Analysis of the Antioxidant and Antibacterial Activities of Fenghuang Dancong Tea

Hongbing QI, Youyuan MO

School of Life Science & Technology, Lingnan Normal University, Zhanjiang 524048, China

Abstract [**Objectives**] To investigate the concentration of tea polyphenols in the tea infusion, evaluate their antioxidant and antibacterial activities, optimize the extraction conditions of tea polyphenols, and provide a foundation for their development as natural antioxidants and antibacterial agents. [**Methods**] Single-factor tests and orthogonal experiments were performed to examine the effects of brewing temperature, duration, and frequency on the extraction of tea polyphenols. Antioxidant activity was assessed using the DPPH radical scavenging assay, reducing power assay, and hydroxyl radical scavenging assay. The antibacterial activities of the tea infusion against *Escherichia coli*, *Bacillus thuringiensis*, *Penicillium*, and *Saccharomyces* were evaluated through spectrophotometry. [**Results**] The concentration of tea polyphenols increased with rising brewing temperature and duration but decreased with an increase in brewing frequency. The relative influence of these factors was ranked as follows: brewing duration > brewing frequency > brewing temperature. The optimal brewing conditions were determined to be 100 ℃, 15 min, and a single brewing cycle. Under these conditions, the tea polyphenol concentration reached 0.788 4 mg/mL. Additionally, the DPPH free radical scavenging rate, reducing power, and hydroxyl radical scavenging rate of the tea infusion all exhibited a positive correlation with tea polyphenol concentration. The antibacterial activity of the tea infusion against four microorganisms, in descending order of efficacy, was *B. thuringiensis*, *E. coli*, *Penicillium*, and *Saccharomyces*. [**Conclusions**] The extract of Fenghuang Dancong tea exhibits significant antioxidant and antibacterial properties. Its efficacy is positively correlated with the concentration of tea polyphenols, demonstrating a stronger inhibitory effect on bacteria compared to fungi.

Key words Fenghuang Dancong tea, Tea polyphenols, Antibacterial activity, Antioxidant activity

0 Introduction

Tea, recognized as one of the world's three primary non-alcoholic beverages, has garnered considerable attention owing to its abundant bioactive constituents and notable health benefits. Among these constituents, tea polyphenols (polyphenolic compounds predominantly composed of catechins) represent the most significant functional components in tea. They constitute approximately 15% - 35% of the dry weight of tea leaves and exhibit diverse biological activities, including antioxidant, antibacterial, anti-inflammatory, and anticancer effects[1]. Regarding antioxidative mechanisms, tea polyphenols directly scavenge free radicals by donating hydrogen atoms or electrons. Concurrently, they activate endogenous antioxidant enzyme systems, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), thereby effectively inhibiting lipid peroxidation and oxidative stress responses[2]. These actions are highly significant for delaying aging and preventing cardiovascular and neurodegenerative diseases. In the domain of antibacterial activity, tea polyphenols have been demonstrated to disrupt bacterial cell membrane structures, impede biofilm formation, and inhibit bacterial quorum sensing systems. These actions result in significant inhibitory effects against common pathogenic bacteria, including *Escherichia coli* and *Staphylococcus aureus*[3-4].

Currently, the predominant research methods employed in the antioxidant activity of tea include the DPPH radical scavenging assay, the ABTS cation radical scavenging assay, the FRAP (ferric reducing antioxidant capacity) assay, and the oxygen free radical scavenging assay. These methods simulate free radical generation environments to assess the free radical scavenging capacity of the samples[5]. Studies investigating antibacterial activity commonly utilize the agar diffusion method and the micro-broth dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), thereby evaluating the inhibitory and bactericidal effects of samples[6].

In recent years, both domestic and international scholars have conducted extensive research on the antioxidant and antibacterial properties of tea. Initial studies primarily concentrated on common tea varieties, including green tea and black tea. However, with the comprehensive exploration of tea germplasm resources, the biological activities of distinctive tea types, such as oolong tea and dark tea, have increasingly become focal points of research. Existing studies have demonstrated significant variations in the composition and concentration of active components across different types of tea, with their antioxidant and antibacterial properties being influenced by factors such as variety, origin, and processing methods. However, research on Fenghuang Dancong tea remains limited. Systematic investigations into its antioxidant and antibacterial activities are relatively scarce, and the mechanisms underlying the actions of its active components have not been thoroughly explored. This gap hinders the advancement of its applications in the food and pharmaceutical industries.

Received: April 8, 2026 Accepted: June 12, 2026

Supported by Zhanjiang Non-funded Science and Technology Research Program (2023B01023); University-level Educational and Teaching Reform Project of Lingnan Normal University (LSJW[2022]154).

Hongbing QI, doctoral degree, associate professor, research fields: microbial fermentation.

Fenghuang Dancong tea, a valuable variety of Chinese oolong tea, originates from Mount Phoenix in Chaozhou City, Guangdong Province. It is distinguished by its distinctive "one tree, one fragrance" aroma and its rich nutritional composition. Processing techniques such as shaking green and killing green induce oxidation and polymerization reactions of tea polyphenols, resulting in the formation of a complex phenolic compound system. This complex system may confer unique antioxidant and antibacterial properties to the tea[7].

This study focused on Fenghuang Dancong tea as the research subject, examining the effects of three variables (brewing temperature, brewing duration, and brewing frequency) on the extraction of tea polyphenols. Following optimization through single-factor tests and orthogonal experiments, the antioxidant and antibacterial activities of the resulting tea infusions were evaluated. The findings offer valuable insights for the development of novel natural antioxidants and antibacterial agents, support the advancement of the high-value tea industry, inform scientific tea consumption practices, promote Chinese tea culture, and contribute to the growth of the health industry.

1 Materials and methods

1.1 Materials

1.1.1 Strains and media. *E. coli*, *Bacillus thuringiensis*, *Penicillium*, and *Saccharomyces* species were isolated and preserved by the School of Life Science & Technology, Lingnan Normal University.

Nutrient broth (NB) medium was obtained from Qingdao Hi-Tech Industrial Park Hope Bio-Technology Co., Ltd., and MRS medium was sourced from Beijing Aoboxing Bio-technology Co., Ltd. The PDA medium was prepared using 200 g of potato, 20 g of glucose, 15 – 20 g of agar, and 1 000 mL of distilled water, with the pH maintained at its natural level.

1.1.2 Reagents. The following reagents were utilized in this study: 1, 1-diphenyl-2-picrylhydrazyl radical (analytical grade; TCI Shanghai Development Co., Ltd.), ferric chloride hexahydrate, potassium hexacyanoferrate (III), trichloroacetic acid, disodium hydrogen phosphate, sodium dihydrogen phosphate (all analytical grade; Tianjin Guangfu Fine Chemical Research Institute), anhydrous ethanol (analytical grade; Guangdong Guanghua Science and Technology Co., Ltd.), hydrogen peroxide (Shanghai Lingfeng Chemical Reagent Co., Ltd.), ferrous sulfate (analytical grade; Guangdong Guangshi Reagent Technology Co., Ltd.), salicylic acid (analytical grade; Guangdong Guanghua Science and Technology Co., Ltd.), and potassium sodium tartrate tetrahydrate (analytical grade; Guangzhou Chemical Reagent Factory).

1.1.3 Test instrument. SPX-250B-Z biochemical incubator (Shanghai Bosun Medical Biological Instrument Co., Ltd.), THZ-100 constant temperature shaking incubator (Shanghai Yiheng Scientific Instrument Co., Ltd.), TG16-WS benchtop high-speed centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd.), and V-1200 visible spectrophotometer (Shanghai Chaoyi Instrument Co., Ltd.) were utilized in this study.

1.2 Methods

1.2.1 Activation and cultivation of strains. The strain was selected and inoculated into 100 mL of the appropriate strain medium. The container was sealed with eight layers of gauze and incubated in a shaker at a rotational speed of 160 rpm. Following incubation at 37 ℃ for 24 h, the culture was stored at 4 ℃ for subsequent use[8].

1.2.2 Preparation of filter paper discs. Circular filter paper discs with a diameter of 6 mm were selected and sterilized in an oven at 121 ℃. The crude extract was then poured into a Petri dish containing the filter paper discs, which were placed in a clean bench to soak for approximately 30 min, followed by air drying for subsequent use[9].

1.2.3 Preparation of the extract of Fenghuang Dancong tea. The ratio of material to water was maintained at tea∶water (m∶v) = 1 g∶100 mL. Initially, 100 mL of distilled water was poured into a beaker and heated to the target temperature, after which heating was discontinued. The tea leaves were then added and brewed according to the specified frequency and duration. Upon completion of the brewing period, the tea leaves were filtered, and the tea infusion was collected in a conical flask and stored at 4 ℃ for subsequent use.

1.2.4 Determination of tea polyphenol concentration. (i) Plotting of the standard regression equation curve of tea polyphenols. 10 mg of tea polyphenol powder was accurately weighed and dissolved in distilled water, then diluted to a final volume of 20 mL to prepare a standard solution with a concentration of 0.5 mg/mL. Subsequently, 1, 2, 3, 4, and 5 mL of this 0.5 mg/mL standard solution were transferred into separate volumetric flasks and diluted to 10 mL with distilled water, yielding tea polyphenol solutions with concentrations of 0.05, 0.10, 0.15, 0.20, and 0.25 mg/mL, respectively. To each solution, 5 mL of ferrous tartrate solution was added and mixed thoroughly, followed by the addition of a pH 7.5 buffer solution up to the calibration mark, with subsequent mixing. The mixtures were allowed to stand for 10 min before measuring absorbance at 540 nm, using distilled water as the blank reference. Absorbance measurements were performed in triplicate for each concentration. The absorbance values (Y-axis) were plotted against the corresponding tea polyphenol concentrations (X-axis) to construct the standard calibration curve[10].

(ii) Determination of tea polyphenol concentration in the extract of Fenghuang Dancong tea. 2.0 mL of the filtrate obtained from the extract of Fenghuang Dancong tea was combined with 8.0 mL of water and 5.0 mL of ferrous tartrate solution, followed by thorough mixing. Subsequently, buffer solution was added up to the calibration mark, and the mixture was homogenized. The solution was allowed to stand for 10 min, after which its absorbance was measured at 540 nm. The recorded absorbance value (Y) was then applied to the standard curve regression equation to determine the concentration of tea polyphenols in the Fenghuang Dancong tea extract.

1.2.5 Single-factor tests. Using Fenghuang Dancong tea leaves as the raw material, this study investigated the effects of brewing

temperature, brewing duration, and brewing frequency on the concentration of tea polyphenols in the tea infusion. Each factor was examined at five gradient levels[11].

(i) Effects of brewing temperature on the extraction of tea polyphenols. Controlling for other variables, this study investigated the effects of five brewing temperatures (60, 70, 80, 90, and 100 ℃) on the concentration of tea polyphenols in the tea infusion.

(ii) Effects of brewing duration on the extraction of tea polyphenols. Controlling for other variables, this study examined the effects of five brewing durations (5, 10, 15, 20, and 25 min) on the concentration of tea polyphenols in the tea infusion.

(iii) Effects of brewing frequency on the extraction of tea polyphenols. Controlling for other variables, this study explored the effects of five brewing frequencies (1, 2, 3, 4, and 5 times) on the concentration of tea polyphenols in the tea infusion.

1.2.6 Orthogonal experiments. Three factors [brewing temperature (A), brewing duration (B), and brewing frequency (C)] along with their respective levels, were selected for investigation. An L_9 (3^3) orthogonal experimental design, incorporating three factors each at three levels, was employed, as detailed in Table 1[12].

Table 1 Design of factors and levels in orthogonal experiments

Level	Factor		
	Temperature (A)//℃	Duration (B)//min	Frequency (C)//Times
1	60	5	1
2	80	10	2
3	100	15	3

1.2.7 Determination of antioxidant function. (i) DPPH free radical scavenging assay. The DPPH radical solution exhibits a deep purple color and displays a prominent absorption peak at approximately 517 nm. In the presence of antioxidants, these compounds interact with DPPH radicals, leading to their reduction and consequent loss of free radical characteristics, which results in a lighter solution color. The degree of DPPH radical scavenging can be quantified by measuring the change in absorbance of the reaction mixture at 517 nm. A greater decrease in absorbance corresponds to a higher removal of DPPH free radicals, indicating a stronger antioxidant capacity[13].

7.886 mg of DPPH free radicals was accurately weighed and diluted to 100 mL with anhydrous ethanol to prepare a 0.2 mmol/L DPPH-ethanol solution. The tea infusion, diluted 100-fold (with an undiluted solid concentration of 0.788 4 mg/mL), were used as the stock solution to prepare five gradient dilutions at 0.2, 0.4, 0.6, 0.8, and 1.0 times the original concentration. Equal volumes of tea infusion dilutions and 0.2 mmol/L DPPH ethanol solution were mixed thoroughly and incubated in the dark for 30 min. Using anhydrous ethanol as the blank reference, the absorbance was measured at 517 nm. Each measurement was performed in triplicate, and the average value was calculated. Vitamin C (Vc) at the same concentration was used as the control, and the scavenging rate was calculated according to Formula (1)[14]:

$$\text{DPPH scavenging rate}\ (\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (1)$$

where A_0 denotes the addition of DPPH solutions without any tea infusions; A_1 indicates the addition of both DPPH solutions and tea infusions; and A_2 refers to the addition of tea infusions without DPPH solutions.

(ii) Determination of reducing power. In a system containing phosphate buffer solution and potassium ferricyanide solution, substances possessing reducing power can reduce Fe^{3+} in potassium ferricyanide ($K_3[Fe(CN)_6]$) to Fe^{2+}. Upon the addition of ferric chloride solution, Fe^{2+} and Fe^{3+} react under specific conditions to form Prussian blue-like compounds, which exhibit characteristic absorption at a wavelength of 700 nm. An increase in absorbance corresponds to a greater formation of Prussian blue-like substances, indicating a higher concentration of Fe^{2+} produced through reduction and, consequently, reflecting the stronger reducing power of the sample.

The tea infusion, diluted 10 times, was used as the stock solution to prepare five gradient concentrations corresponding to 0.2, 0.4, 0.6, 0.8, and 1.0 times the original concentration. Precisely 2.50 mL of each tea infusion was transferred and combined with 2.50 mL of phosphoric acid buffer solution (0.2 mol/L, pH 6.6) and 2.50 mL of 1% potassium ferricyanide solution. The mixture was incubated in a water bath at 50 ℃ for 20 min. After cooling to room temperature, 2.50 mL of 10% trichloroacetic acid solution was added, and the sample was centrifuged at 3 000 rpm for 10 min. Subsequently, 2.50 mL of the supernatant was collected, mixed with 2.50 mL of distilled water and 0.50 mL of 0.1% ferric chloride solution. After allowing the sample to stand for 10 min, the absorbance (A) was measured at 700 nm.. Each experimental group was performed in triplicate, with Vc at the same concentration serving as the control[14-15]. The total reducing power was calculated using the following formula:

$$A_{\text{Total reducing power}} = A_{\text{Sample}} - A_{\text{Blank}} \times 100\% \quad (2)$$

where A_{Sample} denotes the addition of tea infusions, A_{Blank} signifies the absence of tea infusions.

(iii) Determination of hydroxyl radical scavenging. Hydroxyl radicals (·OH) are generated via the Fenton reaction, wherein Fe^{2+} reacts with H_2O_2 to produce ·OH. These radicals exhibit potent oxidizing properties and can participate in hydroxylation reactions with salicylic acid, resulting in products that exhibit absorption peaks at approximately 510 nm. Upon the addition of the tea infusion, changes in absorbance can be monitored; a more pronounced decrease in absorbance indicates a greater capacity of the tea infusion to scavenge hydroxyl radicals.

A tenfold diluted tea infusion was used as the stock solution to prepare five gradient concentrations at 0.2, 0.4, 0.6, 0.8, and 1.0 times the original concentration. Precisely 1 mL each of 9 mM $FeSO_4$ solution, 9 mM salicylic acid-ethanol solution, and the tea infusion were combined. Subsequently, 1 mL of 8.8 mM H_2O_2 was added, and the reaction mixture was incubated at 37 ℃ for

30 min. The absorbance (A_x) was measured at 510 nm[16-17], using an equivalent concentration of Vc as the control. The hydroxyl radical (·OH) scavenging rate was calculated according to Formula (3):

$$\text{Hydroxyl radical scavenging rate (\%)} = \frac{A_0 - (A_x - A_{x0})}{A_0} \times 100\% \quad (3)$$

where A_0 denotes the absorbance of the blank control without the addition of tea infusions; A_x denotes the absorbance following the addition of tea infusions; A_{x0} denotes the absorbance after the addition of tea infusions in the absence of the chromogenic agent H_2O_2.

1.2.8 Determination of antibacterial activity. An undiluted tea infusion served as the stock solution, from which five gradient dilutions were prepared at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 times the original concentration. For each dilution, 2 mL of the corresponding liquid culture medium and 1 mL of the tea infusion were combined in a test tube. Subsequently, 0.1 mL of the appropriate bacterial suspension was taken and evenly inoculated. The control group received distilled water in place of the tea infusion. All samples were incubated on a shaker at a constant temperature of 37 ℃ for 24 h. Following incubation, optical density measurements were taken using a spectrophotometer at 600 nm (OD_{600}) for bacteria and 560 nm (OD_{560}) for Saccharomyces, with distilled water serving as the blank reference. The optical density values were used to estimate the biomass. Each experimental group was prepared in triplicate, and the mean value was calculated[18]. The antibacterial rate was determined according to Formula (4):

$$\text{Antibacterial rate (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\% \quad (4)$$

where A_0 denotes the addition of bacterial suspensions without the inclusion of tea infusions; A_1 indicates the addition of both bacterial suspensions and tea infusions; A_2 represents the addition of tea infusions without the inclusion of bacterial suspensions.

2 Results and analysis

2.1 Standard curve of tea polyphenols Standard solutions of tea polyphenols at concentrations of 0.05, 0.10, 0.15, 0.20, and 0.25 mg/mL were prepared. Their absorbance values were measured, and a standard curve was constructed (Fig. 1). The linear regression equation obtained was $y = 2.352x - 0.0286$, with a correlation coefficient of $R^2 = 0.9988$. These results indicate a strong linear relationship between absorbance and concentration within the range of 0.05 to 0.25 mg/mL of tea polyphenols in the tea infusion. Consequently, the concentration of tea polyphenols in the tea infusion can be determined by substituting the measured absorbance into the regression equation.

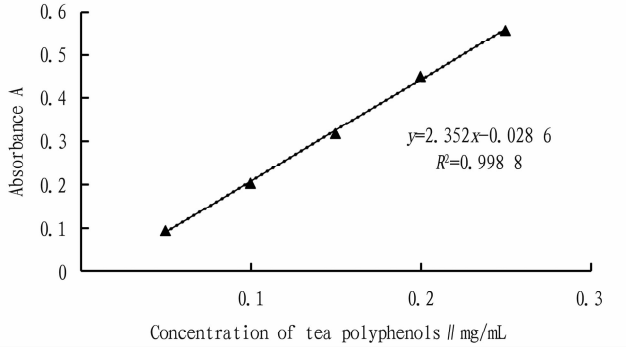
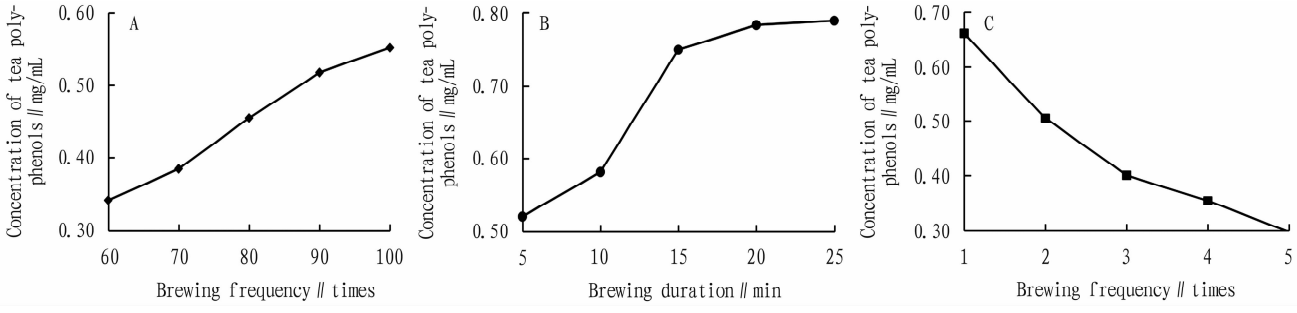


Fig. 1 Standard curve of tea polyphenols

2.2 Single-factor tests

2.2.1 Effects of brewing temperature on the extraction of tea polyphenols. This study investigated the effects of brewing temperatures of 60, 70, 80, 90, and 100 ℃ on the concentration of tea polyphenols in the tea infusion. Within the temperature range of 60 to 100 ℃, the concentration of tea polyphenols increased progressively with rising temperature (Fig. 2). Consequently, orthogonal experiments were performed at three selected temperature levels: 60, 80, and 100 ℃.



NOTE A. Brewing temperature; B. Brewing duration; C. Brewing frequency.

Fig. 2 Effects of single-factor tests on the extraction of tea polyphenols

2.2.2 Effects of brewing duration on the extraction of tea polyphenols. This study examined the effects of brewing durations of 5, 10, 15, 20, and 25 min on the concentration of tea polyphenols in the tea infusion. Within the interval of 5 to 25 min, the concentration of tea polyphenols increased as the brewing duration was extended (Fig. 2). Notably, the most significant increase occurred between 5 and 15 min. Although tea polyphenol concentration continued to rise from 15 to 25 min, the rate of increase diminished considerably. Consequently, orthogonal experiments were performed at three selected durations: 5, 10, and 15 min.

2.2.3 Effects of brewing frequency on the extraction of tea polyphenols. This study investigated the effects of brewing frequency (specifically 1, 2, 3, 4, and 5 times) on the concentration of tea polyphenols in the tea infusion (Fig. 2). As the number of brews increased, the concentration of tea polyphenols consistently decreased. However, the decline followed a diminishing trend, with the rate of reduction becoming progressively smaller. Taking into account both operational convenience and tea polyphenol concentration, orthogonal experiments were subsequently conducted at three levels: 1, 2, and 3 brewing cycles.

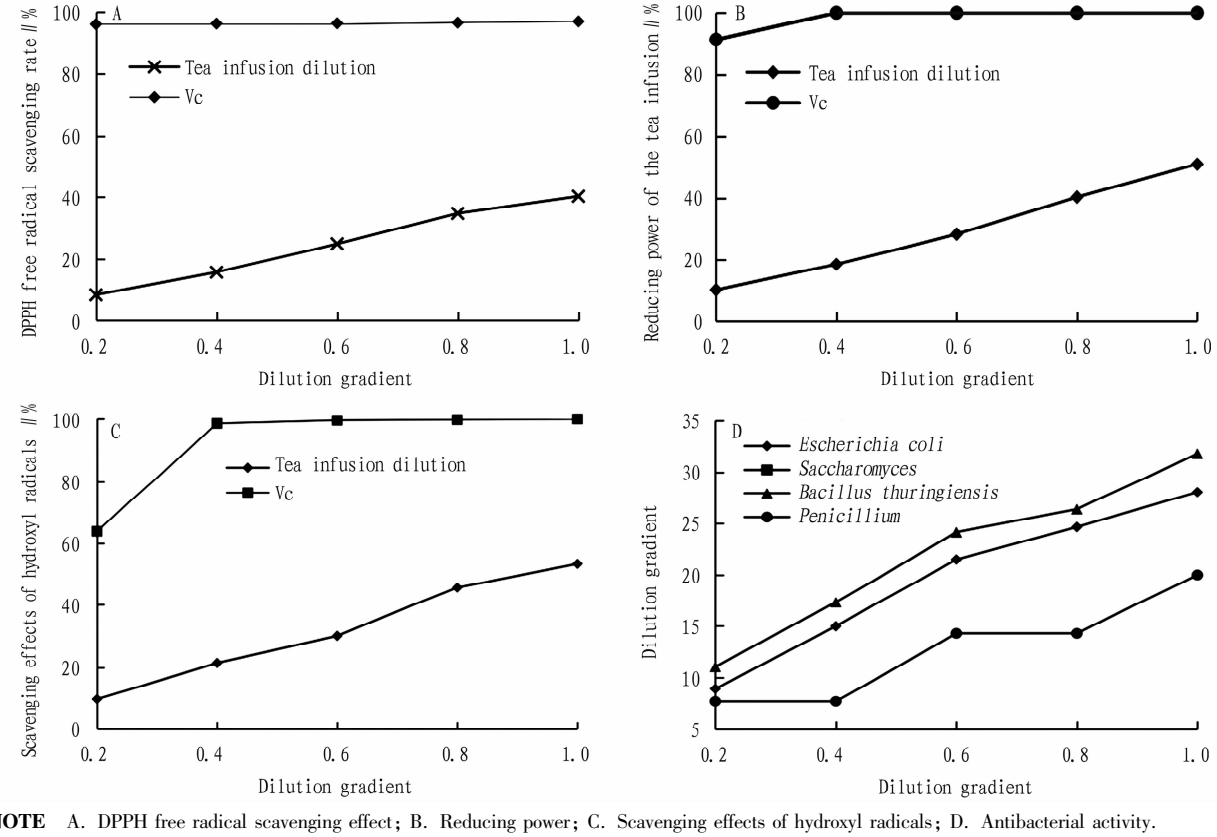
2.3 Orthogonal experiment The $L_9(3^3)$ orthogonal experiment was designed based on the orthogonal factor levels presented in Table 1, with the results summarized in Table 2. As indicated in Table 2, among the three factors [brewing temperature (A), brewing duration (B), and brewing frequency (C)] the magnitude of their effects ranked from greatest to least as follows: brewing duration (B) > brewing frequency (C) > brewing temperature (A). The optimal combination identified was $A_3B_3C_1$, corresponding to a brewing temperature of 100 ℃, a brewing duration of 15 min, and a single brewing cycle. This outcome aligns with the findings of single-factor tests. Therefore, subsequent experiments were conducted using this combination.

2.4 Antioxidant function

2.4.1 Scavenging effects of DPPH free radicals. The effects of Fenghuang Dancong tea on scavenging DPPH free radicals are illustrated in Fig. 3. As the dilution ratio of the tea infusion decreased, corresponding to an increase in the concentration of tea polyphenols, the DPPH free radical scavenging rate increased, demonstrating a strong positive correlation. However, within the dilution range of 0.2 to 0.8 times, the slope of the segmented line remained relatively constant, while between 0.8 and 1.0 times, the slope exhibited a slight decrease.

Table 2 Results of orthogonal experiment

No.	Brewing temperature (A)	Brewing duration (B)	Brewing frequency (C)	Concentration of tea polyphenols mg/mL
1	1	1	1	0.341 6
2	1	2	2	0.429 8
3	1	3	3	0.434 0
4	2	1	2	0.442 4
5	2	2	3	0.486 6
6	2	3	1	0.660 9
7	3	1	3	0.493 7
8	3	2	1	0.631 0
9	3	3	2	0.729 1
K_1	0.401 8	0.425 9	0.544 5	
K_2	0.435 4	0.515 8	0.533 8	
K_3	0.454 3	0.608 0	0.471 4	
R	0.052 5	0.182 1	0.073 1	



NOTE A. DPPH free radical scavenging effect; B. Reducing power; C. Scavenging effects of hydroxyl radicals; D. Antibacterial activity.

Fig. 3 Antioxidant functions of Fenghuang Dancong tea

2.4.2 Reducing power of the tea infusion. The reducing power of the tea infusion is illustrated in Fig. 3. An increase in the concentration of tea polyphenols corresponded with an enhancement in reducing power, as evidenced by the progressively steeper slope. This trend indicates that higher levels of tea polyphenols exert a more pronounced effect on reducing power.

2.4.3 Scavenging effects of hydroxyl radicals. The scavenging effects of the tea infusion on hydroxyl radicals are illustrated in Fig. 3. As the concentration of tea polyphenols increased, the scavenging rate of hydroxyl radicals correspondingly raised, attaining its maximum value at a dilution ratio of 1.0.

2.5 Antibacterial activity As illustrated in Fig. 3, the tea infusion exhibited inhibitory effects on *E. coli*, *B. thuringiensis*, *Penicillium*, and *Saccharomyces*. The antibacterial efficacy ranked from highest to lowest as follows: *B. thuringiensis* > *E. coli* > *Penicillium* > *Saccharomyces*. The antibacterial rates of the tea infusion against *E. coli*, *Penicillium*, and *B. thuringiensis* increased concomitantly with the concentration of tea polyphenols, demonstrating a positive correlation. The maximum antibacterial rate was observed at a dilution gradient of 1.0. In contrast, the antibacterial effect against *Saccharomyces* was considerably lower than that observed for the other three microorganisms. No significant inhibitory effect was detected at dilution gradients below 0.8, and a notable antibacterial effect was only evident at a dilution ratio of 1.0.

3 Conclusions and discussion

3.1 Conclusions (i) The results of single-factor tests and orthogonal experiments indicated that the concentration of tea polyphenols increased with longer brewing durations and higher brewing temperatures, demonstrating a positive correlation. The optimal brewing conditions were identified as 15 min and 100 ℃. Conversely, the concentration decreased with an increase in brewing frequency, showing a negative correlation, with the optimal brewing frequency being one cycle. The relative influence of the three factors on the extraction of tea polyphenols was ranked as follows: brewing duration (B) > brewing frequency (C) > brewing temperature (A). The optimal brewing combination was $A_3B_3C_1$, corresponding to a brewing temperature of 100 ℃, a brewing duration of 15 min, and a single brewing cycle. The orthogonal experimental results corroborated the findings from single-factor tests. Under these conditions, the concentration of tea polyphenols in the tea infusion reached 0.788 4 mg/mL.

(ii) The results of the antioxidant experiment indicated that the DPPH free radical scavenging activity, reducing power, and hydroxyl radical scavenging activity of the tea infusion all increased proportionally with the concentration of tea polyphenols, demonstrating a strong positive correlation.

(iii) The antibacterial assay demonstrated that the tea infusion exhibited a significant inhibitory effect against *E. coli*, *B. thuringiensis*, and *Penicillium*, with the efficacy increasing proportionally to the concentration of tea polyphenols. In contrast, its inhibitory effect on *Saccharomyces* was comparatively weak; negligible inhibition was observed at low concentrations, with only a modest inhibitory effect detected at the highest concentration tested.

3.2 Discussion (i) In single-factor tests, the concentration of tea polyphenols increased with rising brewing temperature; however, the rate of increase in extraction diminished within the temperature range of 80 to 100 ℃. This phenomenon may be attributed to the fact that while elevated temperatures enhance the solubility and extraction rate of tea polyphenols, excessively high temperatures reduce this promoting effect and may even degrade tea polyphenols, thereby decreasing their extraction rate[19].

With the extension of the brewing duration, the concentration of tea polyphenols increased, primarily within the range of 5 to 15 min. This phenomenon may be attributed to several factors: ① as brewing duration increases, the water temperature decreases, and given the temperature-dependent nature of tea polyphenol extraction, this results in a corresponding decline in their concentration; ② once tea polyphenols are fully extracted, the presence of non-polyphenolic substances may interfere with further extraction; and ③ tea polyphenols are susceptible to degradation and oxidation, with the rate of oxidation diminishing over time[19].

An increase in brewing frequency corresponded with a decrease in the concentration of tea polyphenols. This phenomenon can be attributed to the gradual depletion of residual tea polyphenols in the tea leaves[19].

(ii) In the antioxidant assay, the antioxidant activity of the tea infusion increased proportionally with the concentration of tea polyphenols. Tea polyphenols interact with DPPH free radicals and hydroxyl radicals, effectively neutralizing them. As the concentration of tea polyphenols rises, a greater number of free radicals are scavenged, resulting in a decrease in residual free radicals, a reduction in chromogenic substances, lower absorbance values, and consequently, an enhanced antioxidant rate. Additionally, tea polyphenols have the capacity to reduce Fe^{3+} to Fe^{2+}. With increasing concentrations of tea polyphenols, more Fe^{2+} is generated, leading to an increase in chromogenic substances, higher absorbance, and an overall enhancement of reducing power[20-23].

(iii) The antibacterial assay demonstrated that the tea infusion exhibited a significant inhibitory effect on *E. coli*, *B. thuringiensis*, and *Penicillium*, with the efficacy increasing proportionally to the concentration of tea polyphenols. Conversely, its inhibitory effect on *Saccharomyces* was comparatively weak, manifesting only slight inhibition at elevated concentrations. Tea polyphenols reduce bacterial populations by disrupting cellular structures; consequently, higher concentrations result in greater bacterial destruction and enhanced antibacterial activity[24-25].

References

[1] GAO YP, TANG DS, GONG SY. Research of the relationship between antioxidation of tea extractive and tea polyphenols as well as catechins [J]. Journal of Chinese Institute of Food Science and Technology, 2013, 13(6): 40-47. (in Chinese).

[2] ZHAO BL. Free radicals, nutrition, natural antioxidant and aging[J]. Acta Biophysica Sinica, 2010, 26(1): 26 – 36. (in Chinese).
[3] CAI J, YE R, JIA K, *et al.* Review on extraction and antibacterial activity of tea polyphenols[J]. Chemical Reagents, 2020, 42(2): 105 – 114. (in Chinese).
[4] GAO T, YUAN FY, LIU ZW, *et al.* Antibacterial and antiviral effects of tea polyphenols[J]. Progress In Veterinary Medicine, 2022, 43(4): 107 – 111. (in Chinese).
[5] YANG X, CHEN L, LU HM, *et al.* Research progress on extraction and purification methods of tea polyphenols and its functional activities[J]. Science and Technology of Food Industry, 2019, 40(05): 322 – 328, 332. (in Chinese).
[6] DONG JP, LI YQ, HONG SM. Minimum inhibitory concentrations of tea polyphenols (TPP) against eight pathogenic bacteria[J]. Food Science, 1995(1): 6 – 12. (in Chinese).
[7] WEI JH, WANG ML, CHEN L, *et al.* Analysis of gallic acid and catechin contents in Fenghuang Dancong tea[J]. Special Wild Economic Animal and Plant Research, 1 – 8[2025 – 04 – 30]. (in Chinese).
[8] DU JM, ZHANG P, GAO D. Synergistic antibacterial effect and antioxidant activity of the compound liquid with clove essential oil and tea polyphenols[J]. Modern Food Science and Technology, 2021, 37(10): 87 – 95. (in Chinese).
[9] SONG JX, QI HB, PENG L. Study on the antibacterial effect of crude extract from tea[J]. Farm Products Processing, 2022(4): 10 – 14. (in Chinese).
[10] ZHANG YT, XIA YF, HE XX, *et al.* Optimization of extraction process of tea polyphenols from green tea and white tea and comparison of antioxidant activity[J]. Journal of Tea Communication, 2021, 48(3): 507 – 513. (in Chinese).
[11] YANG HB, YANG N, BAI X, *et al.* Research on the extraction process and antioxidant activity of tea polyphenols in white tea[J]. Journal of Chinese Institute of Food Science and Technology, 2014, 14(12): 24 – 31. (in Chinese).
[12] QI T, PEI H, GUO LP, *et al.* Optimization of extraction process of tea polyphenols in four teas by orthogonal experiment[J]. Journal of Zhejiang Agricultural Sciences, 2023, 64(8): 1977 – 1982. (in Chinese).
[13] CHEN BY, CHEN HP, TIAN BM, *et al.* Research progress on the application of tea polyphenols as food additives[J]. China Tea, 2024, 46(11): 33 – 44. (in Chinese).
[14] ZHANG Q, WANG QH, LUO XL, *et al.* Tea polyphenol content and antioxidant activity of Liubao tea from different fermentation years and aromatic[J]. Food Research and Development, 2024, 45(16): 67 – 72. (in Chinese).
[15] LI ML, PENG JL, JIANG HD, *et al.* Study on antioxidant activity and content of main active components of 9 kinds of yellow camellia flowers [J]. Journal of Guangxi Academy of Sciences, 2020, 36(4): 419 – 426. (in Chinese).
[16] CHEN JE, FENG HJ, ZHANG HR. Effects of active ingredients in black tea, green tea and oolong tea on antioxidant capability[J]. Food Science, 2009, 30(3): 62 – 66. (in Chinese).
[17] XU JT, ZHANG CX, ZHANG Y. Extraction and isolation techniques of tea polyphenols and their *in vitro* antioxidant activity[J]. Farm Products Processing, 2024(6): 78 – 85. (in Chinese).
[18] YANG G. Comparison of two measuring methods of bacterial concentration in liquid culture conditions[J]. Journal of Microbiology, 2005(4): 71 – 72. (in Chinese).
[19] ZENG L, HUANG JA, LI CL, *et al.* Research on the bacteriostasic activity and mechanism of catechins[J]. Science and Technology of Food Industry, 2009, 30(5): 89 – 92. (in Chinese).
[20] CHEN JY, LIU CL, LIU W, *et al.* Effects of brewing conditions on polyphenol content and antioxidant activity of Fuding white tea soups [J]. China Tea, 2020, 42(3): 38 – 43. (in Chinese).
[21] MA H, RU X, WANG J, *et al.* Study on the antioxidant capacity of four tea water extracts and tea polyphenols *in vitro*[J]. Food Research and Development, 2019, 40(8): 65 – 70. (in Chinese).
[22] YANG G, ZHANG TW, LI Y, *et al.* Research status of extraction, detection and biological activity of tea polyphenols in tea[J]. Tibet Journal of Agricultural Sciences, 2022, 44(4): 65 – 70. (in Chinese).
[23] ZHAO L, GAO M, MA YF. Anti-oxidation functions of tea polyphenols and their mechanisms[J]. Chinese Journal of Animal Nutrition, 2017, 29(6): 1861 – 1865. 2017, 29(6): 1861 – 1865. (in Chinese).
[24] LIANG WH. Research overview on the antibacterial effects of tea polyphenols[J]. International Journal of Stomatology, 2004(S1): 26 – 28. (in Chinese).
[25] CHEN ZY, LI J, FENG Y, *et al.* Research progress on bioactivity and mechanism of tea polyphenols[J]. Science and Technology of Food Industry, 2024, 45(13): 333 – 334. (in Chinese).