

# Biological Functions of Tea Polyphenols Based on Their Active Substances

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**Abstract.** Tea polyphenols are a type of polyphenolic compounds with a phenolic hydroxyl structure, commonly found in tea trees and with higher concentrations in green tea. They are recognized as key functional elements of tea and exhibit various biological functions, such as antioxidant, metabolic regulation, and antibacterial properties. These characteristics have aroused great interest in different industries, including functional foods, drug research, and food preservation methods. In recent years, research has been conducted on the extraction process of tea polyphenols, exploring their characteristics, biological effects, flavor regulation, mechanism of action, and potential applications. This detailed study provides valuable reference for the development of functional foods, natural therapies, and other related products containing tea polyphenols. The multiple advantages and uses of tea polyphenols highlight their potential to enhance health and well-being in a range of industries. The ongoing research on tea polyphenols emphasizes their importance as natural compounds with encouraging biological effects and functional qualities, which can be used to develop various health enhancing products and applications.

**Keywords:** Tea polyphenols; biological functions; extraction process; health enhancing products.

## 1. Introduction

Tea, one of the most popular beverages around the world, has been recognized for its large number of health benefits for centuries. Tea originated in China, and tea drinking began in China. Chinese people have a long history of drinking tea. Teas have been found to offer a wide range of health benefits, such as providing antioxidants, reducing inflammation, combating carcinogens, and promoting anti-aging effects [1]. Among the many bioactive compounds found in tea, tea polyphenols have attracted much attention due to their potential health promoting properties.

Polyphenols are a group of phytochemicals found in various plant-based foods, including tea leaves. Tea polyphenols are known for their antioxidant properties and play a crucial role in protecting cells from harmful free radical damage. The main types of tea polyphenols found in tea leaves include catechins, flavonoids, and tannins, each having distinct chemical compositions and biological properties.

Catechins, the most primary type of tea polyphenols, are responsible for many of the health benefits associated with tea consumption. Catechins are composed of two benzene rings and a dihydropyran heterocycle with a hydroxyl group. They can esterify with gallate groups to create catechin gallic acid conjugates. Catechins are known for their biological properties when consumed through tea [2]. The primary catechins in tea include (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate (EGCG), each with distinct structures as illustrated in Fig. 1 [3]. EGCG has demonstrated the ability to influence different signaling pathways within the body, potentially offering protective effects against chronic diseases.

Flavonoids, another category of tea polyphenols, play a role in the various biological functions of tea. Quercetin, a natural flavonoid found in a variety of foods like apples, berries, cherries, and citrus fruits [4], is consumed daily in estimated amounts ranging from 37 to 600 mg [5]. Additionally, purified quercetin is utilized as a dietary supplement [6]. Quercetin has been documented to exhibit

antioxidant, anticancer, anti-inflammatory, and anti-allergic properties [4, 7]. The synergistic interactions among various flavonoids in tea form an intricate network of bioactive compounds that work together to produce positive effects on human health.

Tannins are a varied category of polyphenols that are produced as secondary metabolites in plants, encompassing a broad spectrum of oligomeric and polymeric polyphenols. Condensed tannins are known to hinder the digestion of protein and fiber in both humans and non-ruminant animals, as they act within the digestive system by binding to dietary protein and digestive enzymes. Despite this antinutritive effect, condensed tannins have been shown to possess various biological and pharmacological properties that are of interest in human and veterinary medicine. These include the inhibition of lipid oxidation, mutagenicity of carcinogens, and suppression of tumor promotion [8]. The observed health benefits linked to tea consumption and the explanation for the French paradox have both been associated with the advantageous bioactivities of tannins.

Tea polyphenols have attracted interest not just for their antioxidant and anti-inflammatory characteristics but also for their potential to enhance cognitive function and mental well-being. Recent studies indicate that specific polyphenols in tea could offer neuroprotective benefits, supporting brain health and potentially lowering the risk of neurodegenerative conditions like Alzheimer's and Parkinson's. Therefore, studying the biological activity of tea polyphenols not only has medical significance, but also has potential economic benefits. The purpose of this study is to delve deeper into the biological functions of tea polyphenols based on their active substances, examining how these compounds interact with the human body to promote health and potentially prevent diseases.

## **2. Extraction of Tea Polyphenols**

### **2.1. Solvent Extraction**

Tea polyphenols are natural polyphenolic compounds derived from tea leaves, commonly referred to as tea tannins. They are white, non-crystalline powders that dissolve easily in warm water, ethanol, methanol, acetone, and ethyl acetate. While they exhibit low solubility in fats and oils, tea polyphenols are insoluble in ether, chloroform, benzene, and petroleum ether [9]. Additionally, they possess hygroscopic properties and demonstrate excellent heat resistance.

Separate and extract substances based on their different solubility in solvents. Using solvent extraction method to extract tea polyphenols. Extract tea using polar solvents, then perform liquid-liquid extraction to separate the extract, and finally concentrate to obtain the product.

Weigh 2g of tea leaves and place them in a beaker containing 30ml of distilled water. Heat for 30 minutes and filter. Heat the filter residue with 10ml of distilled water for another 10 minutes. Filter and combine the two filtrates. Due to the insolubility of tea polyphenols in trichloromethane, the filtrate can be extracted with trichloromethane to remove other interfering substances from the filtrate. The separating funnel solution has two layers, with the lower layer being chloroform and retained. The upper layer is extracted with ethyl acetate, and tea polyphenols are dissolved in ethyl acetate. At this time, there are two layers in the separating funnel solution, and the upper layer is retained. Pour the upper layer of ethyl acetate into a beaker filled with activated carbon for decolorization. Then transfer the filtrate containing ethyl acetate into a beaker for drying to obtain tea polyphenols.

### **2.2. Supercritical Fluid Extraction**

Compared to other methods, supercritical CO<sub>2</sub> (SCF-CO<sub>2</sub>) extraction is characterized by its cleanliness, non-toxicity, environmental friendliness, mild operating conditions, and suitability for separating heat-sensitive materials [10]. Therefore, during SCF-CO<sub>2</sub> extraction, tea polyphenols are not oxidized, resulting in a high purity product. By simply refining the crude product, tea polyphenols of over 95% purity can be obtained. This represents the trend in the development of plant active ingredient separation technology and serves as an ideal method to address the environmental impact of solvent extraction techniques.

In Li L.'s study, 100g of fine green tea powder was prepared for each extraction using supercritical CO<sub>2</sub>. A fixed separation temperature of 50°C and a CO<sub>2</sub> flow rate of 30L/h were maintained during the process. The experiment employed a Box-Behnken design with three factors and three levels, showing in the Table 1 and 2, which included CO<sub>2</sub> pressure, extraction temperature, and extraction time. After stabilizing the process parameters for 0.5 hours, the separation product was collected to obtain a light-yellow crude tea polyphenol extract. The polyphenol content was measured in a small portion dissolved in water, while the remaining extract was stored at -18°C in the dark. To refine the crude tea polyphenol extract before antioxidant activity testing, chloroform and ethyl acetate were used. The solution was dried in a rotary evaporator at a temperature not exceeding 35°C to achieve a purity of over 95% for the brownish-yellow tea polyphenol product [11].

**Table 1.** Factors and levels of response surface experiment [11]

Levels	Pressure $X_1$ (MPa)	Temperature $X_2$ (°C)	Time $X_3$ (h)
1	28	80	3
0	24	60	2
-1	20	40	1

**Table 2.** Box-Behnken design and experimental result [11]

Test No.	Factors			Extraction rate (%)
	$X_1$	$X_2$	$X_3$	
1	1	-1	0	6.52
2	0	1	1	10.57
3	1	0	1	8.36
4	0	-1	-1	5.15
5	0	0	0	9.43
6	0	0	0	9.10
7	-1	-1	0	5.21
8	-1	0	-1	6.25
9	0	0	0	9.76
10	0	1	-1	7.87
11	-1	0	1	7.38
12	-1	1	0	8.62
13	0	-1	1	5.81
14	1	1	0	10.06
15	1	0	-1	8.07

### 2.3. Microwave Assisted Extraction

Microwave assisted extraction of tea polyphenols enhances the extraction process by ensuring uniform heating of the system. This method offers advantages such as simplicity of operation, lower energy consumption, solvent savings, and time efficiency. It overcomes the shortcomings of traditional extraction methods, which are known for their complexity, time-consuming, and unstable products.

Collect 5g fresh tea leaves and carefully remove any impurities. Use a grinder to crush tea leaves into small particles and increase the extraction surface area. Before adding an appropriate volume of water or ethanol water mixture to form a uniform tea solvent mixture, weigh the finely ground tea leaves accurately. Process the tea solvent mixture in a microwave and adjust parameters such as power, time, and temperature to meet specific extraction requirements. After microwave treatment, carefully cool the mixture to room temperature. Separate the solid residue from the extraction solution using filter paper or centrifuge. Concentrate the obtained liquid to the required strength using a vacuum

evaporator or similar method. Finally, the concentrated extract was dried by spray drying or freeze-drying process to obtain refined pure tea polyphenol powder for extraction.

## **2.4. Ultrasound Assisted Hydrotropic Extraction**

Ultrasonic assisted hydrotropic extraction can improve extraction efficiency, shorten extraction time, enhance the selectivity of required compounds, improve energy efficiency, maintain environmental sustainability, and provide optimization potential through technologies such as response surface methodology.

The experiment conducted by Borah A. et al. involved treating 2g of finely ground green tea leaves with a water-soluble solution ranging from 0.5 mol/L to 2.0 mol/L. The mixture was stirred for 7 hours, and samples were collected at 30-minute intervals. Subsequently, the samples were filtered using Whatman filter paper and analyzed with a UV-visible spectrophotometer to assess the synergistic impact of ultrasound and hydraulic rope on the extraction efficiency of tea polyphenols [4]. This collaborative method combines ultrasound and hydraulic rope. It effectively extracts bioactive compounds from green tea, which is a promising environmentally friendly extraction technology [12]. It can not only obtain high-quality polyphenols but also reduce resource consumption and processing time.

In summary, using different methods to extract tea polyphenols has a series of advantages and limitations, which can meet different extraction needs. Solvent extraction provides simplicity and scalability, while supercritical fluid extraction ensures high purity without oxidation. Microwave assisted extraction provides fast and efficient processing, while ultrasonic assisted water-soluble extraction improves efficiency and sustainability. Understand the unique advantages and challenges of each method, enabling researchers and industry professionals to customize extraction processes based on specific requirements, whether focusing on purity, efficiency, environmental impact, or resource optimization.

## **3. Results and discussion**

### **3.1. Physical and Chemical Properties of Tea Polyphenols**

#### **3.1.1. Catechin.**

Tea is rich in polyphenols, among which catechins are the most common. Catechin is a polyphenolic compound typically composed of two benzene rings and a dihydropyran heterocycle containing one hydroxyl group. It is one of the most important active ingredients in tea, with various health benefits such as antioxidant and anti-inflammatory properties. In a study by Dulloo et al. in 1999, the presence of catechins including (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC), and (–)-epigallocatechin-3-gallate (EGCG) was identified in green tea. EGC, ECG, and EGCG are noted as the primary antimicrobial compounds effective against various pathogenic organisms [13]. Among these catechins, EGCG and EGC are the most abundant in green tea, with both compounds being excreted in bile. Specifically, EGC is later excreted in urine, indicating green tea's antibacterial properties against urinary tract bacterial pathogens [14].

#### **3.1.2. Quercetin.**

Quercetin, with the chemical formula  $C_{15}H_{10}O_7$ , is a flavonoid alcohol known for its natural antioxidant properties. It plays an important role in protecting the body from free radical damage, thereby helping to prevent skin aging. Quercetin is widely considered one of the most popular flavonoids due to its many beneficial properties for human health [15]. One of the main advantages of quercetin is its role as a cardiovascular protective agent. By inhibiting free radicals, it protects the heart and helps prevent potential damage that these reactive molecules may cause to the body. In addition, quercetin is easily available in many inexpensive and readily available food sources, making it a convenient choice to incorporate into the diet for health benefits.

As a potent natural antihistamine and member of the flavonoid group, quercetin inhibits the release of itch- and sting-causing substances, typical symptoms of allergies leading to nasal congestion, eye irritation, and stinging. Acting as red and blue pigments in plants, flavonoids like quercetin offer protective effects to humans and regulate immune responses. With immune-regulating and anti-inflammatory properties, quercetin alleviates nasal congestion, eye itching, and sneezing in allergies, providing relief from discomfort due to pollen and other allergens. Its ability to mitigate allergic reactions makes it a valuable natural remedy for managing allergy-related symptoms. In addition, the flavonol subgroup to which quercetin belongs has anti-cancer properties. Adding quercetin to the diet can reduce the risk of cancer.

### **3.1.3. Tannin.**

Tea tannins, with a molecular formula of  $C_{15}H_{14}O_6$ , are extracted from catechu as colorless crystalline solids, soluble in water, and prone to polymerization into amorphous tannins when heated or in the presence of inorganic acids. While caffeine and theaflavins are essential components in tea, tea tannins are paramount in tea soup. Clinical studies have shown that tea tannins can improve metabolism, enhance fat oxidation, and boost energy consumption, thereby aiding in weight loss by inhibiting obesity, especially targeting visceral fat. This highlights the significant role of tea tannins in promoting weight loss and improving metabolic health [16].

## **3.2. Biological Functions and Applications of Tea Polyphenols**

### **3.2.1. Antioxidant.**

Tea polyphenols act as a potent reducing agent with a low oxidation-reduction potential, effectively neutralizing oxygen free radicals in the body by forming stable compounds. The phenolic hydroxyl groups within tea polyphenols's structure provide hydrogen, undergoing oxidation reactions to generate stable free radicals containing catechol, thus aiding in the removal of harmful free radicals. Studies show that tea polyphenols plays a crucial role in reducing breast epithelial thickness by clearing reactive oxygen species (ROS), lowering cellular malondialdehyde (MDA) content, and lactate dehydrogenase (LDH) activity. Tea polyphenols also boost superoxide dismutase (SOD) activity, reduces cell apoptosis, and diminishes lipid peroxidation, safeguarding bovine mammary epithelial cells against oxidative stress [17]. Furthermore, tea polyphenols work synergistically with vitamin E to enhance free radical elimination, demonstrating similar characteristics in inhibition time, proliferation rate, and antioxidant levels, thereby improving overall body antioxidant capacity.

There is a powerful antioxidant defense system within cells that can directly eliminate free radicals and maintain dynamic balance. Tea polyphenols work synergistically with a series of free radical related substances (such as SOD, CAT, GSH Px, vitamin E, vitamin C, glutathione, etc.) to enhance the antioxidant defense system function and protect the body from oxidative damage. Tea polyphenols inhibit the activities of oxidase such as cyclooxygenase (COX), lipoxygenase (LOX), and xanthine oxidase (XOG). Research has shown that tea polyphenols significantly increase the mRNA expression levels and protein synthesis of SOD, CAT, and GSH Px in cells, increases the activity of these antioxidant enzymes in the culture medium, effectively clears free radicals, and reduces mitochondrial damage caused by oxidative stress [18]. In addition, tea polyphenols affect the antioxidant defense system in the body by regulating the expression of transcription factor genes. Tea polyphenols can also protect the body from oxidative stress damage by regulating signaling pathways such as NF -  $\kappa$  B, Jun, and Fos [19]. Besides, tea polyphenols also have a protective effect on DNA damage, indirectly clearing excess oxygen free radicals by increasing the activity of antioxidant enzymes in the body, changing protein structure and function, etc., effectively reducing DNA damage and protecting cells from the effects of gene mutations and carcinogenesis.

### **3.2.2. Metabolic regulation.**

The polyphenolic substances in tea extracts, such as gallic acid and flavanols, can effectively prevent metabolic disorders induced by high sugar and high fat. This effect may be achieved by upregulating

the expression of antioxidant enzymes and reducing the expression of pro-inflammatory and pro oxidative markers in the body. Insulin resistance is one of the pathogenic factors of metabolic syndrome, and its mechanism involves glucose and lipid metabolism disorders, oxidative stress damage, and inflammatory response. Tea polyphenols can significantly improve the activity of alpha glucosidase, blood glucose content, lipid accumulation, and increase the expression of glucose transporter-4 in insulin resistant rat models, thereby increasing glucose uptake capacity. Tea polyphenols can also improve liver function, lower blood lipid levels, alleviate inflammation, and improve insulin resistance by regulating the liver cell transforming growth factor  $\beta$ /Smad signaling pathway [20]. The comprehensive research results show that tea polyphenols have a significant effect on the prevention and improvement of metabolic syndrome, which can protect liver function, reduce blood lipids, improve glucose metabolism, and alleviate inflammatory reactions through various pathways.

### **3.2.3. Antibacterial and anti-inflammatory.**

Tea polyphenols have good antibacterial properties against various Gram positive and negative bacteria in nature, and are widely used in food preservation and medical and health fields. Its antibacterial activity mainly manifests in disrupting microbial cell membrane permeability, disrupting microbial enzyme activity, inhibiting microbial genetic material synthesis, and inhibiting microbial ATP synthesis. Research has shown that tea polyphenols have significant inhibitory effects on microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* [21].

One of the main antibacterial mechanisms of tea polyphenols is to disrupt the permeability of bacterial cell membranes. Tea polyphenols can increase the permeability of bacterial cell membranes, leading to the efflux of intracellular substances and accelerating cell death. In addition, tea polyphenols also inhibit bacterial proliferation and growth by inhibiting the synthesis of key enzyme proteins in the bacterial body. Tea polyphenols can also inhibit energy synthesis in bacteria, such as inhibiting phosphorus metabolism levels, thereby causing bacteria to lose the necessary energy source for growth and leading to bacterial cell apoptosis [22].

### **3.2.4. Flavor impact.**

The taste and aroma of tea stem from the chemical components present in the tea leaves and are influenced significantly by the tea plant type and the production methods. Different types of tea (such as green tea, black tea, etc.) have unique aromas and flavors. Tea contains components such as tea polyphenols, caffeine, amino acids and stuff, which give tea a unique tea flavor. Catechins are the main phenolic compounds found in tea and are responsible for its bitter taste. This bitterness occurs when catechins react with proteins in the mouth to create impermeable substances, leading to astringency. From a sensory perspective, astringency is also produced by stimulating the tactile nerve endings. And simple catechins mainly produce a refreshing feeling in tea soup. By adding tea polyphenols, products can have a richer flavor and compensate for the lack of flavor caused by low sugar and fat.

## **4. Conclusion**

In summary, this study comprehensively explores the extraction methods, various biological functions, and potential applications of tea polyphenols. Firstly, the significance of this study lies in its contribution to understanding how different extraction methods, such as solvent extraction, supercritical fluid extraction, microwave assisted extraction, and ultrasound assisted hydrophilic extraction, can effectively separate tea polyphenols while considering factors such as purity, efficiency, and environmental impact. By providing detailed insights into these extraction techniques, this study not only supports current applications in industries ranging from pharmaceuticals to food production, but also proposes ways to improve extraction efficiency and sustainability. In addition, the main research findings emphasize that tea polyphenols, including catechins, flavonoids, and

tannins, have significant health benefits such as antioxidant, metabolic regulation, antibacterial, and anti-inflammatory effects. These bioactive compounds contribute to the positive impact of tea or tea products on human health through various mechanisms outlined in research. Although the methods discussed have advantages in purity or efficiency, they also have specific operational complexity and costs. Further research can delve into optimizing these methods to balance effectiveness and practical feasibility in large-scale production environments. Future research can explore the synergistic effects of tea polyphenols with other natural compounds or their specific interactions in human metabolic pathways. Investigating these fields can provide a more detailed understanding of the full potential of tea polyphenols in preventing and treating diseases, and promoting human health.

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