

EFFECT OF WHITE TEA EXTRACT *IN VITRO* AGAINST CARIOGENIC AND PERIODONTAL BACTERIA

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ABSTRACT

Objective: This work aimed to evaluate the *in vitro* antimicrobial properties of white tea maceration extract against pathogenic oral bacteria *Streptococcus mutans* (serotype F), *Porphyromonas gingivalis* (strain ATCC 33277), and *Actinobacillus actinomycetemcomitans* (phenotype A); the properties of this product were also tested against non-pathogenic *Streptococcus sanguis* (ATCC 10556) for comparison.

Methods: A maceration extract was made from white tea collected from West Java, and the infusion was prepared at concentrations of 5–80%. The selected strains of each microbial species were cultured under anaerobic conditions, and equalized standard dilutions of the cultured bacteria were used for testing. Dilution and diffusion tests were conducted to quantify the antimicrobial properties of white tea extract (WTE) against the bacteria.

Results: The dilution test results showed a minimum inhibitory concentration of 40% of WTE against *S. mutans*, *P. gingivalis*, and *A. actinomycetemcomitans* and 80% against *S. sanguis*. The minimum bactericidal concentration was 80% for all tested bacterial strains.

Conclusion: The diffusion test results showed higher inhibition with increasing extract concentration; however, the difference between concentrations of 40% and 80% was small or negligible, except for *A. actinomycetemcomitans*. These results imply the clear, acute antimicrobial effect of WTE against the tested oral anaerobic pathogens *in vitro*. Considering that these effects were concentration dependent and that white tea has the highest content of antimicrobial phenolic compounds among all grades of (green) tea, white tea or WTE may be useful for inhibiting the growth of pathogens involved in the development of caries and/or periodontal disease.

Keywords: White tea extract, Antimicrobial, Cariogenic bacteria, Periodontal bacteria.

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INTRODUCTION

Tea produced by the aqueous infusion of processed leaves of the evergreen shrub *Camellia sinensis* L. is a common beverage worldwide. In addition to the taste and flavor, the popularity of tea may stem from its mildly stimulating effect, which is mainly due to the presence of approximately 50 mg of caffeine per cup. However, before being widely consumed as a beverage, tea was originally used for medicinal purposes. In particular, green tea has been reported to inhibit the growth of pathogenic microbes including species belonging to the genera *Staphylococcus*, *Salmonella*, *Shigella*, *Vibrio*, *Bordetella*, *Clostridium*, *Helicobacter*, and *Pseudomonas* [1-13]. Such antimicrobial effects have also been indicated against cariogenic bacteria and other oral pathogens [14–22]. However, there has been some disagreement on the capacity of tea to inhibit the growth of susceptible and resistant bacterial species due to differences in microbial strains and the types and concentration of tea extracts [1-3,9-22].

Nevertheless, there is a wide range of aqueous plant extracts that are traditionally used for medicinal purposes due to the antimicrobial benefits of various compounds that the plants likely use for innate defenses [23-26].

The antimicrobial properties of tea are thought to be largely due to polyphenolic compounds, which account for approximately 30% of the dry weight of the buds and immature leaves that are picked and processed. The simplest and most important of these compounds are isoflavonoids, which are also known as catechins and account for approximately 10% of the dry weight of ordinary green tea. The catechins of tea mainly consist of epicatechins, epigallocatechins, epicatechin gallates, and epigallocatechin gallates (EGCGs), which occur at concentrations of up to 1 mg/mL of tea [21]. Among these compounds, EGCG is characteristic to tea and is

generally rare or absent in other plants [23]. Although large polyphenolic molecules, such as tannic acid, can be toxic and carcinogenic, this is not the case for the catechins of tea, such as EGCG, which appears to be mainly responsible for the potential benefits of tea. White tea, which is a grade of green tea produced from silvery white, very young leaf buds, has the highest concentration of catechins among all grades of green tea. This work aims to evaluate the antimicrobial properties of a macerated white tea extract (WTE) against select oral bacteria, particularly cariogenic *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*, which are important pathogens in periodontal disease. For comparison, the antibacterial effects of WTE were evaluated for *Streptococcus sanguis*, which is a common but more neutral streptococcal species of the oral cavity.

METHODS

Preparation of WTE

White tea was obtained from PT Perkebunan Nusantara VIII Cisaruni Field, West Java, Indonesia, and maceration extraction was conducted at the LIPI Chemical Laboratory (Tangerang, Banten, Indonesia). In brief, 202 g of dried white tea was soaked in 2 L of 70% ethanol for 24 h. The solution was then filtered and evaporated at 50°C, with the final stage occurring in a vacuum oven. The process was repeated twice for the residual matter. The resulting dry WTE was used in subsequent experiments as an aqueous solution containing 0.8 g/mL of the extract in water plus dimethyl sulfoxide (DMSO). For the experiments, this 80% baseline solution was further diluted as required with water containing DMSO.

Bacterial culture

The bacterial species selected for culturing were *S. mutans* serotype F, *S. sanguis* strain ATCC 10556, *P. gingivalis* strain ATCC 33277, and

Table 1: Dilution test results of various WTE concentrations

Medium/concentration	<i>S. mutans</i> serotype F	<i>S. sanguis</i> ATCC 10556	<i>A. actinomycetemcomitans</i> phenotype A	<i>P. gingivalis</i> ATCC 33277
C(+) no WTE	+	+	+	+
C(-) no bacteria (%)	-	-	-	-
5	+	+	+	+
10	+	+	+	+
20	+	+	+	+
40	-	+	-	-
80	-	-	-	-

(-): No bacterial growth (clear), (+): Bacterial growth observed (turbid). *S. mutans*: *Streptococcus mutans*, *P. gingivalis*: *Porphyromonas gingivalis*, *A. actinomycetemcomitans*: *Actinobacillus actinomycetemcomitans*, *S. sanguis*: *Streptococcus sanguis*, WTE: White tea extract

Table 2: Inhibition zone sizes at various WTE concentrations

Concentration of WTE (%)	<i>S. mutans</i> serotype F (mm)	<i>S. sanguis</i> ATCC 10556 (mm)	<i>A. actinomycetemcomitans</i> phenotype A (mm)	<i>P. gingivalis</i> ATCC 33277 (mm)
5	2	2	2	3
10	2.5	6	6	4
20	7	8	6	4
40	15	16	9	8
80	17	17	14	8

S. mutans: *Streptococcus mutans*, *P. gingivalis*: *Porphyromonas gingivalis*, *A. actinomycetemcomitans*: *Actinobacillus actinomycetemcomitans*, *S. sanguis*: *Streptococcus sanguis*, WTE: White tea extract

A. actinomycetemcomitans phenotype A (standard). Each bacterial sample was transferred into a test tube containing NaCl solution, and the test tubes were shaken until a homogeneous mixture was obtained. Each strain of bacteria was grown in thioglycolate broth medium and incubated in a gas-tight anaerobic jar at 37°C for 72 h. The optical microscopy of Gram-stained bacterial samples was used to confirm the culture results. After culturing, the test tube turbidity level was adjusted to McFarland standard level 1, which corresponds to a cell concentration of approximately 3×10^8 CFU/mL for all bacterial strains. Each bacterial suspension was diluted with NaCl solution to 1:2000 (approximately 1.5×10^5 CFU/mL) for inoculation.

Dilution and diffusion testing

For dilution testing, 0.2 mL of each equalized and diluted bacterial solution was transferred into test tubes containing the selected concentrations of WTE with DMSO. As a positive control, 0.2 mL of each bacterial solution was inoculated into 4 mL of thioglycolate broth medium with DMSO. WTE at a 50% concentration without bacterial inoculation was used as a negative control. The test tubes were incubated in an anaerobic jar for 72 h at 37°C and then evaluated for the presence or absence of bacterial growth. After comparison with the negative control, turbidity in the test tube was taken to indicate bacterial growth (+), whereas the lack of turbidity indicated no bacterial growth (-). To confirm the absence of bacterial growth in the clear test tubes, sampled contents were also cultured on agar plates. The minimum inhibitory concentration (MIC) was defined as the lowest tested concentration to prevent bacterial growth. The minimum bactericidal concentration (MBC) was defined as the concentration that killed 99.9% of the inoculated bacteria. For the disk diffusion test, each equalized and diluted bacterial seed was poured slowly and evenly on an agar plate that was tilted slightly to remove any excess, and the samples were incubated at 37°C for 15 min. Round, 6 mm, sterilized, filter paper disks were soaked in the WTE solutions of preselected concentrations (5%, 10%, 20%, 40%, and 80%) and allowed to dry before placing the discs on the seeded agar plates. The plates were then incubated at 37°C for 72 h in an anaerobic jar, and the mean diameters of the inhibition zones around the paper disks were measured.

RESULTS

The effects of various WTE concentrations in the dilution test for *S. mutans*, *S. sanguis*, *P. gingivalis*, and *A. actinomycetemcomitans* are summarized in Table 1. The results confirmed that sufficient concentrations of WTE had antimicrobial effects against the tested microbes. The MIC was 40% against *S. mutans*, *P. gingivalis*, and

A. actinomycetemcomitans and 80% against *S. sanguis*. The MBC was 80% for all tested bacteria.

The results of the disk diffusion test are summarized in Table 2. In general, all tested extract concentrations showed some effect (diffusion zones) on the tested microbial strains. An increase in the inhibition zone suggested that the inhibitory effect increased with the WTE concentration. The strongest inhibition was found at a concentration of 80%. However, the increase in extract concentration was attenuated toward higher concentrations. The difference between the concentrations of 40% and 80% was small or negligible, except for *A. actinomycetemcomitans*, which still exhibited a considerable increase in inhibition zone size at a WTE concentration of 40–80%. At 80%, the inhibition zone size was >10 mm for all bacteria, except *P. gingivalis*, which had a slightly smaller inhibition zone size of 8 mm.

DISCUSSION

The results of the disk diffusion test showed distinct inhibition zones even at the lowest test WTE concentration (5%) against all tested oral bacteria. Complete inhibition required 40% WTE concentration for all pathogenic strains, that is, *S. mutans* serotype F, *P. gingivalis* strain ATCC 33277, and *A. actinomycetemcomitans* phenotype A. Although an even higher (80%) concentration was needed to inhibit *S. sanguis* (strain ATCC 10556), this bacterial species is not pathogenic but is rather a commensal microbe of the oral cavity. It may also be worth noting that no WTE concentration between 40% and 80% was used. By considering only the tested pathogenic bacteria, a rather modest concentration of WTE appeared to provide significant acute inhibition *in vitro*. The size of the inhibition zone was relatively extensive in the diffusion tests and ranged from 4 to 8 mm at a 20% concentration (Table 2); however, zone size is not necessarily a unique indicator of growth inhibition. The diffusion test results showed that the diffusion zone size tended to sharply increase at WTE concentrations of up to 40%, with relatively little change at higher concentrations, except for *A. actinomycetemcomitans*, which showed a continuous increase in zone size at WTE concentrations between 40% and 80% (Table 2).

The active compounds of the WTE were not further analyzed in terms of chemical composition but presumably contained a high fraction of catechins, which are known to be largely responsible for the antimicrobial properties of green and white tea [13-24].

The dilution test results showed an MIC of 40% WTE for the cariogenic species *S. mutans* (serotype F), *P. gingivalis* (strain ATCC 33277), and *A. actinomycetemcomitans* (phenotype A); *P. gingivalis* and *A. actinomycetemcomitans* are commonly implicated in periodontal disease. The corresponding MIC was 80% for the nonpathogenic streptococcal species *S. sanguis* (strain ATCC 10556). The MBC was 80% for all tested strains. The diffusion test results showed increasing inhibition with the extract concentration, except for *A. actinomycetemcomitans*, which showed a small difference in inhibition between the concentrations of 40% and 80%.

These results support the existing body of evidence on the benefits of green tea on oral health [3-21]. The results are also comparable with the reported effect of green tea extract on other types of pathogenic microbes such as *S. aureus*, *P. aeruginosa*, and *C. albicans*, which cause skin infections. At concentrations of 5 mg/mL of dried hydroethanolic extract dissolved in DMSO, the application of the extract produced an inhibition zone of >10 mm, which increased to approximately 20 mm with a concentration of 10 mg/mL, similar to that of standard antibiotics [27]. For such purposes, stability and penetration of the active compounds can also matter [28].

This evidence implies that the active compounds of green and white teas have broad-spectrum antimicrobial properties when present in sufficient concentrations. In general, the results of the present study imply the clear, acute antimicrobial effect of WTE against the tested oral anaerobic pathogens *in vitro*. Considering that this effect is concentration dependent and that white tea has the highest content of antimicrobial phenolic compounds among all grades of green tea, WTE could be useful for inhibiting the growth of pathogens involved in caries and/or periodontal disease. Further studies are needed to evaluate the parallel effect of WTE *in vivo* with long-term exposure corresponding to the habitual use of white tea or WTE. Given that white tea will remain available in smaller quantities than other main grades, it could also be worth it to consider other means of extracting active compounds, such as EGCG, from minimally processed bulk flush of tea plants.

CONCLUSION

The results of this study showed the clear, acute antimicrobial effect of WTE against the tested oral anaerobic pathogens *in vitro*. Considering that the effect is concentration dependent and that white tea has the highest content of antimicrobial phenolic compounds among all grades of (green) tea, white tea or WTE may be useful for inhibiting the growth of pathogens involved in the development of caries and/or periodontal disease. Further studies are needed to investigate these effects *in vivo* under long-term applications and with extracted active compounds to address cost efficiency.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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