In vitro α-glucosidase inhibitory activity of isolated fractions from water extract of Qingzhuan dark tea

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Abstract

Background: Natural products have being used as potential inhibitors against carbohydrate-hydrolyzing enzymes to treat diabetes mellitus. Chinese dark tea has various interesting bioactivities. In this study, the active compounds from Qingzhuan dark tea were separated and their anti-diabetic activity was examined using an in vitro enzymatic model.

Methods: The chloroform, ethyl acetate, n-butanol, sediment and residual aqua fractions of a Chinese dark tea (Qingzhuan tea) were prepared by successively isolating the water extract with different solvents and their in vitro inhibitory activities against α-glucosidase were assessed. The fraction with the highest inhibitory activity was further characterized to obtain the main active components of Qingzhuan tea.

Results: The ethyl acetate fraction had the greatest inhibitory effect on α-glucosidase, followed by n-butanol, sediment and residual aqua fractions (with the IC₅₀ values of 0.26 mg/mL, 2.94 mg/mL, 3.02 mg/mL, and 5.24 mg/mL, respectively), mainly due to the high content of polyphenols. Among the eight subfractions (QEF1-8) isolated from the ethyl acetate fraction, QEF8 fraction showed the highest α-glucosidase inhibitory potential in a competitive inhibitory manner (the Ki value of 77.10 μg/mL). HPLC-MS analysis revealed that (−)-epigallocatechin gallate (EGCG) and (−)-epicatechin gallate (ECG) were the predominant active components in QEF8.

Conclusion: These results indicated that Qingzhuan tea extracts exerted potent inhibitory effects against α-glucosidase, EGCG and ECG were likely responsible for the inhibitory activity in Qingzhuan tea. Qingzhuan tea may be recommended as an oral antidiabetic diet.

Keywords: Camellia sinensis, Dark tea, Tea extracts, α-Glucosidase, Anti-diabetes

Background

Diabetes has become a major health problem in the world. It is a metabolic disease characterized by a high blood glucose level and can cause other health complications, such as cardiovascular disease, neuropathy, high blood pressure, weakness, gangrene, retinopathy, nephropathy and other dysfunctions [1]. One of the therapeutic approaches is focused on suppressing the glucose production from carbohydrates by inhibiting digestive enzymes, such as α-amylase and α-glucosidase [2, 3]. Acarbose has been used in the clinic therapy as an effective inhibitor of carbohydrate hydrolysis, but has adverse effects including abdominal pain, diarrhea and flatulence [4]. Effective and nontoxic inhibitors of α-amylase and α-glucosidase from natural products have been searched for the treatment of diabetes [5–7].

Tea (Camellia sinensis) is one of the most popular beverages worldwide possessing various pharmacological effects, such as anti-carcinogenic, hypoglycemic and anti-obesity activities [8–10]. Tea is divided into four types depending on the manufacturing process: non-fermented tea (green tea), semi-fermented tea (oolong tea), fermented tea (black tea) and post-fermented tea (dark tea) [11]. Dark tea, a post-fermented tea, is very...
Preparation of Qingzhuan tea extracts and subfraction of the ethyl acetate extract

Qingzhuan tea (50 g) was cut into thin slices and extracted with 1 L of boiling purified water for 7 min. The mixture was filtered through filter paper (Whatman 102, Hangzhou, China) under vacuum condition. The filtrate was collected and the residue was extracted once more under the same conditions. The two filtrates were combined and concentrated to 200 mL by the vacuum rotary evaporator (Jinye RE-52AA, Shanghai, China) at 55 °C under reduced pressure. The concentrated tea extracts (200 mL) were further extracted with 600 mL of chloroform, ethyl acetate and n-butanol separately three times to obtain the chloroform, ethyl acetate and n-butanol fractions. The aqua fraction was precipitated with a three-fold volume of 95 % ethanol (ethanol/water, 95:5 v/v) at room temperature for 24 h to obtain the sediment fraction and the residual aqua fraction. Each fractions was concentrated by vacuum rotary evaporation to remove the organic solvents completely and then freeze-dried (Labogene CoolSafe 110–4, Denmark).

Ethyl acetate fraction (0.2 g) was dissolved in a small amount of methanol and then loaded to a Sephadex LH-20 column (50 × 1.6 cm i.d. GE Healthcare Bio-Sciences AB, Sweden) and eluted successively with 500 mL of water, water/methanol (9:1 v/v, 8:2 v/v, 7:3 v/v, 6:4 v/v, 5:5 v/v, and 4:6 v/v respectively), and water/acetone (1:1 v/v) [15]. The isolated active components (QEF1-8) were concentrated by the vacuum rotary evaporator to remove the organic solvents completely and then further freeze-dried.

Characterization of the Qingzhuan tea extracts and HPLC-MS analysis of QEF8 subfraction

Total polyphenols content in Qingzhuan tea extracts was determined according to the Folin-Ciocalteu method [16]. Water soluble carbohydrates were measured by the anthrone-sulfuric acid method [17]. HPLC analysis of caffeine was performed as described in our previous work [18] using an Agilent TC-C18 column (5 μm, 150 mm × 4.6 mm i.d.). The total theaflavins, thearubigins and theabrowins were measured by following the method of Zhong [19].

The QEF8 was analyzed by high performance liquid chromatography-mass spectrometry (HPLC-MS, Agilent 1100VLO, USA). The HPLC parameters were set according to our previous work with slight modifications [18]. The sample was applied to an Agilent TC-C18 column (5 μm, 250 mm × 4.6 mm i.d.) with a gradient mobile phase consisted of water (0.1 % formic acid, A) and methanol (0.1 % formic acid, B) at a flow rate of 1.0 mL/min. A gradient elution was adopted as follows: 0–2 min, 80–75 % A; 2–6 min, 75–70 % A; 6–10 min, 70–75 % A; 10–13 min, 75–70 % A; 13–20 min, 70 % A; 20–23 min, 70–75 % A; 23–25 min, 75–80 % A; 25–30 min, 80 % A. The injection volume was 5 μL and detection wavelength was 278 nm. The temperature of column oven was set at 35 °C. The MS adopted a positive ion mode and scanned from 100 to 900 m/z. The flow rate of dry gas was 40 L/min at 250 °C. The capillary voltage, ESI voltage and discharge voltage were 3500 V, 10 kV, and 124.8 V, respectively. For the quantitative analysis, pure EC, C, EGC, ECG, CG, ECG, EGCG, GCG and GA were used as standards.
Inhibitory assay of α-glucosidase

α-Glucosidase inhibitory activity was determined as described by Li et al. [20] with slight modifications. Briefly, mixtures of 112 μL potassium phosphate buffer (PBS, pH 6.8), 20 μL enzyme solution (1 unit/mL), and 8 μL of the sample (dissolved in DMSO) were incubated in a 96-well plate at 37 °C for 15 min, followed by the addition of 10 μL of pNPG (2.5 mmol/L) to each well, and incubation at 37 °C for 15 min. The reaction was terminated by adding 80 μL of Na2CO3 solution (0.2 mol/L). Absorbance was determined at 405 nm. The concentration was calculated from the standard curve of the corresponding relation at 405 nm. The reaction was terminated by the addition of 80 μL Na2CO3 solution (0.2 mol/L). Absorbance of PBS + sample + pNPG; ODcontrol was the absorbance of PBS + enzyme + pNPG; OD blank was the absorbance of PBS + pNPG. IC50 was the half inhibition concentration of Qingzhuan tea fractions on α-glucosidase activity.

Determination of the mode of α-glucosidase inhibition

The mixture of 20 μL pNPG substrate solution (at concentrations of 0.2 mmol/L, 0.4 mmol/L, 0.8 mmol/L, 1.0 mmol/L, 1.6 mmol/L, and 2.0 mmol/L), 112 μL PBS, and 8 μL sample (0, 20, 60 and 100 μg/mL) were incubated in a 96-well plate at 37 °C for 15 min, followed by the addition of 20 μL of the enzyme solution (1 unit/mL) to each well and 5 min incubation at 37 °C. The reaction was terminated by the addition of 80 μL of Na2CO3 solution (0.2 mol/L) and the OD values were measured at 405 nm. The concentration was calculated from the standard curve of the corresponding relationship between the OD values and the concentrations of product (p-nitrophenol, pNPG). The Vmax and Km constants were determined using the Lineweaver-Burk plots from the relevant Michaelis-Menten equations.

Statistical analysis

Data were analyzed using SPSS statistical software (SPSS, Chicago, IL, USA). Values were expressed as mean ± standard deviation (SD) of three replications of the experiment. The differences in the mean values were analyzed using the Fisher’s least significant difference (LSD) procedure, and were considered significant at p < 0.05.

Results and discussion

α-Glucosidase inhibitory activity of Qingzhuan tea extracts

α-Glucosidase plays a central role in modulating postprandial hyperglycemia, which breaks down α-1,4-glucosidic linkages of disaccharides, resulting in simpler sugars. A previous study reported the established α-glucosidase inhibitors and their effects on delaying the expeditious generation of blood glucose after food uptake [21].

In the present study, the crude water extract of Qingzhuan tea was divided into five fractions by polarity and the α-glucosidase inhibitory activities of these fractions were detected using pNPG as the reaction substrate. As shown in Table 1, the crude water extract of Qingzhuan tea exerted an obvious inhibitory effect on α-glucosidase, with an IC50 value of 2.47 mg/mL. A previous in vivo study [22] indicated the hypoglycemic effect of dark tea for diabetic mice. In our experiment, no α-glucosidase inhibition was observed in the chloroform fraction of Qingzhuan tea, but the ethyl acetate, n-butanol, sediment and residual aqua fractions exhibited a dose-dependent inhibitory effect on α-glucosidase activity. The IC50 values of the four fractions ranged from 0.26 to 5.24 mg/mL, and followed the sequence of residual aqua fraction (5.24 mg/mL) > ethyl acetate fraction (2.94 mg/mL) > sediment fraction (3.02 mg/mL) > n-butanol fraction (2.94 mg/mL) > ethyl acetate fraction (0.26 mg/mL), indicating that the ethyl acetate fraction had the greatest inhibitory activity. Meanwhile, apart from the residual aqua fraction, the sediment, n-butanol and ethyl acetate fractions could exhibit significantly more potent inhibitory effects than acarbose (an effective inhibitor of α-glucosidase with the IC50 value of 4.64 mg/mL, p < 0.01), suggesting Qingzhuan tea extracts could be potential α-glucosidase inhibitors.

In order to clarify the inhibitory mechanism of α-glucosidase activity, the basic active components in Qingzhuan tea extracts were determined (Table 2). The crude water extract contained 18.25 % polyphenols and 26.83 % tea-pigments (including theaflavins, thearubigins and theabrownins), 14.74 % carbohydrates, and 5.82 %

Table 1 Inhibitory effects of Qingzhuan tea fractions on α-glucosidase

| Extracts                      | IC50 (mg/mL) | Regression equation | R²  |
|-------------------------------|--------------|---------------------|-----|
| The crude water extract       | 2.47 ± 0.30 *| y = 0.120 x + 0.204 | 0.975|
| Chloroform fraction           | No activity  |                     |     |
| Ethyl acetate fraction        | 2.27 ± 0.03 *| y = 2.131 x − 0.057 | 0.984|
| n-Butanol fraction            | 2.94 ± 0.44 *| y = 0.171 x − 0.002 | 0.991|
| Sediment fraction             | 3.02 ± 0.07 *| y = 0.042 x + 0.373 | 0.991|
| Residual aqua fraction        | 5.24 ± 0.11  | y = 0.101 x − 0.029 | 0.987|
| Acarbose                      | 4.64 ± 0.57  | y = 0.045 x + 0.291 | 0.987|

Note: n=3, Mean ± SD

*p > 0.01 when compared to acarbose
cafeine. Due to the special pille-fermenting process, Qingzhuan tea had a high content of tea-pigments, especially thearubigins and theabrownins. Among the five fractions, the ethyl acetate fraction had the highest total polyphenols (62.72 %) and theaflavins (1.70 %), and the second highest amount of total thearubigins (24.21 %). Caffeine was the dominant active compound (70.55 %) in the chloroform fraction. The n-butanol fraction contained a certain amount of polyphenols (31.09 %), carbohydrates (15.56 %), thearubigins (28.00 %), and theabrownins (24.82 %). For the sediment fraction, 16.65 % carbohydrates (15.56 %), thearubigins (28.00 %), and theabrownins accounted for 70.55 %, while the inhibitory effect was weaker than catechins and theaflavins in the Qingzhuan tea extracts. It has been reported that plant phenolic compounds could inhibit α-glucosidase effectively [23]. Different teas (green, oolong and black teas) showed different α-glucosidase inhibitory profiles, which was associated with their major polyphenol contents [24]. Theaflavin was reported to have α-glucosidase inhibitory activity in the following order: theaflavin-3-O-gallate > theaflavine 3,3′-di-O-Gal > theaflavin 3′-O-Gal > theaflavin [25]. It seems that the presences of hydroxyl group and gallate group were closely associated with α-glucosidase inhibitory effects.

Polysaccharides have also been reported to have hypoglycemic activities, partly due to their α-glucosidase or α-amylase inhibitory activity [26, 27]. The present work also showed that the sediment fraction containing a substantial portion of polysaccharides exerted an ability to inhibit α-glucosidase. A similar result was observed that polysaccharides obtained from leaves and flowers of Camellia sinensis by different extraction methods have been reported to have α-glucosidase and α-amylase inhibitory activities [28].

The chemical components change greatly due to the special manufacturing process of Qingzhuan tea. For instance, catechins, the predominant polyphenols in tea, accounted for 70–80 % of total polyphenols [29] and could be oxidized and polymerized to generate theaflavins, thearubigins, theabrownins and even unidentified polyphenols polymers under fungal, damp and hot conditions during postfermentation [30]. Theabrownins were responsible for the characteristic color of Qingzhuan tea and were enriched in the sediment, n-butanol and residual aqua fractions. Although the chemical composition of theabrownins is still unknown, some biological activities have been demonstrated, such as serum lipid-lowering effect, antioxidant property and nitric oxide scavenging ability [31, 32]. It is possible that theabrownins also contribute to the α-glucosidase inhibition, which needs further research. Thearubigins have been reported to have α-glucosidase inhibitory activity, while the inhibitory effect was weaker than catechins and theaflavins [33]. Under the present isolation conditions, the n-butanol fraction had the highest thearubigins, which might also contribute to the α-glucosidase inhibitory activity.

### Inhibitory effect of eight subfractions from ethyl acetate fraction on in vitro α-glucosidase activity

The aforementioned results showed the ethyl acetate fraction had the greatest inhibitory effect on α-glucosidase activity in vitro, and thus it was further separated into eight subfractions on a Sephadex LH-20 column with different mixtures of methanol or acetone with water or water alone as mobile phase. The inhibitory effects of those subfractions on α-glucosidase were investigated, and their IC50 values were listed in Table 3. All subfractions showed α-glucosidase inhibition capabilities, and their IC50 values followed the sequence of QEF1 (12.31 mg/mL) > QEF2 (7.57 mg/mL) > QEF3 (6.22 mg/mL) > QEF4 (5.39 mg/mL) > QEF5 (3.33 mg/mL) > QEF6 (1.61 mg/mL)

| Table 2 | The main constituent of Qingzhuan tea extracts (%) |
|---------|--------------------------------------------------|
|         | Polyphenol | Carbohydrate | Caffeine | Theaflavin | Thearubigin | Theabrownin |
| The crude water extract | 18.25 ± 0.21 cC | 14.74 ± 1.02 bA | 5.82 ± 0.06 bB | 0.15 ± 0.01 cC | 5.51 ± 0.14 bB | 21.17 ± 0.21 bC |
| Chloroform fraction | 0.98 ± 0.06 fF | 1.28 ± 0.33 dC | 70.55 ± 0.01 aA | 0.62 ± 0.00 bB | 0.74 ± 0.00 cC | 20.11 ± 0.00 eE |
| Ethyl acetate fraction | 62.72 ± 2.63 aA | 5.85 ± 1.16 eB | 0.50 ± 0.01 cC | 1.70 ± 0.23 aA | 24.21 ± 3.18 aA | 4.17 ± 0.17 dD |
| n-Butanol fraction | 31.09 ± 0.73 bB | 15.56 ± 0.07 aA | 0.30 ± 0.02 dD | 0.24 ± 0.03 cC | 28.00 ± 1.68 aA | 24.82 ± 1.25 aB |
| Sediment fraction | 9.16 ± 0.35 eE | 16.65 ± 0.04 aA | ND | 0.02 ± 0.01 dD | ND | 27.18 ± 0.80 aA |
| Residual aqua fraction | 11.15 ± 0.31 dD | 17.19 ± 0.20 aA | 0.13 ± 0.02 eE | 0.03 ± 0.03 dD | 0.14 ± 0.19 cC | 19.56 ± 0.05 cC |

Note: At the same column, different capital letters mean a significant difference at p < 0.01 level and small letters at p < 0.05 level compared to each other, ND means not detected.
mL) > QEF7 (0.95 mg/mL) > QEF8 (0.066 mg/mL), indicating that the QEF8 had the greatest inhibitory activity. As shown in Table 3, there was significant difference in the α-glucosidase inhibitory activities among the eight subfractions compared to acarbose (p < 0.01). The IC_{50} values of QEF5, QEF6, QEF7 and QEF8 were significantly lower than that of acarbose, especially QEF8, with IC_{50} value being 70-fold lower than that of acarbose and 4-fold lower than that of the ethyl acetate fraction.

The inhibitory mechanisms of QEF8 were further explored in this study. As shown in Fig. 1, the Lineweaver-Burk plots for various concentrations of QEF8 showed the same intersection on y-axis, indicating that the 1/V_{max} remained unchanged in the absence of the substrate. The QEF8 inhibited the enzymatic activity in a competitive manner. According to Michaelis-Menten equations, the value of the inhibition constant (K_i) was 77.10 μg/mL (Table 4). Therefore, the mode of action of the inhibitory activity of QEF8 on α-glucosidase could be similar to that of acarbose. Acarbose has been shown in an animal study to be a competitive inhibitor of intestinal brush-border α-glucosidase enzymes through the acarviosine moiety binding with high affinity to the active centers of the proteins in this enzyme family [34].

The components in QEF8 were analyzed by HPLC-MS method (Fig. 2). EGCG (m/z 459.2) and ECG (m/z 443.1) were found to be the predominant active compounds in QEF8 (Table 5). To further confirm which components in QEF8 are the most effective as inhibitors, EGCG and ECG were tested on the inhibition of α-glucosidase. As shown in Figs. 3 and 4, EGCG showed greater inhibitory effect than ECG, with IC_{50} values of 59 μmol/L (27.02 μg/mL) and 1626 μmol/L (719.29 μg/mL), respectively. Matsui et al. [25] investigated the α-glucosidase inhibitory activities of catechins (EC, ECG, EGC and EGCG) and found EGCG exhibited greater inhibitory activity of maltase than ECG in rat intestinal acetone powder. Kamiyama et al. [35] further compared the inhibitory activities of ten catechins toward maltase and sucrase in rat brush border membrane vesicles prepared freshly and obtained a similar result. Those results showed that galloylated catechins had higher α-glucosidase inhibitory activities than non-galloylated catechins. The galloyl group bonding at the 3 position of catechins

### Table 3 α-Glucosidase inhibition of various subfractions obtained from the ethyl acetate fraction

| Subfraction | IC_{50} (mg/mL) | Regression equation | R^2 |
|-------------|-----------------|---------------------|-----|
| QEF1        | 12.31 ± 0.60 ** | y = 0.074 x − 0.411 | 0.986 |
| QEF2        | 7.57 ± 0.54 **  | y = 0.075 x − 0.068 | 0.989 |
| QEF3        | 6.22 ± 0.70 **  | y = 0.076 x + 0.027 | 0.968 |
| QEF4        | 5.39 ± 0.58     | y = 0.079 x + 0.074 | 0.987 |
| QEF5        | 3.33 ± 0.11 *   | y = 0.157 x − 0.022 | 0.996 |
| QEF6        | 1.61 ± 0.06 **  | y = 0.200 x + 0.178 | 0.973 |
| QEF7        | 0.95 ± 0.05**   | y = 0.391 x + 0.128 | 0.961 |
| QEF8        | 0.066 ± 0.01**  | y = 0.460 x + 0.193 | 0.968 |
| Acarbose    | 4.64 ± 0.57     | y = 0.045 x + 0.291 | 0.987 |

Note: n = 3, Mean ± SD  
* p < 0.05, ** p < 0.01 when compared to acarbose

### Table 4 K_{in} and V_{max} values of α-glucosidase in the presence of different concentration of QEF8

| Concentration of QEF8 | K_{in} (mmol/L) | V_{max} (mmol/L s) | K_i (μg/mL) |
|-----------------------|-----------------|-------------------|-------------|
| 0.00                  | 0.81 ± 0.01 d   | 0.23 ± 0.005 a    | 77.10       |
| 0.02                  | 1.54 ± 0.05 c   | 0.23 ± 0.007 a    | 77.10       |
| 0.06                  | 2.00 ± 0.08 b   | 0.220 ± 0.003 a   | 77.10       |
| 0.10                  | 2.64 ± 0.10 a   | 0.23 ± 0.006 a    | 77.10       |

Note: At the same column, different small letters mean a significant difference at p < 0.05 level compared to each other

![Fig. 1 Lineweaver-Burk plot of QEF8 towards the substrate pNPG at different concentrations](image1)

![Fig. 2 HPLC chromatogram of individual phenolic compounds in the QEF8](image2)
played an important role in the α-glucosidase inhibitory activity. Among galloylated catechins, the number of the hydroxyl group on the B ring was favorable to the inhibitory activity. Galloylated catechins also had higher inhibitory effects on α-amylase that was another important digestive enzyme, while catechol catechins (CG and ECG) were 2 times more inhibitory than pyrogallol catechins (GCG and EGCG) [36]. Intestinal glucose transporters were responsible for subsequent glucose uptake, and ECG was also more effective against intestinal glucose transport than EGCG [37, 38]. Xu et al. [39] assessed the contribution of seven catechins to the inhibition of carbohydrate digestive enzyme (α-glucosidase and α-amylase) and intestinal glucose transport, and found the inhibitory potency of seven catechins was ranked in a similar order. The inhibitory kinetics of catechins on α-glucosidase has been reported and still remain controversial. Li et al. [40] reported that both EGCG and ECG inhibited Saccharomyces cerevisiae α-glucosidase in non-competitive manners. While the mode of rat intestinal α-glucosidase of EGCG was a mix-competitive manner and the $K_i$ value was 87.8 μg/mL in the study of Xu et al. [39].

Tea has been associated with reducing the risk of diabetes, while the ability of various teas on antidiabetic effects was different. Koh et al. [41] investigated the ability of different teas (green, oolong and black tea) in inhibiting α-glucosidase and α-amylase, and found black tea was most potent in inhibiting α-glucosidase and α-amylase. Theaflavins in black tea were associated with their potent inhibitory effects, and catechins were weaker enzyme inhibitors in contrast to theaflavins. In the study of Yang et al. [24], the inhibitory effect of oolong tea extract was significantly higher than that of green tea and black tea extracts. The difference might be due to different sources of tea, which correlated to their major polyphenol content (theaflavins and catechins). Gomes et al. [42] revealed that black tea extract was more effective in reducing the blood glucose concentration in streptozotocin-induced diabetic rats comparing with green tea extract, while green tea extract was more effective in preventive. Tang et al. [43] performed the comparison between green tea extract and black tea extract in a type II diabetic mouse model, suggesting that different tea extracts might exert hypoglycaemic effects through different pathways. Qingzhuang tea, as a post-fermentation tea, might reduce blood glucose in a different way. Our previous investigation showed that Qingzhuang tea was more effective than green tea in losing weight, decreasing serum lipids, antioxidation and protecting liver cells of hypolipidemic rats [14]. Cheng et al. [15] tried to identify bioactive components from Qingzhuang tea by successively isolating

| Compound | Regression equation | $R^2$ | Linear range (μg/mL) | LOD (μg/mL) | Content (%) |
|----------|---------------------|-------|----------------------|-------------|-------------|
| GA       | $y = 12.285 x - 8.215$ | 0.9997 | 3.91–31.25           | 0.76        | 0.60 ± 0.03 |
| EGC      | $y = 1.112 x - 8.101$  | 0.9998 | 54.69–437.50         | 9.59        | 0.55 ± 0.23 |
| C        | $y = 2.214 x - 2.175$  | 0.9998 | 9.38–75.00           | 1.34        | 1.29 ± 0.15 |
| EGCG     | $y = 7.721 x - 107.250$ | 0.9989 | 62.50–500.00         | 24.05       | 30.94 ± 0.45 |
| GCG      | $y = 1.530 x - 4.051$  | 0.9999 | 25.00–200.00         | 3.42        | 2.74 ± 0.20 |
| EC       | $y = 20.066 x - 33.854$ | 0.9987 | 9.38–75.00           | 3.84        | 1.18 ± 0.02 |
| ECG      | $y = 9.056 x - 30.433$ | 0.9995 | 18.75–150.00         | 4.9         | 10.61 ± 0.15 |
| CG       | $y = 8.725 x - 8.939$  | 0.9993 | 4.69–37.50           | 1.40        | 0.53 ± 0.04 |

Note: LOD (limit of detection) was defined as the value equal to a signal-to-noise ratio of three.
the water extract and found QEF8 was the most active subfraction based on the in vitro antioxidant and α-amylase inhibitory activity, which is consistent with the present results. HPLC chromatographic separation of QEF8 revealed that almost half of the subfraction was catechins. It can concluded that catechins were an important factor in contributing to the antioxidant and digestive enzymes inhibitory activity of Qingzhuan tea. While during the process of Qingzhuan tea, the post-fermentation stage process decreased the contents of catechins and formed some novel catechins derivatives. These unidentified derivatives probably contributed to the anti-hyperglycemic potential, which still requires further research.

Conclusion

α-Glucosidase plays an important role in carbohydrate digestion and is considered as one of the targets for regulating postprandial hyperglycemia in the therapy of diabetes type II. The results from this study indicated that Qingzhuan tea extracts exerted potent inhibitory effects against α-glucosidase, and EGCG and ECG were found to be responsible for the inhibitory activity, implying the potential of Qingzhuan tea as an oral antidiabetic diet.

Abbreviations

C: (+)-catechin; GG: (-)-catechin gallate; EC: (-)-epicatechin; ECG: (-)-epicatechin gallate; EGCG: (-)-epigallocatechin gallate; GA: Gallic acid; GC: (-)-gallocatechin; GCG: (-)-gallocatechin gallate; HPLC-MS: High performance liquid chromatography-mass spectrometry; LOD: Limit of detection; LSD: The Fisher's least significant difference; PBS: Potassium phosphate buffer; pNP: p-nitrophenol; pNP-G: p-nitrophenyl-α-D-glucopyranoside; QEF1-8: The eight subfractions isolated from the ethyl acetate fraction; SD: Standard deviation

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Availability of data and materials

All data on which the conclusions of the manuscript was presented in the main paper.

Authors’ contributions

YC conceived and designed the study, ZY and SL interpreted data, drafted and revised the manuscript. HZ and WZ conducted the experiments and organized the data analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publish

Not applicable.

Ethics approval and consent to participate

Not applicable.
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