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Disinfection performance and synthesis conditions of the EGCG–Cu complex

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ABSTRACT

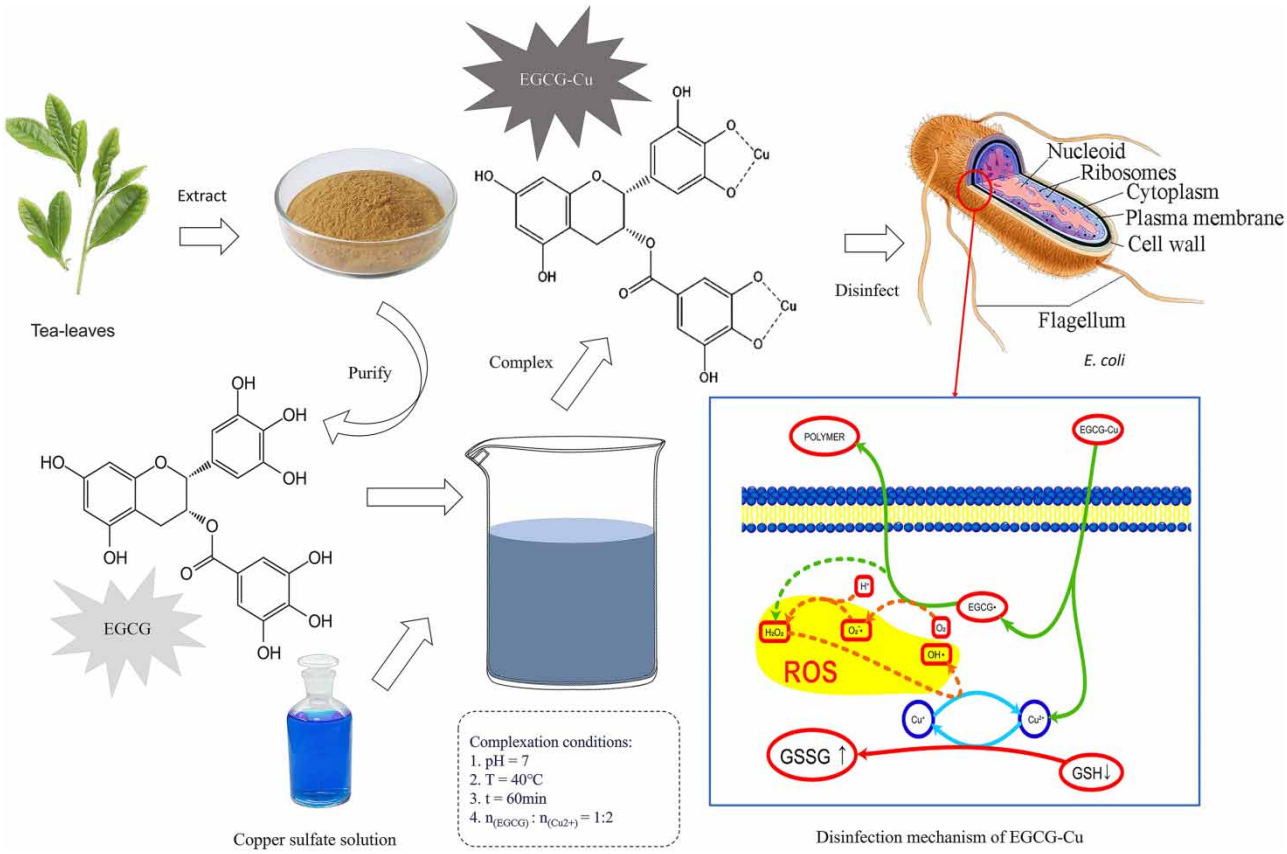
Adopting the yield of the epicatechin gallate (EGCG)–Cu complex as an indicator, the impact of four conditions, such as pH, reaction time, temperature and reactant ratio, on the synthesis of the target complex was analyzed to identify the optimal conditions on synthesizing the compound. The reactant ratio between EGCG and Cu^{2+} was explored, and the characteristic peaks and functional groups of the EGCG–Cu complex were scrutinized by UV–Visible and Fourier-Transform Infrared spectrophotometers. Finally, the efficacy difference of bactericidal properties against *Escherichia coli* suspensions between EGCG and its complex was evaluated as the criteria. Furthermore, comparative studies were performed to gauge the antimicrobial activity of EGCG and its complex at equivalent concentrations. The results demonstrated that the optimal experimental conditions for the complex reaction were a pH of 7, at 40 °C, in a reaction time of 60 min and a reactant ratio of 1:2. By the molar ratio method, the reactant ratio was determined as $n_{(EGCG)}$:$n_{(Cu2+)}$ = 1:2, and the complex reaction was at the phenolic hydroxyl group on the benzene ring of EGCG. Compared to EGCG, the complex demonstrated significant enhancement in bactericidal properties against *E. coli* and has lower chromaticity.

Key words: complex, copper ion, disinfection, drinking water, EGCG, molar ratio

HIGHLIGHTS

- The optimal parameters for complexation were determined.
- The absorbance curve showed that the complex ratio of EGCG and Cu^{2+} was 1:2.
- The changes in absorption peaks of functional groups indicated that EGCG's phenolic hydroxyl groups on the benzene ring formed complexes.
- Complexing Cu^{2+} to EGCG enhances its antibacterial properties.
- The EGCG–Cu complex solution has lower chromaticity than EGCG during disinfection.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Tea polyphenols serve as natural antimicrobial agents, displaying evident inhibitory effects on waterborne pathogenic bacteria including coliforms, *Staphylococcus aureus*, *Proteus* and *Shigella flexneri* (Feng *et al.* 2023; Zhang *et al.* 2023b). Numerous studies indicate that tea polyphenols possess antioxidant, anti-tumor, anti-hyperlipidemia and potent broad-spectrum antibacterial properties (Malik *et al.* 2003; Wen *et al.* 2023). Additionally, recent investigations have demonstrated that polyphenols and catechins can effectively suppress the proliferation of antibiotic resistance genes in aquatic environments (Yu *et al.* 2023). As such, tea polyphenols with their economic, eco-friendly and environmental friendly attributes have been proposed as novel disinfectants for drinking water (Cuimin *et al.* 2016). Catechin, constituting over 70% of total tea polyphenols, harbors epicatechin gallate (EGCG) a main ingredient with substantial bacteriostatic function (Yang *et al.* 2003, 2014). EGCG is acknowledged for its various therapeutic properties, including antibacterial, anticancer, antihypertensive, anticoagulant and antiulcer effects (Azam *et al.* 2004; Ganeshpurkar & Saluja 2020; Liczbiński & Bukowska 2022).

Nature always provides rich inspiration for the design of functional materials (Li *et al.* 2022; Zhang *et al.* 2023a). Polyphenols can affect cellular behavior due to their inherent functional characteristics such as antioxidant and photothermal behavior (Cao *et al.* 2022). In recent years, alongside evaluations of EGCG impact on organic substances, studies focused on the physiological ramifications between EGCG and metal ions. The structure of flavonoid is of a high degree of super-delocalization, and its expansive π conjugate structure renders it an ideal spatial configuration for metal ion chelation. When catechins interact with metal ions, complexation or redox reactions transpire, leading to alterations in their chemical structure. These transformations equip catechins with additional physiological and biochemical properties. The multi-metal complex plays a significant role in biological activities or contributes to new biological properties of catechins. Research indicates that Mn (II)–EGCG can push up EGCG and inhibit α-glucosidase, thereby reducing the risk of type 2 diabetes mellitus (Li *et al.* 2023). Fe^{3+}, as a catalytic medium in the Ni^{2+}-EGCG network, exhibits significant bactericidal effects and the ability to eradicate pathogenic biofilms (Chen *et al.* 2021). The interaction between EGCG and copper ions is primarily responsible

for cytomembrane damage in prostate cancer cells (Yu *et al.* 2007). Yoshioka *et al.* (2001) utilized spin trapping to identify the formation of hydroxyl radicals in the complexation system of EGC, EGCG, EC and copper ions, revealing that EGCG forms a complex with Cu^{2+}, which made the system divalent copper reduced to monovalent copper. This monovalent copper can react with both oxygen and hydrogen peroxide present in the solution, leading to the creation of highly reactive free radicals. These radicals demonstrate strong oxidizing capacity and are capable of inducing the DNA molecules' degradation through oxidative processes (Sun *et al.* 2011). Kumamoto *et al.* (2001) investigated the antioxidant activities of 13 metal ions after complexing with EGCG and discovered that catechins can be oxidized automatically in the presence of copper ions, while the complex of EGCG and Cu^{2+} exhibits enhanced antioxidant capacities under acidic conditions. Similarly, Hayakawa *et al.* (2004) found significant differences in the antioxidant capacities of EGCG and EGCG in the presence of Cu^{2+}. This disparity to the complexation ability of EGCG with metal ions forms a relatively stable complex and inhibits reactive oxygen species production. Holloway *et al.* (2015) observed that the heat-treated complex of catechins and Cu^{2+} demonstrated superior bactericidal efficacy than the non-heated complex. Therefore, this study selected Cu^{2+} as the central ion and EGCG as the ligand, employing the hydrothermal synthesis to investigate the bactericidal effects of synthesized substances on *Escherichia coli*, an indicator bacterium in drinking water, aiming to pave the path for the potential application of tea polyphenols and EGCG in the field of drinking water disinfection.

2. MATERIALS AND METHODS

The EGCG utilized in this experiment was purchased from Nanjing Guang-run Biological Products Co., Ltd, No. GR0799, with a molecular weight of 458.4 and purity over 98%, and was white-like dry powder. The strain of *E. coli* was from China Industrial Microbial Culture Collection Management Center, No. 10004. Copper sulfate, sodium bicarbonate, boric acid buffer, potassium bromide (spectrum pure), ethanol, sodium chloride and nutrient agar were also used for this experiment.

2.1. Determination of tea polyphenol concentration

The concentration of tea polyphenols in the clear liquid was tested using the ferrous tartrate method. The principle is that the ferrous tartrate solution will undergo complexation with tea polyphenols, forming a stable blue-purple complex. The color of the complex solution is related to the concentration of tea polyphenols. Based on this, a concentration–absorbance standard curve was created by tea polyphenol solutions of different concentrations. The absorbance was measured by a DR6000 spectrophotometer, the wavelength was set at 540 nm and a single-wavelength scan was performed. According to the absorbance at different concentrations of tea polyphenol solution in 0–1.0 mg/mL, a standard curve was created as shown in Figure 1.

2.2. Investigation of complexation conditions

Tea polyphenols (TPs), in which EGCG is the most abundant component substance, were chosen as a more cost-effective substitute to identify suitable complexation conditions. The desired complex was synthesized by the hydrothermal method. First, 1 mol/L of sodium bicarbonate buffer was prepared. Then 229.2 mg (0.5 mmol) of EGCG was dissolved in 30 mL of distilled water. The prepared sodium bicarbonate solution was used to adjust the pH to 7. One mmol of copper sulfate was dissolved in 10 mL of distilled water and stirred for 1 h in a water bath of 40°C. The mixed solution was filtered by vacuum (the pore size of filter membrane is 0.45 μm) and rinsed twice using a mixture of water and ethanol in the ratio of 1:1. The filter membrane containing the complex was put into a clean Petri dish, frozen at −50 °C for 30 min and then put into a vacuum freeze-drying oven. After overnight precipitation, the remaining TP content in the supernatant was used to calculate the yield of the complex. The calculation method is as follows:

$$\text{Complex yield} = (M - m)/M \times 100\%$$

where M represents the total mass of TPs in the solution and m represents the mass of remaining TPs in the supernatant after the reaction, which is calculated based on the concentration line of TPs in Figure 1.

2.3. Determination of the reactant ratio between EGCG and copper ions

The reactant ratio between EGCG and Cu^{2+} was examined by the molar ratio method (Liu *et al.* 2021). EGCG in 11.5 mg (0.025 mmol) was dissolved in 25 mL of distilled water and then 0.05 mol/L of borax solution and 0.2 mol/L of boric acid solution were prepared. Borate buffer with pH of 7.6 was composed of 1.5 mL of borax solution and 8.5 mL of boric acid

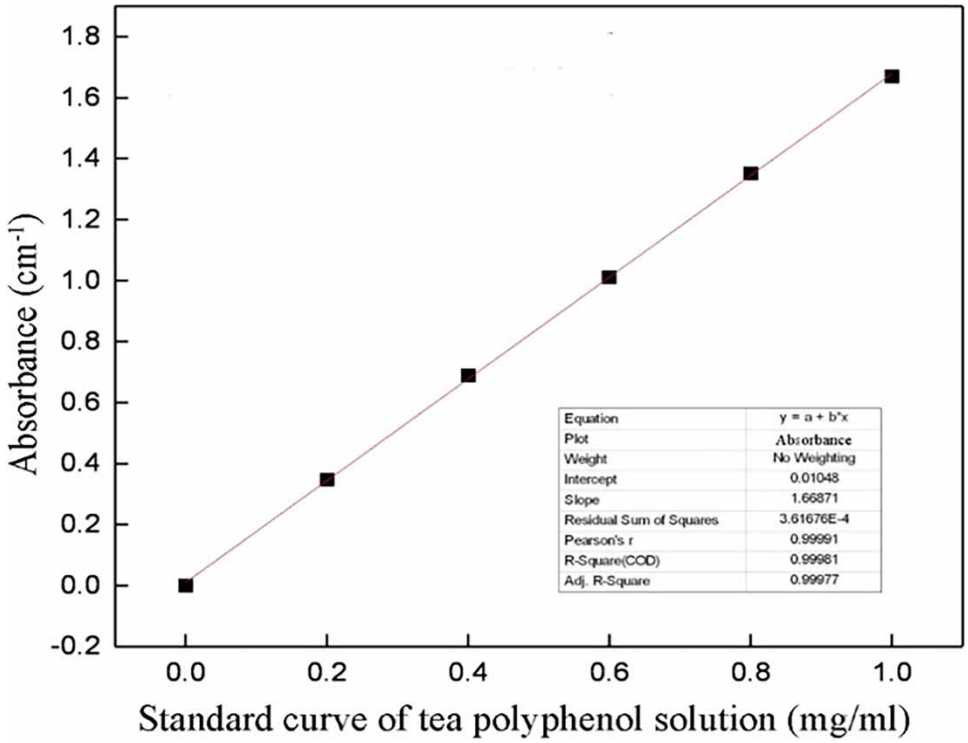


Figure 1 | Standard curve of TP solution.

solution. About 0.5 mL of EGCG stock solution was diluted to 10 mL of borate buffer, and 3 mL of the diluted solution was transferred to the quartz tube. Subsequently, the full spectrum of ultraviolet radiation was determined using a scanning interval of 190–600nm, the ABS scanning mode was selected, and the scanning step was set at 1 nm, the EGCG solution was scanned with a UV spectrophotometer at full wavelength, and 0.0025 mol/L $CuSO_4$ solution 20 μL, full band UV scanning was performed after each addition.

2.4. Analysis of the EGCG–Cu complex and the EGCG functional group

Infrared spectroscopy was carried out by the KBr pellet method (Ridgway & Aulton 1974). EGCG–Cu complex powder of 1–2 mg and spectral pure KBr of 200 mg were mixed evenly and made into a translucent sample by the tablet press, which was scanned by an infrared spectrometer with the air collected as the background, and the data were processed by OMNIC spectrum software.

2.5. Total number of colonies

According to the *Standard examination methods for drinking water – Microbiological parameters* (GB/T 5750.12-2023), the bacterial total count was determined by the plate counting method after serial dilution.

3. RESULTS AND DISCUSSION

3.1. Research on optimal complexation conditions

3.1.1. Effect of reaction pH on complexation

To probe the impact of reaction pH on complexation, the molar ratio of the reactants was maintained at 1:1. The pH of the solution was altered to 4.0, 5.0, 6.0, 7.0 and 8.0 with sodium hydrogen carbonate, and the reaction mixture was stirred for 30 min at room temperature by a magnetic stirrer.

According to the results presented in Figure 2, the conversion rate of the complex changed with the reaction pH within the experimental range. When the pH was lower than 7, the conversion rate of the complex rapidly increased with increasing pH, while when the pH was around 7, the conversion rate of the complex reached its highest value, which was 60.2%. Continuing

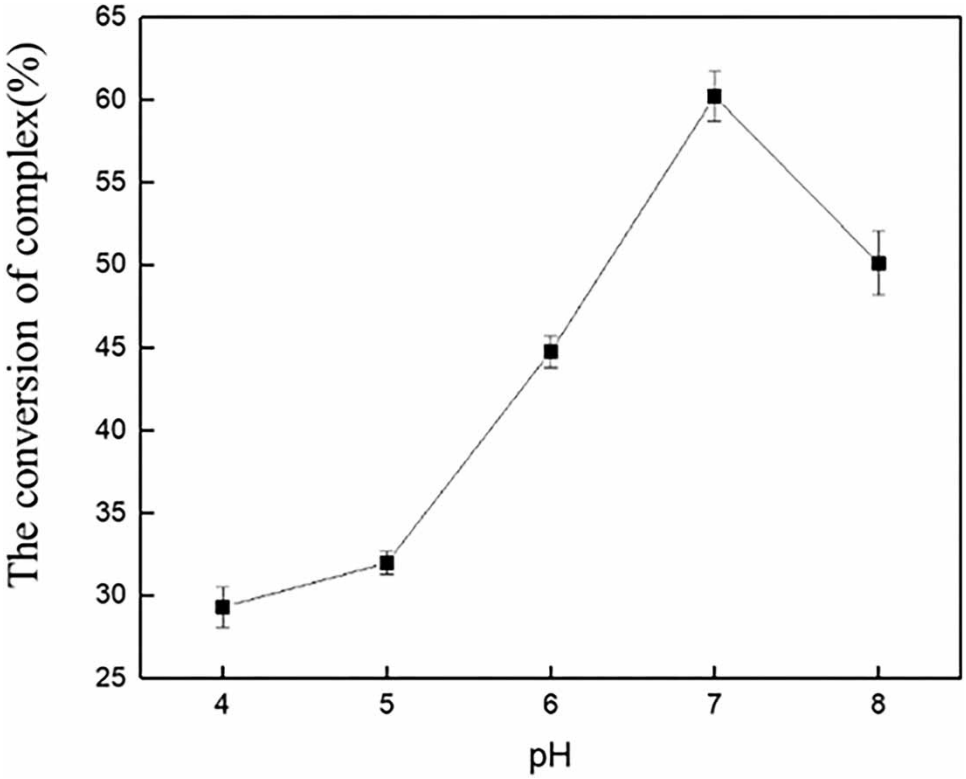


Figure 2 | Effect of reaction pH on the conversion rate of the complex.

to increase the pH of the TP solution, the conversion rate of the complex decreased, as the complexation of tea polyphenols with metal ions is mainly through the hydroxyl group on its benzene ring, and its phenolic hydroxyl group has an equilibrium state in water as shown in Figure 3. The complexation of TPs with metal ions primarily arises from the phenolic hydroxyl group on the benzene ring. The phenolic hydroxyl group in water exhibited the equilibrium: at low pH, the equilibrium was stable and toward to the left. The phenolic hydroxyl groups on TPs were in the undissociated state, making it difficult for TPs to act as electron donors and participate in reactions, and making it difficult for them to complex with metal ions. As pH increased, the equilibrium began to shift to the right, the dissociation degree of TPs in the solution increased and the complexation with metal ions also increased. However, when the alkalinity was too high, copper ions in the solution were easily converted to copper hydroxide, and the concentration of copper ions participating in the complexation reaction decreased, while the A-ring of catechins began to be oxidized to quinone-like substances (Youying 2010), leading to a reduction in the amount of TPs participating in the complexation reaction, which reflected as a decrease in complex yield. Therefore, in order to ensure a high conversion rate of the complex, too high or too low a pH level should be avoided as much as possible. Based on the experimental results, a pH of around 7 should be chosen.

3.1.2. Effect of reaction temperature on complexation

Controlling the molar ratio of reactants at 1:1 and maintaining a reaction pH of 7, the effect of reaction temperature on complex formation was investigated at 30, 40, 50, 60 and 70 °C with magnetic stirring for 30 min. The results are shown as follows.

It is evident from Figure 4 that the temperature does not significantly affect the conversion rate of the complex. However, the conversion rate of the complex was the highest at 48.96% at 40°C. As the temperature increased beyond 40°C, the conversion rate showed a slightly declined tendency. This could be due to the fact that both hydrolysis and ionization reactions of TPs in water are endothermic processes. A slight increase in temperature was beneficial for the dissociation of TPs, increasing the chances of complexation with copper ions. As a result, the chelate complex conversion rate was significantly elevated as compared to room temperature. Simultaneously, the entropy value of the system increased with an increase in temperature.

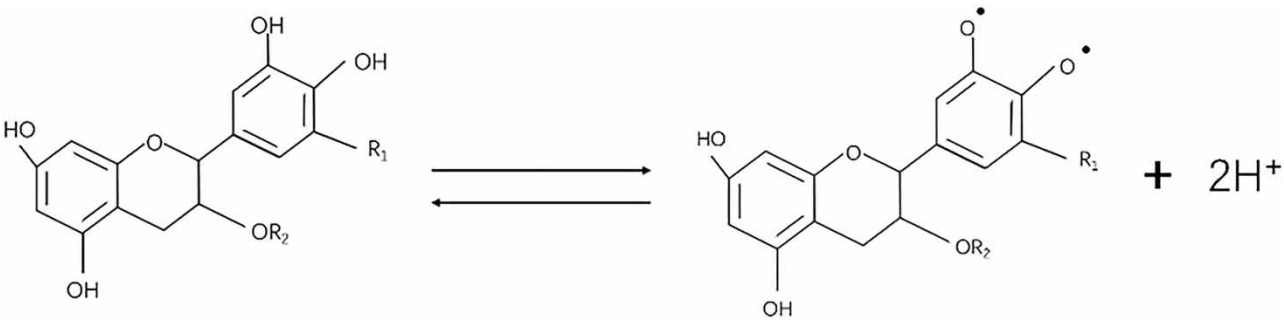


Figure 3 | Equilibrium of TPs in water.

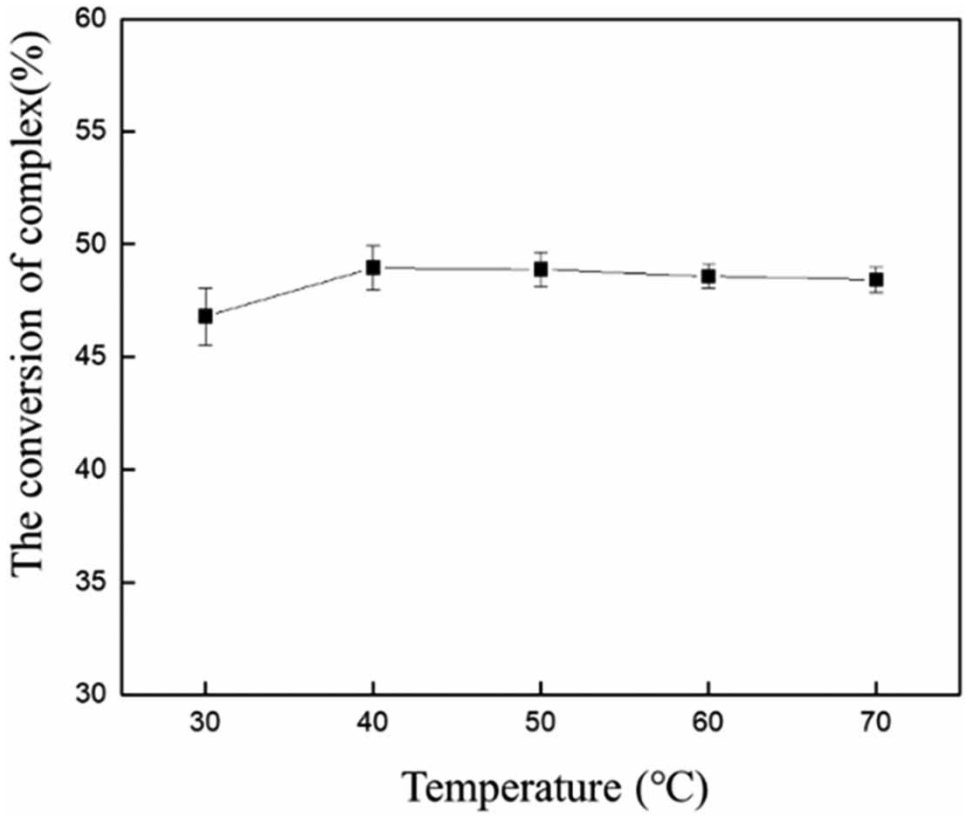


Figure 4 | Effect of reaction temperature on the conversion rate of the complex.

The ion-vibration degree in solution was exacerbated, making it difficult for the complexation reaction to occur under high-temperature conditions. Therefore, to facilitate the complexation reaction efficiently, based on the experiment results presented, it is recommended to select 40 °C as the most suitable reaction temperature.

3.1.3. Effect of reaction time on complexation

The molar ratio of the limiting reagent was 1:1, and the reaction pH was 7. The reaction was carried out under 40 °C with magnetic stirring for 10, 20, 30, 60, 120 and 240 min, and the effect of reaction time on the complexation was explored with the following results.

It can be seen from Figure 5 that the conversion rate of the complex increased during the period of 10–60 min, and the conversion rate reached the highest at 60 min. Thereafter, as time goes on, the conversion rate remained basically the

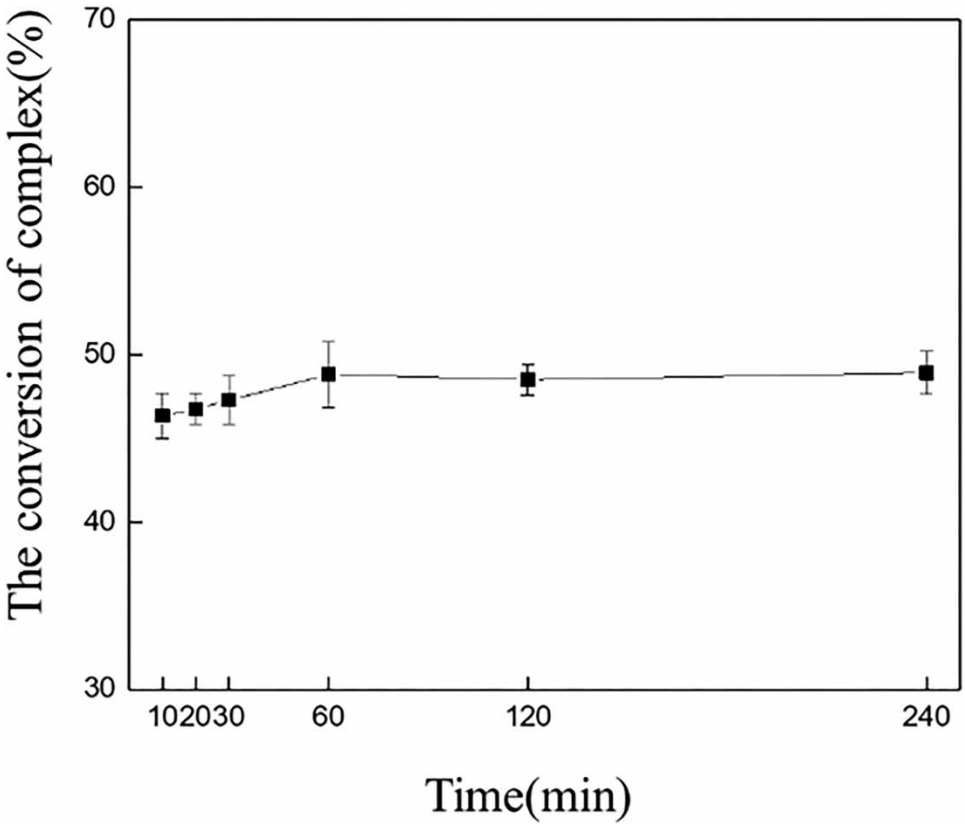


Figure 5 | Effect of reaction time on the conversion rate of the complex.

same, even a slight decrease. It can be seen that under this condition, the complexation of TPs with copper ions was completed at about 60 min, and there was no need to extend the reaction time since the local oxidation of TPs intensified gradually, causing a reduction of the complexing products. Therefore, determining a reaction time of 60 min as the optimal duration for the complexation reaction not only ensures a high conversion rate of the complex but also offers excellent economic efficiency.

3.1.4. Effect of reactant molar ratio on complexation

The molar ratio of the reactant was $n_{(Cu2+)}$:$n_{(EGCG)}$ = 1:2, 1:1, 2:1, 3:1 and 4:1, the reaction pH was kept at 7, and the reaction was stirred for 60 min at 40 °C. The results were presented as follows.

It can be seen from Figure 6 that when $n_{(EGCG)}$:$n_{(Cu2+)}$ was less than 1:2, the conversion rate of the complex increased at a faster rate and the conversion rate of the complex was greater than 1:2, the growth rate was slower and the total increase was about 1.5%. The observed phenomenon can be explained by the complexation between copper ions and TPs in specific proportions during the formation of the complex. Once the complexation was complete, the excess copper ions did not react further with TPs in the solution. Therefore, the optimal reaction conditions were achieved with a molar ratio of 1:2 for TPs and copper sulfate.

3.1.5. Synthesis method for complex formation

To synthesize the necessary complex, a hydrothermal method was employed; 0.5 mmol of EGCG was dissolved in 30 mL of distilled water. Then, 1 mmol of copper sulfate was prepared as a solution and gradually added. After mixing thoroughly, the pH level was adjusted to 7 by 1 mol/L of sodium bicarbonate. The mixture was stirred by a constant temperature magnetic stirrer for 1 h at 40 °C to ensure the complete complex formation. The resulting EGCG–Cu was washed multiple times with a 1:1 ethanol–water solution and finally dried through vacuum freeze-drying overnight.

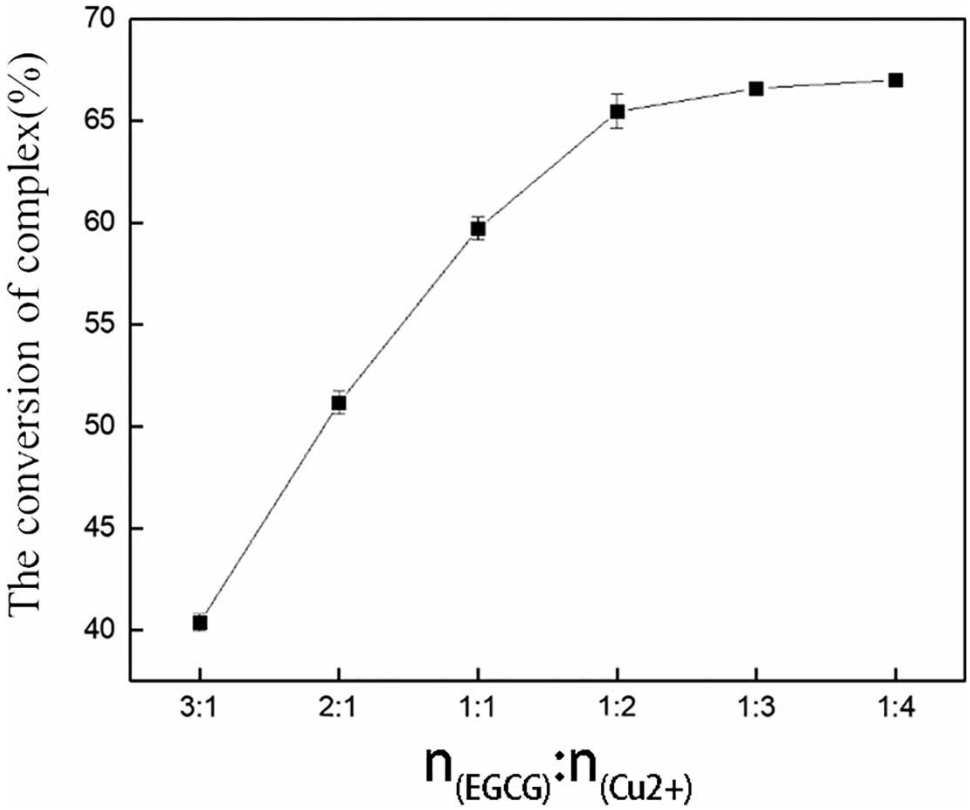


Figure 6 | Effect of reactant ratio on the conversion rate of the complex.

3.2. Determination of the complex ratio of EGCG to copper ion complexation

The complex ratio of EGCG to Cu^{2+} was studied by the molar ratio method. The approach taken by Shengrong (2003) is improved upon, whereby the concentration of the ligand EGCG remains constant and the mole ratio is controlled by increasing the amount of Cu^{2+}. The results are shown in Figure 7.

As can be seen from Figure 7, the molar ratio of EGCG to Cu^{2+} in the cuvette was constantly changed. The waveform of the ultraviolet (UV) scan curve of the measured solution in boric acid changed significantly, and its characteristic peak position at 300 nm began to shift backwards, eventually stabilizing at a wavelength of approximately 320 nm. This indicated that the complexation reaction of the EGCG in solution gradually occurred with the addition of Cu^{2+}, generating a complex with a specific structure, resulting in a red shift of the absorption wavelength of the characteristic peak by 20 nm.

According to Table 1, the characteristic peak of EGCG at 300 nm showed significant changes as Cu^{2+} solution was continuously added to the EGCG solution, gradually decreasing its absorption value and then showing an upward trend. The absorbance value at 320 nm gradually increased with the addition of Cu^{2+}. When the changes in the absorption value of EGCG at 320 nm with the increasing molar ratio of Cu^{2+} to EGCG were plotted in Figure 8, it was found that the absorbance value at 320 nm rapidly increased with the addition of Cu^{2+} and then gradually slowed down as more Cu^{2+} was added. The points with similar changes were divided into two groups, and two straight lines were obtained by fitting the data. The x-value of the intersection was calculated by the equation to be 2.0026787, which was approximately equal to 2, indicating that the stoichiometry of EGCG and Cu^{2+} was 1:2 under these conditions. The results were consistent with the findings reported by Sun *et al.* (2011), and the binding sites may be on the o-catechol group of the B and D rings of the EGCG.

3.3. Characterization of the EGCG–Cu complex

3.3.1. UV characteristic peak of the EGCG–Cu complex and EGCG

A specific amount of EGCG powder and dried complex powder were precisely weighed and diluted with distilled water to produce an extremely dilute solution of EGCG and EGCG–Cu. Distilled water served as the blank control group. The

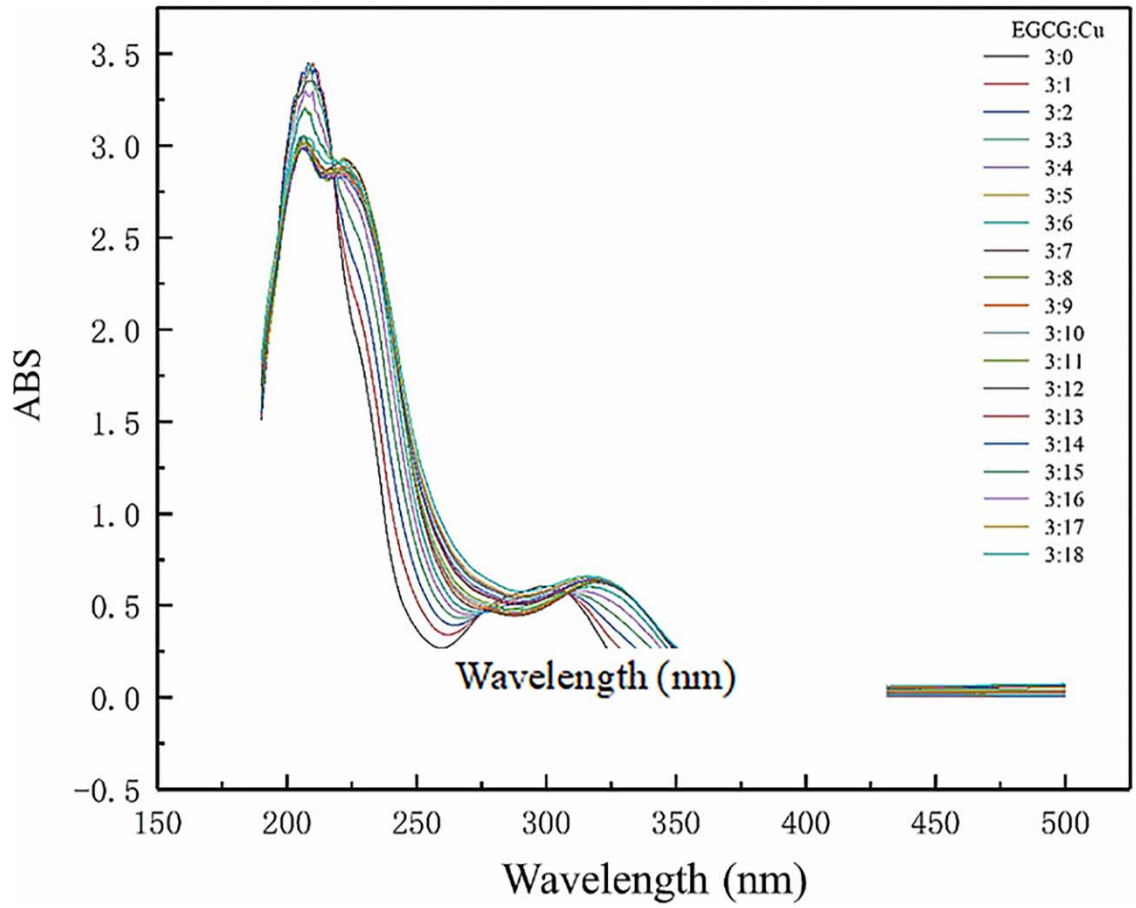


Figure 7 | UV–Visible spectra of EGCG–Cu.

Table 1 | The absorbance of EGCG at 300 and 320 nm after adding Cu^{2+}

Cu^{2+}:EGCG (mol/mol)	ABS (300 nm)	ABS (320 nm)	Cu^{2+}:EGCG (mol/mol)	ABS (300 nm)	ABS (320 nm)
0.0	0.605	0.346	3.3	0.515	0.628
0.3	0.581	0.408	3.7	0.526	0.629
0.7	0.561	0.461	4.0	0.547	0.636
1.0	0.542	0.516	4.3	0.552	0.637
1.3	0.522	0.56	4.7	0.561	0.636
1.7	0.511	0.596	5.0	0.577	0.646
2.0	0.515	0.615	5.3	0.581	0.647
2.3	0.502	0.627	5.7	0.587	0.651
2.7	0.512	0.631	6.0	0.604	0.657
3.0	0.514	0.630			

solution was subsequently transferred into a clean quartz colorimetric dish for UV–Visible scanning. The result is shown in Figure 9.

Upon the formation of a metal complex with copper ions, the UV spectrum of EGCG underwent significant changes, as shown in Figure 9. The UV absorption of EGCG was observed in the B band of the benzene ring in the A and B rings. In addition to E band absorption near 200 nm, there was a characteristic peak at 275 nm in the UV region that did not

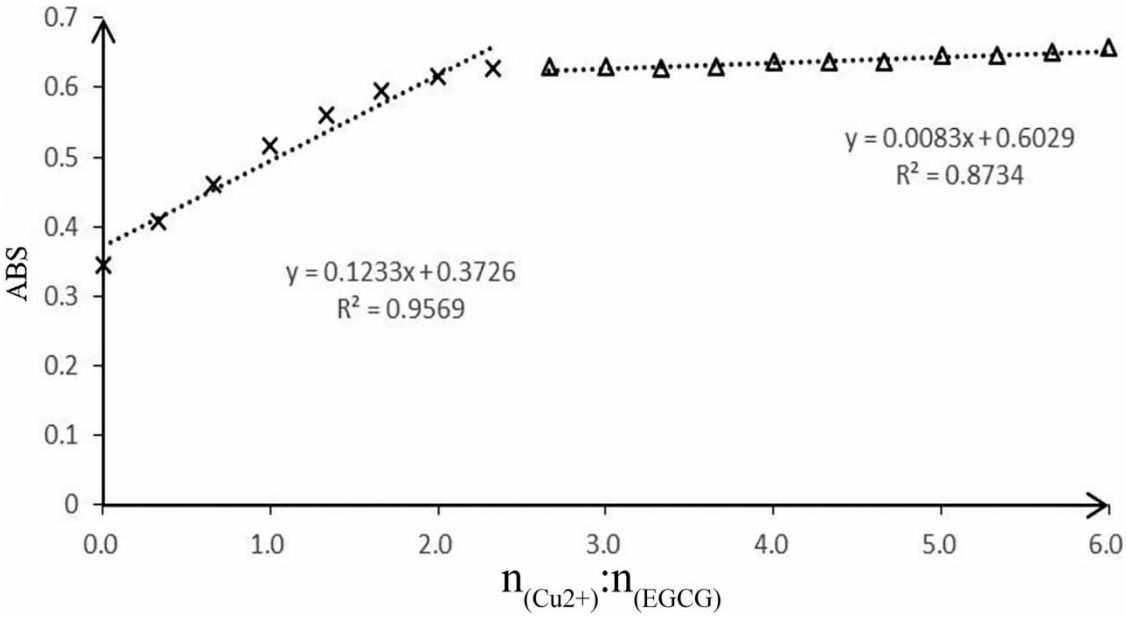


Figure 8 | Comparison of the complex ratio of EGCG to Cu^{2+}.

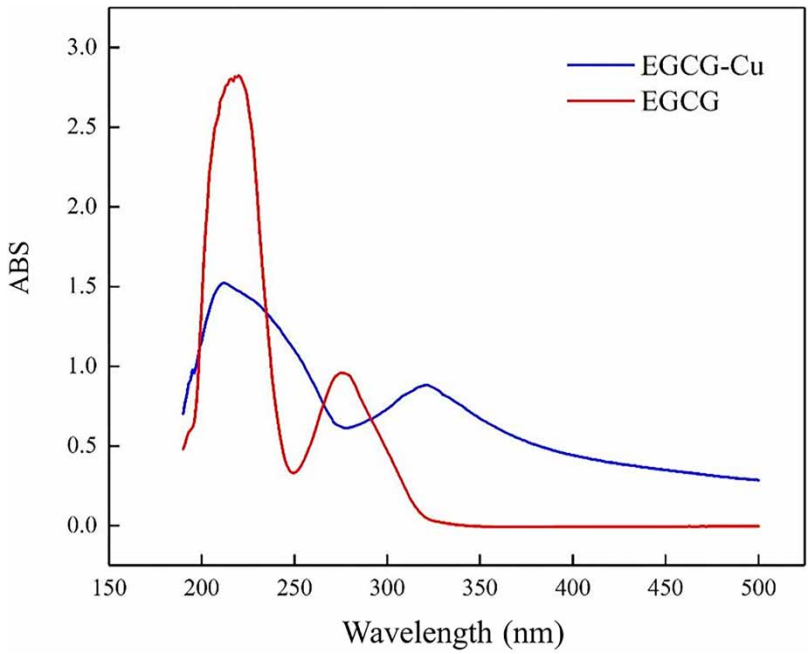


Figure 9 | UV–Visible absorption spectra of EGCG–Cu and EGCG.

change with solubility. The characteristic peak at 275 nm showed a significant shift due to complexation, as a portion of the phenolic hydroxyl groups reacted with the metal ions to form coordination bonds. The lone pairs of electrons on phenolic hydroxyl groups and the pi-electron system of the benzene ring formed p–π conjugation, which was strengthened by the coordination bonds, leading to a shift toward a longer wavelength (Jing 2011). Yue (2016) explored the UV spectra of the dye lignin and copper ion complex and found that the characteristic peak of the dye lignin increased from 336 to 423 nm after complexation. This was attributed to the formation of coordination bonds between copper ions and the phenolic hydroxyl groups of dye lignin. The complexed molecule was planar, and the conjugated system was enlarged, resulting in

an increase in absorption intensity and a redshift of the characteristic peak. This demonstrates that structural changes at the molecular level can be inferred from changes in the characteristic peak. The formation of the complex between EGCG and copper ions indeed caused a change in the overall structure of EGCG, confirming the success of the complexation reaction.

3.3.2. Analysis of the EGCG–Cu complex and the EGCG functional group

EGCG–Cu and EGCG infrared absorption peaks were tested, and the results are shown in Figure 10 and Table 2. The comparison between the infrared spectra of EGCG and EGCG–Cu revealed that the overall shape of the spectra was similar, resulting from the similar molecular skeleton structure of EGCG and EGCG–Cu, and vibrations such as O–H stretching and deformation, as well as benzene ring vibrations. However, the spectrum at certain wavelengths for EGCG–Cu compared to EGCG changed with the addition of copper ions. The O–H vibration frequency was approximately 3,300–3,500 cm^{-1}. The absorption peaks of EGCG at 3,477 and 3,357 cm^{-1} were related to the stretching vibration of the hydroxyl group. However, in the spectrum of the complex, the absorbance intensity of the peak decreased and shifted to 3,416 cm^{-1}, overlapping with the peak of the crystal water. This shift toward lower frequency indicated that the metal ions and EGCG were involved in a

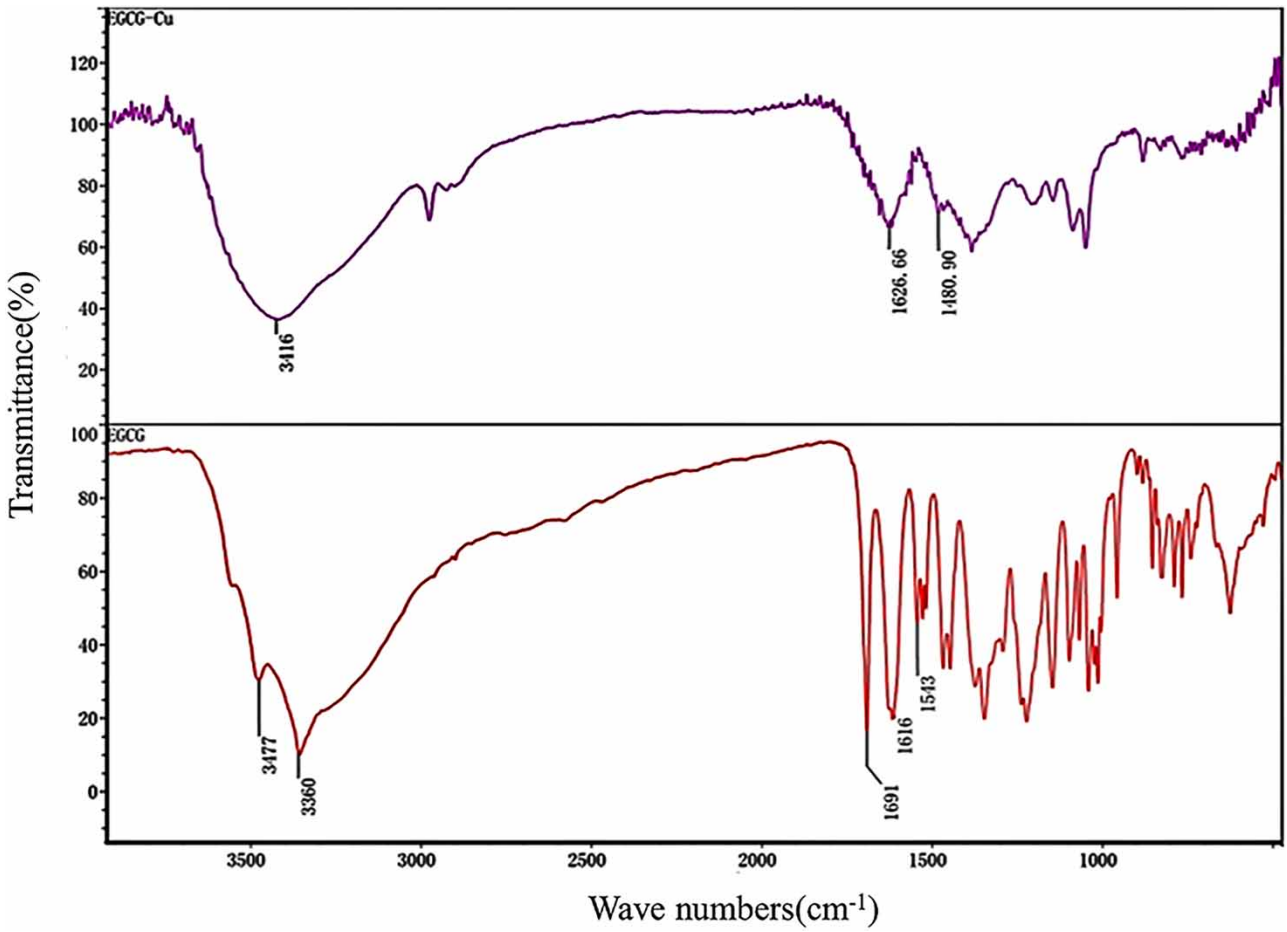


Figure 10 | Infrared spectra of EGCG (red) and EGCG–Cu complex (purple).

Table 2 | Absorption frequency of main functional groups of EGCG and its complex

Group	O–H	C = C	C = O
EGCG (cm^{-1})	3,477	1,616, 1,543	1,691
EGCG–Cu (cm^{-1})	3,416	1,480	1,627

complex reaction with the phenolic hydroxyl group of EGCG. The hydrogen of the phenolic hydroxyl group was replaced by the metal ions, transforming the O–H bond into an O–Cu bond. Furthermore, the electrons in the oxygen atom of the hydroxyl group were stripped away due to the greater polarity of copper, resulting in a decrease in the electron density of the entire hydroxyl group, further causing a shift toward lower wave numbers.

3.4. Water quality when the EGCG–Cu complex is a disinfectant

3.4.1. Antibacterial effects of the EGCG–Cu complex and EGCG

The effects of EGCG–Cu complex and EGCG on the growth of *E. coli* at the same concentration were compared. The results were as follows.

According to Figure 11, the EGCG–Cu complex showed a significant inhibitory effect on the growth of *E. coli*, and its antibacterial effect was much higher than that of EGCG at the same concentration. When the concentration was 100 mg/L, EGCG appeared to have almost no inhibitory effect on *E. coli*, whereas the antibacterial rate of the EGCG–Cu complex was 96%. At a concentration of 200 mg/L, the antibacterial rate of EGCG was approximately 5%, whereas that of the EGCG–Cu complex was as high as 100%. This indicated that *E. coli* was highly sensitive to the complex, and the antibacterial effect of a small amount of complex was more ideal than that before complexation. This may be because the copper ions enhanced the conjugated structure of EGCG after complexation, resulting in an increase in its liposolubility, and easier entry into cells to bind with proteins and DNA for bactericidal effects. Research has also shown that copper ions have antibacterial properties, and in the complex, Cu^{2+} and EGCG acted synergistically to increase the antibacterial activity of the complex compared to EGCG alone. Sun *et al.* (2011) found that EDTA can inhibit the antibacterial activity of the EGCG–Cu complex, which further proved the synergistic effect of Cu^{2+} in the process of EGCG killing of *E. coli*. Therefore, modifying EGCG through copper ion complexation can help to enhance its antibacterial ability.

3.4.2. Release of Cu^{2+} from the EGCG–Cu complex

In order to investigate the relationship between the released Cu^{2+} and the antibacterial disinfectant properties of the EGCG–Cu complex, an inductively coupled plasma (ICP) spectrometer was introduced. The complex solid 250 mg was added to pure water, and samples were taken at different time intervals to explore the existence form of Cu^{2+} in the solution. It is expected that the antibacterial mechanism of the complex will be revealed along with the regularity of Cu^{2+} release from the complex. The release of Cu^{2+} from the complex in pure water over different time periods is shown in Figure 12.

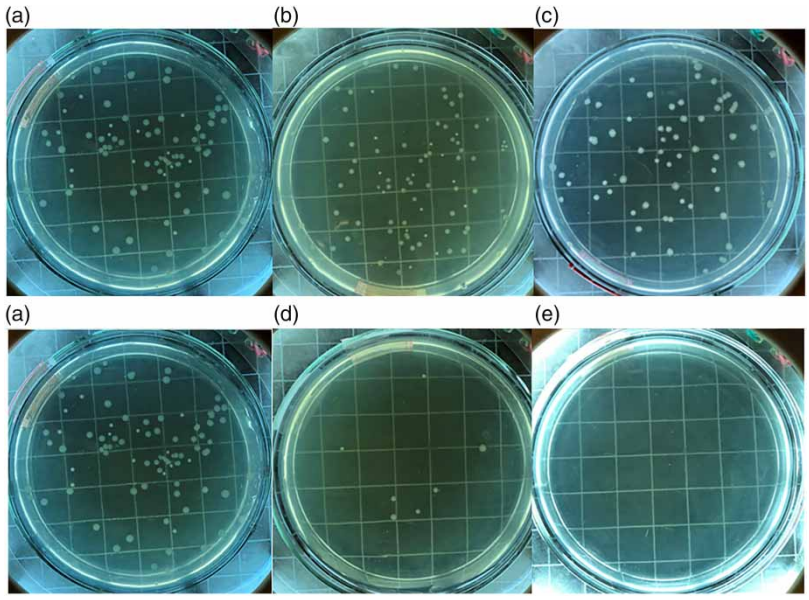


Figure 11 | Comparison of antibacterial activity of the EGCG and EGCG–Cu complex on agar plates. (a) Blank group without added bacteriostatic agent; (b, c) EGCG treatment group, 100 and 200 mg/L, 24 h; (d, e) EGCG–Cu complex treatment group, 100 and 200 mg/L, 24 h).

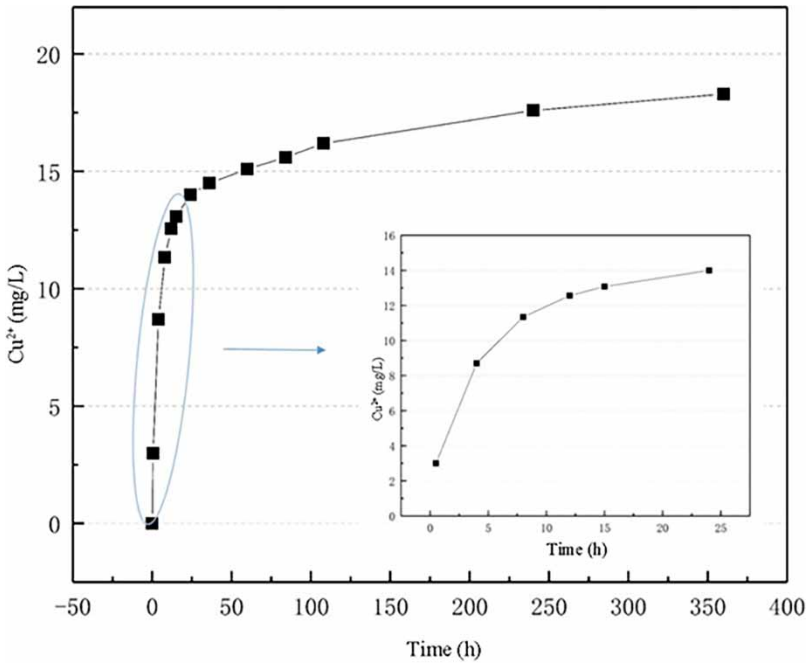


Figure 12 | Cu^{2+} concentration released from EGCG–Cu.

The Cu^{2+} concentration measured in water continuously increases with time, indicating that Cu^{2+} in the complex is continuously released into the aqueous solution. Within the first 24 h, the release of Cu^{2+} increases rapidly from 0 to 14 mg/L. Subsequently, a rapid increase of Cu^{2+} concentration becomes slower, especially after 100 h. So, EGCG–Cu can maintain its bactericidal effect within a relatively long time.

3.4.3. Determination of bactericidal concentration of Cu^{2+} on *E. coli*

To investigate why EGCG–Cu has a more significant antibacterial effect than EGCG, the antibacterial property of Cu^{2+} was studied. Cu^{2+} was provided by the copper sulfate solution, and the interaction between copper ions of different concentrations and *E. coli* was investigated. The concentrations of Cu^{2+} in copper sulfate solution were 4, 2, 1, 0.5, 0.25, 0.125 and 0 mg/L, and a high-dose group with a concentration of 20 mg/L Cu^{2+} was included. The variation of the antibacterial rate of *E. coli* for different concentrations of Cu^{2+} with time is shown in Figure 13.

In the blank group, a few bacteria deaths occurred at 48 h, indicating that *E. coli* entered the decline phase as the nutrients in the water were depleted. When the Cu^{2+} concentration was 0.125 mg/L, the antibacterial rate increased with the contact time. At 24 h of contact time, the antibacterial rate reached its maximum, at around 41%, and it almost did not increase further after 24 h, indicating that *E. coli* is highly sensitive to Cu^{2+}. When the Cu^{2+} concentration increased to 0.5 mg/L, the antibacterial rate still showed a positive correlation with time, reaching a peak of 84% at 24 h. As the Cu^{2+} concentration increased, the bactericidal effect on *E. coli* became more pronounced, and this effect became more evident with time. At 4 mg/L of Cu^{2+}, the antibacterial rate reached nearly 100% at 18 h. At 20 mg/L, the antibacterial rate had already reached over 80% at 2 h, and it achieved a 100% antibacterial effect after 6 h of contact. Overall, both the Cu^{2+} concentration and the contact time are positively correlated with the antibacterial rate. The higher the Cu^{2+} concentration and the longer the contact time, the more pronounced the inhibitory effect on *E. coli*.

3.4.4. Effect of EGCG–Cu disinfection on the chroma of drinking water

Figure 14 shows the chroma status of the bacterial solution after 2 weeks of standing, which is the blank group, 200 mg/L of the EGCG–Cu complex group, and 200 mg/L of the EGCG group, respectively.

The blank group bacteria multiplied significantly and caused the solution to be turbid. The EGCG group was clear but visibly tea red, as EGCG easily undergoes oxidation and polymerization reactions, forming colored quinone polymers. The EGCG–Cu group is clear without turbidity or odor, indicating that the *E. coli* in the solution is completely inhibited and

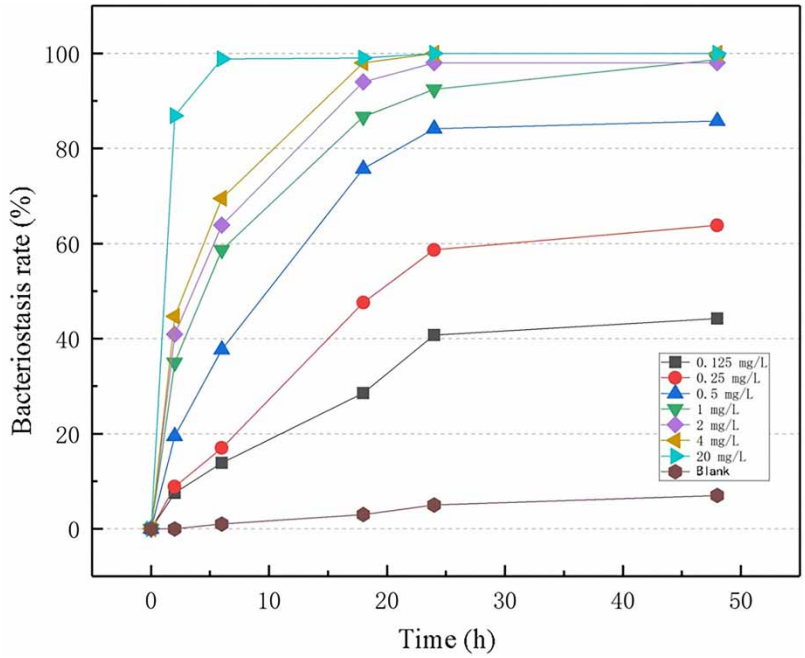


Figure 13 | Antibacterial rate of *E. coli* in different concentrations of Cu^{2+}.

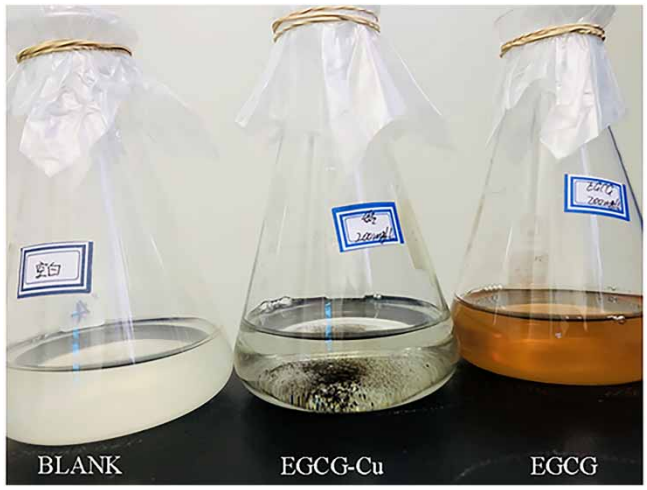


Figure 14 | Chroma status of bacteria solution after 2 weeks.

never grown within 14 days. The black precipitate is the complex, which proves that the complex can exist in the solution for a long time, and even a small amount of dissolved complex can achieve a considerable disinfection effect. Furthermore, due to the chelation between the phenolic hydroxyl groups in the complex and copper ions, it does not undergo electron-loss reactions without colored quinones like EGCG. Therefore, the chromaticity of the solution treated with EGCG–Cu is less than 15, which is very favorable for its application as a disinfectant.

4. CONCLUSIONS

Utilizing complex yield as the determinant factor, the optimal conditions for complexation were established in this study. The complexation ratio between EGCG and Cu^{2+} was investigated by employing the molar ratio method. The examination of characteristic peaks and functional group variations in EGCG and the complex was carried out by UV and infrared

spectroscopy. In addition, the antibacterial effect of the complex was evaluated through its influence on a bacterial suspension of *E. coli*. The following conclusions were drawn:

(1) The optimum experimental conditions for the complexation reaction were determined by experiments: the pH of the complexation reaction was 7; the reaction temperature was 40 °C; the reaction time was 60 min and the ratio of the reactants was 1:2. The results showed that the amount of complex formation was the highest under this condition.
(2) The molar ratio of the complexation of EGCG and Cu^{2+} was explored by the mole ratio method. The absorbance value of the new substance's characteristic peak was used to fit the line, revealing that the complexation ratio of EGCG and Cu^{2+} was 1:2. UV characteristic peak displacement proved that the benzene ring structure of EGCG has changed, resulting in the production of a new substance. The absorption peak changes of various functional groups on the infrared spectrum indicated that the complexation reaction occur on the phenolic hydroxyl group of EGCG's benzene ring.
(3) The relationship between the released Cu^{2+} and the disinfection performance of the EGCG–Cu complex was studied by the ICP spectrometer. The results showed that Cu^{2+} in the EGCG–Cu complex is continuously released into the aqueous solution, which can maintain a long-term bactericidal effect.
(4) Through testing the bactericidal activity of *E. coli*, copper ion complexation modification of EGCG was shown to significantly enhance its antibacterial properties, and the chromaticity of the EGCG–Cu complex antibacterial solution is less than that of EGCG, which is very favorable for its application as a disinfectant.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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