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*Research Article*

# Effect of Brewing Water on the Antioxidant Capacity of Green Tea Infusion with DPPH Assay

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Brewing water plays a crucial role in flavor and potential healthy functions of tea infusion. In this study, seven water samples with different physicochemical properties were selected to brew green tea. Results showed that the brewing water with higher minerals level and pH value would reduce the yield of catechins in tea infusion, which in turn caused the decrease of antioxidant activity to a large extent. Besides, it was found that EGCG, as a major contributor to the antioxidant activity of green tea infusion, was influenced differently by different metal ions, among which $Ca^{2+}$/$Mg^{2+}$ could enhance the antioxidant activity of EGCG solutions with different concentration through synergistic effect, particularly $Ca^{2+}$, and the effect was more markable at a higher EGCG concentration. These results offered theoretical direction to the selection of tea brewing water for consumers and gave a new sight to the effects of metal ions on the antioxidant capacity of EGCG.

## 1. Introduction

Now, apart from water, tea has become the most widely consumed beverage all over the world [1]. Green tea is a kind of unfermented tea and one of the most popular kinds of tea due to its distinct flavor and powerful healthcare functions [2–4]. The health benefits of green tea owe a great deal to its bioactive substances, such as theanine and catechins, which attract more and more researchers to study their various potential in healthy benefits [5, 6]. As a kind of efficient antioxidant, catechins have been applied to food preservation and medical treatment [7]. Antioxidant capacity of green tea is mostly originated from the total catechins, among which EGCG plays a dominant role.

However, the antioxidant activity of green tea is susceptible to various environmental conditions during processing, storage, and brewing, such as pH, temperature, and ions in brewing water [8–14]. This susceptibility is mainly due to the instability of catechins, including degradation, oxidation, and epimerization [9, 15].

On the one hand, pH is an important factor affecting the stability of catechins during tea brewing. Through the study of the reaction kinetics on the catechins of green tea infusion, Komatsu et al. [16] found that the catechins would degrade when pH was above 6.0, and they were more stable when pH was below 5.0. It indicated that the stability of catechins in aqueous solutions was pH-dependent to a great extent [9]. Usually, catechins were stable at weak acid condition (pH 3.0–5.0); when pH was lower or higher, the stability of catechins would be affected [17].

On the other hand, metal ions also act on catechins stability and antioxidant capacity of tea infusion. It was found that both pH and conductivity of brewing water could influence the antioxidant capacity of tea infusions negatively

[10]. Significant negative correlations between mineral concentration and the extraction yield of polyphenols in tea infusion were reported by Mossion et al. [18]. But the study pointed that the effect of metal ions on the antioxidant activity of EGCG solution was very limited.

This study aimed to investigate the effect of different types of water on the catechins concentration and the antioxidant capacity of green tea infusion and further explore the effect of metal ions on the antioxidant capacity of EGCG solution. Seven types of water with different pH value and metal ions were chosen for tea brewing. The differences of total catechins extraction yield and antioxidant capacity of green tea infusion brewed with different waters were compared. Afterwards, the addition of different metal ions was performed to investigate their influences on the antioxidant capacity of EGCG monomer solution. The results would reveal the influence of metal ions on the antioxidant capacity of catechins and tea infusion, which could provide knowledge and guidance on the selection of water when brewing green tea.

## 2. Materials and Methods

*2.1. Reagents.* 1,1-Diphenyl-2-picrylhydrazyl (DPPH), (−)-epigallocatechin gallate (EGCG), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma (Shanghai, China). Methanol, dried $CaCl_2$, $MgCl_2$, KCl, and NaCl were purchased from Shanghai Suke Chemical Co., Ltd. (Shanghai, China). Green tea was obtained from the Tea Research Institute of the Chinese Academy of Agricultural Sciences. Seven types of water used for the present study were obtained from different water sources: distilled water (DW) from Guangzhou Watson's Food & Beverage Co., Ltd. (Guangzhou, China); pure water (PW) from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China); tap water (TW) from Hangzhou; natural water (NW), Nongfu Spring, from Nongfu Spring Co., Ltd. (Hangzhou, China); mountain spring water (MSW), Hupao Cool Spring, from Hangzhou Tongsheng Water Co., Ltd. (Hangzhou, China); mineral water 1 (MW1) from Kunlun Mountains Mineral Water Co., Ltd. (Geermu, China); and mineral water 2 (MW2) from Tibet 5100 Water Resources Holdings Ltd. (Dangxiong, China).

*2.2. Preparation of Green Tea Infusion.* According to the Chinese national standards of tea sensory evaluation [19], the tea leaves were brewed with a leaf/water ratio of 1 : 50 (w/w) by using different boiling water (DW, PW, TW, NW, MSW, MW1, and MW2) for 5 min at room temperature (RT, $25 \pm 2°C$). The leaves were removed by filtration, and the infusion was quickly cooled in a cooling tank. Subsequently, the pH value, catechins concentrations, and antioxidant capacity of the tea infusion were determined. All treatments were performed in triplicate.

*2.3. Preparation of Metal Ions or EGCG Solutions with Different Concentrations.* The mixed solutions of EGCG (50 mg/L) and different metal ions (5, 10, 20, 40, and 80 mg/L) were used to study the effect of the types and concentrations of metal ions on the antioxidant activity of EGCG solutions.

The mixed solutions of EGCG (10, 20, 40, 50, 80, and 100 mg/L) and $Ca^{2+}/Mg^{2+}$ ions (10, 20, 40, and 80 mg/L) were used to study the effect of $Ca^{2+}/Mg^{2+}$ ions on the antioxidant activity of EGCG solution with different concentrations.

All the concentrations mentioned above were the final concentrations in (mixed) solutions, and all the sample solutions were prepared with pure water. The sample solutions obtained were used for conducting the analysis of antioxidant activity using DPPH assay in triplicate.

*2.4. Determination of Metal Ions and pH.* The metal ions in these seven types of water were determined by using Inductively Coupled Plasma Mass Spectrometry with a charge-injection device detector (ICP-MS, Thermo Jarrell Ash Corp., USA), as described in our previous work [20]. The detailed conditions were as follows: the maximum integration times of the low wavelength and high wavelength were set as 15 s and 5 s, respectively. The nebuliser pressure was set at 28 psi. The pump speed was 100 r/min. The auxiliary gas flow was set as medium (1 L/min), and the RF power was 1150 W.

The pH values of different waters and tea infusions were determined using a pH meter (SG2, Mettler-Toledo Instruments (Shanghai) Co., Ltd., Shanghai, China). The buffer solutions at pH 4.01 and 7.01 (Mettler-Toledo) were used to calibrate the pH meter.

*2.5. Determination of Catechins Concentration.* Catechins concentration in different tea infusions was determined by using high performance liquid chromatography (HPLC, Shimadzu LC-20A) with an ultraviolet detector (SPD-10A, Shimadzu Suzhou Corporation, Suzhou, China) [21]. The HPLC conditions were as follows: column: Diamonsil™ C18 column ($4.6 \times 250$ mm, 5 $\mu$m, Waters); injection volume: 10 $\mu$L; temperature: 35°C; mobile phase A: 2% acetic acid; mobile phase B: acetonitrile 100%. The following elution gradient plan was adopted: 0–16 min, 6.5% B; 16–25 min, 15% B; and 25–30 min, 6.5% B. Post-run-time was 5 min. The flow rate was 1 mL/min. The detection wavelength was set as 280 nm. Before injection, the cooled tea infusion should be filtered through the 0.22 $\mu$m Millipore filter.

*2.6. Determination of Antioxidant Activity.* The antioxidant capacity of the tea infusion or EGCG solution was determined using DPPH assay, following the method reported by Xu et al. [10]. The sample or Trolox standard of 100 $\mu$L was added to 3.9 mL of a DPPH stock solution ($6 \times 10^{-5}$ mol/L, in methanol), and the reaction was left for 2 h in the dark at RT. The absorbance of the reaction mixture was determined at 515 nm using a spectrophotometer (UV 2550, Shimadzu Suzhou Corporation, Suzhou, China). Methanol was used as the blank. The total antioxidant capacity was expressed as mg Trolox equivalents/L.

*2.7. Statistical Analysis.* All analyses were carried out in triplicate, and the results were recorded as mean ± standard deviation (SD). The analysis of significant differences ($p < 0.05$) between the means was performed by one-way analysis of variance (ANOVA) using SPSS statistics (version 16, SPSS Inc., Chicago, IL, USA), and all the figures were plotted by GraphPad Prism (version 9.00, GraphPad Software Inc., San Diego, CA).

## 3. Results and Discussion

*3.1. The Physicochemical Properties of Water Samples.* Seven types of water were chosen to brew green tea to understand the relationship of water and tea infusion in the present study. These types of water varied greatly in the mineral composition and pH, as shown in Table 1.

The types of water contained metal ions $Ca^{2+}$, $Cu^{2+}$, $K^{+}$, $Mg^{2+}$, and $Na^{+}$ with different concentrations, among which $Cu^{2+}$ was very little and could be ignored, whereas $Ca^{2+}$ was the dominant ion except only for MW2 (mineral water 2). It could be found that MW1 (mineral water 1) and MW2 had the most minerals with total ions concentrations of above 90 mg/L, followed by TW (tap water). MW1 had the highest concentrations of $Ca^{2+}$ and $Mg^{2+}$, while MW2 had the highest concentrations of $K^{+}$ and $Na^{+}$. The concentrations of these cations were almost all above 40 mg/L. TW also contained quite a lot of $Ca^{2+}$ with a concentration of 25.43 ± 1.17 mg/L. However, for DW (distilled water), the concentrations of above ions were very low and even zero, similar to PW (purified water).

The pH values of MW1 and MW2 were higher than 7.5, while those of DW and MSW (mountain spring water) were below 6.0, and other types of water were between 6.0 and 7.0. It was not difficult to find that the trend of pH was strikingly similar to the minerals content mentioned above. There were significant positive correlations between the pH values and the concentration of total ions ($r = 0.909$, $p < 0.01$), $Ca^{2+}$ ($r = 0.830$, $p < 0.05$), and $Na^{+}$ ($r = 0.852$, $p < 0.05$). We supposed that the obvious differences of pH could partly be attributed to such alkali ($K^{+}$ and $Na^{+}$) and alkaline earth ($Ca^{2+}$ and $Mg^{2+}$) metal ions in the types of water, and their existence could promote the alkalinity in aqueous solution. Plusquellec et al. [22] also found that higher pH would be obtained if alkali ions such as $Na^{+}$ and $K^{+}$ were present in the pore solution of concrete.

*3.2. Effect of Different Water Samples on Catechins Concentration and Antioxidant Capacity of Green Tea Infusion.* Just as mentioned above, the types of water selected in the present study varied greatly from each other in pH value, ranging from 5.17 to 7.97 (see Table 1). After tea brewing, the pH value of their corresponding tea infusion changed significantly and ranged from 6.06 to 6.96 (see Figure 1). It indicated that the gap between the pH values of different types of water was narrowed greatly after tea brewing. This phenomenon of neutralization may be caused by the tea brewing process. For one thing, the pH of water itself can get rise after heating, especially boiling, due to the substantial lessening of $CO_2$ dissolved in it [18]. For another, the extraction of some components from green tea, such as amino acids, phenols, and alkaloids, can also affect final pH of tea infusion differently. This may be attributed to the pH buffering capacity of green tea according to Vuong et al. [17], and similar results for black tea were also reported by Liang and Xu [23].

The concentrations of total catechins and EGCG in green tea infusion brewed with above types of water were much different (see Figures 2(a) and 2(b)). On the whole, the higher pH value of the water and the lower concentrations of the total catechins and EGCG in green tea infusions were found. In particular, the concentrations of total catechins and EGCG in the tea infusions brewed by MW1 and MW2 were greatly lower than those brewed by the other types of water. Similar results were also found in green tea infusion, oolong tea infusion, and black tea infusion brewed by mineral water [10], as well as even all the six types of traditional tea in China [24]. On one hand, it was due to the fact that the stability of catechins is pH-dependent [9, 10]. Because of the epimerization and degradation reaction at higher pH value [25], usually the catechins were unstable when pH > 6.0, and lowering the pH of tea infusion could help increase the stability of catechins in tea infusion [26–28]. On the other hand, metal ions in water also affected the existence of catechins. There were significant negative correlations between metal ions concentration and extraction yield of total catechins ($Ca^{2+}$: $r = -0.757$, $p < 0.05$; $Mg^{2+}$: $r = -0.939$, $p < 0.01$) and EGCG ($Ca^{2+}$: $r = -0.755$, $p < 0.05$; $Mg^{2+}$: $r = -0.949$, $p < 0.01$). Mossion et al. [18] reported that the higher the mineral content in the water, the lower the extraction yield of total polyphenols in tea infusion. Yasuda et al. [29] studied the HPLC peaks of catechins in the absence and presence of metal cations ($Cu^{2+}$, $Fe^{2+}$, and $Fe^{3+}$) and found that HPLC intensities of esterified catechins reduced markedly with the increasing of metal ions.

The results of total antioxidant activity, analyzed in DPPH assay, of tea infusions, are shown in Figure 2(c). Similarly, the significant differences ($p < 0.05$) between the tea infusions brewed with different types of water could also be found, and there was significant positive correlation ($r = 0.836$, $p < 0.05$) between the antioxidant capacity and the concentrations of the total catechins in green tea infusions, which indicated that the catechins were the main antioxidant components of the green tea infusion. Catechins, accounting for 70–80% of tea polyphenols, had been demonstrated to be the main antioxidant components of green tea infusion in previous studies [9, 10, 30].

However, it was worth noting that there were a few contradictions between the results of total catechins (see Figure 2(a)) and antioxidant activity (see Figure 2(c)), particularly for MW1 and MW2. The tea infusion of MW2 contained much more total catechins than MW1, while its antioxidant activity was significantly lower than that of MW1. It implied that the antioxidant activity of green tea was originated from catechins though, affected greatly by other factors as well, such as pH or ions of solution system. The influence of pH and metal ions of sample solution on the antioxidant capacity determined by DPPH assay had been

TABLE 1: The mineral concentrations (mg/L) and pH value of different types of water.

| Water | $Ca^{2+}$ | $Cu^{2+}$ | $K^{+}$ | $Mg^{2+}$ | $Na^{+}$ | pH |
|---|---|---|---|---|---|---|
| DW | $0.04 \pm 0.00^{f}$ | $0.00 \pm 0.00^{b}$ | $0.00 \pm 0.00^{f}$ | $0.08 \pm 0.04^{f}$ | $0.00 \pm 0.00^{f}$ | $5.17 \pm 0.07^{f}$ |
| PW | $0.08 \pm 0.04^{f}$ | $0.01 \pm 0.01^{b}$ | $0.01 \pm 0.01^{f}$ | $0.01 \pm 0.00^{g}$ | $0.00 \pm 0.00^{f}$ | $5.94 \pm 0.03^{d}$ |
| TW | $25.43 \pm 1.17^{b}$ | $0.05 \pm 0.00^{a}$ | $6.46 \pm 2.42^{b}$ | $3.49 \pm 0.20^{c}$ | $8.50 \pm 0.18^{c}$ | $6.83 \pm 0.08^{c}$ |
| NW | $13.22 \pm 0.61^{d}$ | $0.00 \pm 0.00^{b}$ | $1.04 \pm 0.01^{d}$ | $2.23 \pm 0.06^{d}$ | $1.92 \pm 0.03^{d}$ | $6.95 \pm 0.07^{c}$ |
| MSW | $3.72 \pm 0.17^{e}$ | $0.00 \pm 0.00^{b}$ | $0.22 \pm 0.13^{e}$ | $1.49 \pm 0.03^{e}$ | $0.83 \pm 0.01^{e}$ | $5.57 \pm 0.03^{e}$ |
| MW1 | $34.00 \pm 0.46^{a}$ | $0.00 \pm 0.00^{b}$ | $1.59 \pm 0.54^{c}$ | $38.06 \pm 0.73^{a}$ | $18.91 \pm 0.16^{b}$ | $7.63 \pm 0.08^{b}$ |
| MW2 | $18.87 \pm 1.02^{c}$ | $0.00 \pm 0.00^{b}$ | $28.48 \pm 0.42^{a}$ | $12.64 \pm 0.43^{b}$ | $32.60 \pm 0.42^{a}$ | $7.97 \pm 0.05^{a}$ |

DW: distilled water; PW: purified water; TW: tap water; NW: natural water; MSW: mountain spring water; MW1: mineral water 1; MW2: mineral water 2. Data are means (±SD) of three replicates. $^{a, b, c, d, e, f}$Different letters in the same column indicate significant differences between mean values ($p < 0.05$).

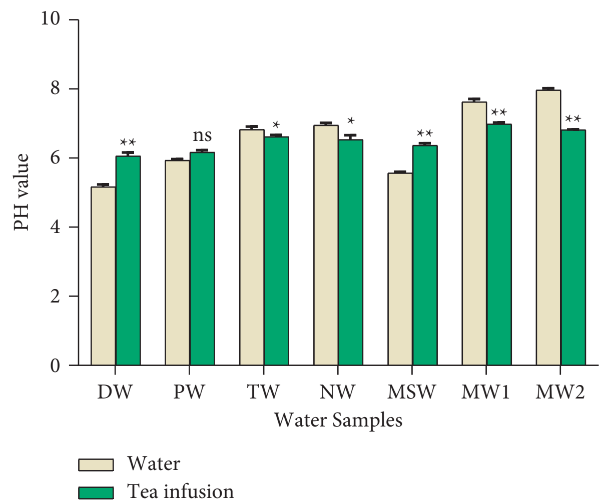



FIGURE 1: The pH values of types of water and tea infusions with corresponding water. DW: distilled water; PW: purified water; TW: tap water; NW: natural water; MSW: mountain spring water; MW1: mineral water 1; MW2: mineral water 2. The marks above the column show the significance of differences between water and tea infusion, ns indicates $p > 0.05$, $^{*}$indicates $p < 0.05$, and $^{**}$indicates $p < 0.01$.

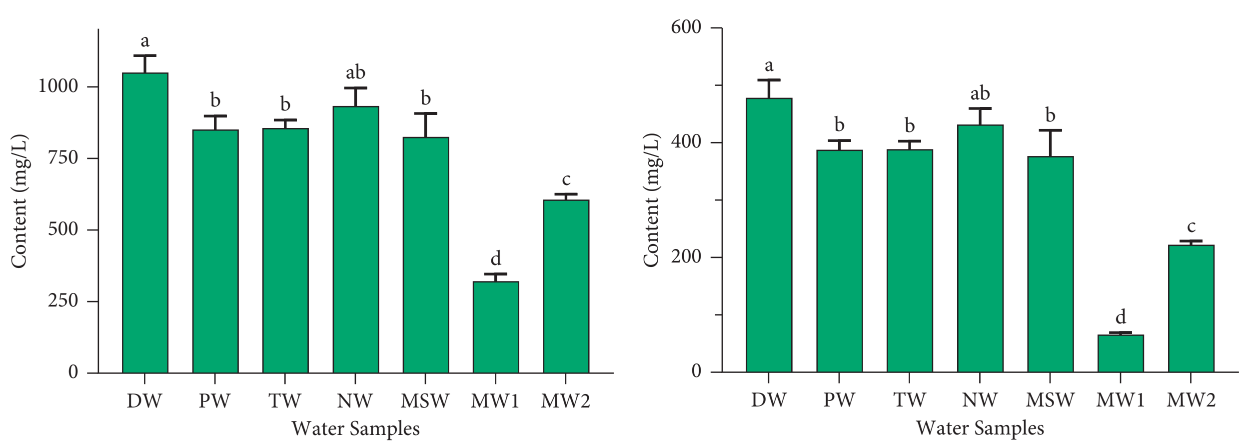
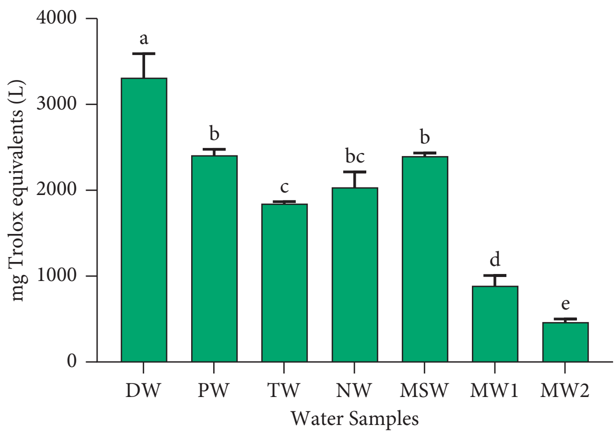



FIGURE 2: Continued.




(c)

Figure 2: The contents of total catechins (a), EGCG (b), and antioxidant activity (c) of tea infusions brewed with different types of water. DW: distilled water; PW: purified water; TW: tap water; NW: natural water; MSW: mountain spring water; MW1: mineral water 1; MW2: mineral water 2. [a,b,c,d]Different letters above the column indicate significant differences between tea infusions brewed with different types of water ($p < 0.05$).

reported widely [12, 31, 32]. Our study found that there were significant negative correlations between the DPPH antioxidant activity of tea infusions and the pH values of types of water ($r = -0.957$, $p < 0.001$), which confirmed the effect of pH. When it came to ions, the total ions concentrations of MW1 and MW2 did not show difference ($p > 0.05$) yet. But the dominant ions were $Ca^{2+}$ and $Mg^{2+}$ in MW1, whereas the dominant ions were $Na^{+}$ and $K^{+}$ in MW2 as mentioned above. Maybe different ions would exert different effects on the antioxidant activity. To illustrate it, here taking EGCG monomer as an example, the specific effect of different metal ions on the antioxidant activity of EGCG solution was further studied.

*3.3. Effect of Different Types of Metal Ions and Their Concentrations on the Antioxidant Capacity of EGCG Solutions.* Metal ions $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, and $K^{+}$ were selected to investigate their effects on the antioxidant capacity of EGCG solution of 50 mg/L, which was the dominant composition of catechins and one of the major antioxidant components in green tea [9, 10]. As shown in Figure 3, addition of $Ca^{2+}$ and $Mg^{2+}$ could enhance the antioxidant capacity of EGCG solutions of 50 mg/L with a dose effect, especially $Ca^{2+}$. The antioxidant capacity of EGCG solutions increased by 16% and 10% with addition of 80 mg/L $Ca^{2+}$ or $Mg^{2+}$. Similar results were also concluded by Kumamoto et al. [12]. They found that $Al^{3+}$, $Mg^{2+}$, $Mn^{2+}$, and $Cu^{2+}$ with $8.06 \times 10^{-6}$ M could increase the antioxidant capacity of EGCG solutions. However, the influence of different concentrations of metal ions was not reported in their study. Addition of $Na^{+}$ and $K^{+}$ was found to have little influence on the antioxidant capacity of EGCG solutions. The results could explain well the contradictions in Figure 2.

The differences in the effect of metal ions on the antioxidant capacity of EGCG solutions may be due to the formation of different metal complexes with catechins and the change in oxidation potentials [12]. The existence of metal ions (such as $Ca^{2+}$ and $Mg^{2+}$) would influence the correct evaluation for the antioxidant capacity of EGCG solutions or tea infusions. The higher concentrations of metal ions may result in higher antioxidant capacity.

*3.4. Effect of $Ca^{2+}$ and $Mg^{2+}$ on the Antioxidant Capacity of EGCG Solutions with Different Concentrations.* It seemed that $Ca^{2+}$ and $Mg^{2+}$ had potential for improving the antioxidant activity of EGCG solution. To confirm it, the effect of the two cations on the antioxidant activity of EGCG solutions with different concentrations (ranging 0–100 mg/L) was further investigated.

The cations of $Ca^{2+}$ and $Mg^{2+}$ themselves possessed certain antioxidant capacity, which increased with their concentrations (see Figures 4(a) and 4(b)). There were significant positive correlations between the DPPH values and the concentrations of $Ca^{2+}$ ($r = 0.982$, $p < 0.01$) and $Mg^{2+}$ ($r = 0.987$, $p < 0.01$). The significant positive correlations ($Ca^{2+}$: $r = 0.963$, $p < 0.01$; $Mg^{2+}$: $r = 0.904$, $p < 0.05$) still existed when EGCG was added to them, respectively. It indicated that the potential of $Ca^{2+}$ and $Mg^{2+}$ to improve the antioxidant capacity of EGCG may be just due to their additive effect with EGCG.

The effects of $Ca^{2+}$ and $Mg^{2+}$ at 40 mg/L on the antioxidant capacity of EGCG with different concentrations were also analyzed, as shown in Figure 5. It could be found that addition of $Ca^{2+}/Mg^{2+}$ (40 mg/L) greatly enhanced the antioxidant capacity of EGCG solution with different concentrations, particularly $Ca^{2+}$. The antioxidant capacity of EGCG solution rose by $51.58 \pm 6.02\%$ and $39.83 \pm 9.42\%$ on average due to the addition of $Ca^{2+}$ or $Mg^{2+}$, respectively. The higher the EGCG concentration was, the higher the rise rate was found. The results implied that $Ca^{2+}$ or $Mg^{2+}$ improved the antioxidant capacity of EGCG solution through synergistic effect rather than additive effect (Figure S1). Besides, the concentration of EGCG played a dominant role during the binary interactions. The

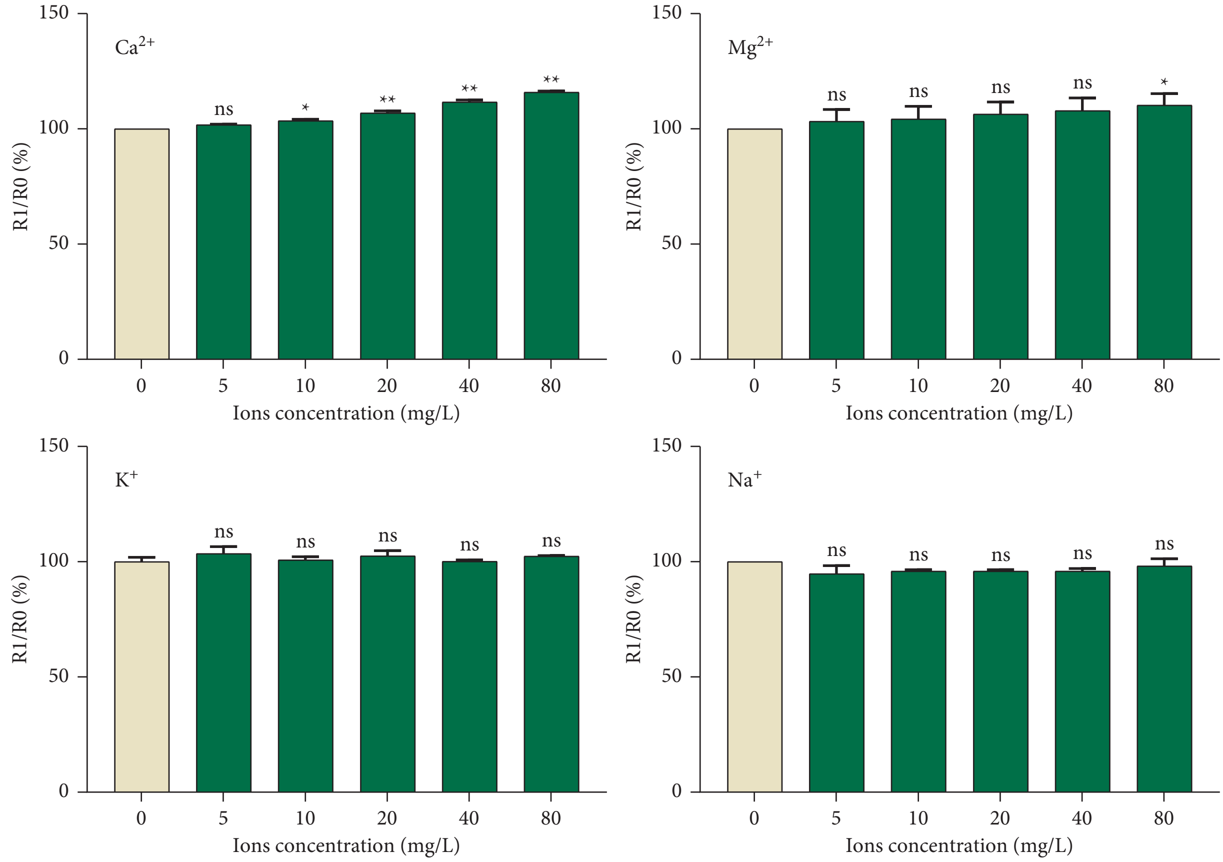



FIGURE 3: Effect of different metal ions on the antioxidant activity of EGCG solutions of 50 mg/L. $R1/R0$: the antioxidant activity of EGCG solution added with/without metal ion, respectively. The marks above the column show the significance of differences, ns indicates $p > 0.05$, $^{*}$indicates $p < 0.05$, and $^{**}$indicates $p < 0.01$.

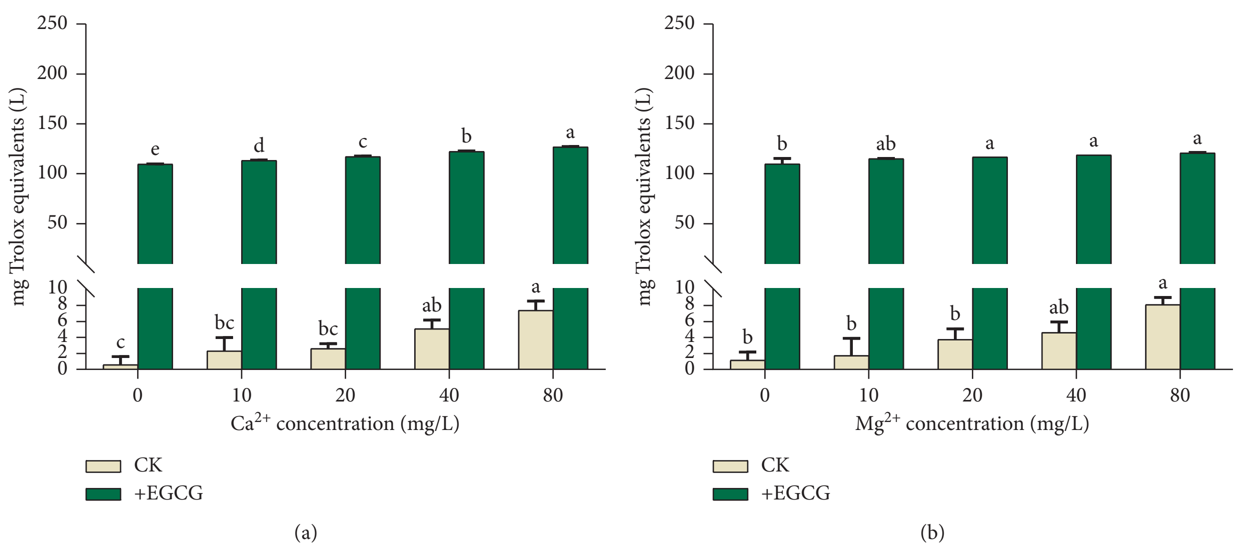



FIGURE 4: Effect of $Ca^{2+}$ (a) and $Mg^{2+}$ (b) with different concentrations on the antioxidant activity of EGCG solutions of 50 mg/L. CK: control check, ion solution without EGCG; +EGCG: ion solution with EGCG. [a,b,c,d]Different letters above the column indicate significant differences among different concentrations of ions ($p < 0.05$).

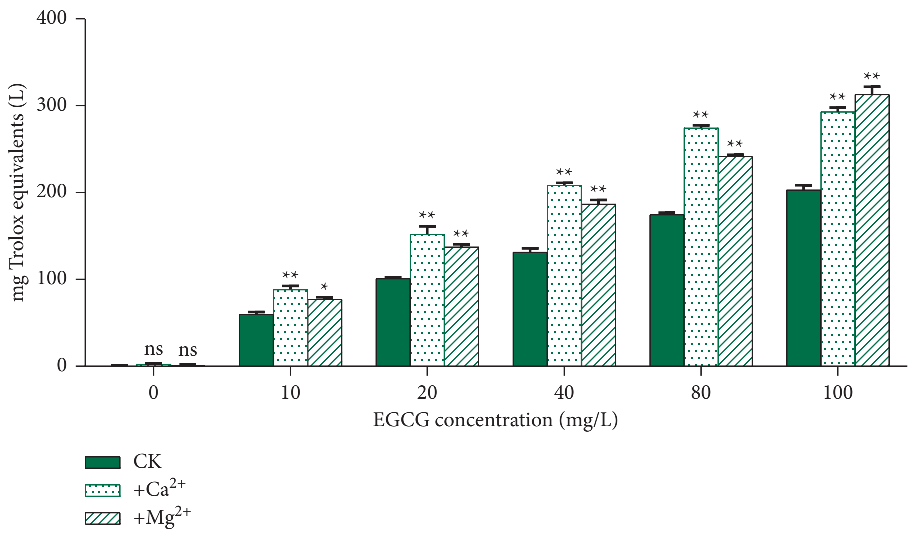



FIGURE 5: Effect of $Ca^{2+}$ and $Mg^{2+}$ of 40 mg/L on the antioxidant activity of EGCG solutions with different concentrations. CK: control check, EGCG solution without ion; +$Ca^{2+}$: EGCG solution with $Ca^{2+}$; +$Mg^{2+}$: EGCG solution with $Mg^{2+}$. The marks above the column show the significance of differences between CK with +$Ca^{2+}$ or +$Mg^{2+}$, ns indicates $p > 0.05$, $^*$indicates $p < 0.05$, and $^{**}$indicates $p < 0.01$.

synergistic effects were more remarkable at a higher concentration of EGCG.

## 4. Conclusions

The green tea infusions, prepared with different types of water, showed different antioxidant capacities. On one hand, brewing water affected the yield of total catechins, particularly EGCG, to impact on the antioxidant capacity of tea infusion indirectly. On the other hand, the antioxidant activity itself was influenced directly by various minerals in the brewing water. The results, from the monomer experiment of EGCG, showed that metal cations could act on the antioxidant capacity of EGCG solution in varied effects, among which $Ca^{2+}$ and $Mg^{2+}$ possessed the potential for enhancing antioxidant capacity of EGCG. Besides, $Ca^{2+}$ and $Mg^{2+}$ improved the antioxidant capacity of EGCG through synergistic effect, which was dominated by EGCG concentration. These results helped to understand the influence factors on the antioxidant capacity of catechins and tea infusion, which can guide the selection of brewing water for tea consumers.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

## Authors' Contributions

Qing-Qing Cao and Yan-Qing Fu contributed equally to this study.

## Acknowledgments

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## Supplementary Materials

Figure S1 is available in additional document of Supplementary Materials and it shows the synergistic effects of $Ca^{2+}$ and $Mg^{2+}$ of 40 mg/L on the antioxidant activity of EGCG solutions with different concentrations. (*Supplementary Materials*)

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