ABSTRACT

Background: Inhibition of glycosidase activity is an effective diabetes therapy and prevention strategy. In this study, we aimed to explore the effects of quercetin and its derivatives extracted from Potentilla bifurca on \( \alpha \)-glycosidase inhibited vitality. Materials and Methods: Various chromatographic methods and separation techniques were used to separate quercetin and quercetin derivatives. An enzyme reaction system was established to determine the inhibitory activity of \( P. \) bifurca compounds against \( \alpha \)-glycosidase and the type of enzyme inhibitor reaction. Results: The changes in activity between quercetin and its derivatives is observed in the following areas: (1) The action of flavonoids is expected to be influenced by the substituents of different molecules. Glycosylation of quercetin reduces its hypoglycemic effect, and the molecule created after adding penta-glycoside is larger than that formed after adding hexa-glycoside. (2) The acylated drugs can possibly reduce their activity. Conclusion: \( P. \) bifurca has been shown to inhibit glycosidase activity, making it effective in diabetes treatment.

Key words: \( \alpha \)-glycosidase, derivatives, diabetes, \( P. \) bifurca, quercetin

INTRODUCTION

Diabetes mellitus (DM) is related to a group of metabolic disorders characterized by high blood glucose levels, one of the major global public health problems.[1] The latest available statistics from the International Diabetes Federation reveal that 63 million people had DM worldwide in 2019 and this number is expected to be 700 million by 2045.[2] Glycosidases, which as some of the most specific and proficient catalysts, have gained prominence.[3] Some research suggests that inhibition of the activity of glycosidase is an effective method for the treatment and prevention of diabetes.[4] The \( \alpha \)-glycosidase enzyme, also known as a small intestinal digestive enzyme, is one of the main metabolic enzymes in the body. It can quickly degrade dietary starches, leading to a quick increase in blood glucose levels.[5] Inhibition of \( \alpha \)-glucosidase delays the digestion and absorption of carbohydrates, resulting in a hypoglycemic effect by controlling postprandial hyperglycemia.[6] The filtering strategy based on \( \alpha \)-glucosidase inhibition has been widely applied in the discovery of anti-diabetic drugs.[7] Therefore, developing efficient inhibitors is very important to prevent diabetic complications.[8]

Natural \( \alpha \)-glucosidase inhibitor (AGI) is considered an effective drug to treat type 2 diabetes (T2DM). At present, researchers have screened out several AGIs from natural sources. For example, the triterpenoids from \textit{Actinidia arguta} that contained phenylpropanoid constituent unit showed inhibitory activity on \( \alpha \)-glucosidase.[9] The anthocyanins from the fruits of \textit{Cinnamomum camphora} tree inhibit \( \alpha \)-glucosidase.[10] Polyphenolic fraction derived from citrus has been confirmed to have an anti-glycation effect and has inhibitory activity on the \( \alpha \)-glycosidase.[11] \textit{Potentilla bifurca} (\textit{Potentilla bifurca} var. humilior) is a variety of \textit{Potentilla} in Rosaceae. A genus of \( \geq 500 \) species worldwide, it has annual or perennial shrubs or herbs with a large distribution in northern temperate regions.[12] The plant is short and is scattered on the surface. It is distributed in Qinghai, Inner Mongolia, Shanxi, Ningxia, Xinjiang, and Tibet in China. It is common in hillside grasslands, river beach sandy...
lands, and arid grasslands at 2300–4500 m above sea level. Quercetin and its derivatives from *P. bifurca* were studied to discuss the difference of activity of α-glucosidase in four compounds.

**MATERIALS AND METHODS**

**Extraction of four compounds**

The four compounds were developed by following these steps: The powdered and dried *P. bifurca* was extracted with petroleum ether and ethyl acetate at room temperature. With 100% methanol at 65°C for three times, the compounds were separated by chromatography using Sephadex LH-20, MCI-gel, and RP-18. Various chromatographic methods and separation techniques were used to separate quercetin and its derivatives.

**α-glucosidase inhibitory activity assay**

The α-glucosidase activity was assayed using 100 µL PBS buffer (Beijing Solarbio Science & Technology Co., Ltd.), 20 µL sample, 20 µL AG (0.05 U mL⁻¹, SigmaAldrich, USA), and 20 µL PNPG (8.92 × 10⁻³ mol L⁻¹, Sigma, USA) was used as substrates. Further, the enzyme control well was assayed using 120 µL PBS buffer, 20 µL AG (0.05 U mL⁻¹), and 20 µL PNPG (8.92 × 10⁻³ mol L⁻¹) was used as substrates. The color of the sample and the substrate affects the measurement results. To eliminate these effects, the sample control group (with 140 µL PBS buffer, 20 µL AG, and 20 µL PNPG) were both assayed. The whole enzymatic reaction was performed at 37°C for 30 min, and the absorption at 400 nm was monitored on a microplate spectrophotometer (Bio-Rad Laboratories, China). Each group was tested for 30 min, and the inhibition rate curve was made using Origin 9.1 package, and the double reciprocal curve was made by Microsoft Excel.

**α-glucosidase inhibitory type determination**

The enzyme reaction was performed under the abovementioned reaction conditions with inhibitors of various concentrations. The substrate (0.1254 mg mL⁻¹) was diluted to different concentrations of 0.0249 mg mL⁻¹, 0.0124 mg mL⁻¹, 0.0083 mg mL⁻¹, and 0.0062 mg mL⁻¹. According to reactions with compounds of different concentrations, the reaction rates were calculated. Inhibition types for the inhibitors were determined by Lineweaver–Burk plots.

**RESULTS**

**Separation and identification of chemical components**

Figure 1 shows the structure of the four compounds. The characteristics of the compounds are identified as follows:

Quercetin-3-O-β-D-glucopyranoside (QC3G), yellow powder. 

1H NMR (500 MHz, CD₃OD): 8.73 (1H, d, J = 1.8 Hz, H-2'), 7.68 (1H, dd, J = 8.7, 1.8 Hz, H-6'), 7.27 (1H, d, J = 8.7 Hz, H-5'), 6.36 (1H, d, J = 1.9 Hz, H-8), 6.16 (1H, d, J = 2.0 Hz, H-6), 4.91 (1H, s, H-1'), 3.36 ~ 3.49 (m, Glc-H2,3,4,5), 3.93 (1H, dd, J = 2.0, 12.1 Hz, Glc-H6a), 3.73 (1H, dd, J = 5.4, 12.1 Hz, Glc-H6b). 13C NMR (500 MHz, CD₃OD): δ 158.2 (C-2), 137.9 (C-3), 177.4 (C-4), 162.5 (C-5), 99.3 (C-6), 165.7 (C-7), 94.5 (C-8), 148.1 (C-9), 104.6 (C-10), 121.3 (C-1'), 116.5 (C-2'), 146.8 (C-3'), 117.6 (C-5'), 127.6 (C-6'); 103.4 (C-1), 74.8 (C-2), 78.4 (C-3), 71.3 (C-4), 77.5 (C-5), 62.4 (C-6).

Quercetin-3-O-β-D-xylose (QC3X), yellow powder. 

1H NMR (500 MHz, CD₃OD): δ 7.72 (1H, d, J = 1.9 Hz, H-2'), 7.63 (1H, dd, J = 8.5, 1.9 Hz, H-6'), 7.14 (1H, d, J = 8.7 Hz, H-5'), 6.39 (1H, brs, H-8), 6.18 (1H, brs, H-6), 4.87 (1H, s, H-1'), 3.96 (1H, m, H-5'), 3.60 (1H, m, H-4'), 3.54 (1H, m, H-3'), 3.48 (1H, m, H-3'), 3.42 (1H, m, H-5').

Quercetin-3-O-trans-p-coumaroyl-β-D-glucoside (QC3GP), yellow powder. 

FAB-MS m/z: 609 [M-H]; 1H NMR (600 MHz, CD₃OD): δ 7.77 (2H, d, J = 7.6 Hz, H-2'), 7.69 (1H, d, J = 8.4 Hz, H-6'), 7.16 (2H, d, J = 9.0 Hz, H-5'), 6.81 (1H, t, J = 6.0 Hz, H-3''), 6.39 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 1.8 Hz, H-6), 6.08 (1H, d, J = 15.6 Hz, H-8'), 5.02 (1H, d, J = 7.2 Hz, H-1'), 4.35 (1H, dd, J = 2.4, 1.8 Hz, H-6'), 3.49 (2H, m, H-3', 4').

13C NMR (600 MHz, CD₃OD): δ 177.64 (C-4), 165.87 (C-7), 162.70 (C-5), 158.41 (C-2), 158.37 (C-9), 148.11 (C-4'), 146.97 (C-3'), 138.15 (C-3), 127.82 (C-6'), 121.27 (C-1'), 115.77 (C-2'), 116.73 (C-5'), 104.72 (C-10), 103.98 (C-1'), 99.46 (C-6), 94.59 (C-8), 77.36 (C-3'), 74.72 (C-2'), 71.12 (C-4'), 67.17 (C-5').

Quercetin-3-O-(6'-O-trans-p-coumaroyl)-β-D-glucoside (QC3GP), yellow powder. 

1H NMR (500 MHz, CD₃OD): δ 7.77 (2H, d, J = 7.6 Hz, H-2'), 7.69 (1H, d, J = 8.4 Hz, H-6'), 7.16 (2H, d, J = 9.0 Hz, H-5'), 6.81 (1H, t, J = 6.0 Hz, H-3'''), 6.39 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 1.8 Hz, H-6), 6.08 (1H, d, J = 15.6 Hz, H-8''), 5.02 (1H, d, J = 7.2 Hz, H-1'), 4.35 (1H, dd, J = 2.4, 1.8 Hz, H-6''), 3.49 (2H, m, H-3', 4');

13C NMR (600 MHz, CD₃OD): δ 177.65 (C-4), 165.87 (C-7), 162.70 (C-5), 158.41 (C-2), 158.37 (C-9), 148.11 (C-4'), 146.97 (C-3'), 138.15 (C-3), 127.82 (C-6'), 121.27 (C-1'), 115.77 (C-2'), 116.73 (C-5'), 104.72 (C-10), 103.98 (C-1'), 99.46 (C-6), 94.59 (C-8), 77.36 (C-3'), 74.72 (C-2'), 71.12 (C-4'), 67.17 (C-5').

Statistical analysis

IC₅₀ was calculated and analyzed using statistical software, SPSS 17.0, and the inhibition rate curve was made using Origin 9.1 package, and the double reciprocal curve was made by Microsoft Excel.
Quercetin (QC), yellow powder. 1H NMR (600 MHz, CD3OD): δ 7.73 (1H, d, J = 2.1 Hz, H-2'), 7.63 (1H, dd, J = 2.1, 8.5 Hz, H-6'), 6.88 (1H, d, J = 8.4 Hz, H-5'), 6.38 (1H, d, J = 2.0 Hz, H-8), 6.17 (1H, d, J = 2.0 Hz, H-6).

α-glucosidase inhibitory activity

Our study showed that all four compounds could inhibit the hydrolysis of AG. In the concentration of 0.031, 0.0781, 0.1563, 0.3125, 0.625, 1.25 and 2.5 mg/mL−1, the inhibition rate gradually increased with the increase of concentration. The inhibition rates were all ≥70% at the maximum concentration, and the IC50 was 0.318, 0.082, 0.695, and 0.065 mmol/L−1. In the concentration of 0.19531, 0.3906, 0.781, 1.5625, 3.125, 6.25, and 12.5, 25 mg·mL−1, the inhibition rate of acarbose showed a dose-effect relationship with the concentration, and the IC50 was 4.509 mmol·L−1. Compared with acarbose, all four compounds had higher AG inhibitory activity [Figure 2 and Table 1].

α-glucosidase inhibitory type

To examine the inhibitory mechanism of P. bifurca compounds, the AGI type of four compounds of P. bifurca was determined. Vm is the reaction rate at which the enzyme is completely saturated with the substrate. The Km value is equal to the substrate concentration at which the enzymatic reaction rate is half the maximum rate. The Km value of the competitive inhibitor varies with the concentration, while the Vm value remains the same. The Km value of the non-competitive inhibitor did not change with the concentration, but the Vm value decreased. The Vm of compounds QC3G, QC3X, QC3GP remained unchanged; the Km increased with the increase of concentration and conformed to the reaction characteristics of competitive inhibitors. Km of compound QC increased with compound concentration and the Vm gradually decreased. Thus compounds QC3G, QC3X, QC3GP were competitive inhibitor types, and QC was a typical non-competitive inhibitor type [Figure 3 and Table 2].

DISCUSSION

Quercetin, a strong antioxidant, possesses anti-oxidative, anti-inflammatory, anti-aggregatory, anti-cancer, and anti-vasodilation effects. Several studies show that quercetin can attenuate diabetes. Based on the hypoglycemic activity of quercetin, the effect of the structural difference of quercetin and its derivatives on the inhibitory activity of α-glucosidase was studied. QC compared with QC3X, the hydroxy of position 3 was replaced by penta-glycoside and the hydroxyl shifted from 3’ position to 5’ position. QC compared with QC3G, the hydroxy of position 3 was replaced by hexose, and the hydroxyl shifted from 3’ position to 5’ position. QC compared with QC3GP, the hydroxy of position 3 was replaced by hexose, and the hydroxyl shifted from 3’ position to 5’ position. Our study showed that the inhibitory activity of α-glycosidase has some relationship with the glycosyl and acyl substitution of flavonoids. In the in vitro assay of inhibitory activity on α-glucosidase, QC showed the highest efficacy as inhibitors of α-glucosidase activity, QC3X showed higher efficacy as inhibitors of α-glucosidase activity, QC3G and QC3GP showed lower than QC and QC3X [Table 2]. Although the position of quercetin and its derivatives 3 and 5 were different, we suspected that this difference did not affect quercetin activity. The substituents of different compounds may affect the activity of flavonoids. Quercetin glycosylation can lead to the decrease of hypoglycemic activity, and the compound after adding penta-glycoside is larger than that after adding hexa-glycoside. In addition, the acylation of compounds can reduce the activity of the original compounds.

**Table 1: Inhibitory activity of different compounds against α-glucosidase (X±SD)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Max concentration (mg·mL⁻¹)</th>
<th>Inhibition rate (100%)</th>
<th>IC50 (mmol·mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC3G</td>
<td>1.250</td>
<td>77.324±0.092</td>
<td>0.318</td>
</tr>
<tr>
<td>QC3X</td>
<td>2.50</td>
<td>92.065±0.064</td>
<td>0.082</td>
</tr>
<tr>
<td>QC3GP</td>
<td>1.250</td>
<td>88.156±0.045</td>
<td>0.695</td>
</tr>
<tr>
<td>QC</td>
<td>2.500</td>
<td>78.839±0.122</td>
<td>0.065</td>
</tr>
<tr>
<td>acarbose</td>
<td>25.000</td>
<td>78.85±0.034</td>
<td>4.509</td>
</tr>
</tbody>
</table>

QC3G: quercetin-3-O-β-D-glucopyranoside; QC3X: quercetin-3-O-β-D-xylose; QC3GP: quercetin-3-O-(6''-O-trans-p-coumaroyl)-β-D-glucoside; QC: quercetin

**Figure 2: AG inhibition rate curve of each compound**
CONCLUSION
Overall, the compounds from *P. bifurca* were found as potent α-glucosidase inhibitors, and QC exhibited the strongest inhibitory activity on α-glucosidase. This study suggested that quercetin and quercetin derivatives are the suitable lead compounds for designing new patent α-glucosidase inhibitors of this kind.

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Conflicts of interest
There are no conflicts of interest.

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