in vitro. Crit Rev Food Sci Nutr. 2016; 56(9):1428-39.
8. Jose P, Sanjeev K, Sekar M. Effect of Green and White Tea Pretreatment on Remineralization of Demineralized Dentin by CPP-ACFP-An Invitro Microhardness Analysis. J Clin Diagn Res. 2016; 10(4):Zc85-9.
9. Simpson A, Shaw L, Smith AJ. The bio-availability of fluoride from black tea. J Dent. 2001; 29(1):15-21.
10. Sounah SA, Madfa AA. Correlation between dental caries experience and the level of Streptococcus mutans and lactobacilli in saliva and carious teeth in a Yemeni adult population. BMC Res Notes. 2020; 13(1):112.
11. Higuchi T, Suzuki N, Nakaya S, Omagari S, Yoneda M, Hanioka T, et al. Effects of Lactobacillus salivarius WB21 combined with green tea catechins on dental caries, periodontitis, and oral malodor. Arch Oral Biol. 2019; 98:243-7.
12. Nanda A, Saravanan M. Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. Nanomedicine. 2009; 5(4):452-6.
13. CLSI (Ed.), 2020. Performance Standards for Antimicrobial Susceptibility Testing; 30$^{th}$ ed. CLSI Supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
14. Zarei M, Jamnejad A, Khajehali E. Antibacterial effect of silver nanoparticles against four foodborne pathogens. Jundishapur J Microbiol. 2014; 7(1):e8720.
15. Taguri T, Tanaka T, Kouno I. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biol Pharm Bull. 2004; 27(12):1965-9.
16. Fukai K, Ishigami T, Hara Y. Antibacterial activity of tea polyphenols against phytopathogenic bacteria. Agric Biol Chem. 1991; 55(7):1895-7.
17. Nakahara K, Kawabata S, Ono H, Ogura K, Tanaka T, Ooshima T, et al. Inhibitory effect of oolong tea polyphenols on glycosyltransferases of mutans Streptococci. Appl Environ Microbiol. 1993; 59(4):968-73.
18. Tagashira M, Uchiyama K, Yoshimura T, Shirota M, Uemitsu N. Inhibition by hop bract polyphenols of cellular adherence and water-insoluble glucan synthesis of mutans streptococci. Biosci Biotechnol Biochem. 1997; 61(2):332-5.
19. Taylor PW, Hamilton-Miller JM, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull. 2005; 2:71-81.
20. Rasolifard MH, Nozohor Y. The Effect of Aqueous Extract of White Tea on the Levels of Antioxidant Enzymes of Rats' Liver Tissue Exposed to Arsenic. J Ilam Uni Med Sci. 2018; 25(5):146-53.
21. Ranjbar F, Eslami G, Ghahremanloo A, Taheri S, Ayatollahi M, Eslami S. Antibacterial effect of green tea extract on Streptococcus mutans and Enterococcus faecalis isolated from dental plaque. J Mashhad Dent Sch. 2015; 39(4):335-42.