Antifungal effect of tea extracts on *Candida albicans*

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Determining whether tea extracts are effective in removing *Candida albicans* (*C. albicans*) from dentures is of interest. This study aimed to investigate the antifungal effect of tea extracts on *C. albicans*. One green tea (Anji white tea, AGW) and 2 oolong teas (Tie Guan Yin, TGY; Da Hong Pao, DHP) of different concentrations were tested. *C. albicans* suspensions were inoculated on the plates and the numbers of colony-forming units (CFU) in the culture medium were used to screen for the optimum tea extracts. Polymethyl methacrylate (PMMA) specimens that contained *C. albicans* biofilms were then treated with the tea extracts and the numbers of CFU were counted. The antifungal activities of the tea extracts were not significantly correlated with their catechin concentrations. Although AGW at 10.0 mg/mL and DHP at 2.5 mg/mL significantly inhibited *C. albicans* in the culture medium, the extracts failed to exert inhibitory effects against *C. albicans* biofilms on the PMMA surfaces.

Keywords: Tea extracts, Antifungal, *C. albicans*, Biofilm, Catechins

INTRODUCTION

Removable dentures are a common treatment for complete and partial tooth loss^{1,2}. Owing to decreased dexterity with age, the majority of elderly people with dentures fail to keep their denture clean and generally have poor oral hygiene³. Denture-related stomatitis (atrophic chronic candidiasis), an inflammatory lesion of the denture-bearing mucosa, is the most common oral fungal infection in denture wearers^{4,5}. Although there may be systemic factors related to denture-related stomatitis, microbial plaques that form on the denture surface have been considered a critical factor favoring denture-related stomatitis⁶. *Candida* is a commensal fungus that inhabits the oral cavity in approximately 50% of people, and *Candida albicans* (*C. albicans*) was found in 81.7% of denture plaques^{7,8}. Thus, denture-related stomatitis is, at least to some extent, attributed to the proliferation of *C. albicans* in plaques attached to the dentures.

Currently, there are 3 methods for removing plaque and debris from dentures: mechanical, chemical, and thermal methods⁹. The mechanical methods include the use of denture brushes and ultrasonic devices. As the most widely used mechanical method, brushing with a soft denture brush and formulated toothpaste may be able to remove *C. albicans* biofilms from the dentures¹⁰. However, the inappropriate motion of the denture brush, especially when using toothpaste, might scratch the dentures and cause surface irregularities, which could further accelerate adherence of the microflora¹¹. The chemical method involves immersion of the denture in chemical solutions, such as alkaline peroxides, sodium hypochlorite, acids, enzymes, and neutral enzymatic peroxides solutions, for a certain period of time¹². Vieira

*et al.*¹³ compared the effects of alkaline peroxides, 0.5% sodium hypochlorite (NaClO) and distilled water on the removal of *C. albicans* biofilms in a laboratory study. The results suggested that NaClO was the only treatment that removed biofilms efficiently. However, NaClO may corrode metal and alter color, increase the surface roughness, reduce the flexural strength and cause color changes in acrylic resins¹⁴⁻¹⁶. In addition, such solutions may have a disagreeable smell and taste for patients¹⁷. Recently, a thermal method that uses microwave energy and ultraviolet C light ovens has been proven effective in denture cleaning⁹. As a newly established thermal cleaning method, the use of microwave energy was found to be useful in removing *C. albicans* biofilms from dentures. However, dentures are likely to undergo harmful dimensional changes, particularly after repeated, long-term microwave energy exposure¹⁸. Therefore, it is of interest to find alternative antifungal methods for removing *C. albicans* from dentures.

After water, tea (*Camellia sinensis*) is the second most popular drink worldwide and has recently received the attention of the pharmaceutical and scientific communities due to the plethora of associated natural therapeutic compounds^{19,20}. Tea is generally harmless and nontoxic and has been proven to have physiological as well as antioxidant and anti-inflammatory effects²¹. Depending on the manufacturing process, teas are classified into 3 main categories: nonfermented (green and white tea), semifermented (oolong tea), and fermented (black and red tea), although all teas come from the same plant²⁰. The antimicrobial activity of tea polyphenols has been studied in many previous investigations^{22,23}, although only a few studies have investigated the antifungal activity of these compounds. Chen *et al.*²⁴ studied the activity of 23 tea extracts

against *C. albicans* and found that Anji white tea (white tea), Tie Guan Yin (oolong tea), and Da Hong Pao (oolong tea) have antifungal activity, with a minimum inhibitory concentration of 1.25 mg/mL. Similarly, black tea polyphenols (catechins and theaflavins) also exhibited an ability to inhibit *C. albicans*²⁰. However, the antimicrobial activity of tea extracts against biofilms must be investigated. The bacteria in a biofilm are much more tolerant to antimicrobial agents than the corresponding planktonic cells²⁵. Antunes *et al.*²⁶ concluded that green tea extractions led to a reduction in the number of viable fungal cells in biofilms formed on acrylic resin. However, conflicting results have also been reported²⁷.

The major tea catechins (epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG)) account for as much as 30% of the dry weight of fresh tea leaves²⁰. The antifungal ability of tea extracts may be related to catechins, such as EGCG²⁸. These compounds may prevent ergosterol synthesis by disturbing folic acid metabolism in *C. albicans*²⁹. However, limited information is available regarding the relationship between the antifungal activity of tea extracts and their catechin contents.

Therefore, the purpose of this study was to investigate the effects of tea extracts on *C. albicans* in culture medium and in biofilms on polymethyl methacrylate (PMMA) resin surfaces. The following null hypotheses were tested: 1) that there would be no differences in activity against *C. albicans* in the culture medium among the tea extracts tested, 2) that there would be no correlation between the antifungal activities and the catechin contents of the tea extracts, and 3) that there would be no differences in the activity against *C. albicans* biofilms on PMMA resin surfaces among the tea extracts tested.

MATERIALS AND METHODS

Tea extract preparation

One green tea (Anji white tea: AGW; Anji, Zhejiang, China) and two oolong teas (Tie Guan Yin: TGY; Anxi, Fujian, China; and Da Hong Pao: DHP; Wuyishan, Fujian, China) were used in this study. Twenty grams of tea were incubated separately with 100 mL of boiled water at 82°C for 10 min, respectively²⁴. The supernatant was filtered through a 0.2-µm filter, and the final concentration of the tea extracts was adjusted to 200 mg of solid tea/mL.

Catechin measurement

The catechin contents (ECG, EGCG, EC, and EGC) of the tea extracts were measured by high-performance liquid chromatography (HPLC)³⁰. Concentrations of 1.25, 2.50, 5.00, 10.00, and 20.00 mg/mL of the AGW, TGY, and DHP extracts were prepared by diluting the tea extracts with distilled water. Catechin standards of ECG, EGCG, EC, and EGC (Sigma-Aldrich, St. Louis, MO, USA) were prepared by accurately weighing 2 mg of the respective solid in tubes and diluting the solids with chloroform.

The final concentrations of the catechin standards were 5.0, 12.5, 25.0, 50.0, 100.0, 200.0, and 600.0 mg/L. The chromatographic separations were carried out on a ZORBAX SB-C18 column (15 cm×4.6 mm) with an oven temperature of 30°C, a detector UV wavelength of 280 nm and a flow rate of 1 mL/min. The mobile phase was 1% acetonitrile-methanol. All analyses were performed in triplicate for each sample.

Effects on *C. albicans* in culture medium

C. albicans (ATCC90029, Shanghai Rui Chu Biological Technology, Shanghai, China) was subcultured on Sabouraud's dextrose agar (Difco, Detroit, MI, USA) plates and incubated at 37°C for 48 h. To make the suspension, an individual colony of *C. albicans* was placed in 5 mL of Sabouraud's broth medium and incubated for 24 h at 37°C. A standardized *C. albicans* suspension containing 1×10⁴ colony-forming units (CFU)/mL was prepared in sterile phosphate-buffered saline (PBS; Ding Guo Chang Shen Biological Technology, Beijing, China). The tea extracts were combined with melted Sabouraud's dextrose agar to produce plates with different concentrations (1.25, 2.50, 5.00, 10.00, and 20.00 mg/mL). The antifungal activity of each tea extract plate was then determined by using distilled water plates for comparison. Aliquots (5 µL) of *C. albicans* standardized suspensions were planted on the abovementioned plates and were incubated at 37°C for 48 h. Finally, the numbers of CFU in the culture media were measured and used to screen for the optimum tea extract against *C. albicans*.

Effects on *C. albicans* biofilms on PMMA surfaces

Twenty-four PMMA acrylic resin specimens (20×10×2.5 mm) (Vertex Castavaria, Vertex, the Netherlands) were prepared according to the manufacturer's introductions using a customized steel mold. After polymerization, the specimens were finished with silicon carbide abrasive papers with grit sizes of 600, 800 and 1,000 under water cooling. The specimens were then sterilized under ultraviolet radiation (Dong Guan Xin Heng Technology, China) for 24 h and immersed in sterilized artificial saliva for 1 h. *C. albicans* standardized suspensions containing 1×10⁶ CFU/mL were prepared. Afterwards, the specimens were contaminated *in vitro* by immersion in 4 mL of Sabouraud's broth inoculated with 1 mL of the standardized suspension. The specimens were incubated at 37°C for 24 h, in the vertical position, for biofilm formation³¹. PMMA specimens that contained *C. albicans* biofilms were randomly treated with tea extracts (as determined above), with 0.5% NaClO and distilled water used as positive and negative controls (*n*=6), respectively. Each specimen with *C. albicans* biofilms was immersed with 2.5 mL of the test solution for 10 min. After immersion, the specimens were washed with 10 mL of sterile PBS to eliminate the test substance. The specimens were then transferred to tubes containing 3 mL of sterile PBS. Agitation of these tubes for 10 min in a vortex-type agitator allowed the dispersion of the *C. albicans* adhered to the acrylic resin plates²⁶. From the

suspension at the initial concentration, serial dilutions were made in PBS. Aliquots (5 µL) of the dilutions were plated on plates with Sabouraud's dextrose agar and incubated at 37°C for 48 h. The number of CFU per PMMA specimen (CFU/mL) was calculated.

Statistical analyses

Correlations between the antifungal activity and catechin contents of the tea extracts were explored using Pearson correlation analysis. Normal distribution of the data was confirmed with the Kolmogorov-Smirnov test. The data were statistically analyzed by two-way analysis of variation (ANOVA) and the least significant difference (LSD) test. All statistical analyses were performed using SPSS 19.0 software (IBM, Chicago, IL, USA) at a significance level of 5%.

RESULTS

A comparison of the concentrations of catechins, as shown in Table 1, revealed that the rank was as follows: EGCG>EGC>EC>ECG. The AGW showed the highest values for the concentrations of total catechins among the 3 types of tea extracts, and the TGY showed the lowest values. The results indicated that catechin concentrations were significantly influenced by 2 factors: "concentration of the tea extract" and "type of tea" (all $p<0.05$). Pearson correlation analysis showed that the antifungal activity of the tea extracts was not significantly correlated with catechins ($p=0.656$ for EGC, $p=0.621$ for EGCG, $p=0.482$ for EC, and $p=0.334$ for ECG).

The antifungal activity of the 3 tea extracts at 5 different concentrations is shown in Fig. 1. In general,

the tea extracts reduced the counts of *C. albicans* (up to 26%) in the culture medium. The antifungal effects of the AGW at 10.0 mg/mL and DHP at 2.5 mg/mL were significantly greater ($p=0.025$ and $p=0.046$, respectively) than the other tea extracts.

AGW at 10.0 mg/mL, DHP at 2.5 mg/mL, 0.5% NaClO, and sterile distilled water were further investigated to determine their effects on *C. albicans* biofilms formed on PMMA surfaces (Table 2). No significant differences in colony counts were found between the tea extracts and the negative controls ($p=0.979$ for AGW and $p=0.652$ for DHP), whereas no colonies were observed on the specimens treated with 0.5% NaClO.

Fig. 1 Means and standard deviations of the number of *C. albicans* colonies in the culture medium with different tea extracts. The dotted line indicates the number of *C. albicans* colonies on the control plates (1.05×10^4 CFU/mL).

Table 1 Means and standard deviations of the catechin concentrations (mg/L) in the tea extracts

Group	Concentration of tea extract (mg/mL)	EGC	EGCG	EC	ECG
AGW	1.25	14.93(0.25) ^{A,a}	31.23(0.37) ^{A,b}	8.91(0.18) ^{A,c}	7.21(0.30) ^{A,d}
	2.50	29.81(1.06) ^{B,a}	62.93(1.64) ^{B,b}	17.83(1.12) ^{B,c}	13.98(0.80) ^{B,d}
	5.00	59.73(1.15) ^{C,a}	125.91(1.58) ^{C,b}	35.41(0.52) ^{C,c}	28.00(0.46) ^{C,d}
	10.00	119.35(0.97) ^{D,a}	251.90(0.37) ^{D,b}	71.27(0.45) ^{D,c}	56.04(0.95) ^{D,d}
	20.00	238.87(1.84) ^{E,a}	503.82(2.54) ^{E,b}	142.60(1.23) ^{E,c}	112.10(0.97) ^{E,d}
TGY	1.25	13.17(0.33) ^{A,a}	10.41(0.77) ^{A,b}	4.16(0.42) ^{A,c}	2.93(0.06) ^{A,d}
	2.50	25.72(0.43) ^{B,a}	20.72(0.41) ^{B,b}	8.31(0.78) ^{B,c}	5.85(0.75) ^{B,d}
	5.00	52.17(0.36) ^{C,a}	41.97(0.65) ^{C,b}	16.51(0.06) ^{C,c}	11.70(0.09) ^{C,d}
	10.00	105.22(0.41) ^{D,a}	83.67(0.66) ^{D,b}	33.21(0.37) ^{D,c}	23.46(0.59) ^{D,d}
	20.00	210.65(1.43) ^{E,a}	167.85(2.56) ^{E,b}	66.63(0.96) ^{E,c}	46.91(0.87) ^{E,d}
DHP	1.25	4.96(0.07) ^{A,a}	11.00(0.04) ^{A,b}	5.01(0.03) ^{A,c}	4.02(0.08) ^{A,d}
	2.50	9.87(0.77) ^{B,a}	21.83(0.28) ^{B,b}	10.13(0.79) ^{B,c}	8.37(0.08) ^{B,d}
	5.00	18.72(0.83) ^{C,a}	43.73(0.64) ^{C,b}	20.05(0.43) ^{C,c}	16.68(0.11) ^{C,d}
	10.00	39.21(0.43) ^{D,a}	87.81(0.60) ^{D,b}	41.24(0.62) ^{D,c}	33.42(0.56) ^{D,d}
	20.00	79.26(0.85) ^{E,a}	176.03(2.49) ^{E,b}	80.21(1.63) ^{E,c}	66.87(0.67) ^{E,d}

Values marked with the same lowercase letter were not significantly different within each row ($p>0.05$); values marked with the same uppercase letter were not significantly different within each column for each tea extract tested ($p>0.05$).

Table 2 Means and standard deviations of the number of *C. albicans* colonies (10^4 CFU/mL) attached to PMMA immersed in different solutions

Test solution	Number of <i>C. albicans</i> colonies
Sterile distilled water	1.42±0.29 ^a
AGW at 10.0 mg/mL	1.57±0.54 ^a
DHP at 2.5 mg/mL	1.32±0.49 ^a
0.5% NaClO	<0.02 ^b

Values marked with the same lowercase letter were not significantly different ($p>0.05$).

DISCUSSION

Based on the current findings, the null hypothesis that there would be no effects of the tea extracts on *C. albicans* in the culture medium was rejected. The null hypotheses that there would be no correlation between the antifungal activities and catechin contents of the tea extracts and that there would be no effects of the tea extracts on *C. albicans* biofilms on the PMMA surfaces were accepted.

Previous researchers attributed the antifungal activity of tea extracts to the high content of polyphenols, mainly catechins, which have shown strong antioxidant properties *in vivo* and *in vitro*²⁰. Although limited information is available regarding the mechanism of the antifungal effect of each tea catechins, the antibacterial activities of catechins are predominantly related to the gallic acid moiety and the hydroxyl group member^{28,32}. Moreover, tea catechins may prevent ergosterol synthesis by disturbing folic acid metabolism in *C. albicans*³³. However, correlation of the antifungal activities of tea extracts with their catechin concentrations and the impact of tea extracts on *C. albicans* biofilm removal have not been thoroughly studied. In the present study, the concentrations of EGCG, EGC, ECG, and EC in the tea extracts were measured, as EGCG (32.77–75.05%), EGC (2.34–47.68%), ECG (5.75–27.03%) and EC (4.14–11.01%) account for 96.45% of total catechins³⁴. In general, catechin levels decreased when the extent of tea fermentation increased, which is consistent with the results reported in previous studies^{20,35}. Moreover, the more concentrated the tea extract is, the higher the concentration of catechins in the tea extract. Compared with a previous study, the present study found similar concentrations of catechins in the tea extracts, as follows: EGCG>EGC>EC>ECG³⁶. Although catechins are considered the main representative of the class of biologically active polyphenols in tea extracts and have a wide range of functional activities, especially due to their antioxidant activity³⁷, it is important to note that there is no consensus on the specific dose of catechins that could benefit human health.

Okubo *et al.*³⁸ reported that a 2.5% black tea extract completely inhibited the growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum*, but a 10% extract had no effect on *C. albicans*. Hirasawa and

Takada²⁸ also confirmed the inhibitory effects of green tea on *C. albicans*. Chen *et al.*²⁴ reported that tea extracts have antifungal activity, with an MIC of 1.25 mg/mL. However, of the 5 concentrations and 3 types of tea extracts tested in the current study, only AGW at 10.0 mg/mL and DHP at 2.5 mg/mL yielded significant inhibitory effects against *C. albicans* in the culture medium. Similar inhibitory effects were observed for the treatment with nystatin and denture microwave, which reduced both the clinical and microbiological signs of denture stomatitis³⁹. The degree of antifungal activity found in the present study may be considered effective for clinical application. The different antifungal activities of tea extracts may be related to variations in methodology and the teas investigated. Notably, even among teas originating from the same plant, each type of tea may have a unique character, taste, and chemical profile due to different processing methods and agricultural conditions²⁰. Notably, no correlation was found between the antifungal activities and catechin concentrations of the tea extracts. Possibly, factors other than catechin concentrations may influence the antifungal activities of tea extracts. Studies have shown that the effect of tea extracts on *C. albicans* biofilms on PMMA surfaces may be related to the adhesion of these biofilms, soaking time, and pH values of the tea extracts^{28,40–42}. Given that limited information is available in the literature, further studies are needed to clarify this issue.

In addition to antifungal activity in the culture medium, the effects of tea extracts on *C. albicans* biofilms on PMMA resin surfaces were also investigated in this study, which was designed to imitate clinical conditions. In the current experimental setting, *C. albicans* was grown in the form of a biofilm. Biofilms are heterogeneous structures composed of bacterial cells surrounded by a matrix. Given that the bacteria in the biofilm are 100 to 1,500 times more tolerant to antimicrobial agents than the corresponding planktonic cells²⁸, it is not surprising that no effect of the tea extracts on *C. albicans* biofilms on PMMA surfaces was found. Moreover, irregularities and porosities in the denture surfaces offer a favorable niche for the retention of bacterial biofilms⁴³. Furthermore, it is possible that the effect of green tea extracts on the number of viable fungal cells in biofilms formed on acrylic resin is time-dependent²⁶. Only a 10 min immersion in the tea extracts was evaluated, and this

could be considered a limitation of this study. Whether the immersion time affects the antifungal activity of tea extracts warrants further study.

In this study, the choice of tea extracts was based on increasing popular consumption and the antifungal activities previously described in the literature. *C. albicans* was chosen due to its strong association with denture stomatitis and other forms of oral candidiasis and its strong resistance to various therapies^{44,45}. The PMMA resin specimens were fabricated to simulate the formation of biofilms of *C. albicans* on a prosthesis intraorally. Moreover, the specimens were prepared following the same procedures used to fabricate dentures, and the agar dilution method was used as suggested in previous studies^{46,47}.

Easy access, cost-effectiveness, a long shelf life, low toxicity and a lack of antimicrobial resistance are the main benefits of natural products²¹. Therefore, the purpose of the current study was to develop a new type of denture cleanser based on natural products. The current findings suggest that AGW at 10.0 mg/mL and DHP at 2.5 mg/mL significantly inhibited *C. albicans*. Given that tea is a widely consumed beverage and has no known adverse effects, future research is warranted to develop these extracts as commercially viable denture cleaning products. Although it has been suggested that the antifungal activity of tea extracts is correlated with their polyphenol content²⁴, the present study failed to establish this type of correlation. The present study did not completely simulate clinical behavior because the antifungal effect was tested *in vitro*. Further studies are needed to optimize and confirm the antifungal effect of these tea extracts in an oral environment.

CONCLUSIONS

Within the limitations of the present study, the following conclusions can be drawn:

1. The tea extracts significantly reduced the growth of *C. albicans* in the culture medium but had no effect on *C. albicans* biofilms on the PMMA resin surface.
2. There was no correlation between the antifungal activities of the tea extracts and their catechin concentrations.

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