

# Exploring the Optimal Extraction Conditions and Antibacterial Effects of Tea Polyphenols

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**Abstract.** Tea polyphenols are a general term for polyphenols in tea, which are a white amorphous powder that is easily soluble in water. It is well known that it has many functions, such as antioxidant activity, antibacterial activity, inhibitory enzyme activity and other physiological activities. And as a natural compound, tea polyphenols have good environmental friendliness, and as additives, they can help improve the current environmental pollution situation. Tea polyphenols have extensive application value in various industries, but there is currently a lack of research on their antibacterial properties when combined with daily chemical products. Due to the current lack of research in this area, this experiment used shower gel as an example to study the antibacterial effect of daily chemical products which are added tea extract. This article includes two experiments. One is study on the optimal extraction conditions for tea polyphenol, including two variables on temperature and extraction agent concentration. The other one is study on antibacterial effect of tea polyphenol, by combining variables on store temperature and concentration of tea extract solution, a total of 14 experimental conditions are set up, including the extract acting alone, and acting together with diluted shower gel, and the control group (water, diluted shower gel). The experimental study shows that the inherent content of tea polyphenols varies among different tea varieties and tea extracts is with good antibacterial properties. Generally speaking, the extraction effect at 80 degrees Celsius is significantly better than those at 50 degrees Celsius, and the content also changes with the extraction time. And tea extracts extracted with suitable extraction conditions shows the best antibacterial effect when diluted 100 to 200 times. This article aims to find better extraction methods and antibacterial effect, providing a new approach for the industrial application of tea polyphenols as natural bacteriostat.

**Keywords:** Tea chemistry; tea polyphenols; bacteriostat; additive; environmentally friendly.

## 1. Introduction

Tea has now become a popular beverage in the world, with its consumption ranking second globally, tea is a natural beverage mainly made from tea plant (*Camellia sinensis* L.) leaves [1]. There is a very important chemical substance in tea called tea polyphenols, they are a kind of polyhydroxy compounds that can be extracted from tea [2].

According to reports, tea polyphenols have various different effects, among which the antibacterial effect has received widespread attention. In the past decade, there has been a high incidence of foodborne diseases, and people's awareness of hygiene has increased. There has been a new pursuit for safe and pollution-free antibacterial agents. Foodborne pathogens, including *Salmonella* [3, 4], *Listeriosis* [5, 6], *Staphylococcus aureus* [7, 8], *Escherichia coli* [9], *Campylobacter* isolated [10], *Aflatoxin* [11], *Bacillus cereus* [12] are the major cause of foodborne illnesses, controlling the reproduction of these pathogens can help reduce the occurrence of foodborne diseases. Developing a natural non-toxic antibacterial agent can help reduce the occurrence of such situations. Tea polyphenols, due to their low price and broad-spectrum antibacterial properties, can be used as a new generation of antibacterial agents. According to literature reports, tea polyphenols have the potential as food preservatives and antibacterial agents, as well as their good antioxidant properties and certain inhibitory effects on cancer cells. They are a widely used and highly potential substance for development. The research on the antibacterial characteristics of tea polyphenols is mainly focused on preservatives and dental caries, and there is no research on tea polyphenols as additives added to hand sanitizers or shower gels. Therefore, this study has made relevant innovations.

This study mainly focuses on common bacteria such as *Escherichia coli*, *Staphylococcus aureus* and other bacteria commonly found in tap water and in daily life, and verifies that tea polyphenols can bring good bacteriostatic effect to washing products (shower gel). It can provide new ideas for controlling the occurrence of Foodborne illness. Moreover, this study also verifies that tea polyphenols can provide good long-term (24 to 48 hours) antibacterial performance at higher dilution ratios (100-200 times, volume dilution ratio). The experiment also shows that the extracting and applying of tea polyphenols are simple and good operability. There is the highly possibility of becoming industrial production.

## 2. Materials and Method

### 2.1. Materials

The experimental materials required for exploring the optimal extraction method of tea extract (with tea polyphenols as the main indicator) include Oolong and Phoenix Dahongpao tea produced by Fujian Anxi Tea Xiaoshi Tea Industry Co., Ltd. and black tea produced by Fujian Tianhaoyuan Tea Industry Co., Ltd. Ferrous Tartaric acid produced by Shanghai Ziyi Reagent Factory, Iron(II) sulfate produced by Langfang Kaiwei Chemical Technology Co., Ltd., disodium hydrogen sulfate produced by Shandong Denuo New Material Technology Co., Ltd., and disodium hydrogen phosphate produced by Qingzhou Xinsheng Chemical Co., Ltd and anhydrous ethanol produced by Shandong Taixi Chemical Co., Ltd. The precision electronic balance produced by Shenyang Shenyu Longteng Balance Co., Ltd., the water bath pot produced by Nanjing Tongchuang Technology Co., Ltd., the ultraviolet spectrophotometer produced by Shanghai Jingke Industrial Co., Ltd., and the Cell disruption instrument produced by Shanghai Daluo Scientific Instrument Co., Ltd.

The reagents and equipment used in the experiment to explore the antibacterial effect of tea polyphenols. Lipton green tea bag, Safeguard pure white and fragrant shower gel, tap water, laboratory made beef extract peptone solid culture medium, anhydrous ethanol produced by Shandong Taixi Chemical Co., Ltd. The precision electronic balance produced by Shenyang Shenyu Longteng Balance Co., Ltd., the water bath pot produced by Nanjing Tongchuang Technology Co., Ltd., and Haier produces refrigerated cabinets and Canon digital cameras.

### 2.2. Determination of Tea Polyphenol Content

#### 2.2.1 Preparation of tea extract

Accurately weigh 1.0 g of tea leaves and grind them into fine pieces. Place them in a 200 mL triangular flask, add 90 mL of extraction agent (ethanol concentration is 50% and 80%), and perform ultrasonic crushing (crushing parameter: 1200 w, 5 minutes, crushing for 5 seconds, termination for 5 seconds as a cycle). Soak and extract at 50 °C, 80 °C for 30 minutes. Filter the reactants after extraction, keep the filtrate and seal it for storage. Three different types of tea leaves are selected and numbered. Group A is Oolong tea, Group B is black tea, Group C is phoenix dahongpao tea. There are two different variables which are concentration of ethanol and water bath temperature. In group A, A1 is treated by ethanol in 50% concentration with 50°C water bath. A2 is treated by ethanol in 50% concentration with 80°C water bath. A3 is treated by ethanol in 80% concentration with 50°C water bath. A4 is treated by ethanol in 80% concentration with 80°C water bath. In group B, B1 is treated by ethanol in 50% concentration with 50°C water bath. B2 is treated by ethanol in 50% concentration with 80°C water bath. B3 is treated by ethanol in 80% concentration with 50°C water bath. B4 is treated by ethanol in 80% concentration with 80°C water bath. In group C, C1 is treated by ethanol in 50% concentration with 50°C water bath. C2 is treated by ethanol in 50% concentration with 80°C water bath. C3 is treated by ethanol in 80% concentration with 50°C water bath. C4 is treated by ethanol in 80% concentration with 80°C water bath.

### 2.2.2 Total phenol content by ferrous tartaric acid method

After preparing the tea extract, the reagent required for determination of total phenol content by ferrous Tartaric acid method is prepared. The Tartaric acid ferrous method is used to test the concentration of tea polyphenols in the solution. This reaction can be used to detect the content of tea polyphenols, because the reaction shows different shades of blue purple, which reflects the different content of tea polyphenols, leading to the different content of blue purple complexes produced by the reaction of ferrous Tartaric acid and tea polyphenols.

The content of tea polyphenols can be determined by spectrophotometry. First, prepare ferrous Tartaric acid solution, weigh 0.50 g Iron (II) sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) and 2.50 g Potassium sodium tartrate ( $\text{C}_4\text{H}_4\text{O}_6\text{NaK} \cdot 4\text{H}_2\text{O}$ ), mix them, add distilled water to dissolve them, and fix the volume to 500 mL. Next, prepare a pH 7.5 phosphate buffer solution, weigh 30.10g of disodium hydrogen sulfate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) and 2.50 g of disodium hydrogen sulfate ( $\text{NaH}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$ ), mix and dissolve in distilled water to a constant volume of 500 mL.

### 2.2.3 Determination of total phenolic content in extracts

For the convenience of quantifying data, we use the following method for calculation. The calculation formula is shown in formula (1). Accurately suck 0.5 mL of the above tea extract solution, add 2 mL of distilled water and 2.5 mL of ferrous Tartaric acid solution, and add 7.5 mL of phosphoric acid buffer solution (pH=7.5) after full mixing. The total volume of the above four reagents is L1 as the test solution. Using a 10 mm colorimetric cup with a volume of 4 mL, the amount of test solution used for determination is L2, the mass is M, and the dry matter percentage of the sample (calculated as 95%) is m. At the wavelength of 540nm, the absorbance A is measured using the reagent blank solution as a reference. The value of 1.957 in the formula is equivalent to 1.957 mg of tea polyphenols per milliliter of tea extract when the absorbance is equal to 0.50 using the same 10mm colorimetric cup. If the requirements for repeatability are met, the arithmetic mean of the two measurements shall be taken as the result. The repeatability requirement is the difference between two measurements of the same sample, which should not exceed 0.5 g per 100 g of sample.

$$\text{Tea polyphenols(\%)} = \frac{A \times 1.957 \times 2}{1000} + \frac{L1}{L2 \times M \times m} \times 100\% \quad (1)$$

## 2.3. Determination of Antibacterial Effect of Tea Polyphenols

### 2.3.1 Production of tea extract solution

Grind the tea leaves in the green tea bag to a fine powder state, weigh 3g of green tea fine powder with an electronic balance, and add it to the 100 mL of 80% ethanol (volume concentration), soak in water at 80°C for 30 minutes to obtain the original solution of tea extract. The main component in tea extract is tea polyphenols, which can be considered as tea polyphenol extract in this experiment. Sealed and stored at room temperature (25 °C) and in refrigerator (4 °C). To simulate the actual production situation and verify the effect of storage time on the activity of tea polyphenols, the original solution is stored for one month with the temperature above before next step.

### 2.3.2 Dilute tea extract solution and shower gel

To introduce conventional bacteria and verify their antibacterial effects, the water mentioned in this experiment is tap water. Dilute the original tea extract solution stored at different temperatures with tap water in volume to 200 times, 100 times, and 70 times the extraction solution. The shower gel is selected from the commonly seen ones on the market that do not contain antibacterial ingredients, and this time it is a Safeguard brand. Dilute 1:1 by volume with tap water. And keep the two diluted matters at room temperature for sample preparation in short time.

### 2.3.3 Sample preparation

Because this experiment involves multiple sets of variables, including temperature and concentration, there are fourteen sets of samples. The samples are divided into two groups: diluted tea extract solution group (six groups, numbered 1~6), diluted tea extract solution with diluted shower

gel (six groups, numbered 13~18). Diluted shower gel and tap water are control group (numbered 20&21 respectively).

Method for configuring corresponding numbered samples: No.1 take 1 mL of refrigerated tea extract solution diluted 200 times and add 0.15 mL of water, mix fully; No.2 take 1 mL of room temperature stored tea extract solution diluted 200 and add 0.15 mL of water, mix fully; No.3 Take 1 mL of refrigerated tea extract solution diluted by 100 times and add 0.15 mL of water, mix fully; No.4 take 1 mL of room temperature stored tea extract solution diluted 100 times and add 0.15 mL of water, mix fully; No.5 take 1 mL refrigerated tea extract solution diluted 70 times with 0.15 mL of water; No.6 take 1 mL of room temperature stored tea extract solution tea extract solution diluted 70 times and add 0.15 mL of water, mix fully; No.13 Take 1 mL of refrigerated tea extract solution diluted 200 times and add 0.15 mL diluted shower gel, mix fully; No.14 take 1 mL of room temperature stored tea extract solution diluted 200 times and add 0.15 mL diluted shower gel, mix fully; No.15 take 1 mL of refrigerated tea extract solution diluted 100 times and add 0.15 mL diluted shower gel, mix fully; No.16 take 1 mL of room temperature stored tea extract solution diluted 100 times and add 0.15 mL diluted shower gel, mix fully; No.17 take 1 mL of refrigerated tea extract solution diluted 70 times and add 0.15 mL diluted shower gel, mix fully; No.18 take 1 mL of room temperature stored tea extract solution diluted 70 times and add 0.15 mL diluted shower gel, mix fully; No.20 take 1 mL of water and add 0.15 mL of diluted shower gel, mix fully; No. 21 take 1 mL of water and add 0.15 mL of water, mix fully.

The 14 groups of samples are applied to Petri dish respectively immediately after prepared. These Petri dishes are stored at room temperature and observed the proliferation status of bacterial colonies at 24 hours and 48 hours. And recorded visually with a camera.

### 3. Result and Discussion

#### 3.1. Total Phenol Content

Table 1, Table 2, and Table 3 show the different presentation forms of two sets of data, clearly comparing the differences between the data. First, under the condition of the same concentration and temperature of extractant, the content of total polyphenols in tea is Oolong<black tea< Phoenix Dahongpao tea; Secondly, under the same concentration of extractant, the higher the temperature, the generally higher the total phenolic content; Finally, at the same temperature, the total phenolic content of Phoenix Dahongpao tea increases with the increase of the concentration of the extractant, while the total phenolic content of black tea decreases with the increase of the concentration of the extract.

In this experiment, author studied the effects of three variables: extractant concentration, temperature and different types of tea. After experimental proof, all variables can lead to different results in actual experimental processes. These results contribute to the application in large-scale industrial production. It can further save energy and resources, reduce unnecessary waste, and reduce pollution caused by waste disposal. This study only conducted a simple control experiment here, and further detailed experiments are needed to find the optimal extraction concentration and extraction temperature. In addition to the content of tea polyphenols, the activity of tea polyphenols is an important judgment basis in this experiment. The activity of tea polyphenols shows the quality of tea polyphenols. Under appropriate extraction temperature and concentration conditions, tea polyphenols with higher activity are also a topic worth exploring. Due to laboratory limitations, the author was unable to proceed with the next experiment and therefore did not discuss this part.

**Table 1.** Determination of total phenol content: Comparison of tea types and contents under the same extraction conditions.

First	[Extract]	Temperature	Tea polyphenol (%)	Second	[Extract]	Temperature	Tea polyphenol (%)
A1	50%	50°C	0.448	A1	50%	50°C	0.466
B1	50%	50°C	0.478	B1	50%	50°C	0.493
C1	50%	50°C	0.547	C1	50%	50°C	0.675
A2	50%	80°C	0.409	A2	50%	80°C	0.432
B2	50%	80°C	0.7	B2	50%	80 °C	0.769
C2	50%	80°C	0.711	C2	50%	80°C	0.807
A3	80%	50°C	0.276	A3	80%	50°C	0.313
B3	80%	50°C	0.359	B3	80%	50°C	0.458
C3	80%	50°C	0.732	C3	80%	50°C	0.840
A4	80%	80°C	0.455	A4	80%	80°C	0.462
B4	80%	80°C	0.556	B4	80%	80°C	0.698
C4	80%	80°C	0.775	C4	80%	80 °C	0.891

**Table 2.** Determination of total phenol content: Comparison of content when the concentration of extractants is the same.

Firs t	[Extract ]	Temperature	Tea polypheno l (%)	Secon d	[Extract ]	Temperature	Tea polypheno l (%)
A1	50%	50°C	0.448	A1	50%	50°C	0.466
A2	50%	80°C	0.409	A2	50%	80°C	0.432
B1	50%	50°C	0.478	B1	50%	50°C	0.493
B2	50%	80 °C	0.700	B2	50%	80°C	0.769
C1	50%	50°C	0.547	C1	50%	50°C	0.675
C2	50%	80°C	0.711	C2	50%	80°C	0.807
A3	80%	50°C	0.276	A3	80%	50°C	0.313
A4	80%	80°C	0.455	A4	80%	80°C	0.462
B3	80%	50°C	0.359	B3	80%	50°C	0.458
B4	80%	80°C	0.556	B4	80%	80 °C	0.698
C3	80%	50 °C	0.732	C3	80%	50°C	0.840
C4	80%	80 °C	0.775	C4	80%	80 °C	0.891

**Table 3.** Determination of total phenol content: Comparison of content at the same extraction temperature.

First	[Extract]	Temperature	Tea polyphenol (%)	Second	[Extract]	Temperature	Tea polyphenol (%)
A1	50%	50°C	0.448	A1	50%	50 °C	0.466
A3	80%	50°C	0.276	A3	80%	50°C	0.313
B1	50%	50°C	0.478	B1	50%	50°C	0.493
B3	80%	50 °C	0.359	B3	80%	50 °C	0.458
C1	50%	50°C	0.547	C1	50%	50°C	0.675
C3	80%	50°C	0.732	C3	80%	50°C	0.840
A2	50%	80°C	0.409	A2	50%	80°C	0.432
A4	80%	80°C	0.455	A4	80%	80°C	0.462
B2	50%	80°C	0.700	B2	50%	80°C	0.769
B4	80%	80°C	0.556	B4	80%	80°C	0.698
C2	50%	80°C	0.711	C2	50%	80°C	0.807
C4	80%	80°C	0.775	C4	80%	80°C	0.891

### 3.2. Tea Polyphenols are Antibacterial

The Table 4 is a textual description of the experimental results of this experiment, clearly comparing the changes in the 24 h and 48h Petri dish. The result shows that the tea extract has a significant inhibitory effect on bacterial proliferation within 24 hours, the antibacterial effect of tea extract is related to its concentration and under the extraction condition in this experiment, the dilution ratio between 100-200 times achieves good antibacterial effects, the combination of tea extract and shower gel has the better antibacterial. In addition, this experiment also proves that the antibacterial effect of tea extract has little correlation with its storage temperature and the tea extraction can still maintain good activity after being stored in a sealed state for one month.

The above experiments have shown that the production of tea polyphenol extract is not difficult and can be industrialized on a large scale. The author focused on exploring the antibacterial effect of tea polyphenols in this experiment. Tea polyphenol extract has good ability as an antibacterial agent. As a natural additive for daily chemical products, it has better environmental friendliness compared to for the antibacterial additives currently used. Due to laboratory limitations, the author only conducted preliminary experiments and achieved visible results to the naked eye. This experiment can further use the dilution coating plate method to count the number of colonies and obtain further experimental results. Alternatively, specific bacteria can be introduced to verify the inhibitory properties of tea polyphenols against a specific type of bacteria. The further experiments mentioned above can promote the possibility of using tea polyphenols as natural additives in daily chemical products, and promote the use of other environmentally friendly antibacterial agents in daily chemical products.

**Table 4.** The experimental results of the antibacterial effect of tea polyphenols.

	Experimental grouping	Antibacterial effect in 24H	Antibacterial effect in 48H
Cold storage group	1	Small number of bacterial colonies	Bacterial colonies increase
	3	Small number of bacterial colonies	Bacterial colonies increase
	5	Multi numbers of bacterial colonies	Bacterial colonies increase
	13	No obvious bacterial colony	Bacterial colonies increase
	15	No obvious bacterial colony	Bacterial colonies increase
	17	Small number of bacterial colonies	Bacterial colonies increase
Room temperature storage group	2	Small number of bacterial colonies	Bacterial colonies increase
	4	Small number of bacterial colonies	Bacterial colonies increase
	6	Multi numbers of bacterial colonies	Bacterial colonies increase
	14	No obvious bacterial colony	Bacterial colonies increase
	16	No obvious bacterial colony	Bacterial colonies increase
	18	Small number of bacterial colonies	Bacterial colonies increase
Control group	20	Small number of bacterial colonies	Full of bacterial colonies
	21	Full of bacterial colonies	Full of bacterial colonies

#### 4. Conclusion

Now environmental protection and people's awareness of health have increased, and more demands have been put forward for antibacterial and disinfection and sterilization products. In this experiment, higher extraction concentration and temperature can increase the content of tea polyphenols in the tea polyphenol extraction solution. However, the relationship between tea polyphenol content and tea types is more closely related, so the research results brought by this experiment cannot summarize all types of tea. Other teams need to pay attention to the types of tea if conducting such studies. For tea polyphenols extracted under certain conditions, the experimental results of preserving the extract at 4 °C and 25 °C are not significantly different. It can be considered that if used in daily chemical products, the effect will not be affected by temperature changes. And when it is added as an additive to daily chemical products, it only needs a very small amount. In this experiment, it was found that using the preparation standard of tea extract in this study as an example, the volume dilution ratio of 100-200 times has significant antibacterial effects, which can meet the needs of daily life for antibacterial effects. Future research teams can refine this experimental plan or specifically explore the inhibitory effect of tea polyphenols on a certain type of bacteria. The relevant achievements can provide some new ideas for social and environmental protection work, and help us build an environmentally friendly society.

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