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# Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analyses*

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**Abstract:** Theaflavins (TFs) are the dimers of a couple of epimerized catechins, which are specially formed during black tea fermentation. To explore the differences among four main TF derivatives (theaflavin ($TF_1$), theaflavin-3-gallate ($TF_2A$), theaflavin-3′-gallate ($TF_2B$), and theaflavin-3,3′-digallate ($TF_3$)) in scavenging reactive oxygen species (ROS) in vitro, their properties of inhibiting superoxide, singlet oxygen, hydrogen peroxide, and the hydroxyl radical, and their effects on hydroxyl radical-induced DNA oxidative damage were systematically analyzed in the present study. The results show that, compared with (−)-epigallocatechin gallate (EGCG), TF derivatives were good antioxidants for scavenging ROS and preventing the hydroxyl radical-induced DNA damage in vitro. $TF_3$ was the most positive in scavenging hydrogen peroxide and hydroxyl radical, and $TF_1$ suppressed superoxide. Positive antioxidant capacities of $TF_2B$ on singlet oxygen, hydrogen peroxide, hydroxyl radical, and the hydroxyl radical-induced DNA damage in vitro were found. The differences between the antioxidant capacities of four main TF derivatives in relation to their chemical structures were also discussed. We suggest that these activity differences among TF derivatives would be beneficial to scavenge different ROS with therapeutic potential.

**Key words:** Theaflavin derivatives, Black tea, Antioxidant capacity, DNA oxidative damage
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## 1 Introduction

There is a 5000-year history of tea as a beverage or a folk medicine. During the past two decades, its therapeutic potential and its effects on human health, such as preventing cancer (Bushman, 1998; Blumberg, 2003; Yang *et al*., 2009; Li *et al*., 2010) and cardiovascular disease (Trevisanato and Kim, 2000; Zhu *et al.*, 2006), have been extensively investigated in vitro and in vivo. The basic protecting mechanism of tea is due to its good antioxidative properties.

Tea and its chemical compounds are regarded as natural antioxidants. Tea polyphenols are particularly good in vivo antioxidants, due to their bipolar properties. Antioxidative properties of catechins have already been shown to inhibit free radical generation, scavenge free radicals, and chelate transition metal ions. In recent years, theaflavins (TFs) formed by the oxidation of a couple of epimerized catechins have been proposed to be prospective antioxidative agents.

TFs are the orange pigments and ‘mouthfeel’ compositions in brewed black tea, and account for 2%–6% of the dry weight of solids (Roberts, 1958; Balentine *et al*., 1997). To date, more than 28 TF derivatives have been isolated, and the most abundant

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TFs in black tea are theaflavin ($TF_1$), theaflavin-3-gallate ($TF_2A$), theaflavin-3′-gallate ($TF_2B$), and theaflavin-3,3′-digallate ($TF_3$) (Fig. 1). TF complexes were generally believed to be the major antioxidant constituents of black tea, inhibiting free radical generation (Miller *et al.*, 1996), inhibiting pro-oxidative enzyme activities (Lin Y.L. *et al.*, 1999; Lin J.K. *et al.*, 2000; Yang *et al.*, 2008), and chelating transition metal ions to prevent lipid peroxidation in vitro and in vivo (Rice-Evans *et al.*, 1997). $TF_3$ has also been shown to possess a higher antioxidative activity than catechins, including (−)-epigallocatechin gallate (EGCG) in HL-60 cells (Lin *et al.*, 2000; Yang *et al.*, 2008). However, the differences among the four main TF derivatives in scavenging reactive oxygen species (ROS) in vitro are still unknown, partly because of their low abundance and technical limitations in their separation process. The mixture of $TF_2A$ and $TF_2B$ was usually used in previous studies, due to the difficulty of separation of $TF_2A$ from $TF_2B$. In the present work, we used the optimized semi-preparative high performance liquid chromatography (HPLC) separation system, and efficiently obtained the four purified individual TFs. The scavenging abilities of these four TF derivatives for ROS (superoxide radical, singlet oxygen ($^1O_2$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (·OH)) were systematically investigated in vitro, and their abilities in preventing DNA damage from ·OH were also studied.

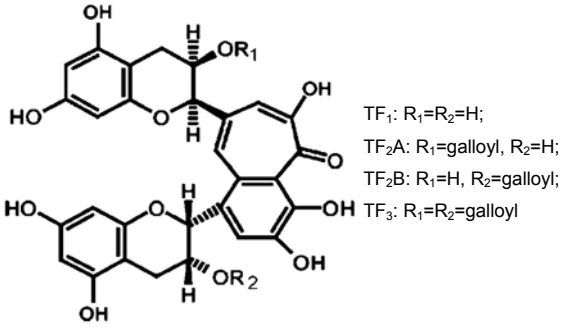


**Fig. 1 Chemical structures of four main TF derivatives**

## 2 Materials and methods

### 2.1 Preparations of $TF_1$, $TF_2A$, $TF_2B$, and $TF_3$

Enzymatic oxidation of immobilized polyphenol oxidase (PPO) on tea polyphenols (80% purity) was used to prepare TF compounds (50%, w/w) (Tu and Xia, 2004; Tu *et al*., 2005; Yang *et al*., 2007). Briefly, 100 ml of sodium alginate solution (2%, v/v) was mixed with 75 ml of PPO (1500 U) solution for 5 min, then injected into 1000 ml of 0.1 mol/L $CaCl_2$ solution. The granules formed after shaking for 30 min and were kept in 0.25 mg/ml glutaraldehyde aqueous solution for further aggregation. The oxidation reaction system contained 500 ml of tea polyphenols (5.95 mg/ml) and 15 g of immobilized PPO, and carried out at pH of 5.6 and 37 °C for 30 min with a stirrer. Then immobilized PPO was removed by filtering and a TF solution was obtained. The TF solution was applied to a Mitsubishi SP-207 resin column (5.5 cm×100 cm) with a gradient elution of 20%–70% aqueous ethanol to obtain a TF mixture of 80% purity. These TF mixtures were further purified with a semi-preparative HPLC following our previous method (Xu *et al.*, 2010). In total, 7 mg of 93.02% $TF_1$, 4 mg of 92.48% $TF_2A$, 4 mg of 92.40% $TF_2B$, and 8 mg of 90.05% $TF_3$ were obtained from 30 mg of TFs of 80% purity.

### 2.2 Scavenging activities of $TF_1$, $TF_2A$, $TF_2B$, and $TF_3$ on different ROS

The ROS-scavenging activity of TF individuals was assayed by the chemiluminescence method, as described previously (Tian *et al*., 2004), with some modifications.

#### 2.2.1 Superoxide radical assay

Superoxide anions were generated from a pyrogallol auto-oxidation system. The reaction mixture was composed of 50 μl pyrogallol (0.3 mmol/L), 650 μl carbonic acid-buffered saline solution (CBSS, pH 10.2) containing 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA), and 50 μl test sample at different concentrations. A total of 280 μl luminol (1 mmol/L) was added to trigger the chemiluminescence reaction. The chemiluminescence intensity (CL) was simultaneously recorded on the Sirius luminometer (Berthold Detection Systems GmbH, Germany) working with a photon counter (370–630 nm) every 6 s (CBSS instead of specimen was present in the control). The scavenging activity (SA) was calculated according to the Eq. (1):

$$SA=[(CL_c-CL_0)-(CL_s-CL_0)]\times 100\%/(CL_c-CL_0), \quad (1)$$

where $CL_c$ is the luminosity of control, $CL_0$ is the luminosity of background, and $CL_s$ is the luminosity of test samples.

2.2.2 Singlet oxygen ($^1O_2$) assay

For assaying the scavenging activity of $^1O_2$, $^1O_2$ was generated by Mallet reaction and measured with the method described by Deby-Dupont *et al*. (1998) and Yu and Zhao (2005). $^1O_2$ was generated chemically by the reaction between NaOCl and $H_2O_2$ in a solution (pH 8.0) at room temperature. The reaction mixture contained 600 μl of 50 mmol/L phosphate buffer (pH 8.0), 50 μl of the test sample or deionized water (as control), and 250 μl of $H_2O_2$ (10 mmol/L). Then the reaction started with the loading of 20 μl of 0.1 mmol/L NaOCl, and luminosity was monitored immediately for 90 s on the Sirius luminometer, and then was integrated. Scavenging activity was calculated by Eq. (1).

2.2.3 Hydrogen peroxide ($H_2O_2$) assay

For assaying the scavenging activity of $H_2O_2$, the reaction mixture contained 200 μl of 50 mmol/L phosphate buffer (pH 8.0), 10 μl of $H_2O_2$ (3.33 mmol/L), and 10 μl of the test sample or deionized water (as control). A total of 50 μl of 0.1 mmol/L luminol was added to trigger the chemiluminescence reaction. Luminescence was measured every 0.6 s. The amounts of luminosity (total counts for 90 s) were integrated. Scavenging activity was calculated by Eq. (1).

2.2.4 Hydroxyl radical (·OH) assay

·OH was generated by a Fenton-type reaction. The reaction mixture included 20 μl $FeCl_2$ (1 mmol/L), 30 μl 1,10-phenanthroline (1 mmol/L), 800 μl CBSS, 50 μl $H_2O_2$ (0.2 mol/L), and 20 μl of the test sample (deionized water as control). The CL was simultaneously recorded in the processor and then was recorded once every 6 s. Scavenging activity was obtained according to Eq. (1).

### 2.3 Efficiencies of four TF derivatives in preventing the hydroxyl radical-induced DNA damage

The efficiency of samples to reduce the extent of DNA damage induced by ·OH in vitro was conducted according to the method of Tian *et al*. (2004). The reaction included 0.5 μl of pUC19 DNA (0.25 μg) in 1.5 μl of 50 mmol/L phosphate buffer (pH 7.4), 1.0 μl of 2 mmol/L $FeSO_4$, and 6.0 μl of the test samples. After 3 μl of 1 mol/L $H_2O_2$ were added, the mixture was incubated at 37 °C for 1 h. Samples were subjected to 0.8% agarose gel electrophoresis for 1 h at 90 V. Experiments were performed three times. The DNA fractions (supercoiled (SC), linear, and open circular (OC)) were stained with ethidium bromide and quantified by scanning the intensity of bands with Quantity One program (Version 4.2.3, BioRad Co., USA). The increase or decrease in the percentage of SC forms compared to that of control was analyzed, representing the protective effects on DNA. All of the experiments were conducted in darkness to avoid the effects of photo-excitation on samples.

### 2.4 Statistical analysis

All experiments were performed in triplicate. SAS 8.0 (SAS Institute, Cary, NC, USA) for windows was used for statistical analysis. The results were expressed as mean±standard deviation (SD). The data were analyzed with a one-way analysis of variance (ANOVA) and differences between treatment means were separated by Turkey's test at the $P<0.05$ and $P<0.01$.

## 3 Results

The abilities of four TF derivatives on ROS inhibition were systematically analyzed with the described methods in Section 2.2. Four purified TF derivatives were prepared at different concentrations for further analysis. EGCG, the most abundant and effective compound in green tea polyphenols, was used as a control. The half maximal inhibitory concentration ($IC_{50}$) was determined from the plotted graph of scavenging activity versus the concentration of test samples, which was used to measure the efficiencies of different compounds in inhibiting ROS.

### 3.1 Effects of TF derivatives on the superoxide radical

Superoxide is the precursor of many excited and toxic oxygen species. Usually, superoxide anions are transformed to $H_2O_2$ by superoxide dismutase (SOD) and SOD-like antioxidants; otherwise oxidative damage would occur. In this work, a pyrogallol auto-oxidation system, used for superoxide anion

generation, and chemiluminescence signal determination were applied for testing the inhibition effects of four main TF derivatives on superoxide anions.

The effects of the test samples on the superoxide radical were analyzed, and the dose-response equations and $IC_{50}$ values for TF derivatives and EGCG are shown in Table 1. The degree of superoxide radical scavenging increased as the sample concentrations increased. Their dose-dependent effects on superoxide anion quenching were detected at the range of 5.00–50.00 μmol/L. Among all test samples, EGCG had the highest $IC_{50}$ value of 45.80 μmol/L, which presented the weakest inhibition of superoxide radical. All TF derivatives had lower $IC_{50}$ values than EGCG. Among the TF derivatives, $TF_1$ was the most efficient superoxide inhibitor with an $IC_{50}$ value of 14.50 μmol/L, only 31.66% that of EGCG. The $IC_{50}$ values of TF derivatives with monogallate and digallate were 40.61% to 58.30% that of EGCG. The monogallate TFs were more efficient than $TF_3$ in superoxide radical scavenging, with the scavenging capacity of $TF_2B$>$TF_2A$>$TF_3$.

### 3.2 Effects of TF derivatives on singlet oxygen

$^1O_2$ is related to oxidation of low-density lipoprotein (LDL) cholesterol and resultant cardiovascular effects (Wang and Xing, 2002). The generation of $^1O_2$ from lipid hydroperoxides involved a cyclic mechanism from a linear tetraoxide intermediate (Miyamoto *et al*., 2003). It is unstable and deleterious, and usually occurs from the degradation of biological systems. Three independent experiments were performed to determine the effect of the test samples on $^1O_2$ over a wide concentration range. All samples could scavenge $^1O_2$ at low concentrations, and their inhibition abilities were detected to increase with the increasing concentration from 0.50 to 10.00 μmol/L (Table 2). The $IC_{50}$ value of EGCG was 0.87 μmol/L, it was almost similar to that of $TF_2A$. $TF_2B$ had the lowest $IC_{50}$ ($^1O_2$) value of 0.55 μmol/L, which was 63.22% that of EGCG. The scavenging ability of $^1O_2$ decreased in the order of $TF_2B$>$TF_1$>$TF_3$>$TF_2A$>EGCG. $TF_2B$ had the strongest scavenging activity of $^1O_2$ among all TF derivatives.

### 3.3 Effects of TF derivatives on hydrogen peroxide and the hydroxyl radical

·OH is one of the most harmful radicals in nature. It can cause damage by cell oxidation, particularly erythrocytes (or red blood cells), DNA, lipids, and proteins. The scavenging activities of TF derivatives on $H_2O_2$ and ·OH were determined

**Table 1 Inhibition capacities of four main TF derivatives on the superoxide radical**

| Sample | Regression equation | $R^2$ | Linear range (μmol/L) | $IC_{50}$ (μmol/L) |
|---|---|---|---|---|
| EGCG | $y$=0.2059ln$x$−0.2875 | 0.9674** | 5.00–50.00 | 45.80 |
| $TF_1$ | $y$=0.2403ln$x$−0.1424 | 0.9936** | 5.00–50.00 | 14.50 |
| $TF_2A$ | $y$=0.2412ln$x$−0.2421 | 0.9858** | 5.00–50.00 | 21.70 |
| $TF_2B$ | $y$=0.2555ln$x$−0.2472 | 0.9956** | 5.00–50.00 | 18.60 |
| $TF_3$ | $y$=0.2551ln$x$−0.3382 | 0.9925** | 5.00–50.00 | 26.70 |

$y$: inhibition rate (%); $x$: corresponding concentrations of different samples. ** $P$<0.01

**Table 2 Inhibition capacities of four main TF derivatives on singlet oxygen**

| Sample | Regression equation | $R^2$ | Linear range (μmol/L) | $IC_{50}$ (μmol/L) |
|---|---|---|---|---|
| EGCG | $y$=0.1768ln$x$+0.5239 | 0.9807** | 0.50–10.00 | 0.87 |
| $TF_1$ | $y$=0.1699ln$x$+0.5535 | 0.9816** | 0.50–10.00 | 0.73 |
| $TF_2A$ | $y$=0.1741ln$x$+0.5268 | 0.9843** | 0.50–10.00 | 0.86 |
| $TF_2B$ | $y$=0.1663ln$x$+0.5982 | 0.9598** | 0.50–10.00 | 0.55 |
| $TF_3$ | $y$=0.1740ln$x$+0.5329 | 0.9809** | 0.50–10.00 | 0.83 |

$y$: inhibition rate (%); $x$: corresponding concentrations of different samples. ** $P$<0.01

with the chemiluminescence assay in vitro (Tables 3 and 4). The effects of four main TF derivatives and EGCG on $H_2O_2$ and ·OH increased in a dose-dependent manner. All TF derivatives efficiently inhibited $H_2O_2$ at low concentrations from 0.50 to 10.00 μmol/L (Table 3), while they were effective on scavenging ·OH at the lowest concentration of 10.00 μmol/L (Table 4). Most TF derivatives had higher scavenging capacities on $H_2O_2$ and ·OH than EGCG. $TF_2B$ was found to be equally effective to $TF_3$ with the $IC_{50}$ value of 0.39 μmol/L for quenching $H_2O_2$. The capacities of scavenging $H_2O_2$ were $TF_3$=$TF_2B$>$TF_2A$>$TF_1$>EGCG (Table 3). Meanwhile, $TF_3$ was found to be the most effective ·OH scavenger, followed by $TF_2B$, $TF_2A$, EGCG, and $TF_1$ (Table 4).

### 3.4 Protective effects of TF derivatives from the hydroxyl radical-induced DNA damage

A ·OH-induced DNA breaking system was applied to test the effects of TF derivatives on DNA oxidative damage in vitro. When the SC plasmid DNA is attacked by a ·OH generated from the Fenton reaction, it will be transformed into three forms: SC, OC, and linear. The OC DNA and linear DNA represent the damaged DNA. The extent of undamaged DNA is represented by the percentage of the SC form in DNA bands. The antioxidant effects of the test samples were demonstrated by comparing the percentages of the SC DNA in the test samples and in the control (DNA treated with ·OH). The conversion of the SC form of DNA to OC DNA and linear DNA has already been used as an index of DNA damage (Lewis *et al*., 1988).

The effects of different TF derivatives on ·OH-induced DNA damage were compared in parallel, and EGCG was also used for comparison (Fig. 2a). pUC19 plasmid DNA (SC form 95.23%) was broken into OC and linear forms by ·OH generated with 35.31% SC form being kept. However, there was no significant damage on DNA when being treated by $H_2O_2$ or $Fe^{2+}$ (Tian and Hua, 2005). Except for $TF_1$, there were significantly protective effects by $TF_2A$, $TF_2B$, and $TF_3$ compared with EGCG ($P$<0.05). The protective effects of TFs ($TF_2A$, $TF_2B$, and $TF_3$) on DNA could be inferred from the higher recovery (50.60%, 54.43%, and 52.07%) of the SC form (Fig. 2b). This result was also consistent with chemiluminescence assay result of their scavenging abilities on ·OH.

**Table 3 Inhibition capacities of four main TF derivatives on hydrogen peroxide**

| Sample | Regression equation | $R^2$ | Linear range (μmol/L) | $IC_{50}$ (μmol/L) |
|---|---|---|---|---|
| EGCG | $y$=0.1598ln$x$+0.5653 | 0.9632** | 0.50–10.00 | 0.66 |
| $TF_1$ | $y$=0.1550ln$x$+0.6101 | 0.9531** | 0.50–10.00 | 0.49 |
| $TF_2A$ | $y$=0.1521ln$x$+0.6227 | 0.9725** | 0.50–10.00 | 0.45 |
| $TF_2B$ | $y$=0.1566ln$x$+0.6484 | 0.9539** | 0.50–10.00 | 0.39 |
| $TF_3$ | $y$=0.1562ln$x$+0.6452 | 0.9500** | 0.50–10.00 | 0.39 |

$y$: inhibition rate (%); $x$: corresponding concentrations of different samples. ** $P$<0.01

**Table 4 Inhibition capacities of four main TF derivatives on the hydroxyl radical**

| Sample | Regression equation | $R^2$ | Linear range (μmol/L) | $IC_{50}$ (μmol/L) |
|---|---|---|---|---|
| EGCG | $y$=0.2866ln$x$−0.5171 | 0.9935** | 10.00–50.00 | 34.77 |
| $TF_1$ | $y$=0.2727ln$x$−0.4917 | 0.9666** | 10.00–50.00 | 37.96 |
| $TF_2A$ | $y$=0.2850ln$x$−0.4921 | 0.9994** | 10.00–50.00 | 32.49 |
| $TF_2B$ | $y$=0.2998ln$x$−0.4972 | 0.9962** | 10.00–50.00 | 27.83 |
| $TF_3$ | $y$=0.3197ln$x$−0.5300 | 0.9826** | 10.00–50.00 | 25.07 |

$y$: inhibition rate (%); $x$: corresponding concentrations of different samples. ** $P$<0.01

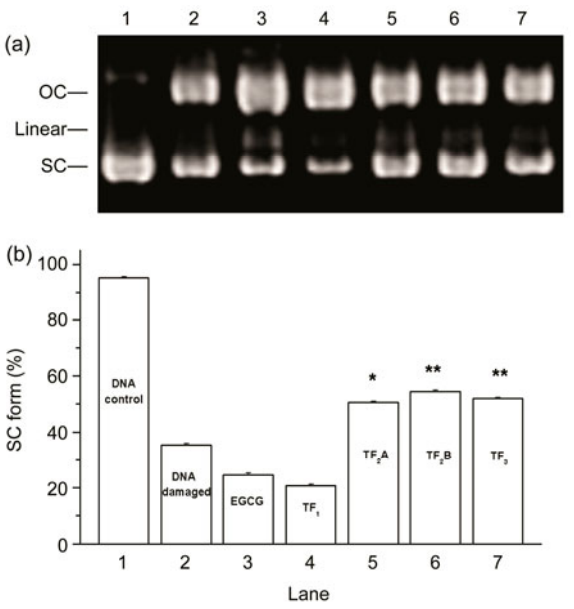


**Fig. 2 Agarose gel electrophoretic analysis of ·OH-induced plasmid DNA damage with different samples**

(a) Agarose gel electrophoresis of different treatments. Lane 1: plasmid DNA; Lane 2: DNA damage control (DNA treated with $FeSO_4$ and $H_2O_2$), in which 0.25 μg of pUC19 DNA was incubated with 2 mmol/L $FeSO_4$ and 1 mol/L $H_2O_2$ at 37 °C for 1 h; Lanes 3–7: DNA treated with $FeSO_4$ and $H_2O_2$ in the presence of EGCG, $TF_1$, $TF_2A$, $TF_2B$, and $TF_3$ at a concentration of 0.025 mmol/L. (b) Comparison of percentages of supercoiled (SC) form in the test samples to DNA damage control. Experiments were performed three times and the values are represented as mean±SD. * $P<0.05$, ** $P<0.01$, compared with EGCG

## 4 Discussion and conclusions

ROS including $H_2O_2$, ·OH, $^1O_2$, and superoxide anions are deleterious when they are excessive and cannot be removed by antioxidant enzymes, such as SOD, catalase, glutathione peroxidase, thioredoxin, and thioredoxin reductase. ROS accelerate membrane damage, DNA base oxidation, DNA strand break, and chromosome aberration. They also play a causative role in aging and several degenerative diseases, such as cancer (Hussain *et al.*, 2003), atherosclerosis (Cooke *et al.*, 2003), and cataract (Finkel and Holbrook, 2000). The supplement of antioxidants, such as vitamin E and vitamin C from foods and beverages has been an attractive therapeutic strategy for reducing the risk of these diseases.

Black tea is widely preferred in India and the Western countries, not only as a popular beverage, but also as an antioxidative agent. It is generally believed that polyphenols such as TFs and thearubigins, as well as catechins, as major constituents of black tea, are mainly responsible for antioxidant actions. Since the abundant constituent of catechins, EGCG has been proved to be the most effective antioxidant among green tea polyphenols. Its superiority is attributed to the numbers of hydroxyl groups in its chemical structure.

TFs, the characteristic compositions in black tea, are formed via the co-oxidation of pairs of epimerized catechins (Fig. 3), one with a vic-trihydroxyphenyl moiety, and the other with an ortho-dihydroxyphenyl structure. Different from epimerized catechins, four main TF derivatives reserve two A-rings, two C-rings from their precursors, and possess a characteristic element of the fused seven-member benzotropolone ring (Fig. 1). It has been suggested that the existence of resonance formed in the benzotropolone moiety might be responsible for electron donation (Jovanovic *et al*., 1997). Jhoo *et al*. (2005) suggested that the benzotropolone moiety of TFs might play an important role in affording antioxidant protection for the preferred oxidation site in the oxidant models of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and $H_2O_2$. Accordingly, we suggest that the higher number of phenyl hydroxyl groups in TF derivatives which could interact with ROS might increase their antioxidant capacities, and their benzotropolone moiety might have played an important role in scavenging the superoxide radical, $^1O_2$, $H_2O_2$, and ·OH in our in vitro models.

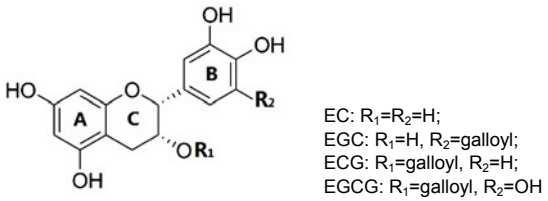


**Fig. 3 Chemical structures of epimerized catechins**

It is noteworthy that four major TF derivatives were detected to have different antioxidant capacities for different ROS. $TF_3$ containing two gallate groups has been reported to be a prior inhibitor on the ABTS radical (Miller *et al*., 1996), DPPH radical (Yang *et al*., 2008), and $Cu^{2+}$-mediated LDL oxidation (Leung

*et al*., 2001). Consistent with previous works, we found that $TF_3$ could efficiently scavenge $H_2O_2$ and ·OH with the lowest $IC_{50}$ values (Tables 3 and 4). Similarly, $TF_2A$ and $TF_2B$, which have one gallate group, had stronger activities than $TF_1$, which has no gallate group. The gallate ester seems to be positive in reacting with $H_2O_2$ and ·OH.

Meanwhile, the difference in antioxidant activities between $TF_2A$ and $TF_2B$ was observed in the present work. $TF_2A$ and $TF_2B$ are a pair of monogallate TFs with the same basic skeleton and one gallate ester in different positions (Fig. 1). They were thought to have equal bioactivities for the similar structure. Actually, $TF_2B$ showed the highest activity in suppressing chemiluminescence signal of $^1O_2$ (Table 2). And it had more effective antioxidant capacities in inhibiting the superoxide radical, $H_2O_2$ and ·OH than $TF_2A$. Therefore, the monogallate ester of 3′-position in TFs seems to play an important role in scavenging ROS, such as $^1O_2$, $H_2O_2$, and ·OH. Although the mechanism is still unknown, these results suggest that $TF_2B$ might possess a higher biological activity in other aspects.

Interestingly, compared with other three TF monomers, $TF_1$ was not so efficient in scavenging ·OH and free radicals arising from $H_2O_2$; however, it showed the most effective inhibition on the superoxide radical among four major TF derivatives and EGCG (Table 1). This result was highly consistent with their reaction rate order with superoxide anions: the reaction rates of TF derivatives with the superoxide radical were found to be almost an order of magnitude higher than that of gallocatechins, and $TF_1$ had the highest reaction rate of $1.0\times10^6$ $(mol/L)^{-1}s^{-1}$ (Jovanovic *et al*., 1997). The benzotropolone skeleton of TF derivatives was thought to be important in scavenging the superoxide radical with the capacities of charge separation and one-electron abstraction. The present work also provided the possibility that TF derivatives inhibit free radical-induced DNA oxidative damage, which is responsible for various diseases (Fig. 2). The preventive effects of TF derivatives on ·OH-induced DNA damage were in accord with their capacities of inhibiting ·OH. It is suggested that the positive role of TF derivatives in scavenging free radicals might contribute to their effects on DNA oxidative damage. Green tea polyphenols have been shown to directly interact with DNA radicals to repair DNA by a mechanism of electron transfer (Anderson *et al*., 2001). The precise mechanism of TF derivatives in preventing DNA oxidative damage is still unclear and worthy of further study.

In summary, the activity differences of four major TF derivatives provide some new insights into the antioxidant potency of these derivatives. More detailed studies of their antioxidant effects on ROS and DNA oxidative damage are required.

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