

# Quercetin of *Potentilla bifurca* 3-Glycosylation Substitution Impact the Inhibitory Activity on $\alpha$ -glucosidase

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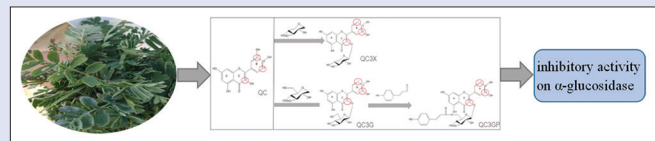
## ABSTRACT

**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  $\alpha$ -glucosidase 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:**  $\alpha$ -glucosidase, derivatives, diabetes, *P. bifurca*, quercetin

## SUMMARY

- At present, there are few studies on the *Potentilla bifurca*, and the pharmacological value of *Potentilla bifurca* needs to be explored. The inhibitory activity and type of  $\alpha$ -glucosidase of the *Potentilla bifurca* compounds were used to explore experiments, and the difference in activity of each compound due to the structure was compared. It proves the potential value of *Potentilla bifurca* in the treatment of type 2 diabetes



**Abbreviations used:** AG:  $\alpha$ -glucosidase; PNP: p-Nitrophenyl  $\alpha$ -D-glucopyranoside; QC3G: Quercetin- 3-O- $\beta$ -D-glucopyranoside; QC3X: Quercetin-3-O- $\beta$ -D-xylose; QC3GP: Quercetin- 3-O-(6"-O-trans-p-coumaroyl)- $\beta$ -D-glucoside; QC: Quercetin.

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## 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.<sup>[1]</sup> 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.<sup>[2]</sup> Glycosidases, which as some of the most specific and proficient catalysts, have gained prominence.<sup>[3]</sup> Some research suggests that inhibition of the activity of glycosidase is an effective method for the treatment and prevention of diabetes.<sup>[4]</sup> The  $\alpha$ -glucosidase 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.<sup>[5]</sup> Inhibition of  $\alpha$ -glucosidase delays the digestion and absorption of carbohydrates, resulting in a hypoglycemic effect by controlling postprandial hyperglycemia.<sup>[6]</sup> The filtering strategy based on  $\alpha$ -glucosidase inhibition has been widely applied in the discovery of anti-diabetic drugs.<sup>[7]</sup> Therefore, developing efficient inhibitors is very important to prevent diabetic complications.<sup>[8]</sup>

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 *Actinidia arguta* that contained phenylpropanoid constituent unit showed inhibitory activity on  $\alpha$ -glucosidase.<sup>[6]</sup> The anthocyanins from the fruits of the *Cinnamomum camphora* tree inhibit  $\alpha$ -glucosidase.<sup>[9]</sup> Polyphenolic fraction derived from citrus has been confirmed to have an anti-glycation effect and has inhibitory activity on the  $\alpha$ -glycosidase.<sup>[10]</sup> *Potentilla bifurca* (*Potentilla bifurca* var. *humilior*) is a variety of *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.<sup>[11]</sup> 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

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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  $\alpha$ -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.

### $\alpha$ -glucosidase inhibitory activity assay

The  $\alpha$ -glucosidase activity was assayed using 100  $\mu$ L PBS buffer (Beijing Solarbio Science & Technology Co., Ltd.), 20  $\mu$ L sample, 20  $\mu$ L AG (0.05 U·mL<sup>-1</sup>, SigmaAldrich, USA), and 20  $\mu$ L PNPG (8.92  $\times 10^{-3}$  mol·L<sup>-1</sup>, Sigma, USA) was used as substrates. Further, the enzyme control well was assayed using 120  $\mu$ L PBS buffer, 20  $\mu$ L AG (0.05 U·mL<sup>-1</sup>), and 20  $\mu$ L PNPG (8.92  $\times 10^{-3}$  mol·L<sup>-1</sup>) 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  $\mu$ L PBS buffer, and 20  $\mu$ L sample) and the substrate control group (with 140  $\mu$ L PBS buffer, and 20  $\mu$ L 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 three times at the same time, the inhibition rate was calculated according to the formula, and the average value was taken for analysis.

$$\text{Inhibition rate (100\%)} = \frac{(Y_1 - Y_3) - (Y_2 - Y_4)}{Y_1 - Y_3} \times 100 \quad (1)$$

where  $Y_1$  and  $Y_2$  denote the original enzyme activity and the enzyme activity after adding sample; respectively,  $Y_3$  and  $Y_4$  represent the PNPG background and sample background.

### $\alpha$ -glucosidase inhibitory type determination

The enzyme reaction was performed under the abovementioned reaction conditions with inhibitors of various concentrations. The substrate (0.1245 mg·mL<sup>-1</sup>) was diluted to different concentrations of 0.0249 mg·mL<sup>-1</sup>, 0.0124 mg·mL<sup>-1</sup>, 0.0083 mg·mL<sup>-1</sup>, and 0.0062 mg·mL<sup>-1</sup>. According to reactions with compounds of different concentrations, the reaction rates were calculated. Inhibition types for the inhibitors were determined by Lineweaver–Burk plots.

## Statistical analysis

IC<sub>50</sub> was calculated and analyzed using statistical software, SPSS 17.0, the inhibition rate curve was made using Origin 9.1 package, and the double reciprocal curve was made by Microsoft Excel.

## 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- $\beta$ -D-glucopyranoside (QC3G), yellow powder. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$ : 7.73 (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''), 3.30 ~ 3.49 (m, Glc-H2,3,4,5), 3.93 (1H, dd,  $J$  = 2.0, 12.1 Hz, Glc-H6 $\alpha$ ), 3.73 (1H, dd,  $J$  = 5.4, 12.1 Hz, Glc-H6 $\beta$ ). <sup>13</sup>C NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$ : 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'), 147.8 (C-4'), 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- $\beta$ -D-xylose (QC3X), yellow powder. <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD):  $\delta$ : 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-2''), 3.48 (1H, m, H-3''), 3.42 (1H, m, H-5''); <sup>13</sup>C-NMR (600 MHz, CD<sub>3</sub>OD):  $\delta$ : 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'), 117.57 (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)- $\beta$ -D-glucoside (QC3GP), yellow powder. FAB-MS  $m/z$ : 609 [M-H]<sup>-</sup>; <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD):  $\delta$ : 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 (2H, t,  $J$  = 6.0 Hz, H-3''', 5'''), 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''); <sup>13</sup>C-NMR (600 MHz, CD<sub>3</sub>OD):  $\delta$ : 177.65 (C-4), 168.92 (C-9'''), 166.09 (C-7), 163.12 (C-5), 161.68 (C-4'), 161.34 (C-4'''), 159.46 (C-2), 158.55 (C-9), 146.98 (C-7'''), 138.16 (C-3), 132.35 (C-2', 6'), 130.52 (C-2'', 6''), 126.77 (C-1'''), 121.27 (C-1'), 117.48 (C-3', 5'), 116.73 (C-3'', 5''), 114.84 (C-8'''), 104.72 (C-10), 103.44 (C-1''), 99.47 (C-6), 94.60 (C-8), 77.82 (C-3'''), 74.83 (C-2''), 73.10 (C-5''), 71.12 (C-4''), 67.17 (C-6'').

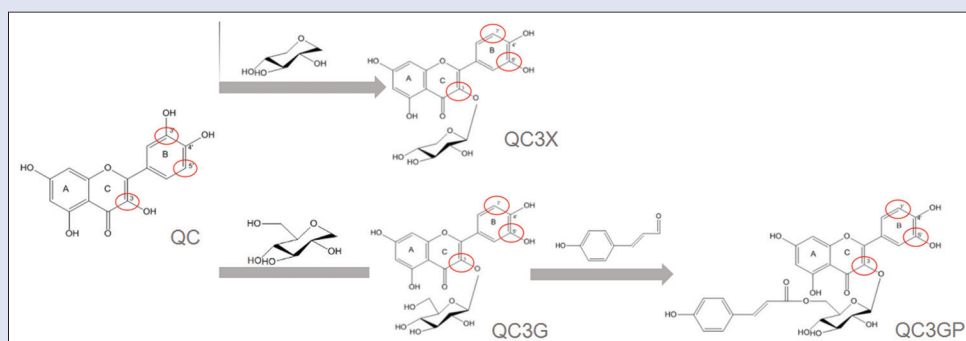


Figure 1: The structure of four compounds

Quercetin (QC), yellow powder.  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  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).

### $\alpha$ -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  $\text{mg/mL}^{-1}$ , the inhibition rate gradually increased with the increase of concentration. The inhibition rates were all  $\geq 70\%$  at the maximum concentration, and the  $\text{IC}_{50}$  was 0.318, 0.082, 0.695, and 0.065  $\text{mmol/L}^{-1}$ . In the concentration of 0.19531, 0.3906, 0.781, 1.5625, 3.125, 6.25, and 12.5, 25  $\text{mg}\cdot\text{mL}^{-1}$ , the inhibition rate of acarbose showed a dose-effect relationship with the concentration, and the  $\text{IC}_{50}$  was 4.509  $\text{mmol}\cdot\text{L}^{-1}$ . Compared with acarbose, all four compounds had higher AG inhibitory activity [Figure 2 and Table 1].

### $\alpha$ -glucosidase inhibitory type

To examine the inhibitory mechanism of *P. bifurca* compounds, the AGI type of four compounds of *P. bifurca* was determined.  $V_m$  is the reaction rate at which the enzyme is completely saturated with the substrate. The  $K_m$  value is equal to the substrate concentration at which the enzymatic reaction rate is half the maximum rate. The  $K_m$  value of the competitive

inhibitor varies with the concentration, while the  $V_m$  value remains the same. The  $K_m$  value of the non-competitive inhibitor did not change with the concentration, but the  $V_m$  value decreased. The  $V_m$  of compounds QC3G, QC3X, QC3GP remained unchanged; the  $K_m$  increased with the increase of concentration and conformed to the reaction characteristics of competitive inhibitors.  $K_m$  of compound QC increased with compound concentration and the  $V_m$  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].

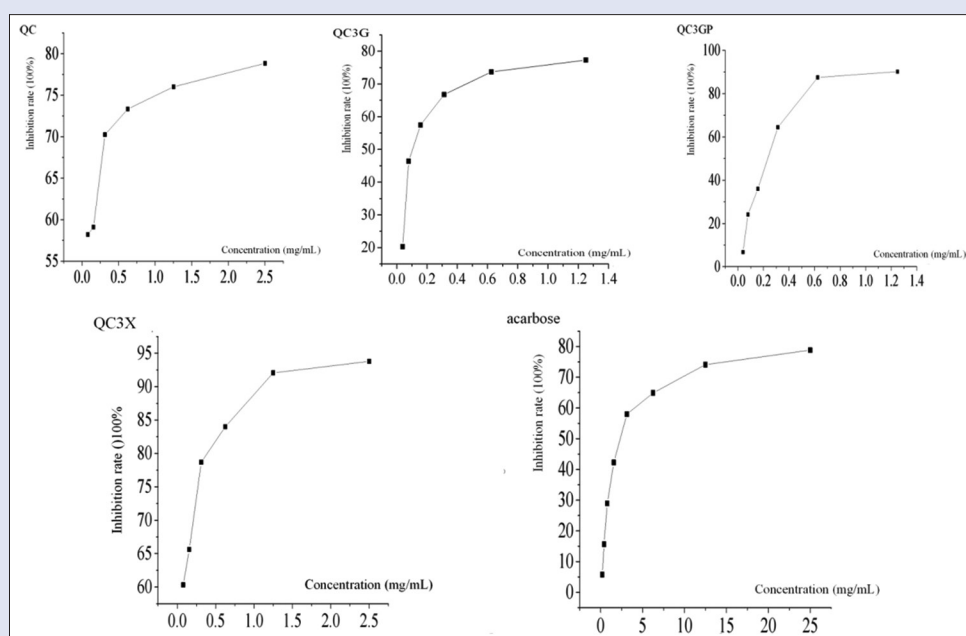
## DISCUSSION

Quercetin, a strong antioxidant, possesses anti-oxidative, anti-inflammatory, anti-aggregatory, anti-cancer, and anti-vasodilation effects.<sup>[12]</sup> Several studies show that quercetin can attenuate diabetes.<sup>[13,14]</sup> Based on the hypoglycemic activity of quercetin, the effect of the structural difference of quercetin and its derivatives on the inhibitory activity of  $\alpha$ -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  $\alpha$ -glucosidase has some relationship with the glycosyl and acyl substitution of flavonoids. In the *in vitro* assay of inhibitory activity on  $\alpha$ -glucosidase, QC showed the highest efficacy as inhibitors of  $\alpha$ -glucosidase activity, QC3X showed higher efficacy as inhibitors of  $\alpha$ -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  $\alpha$ -glucosidase ( $X \pm \text{SD}$ )

Name	Max concentration ( $\text{mg}\cdot\text{mL}^{-1}$ )	Inhibition rate (100%)	$\text{IC}_{50}$ ( $\text{mmol}\cdot\text{mL}^{-1}$ )
QC3G	1.250	77.324 $\pm$ 0.092	0.318
QC3X	2.50	92.065 $\pm$ 0.064	0.082
QC3GP	1.250	88.156 $\pm$ 0.045	0.695
QC	2.500	78.839 $\pm$ 0.122	0.065
acarbose	25.000	78.85 $\pm$ 0.034	4.509

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

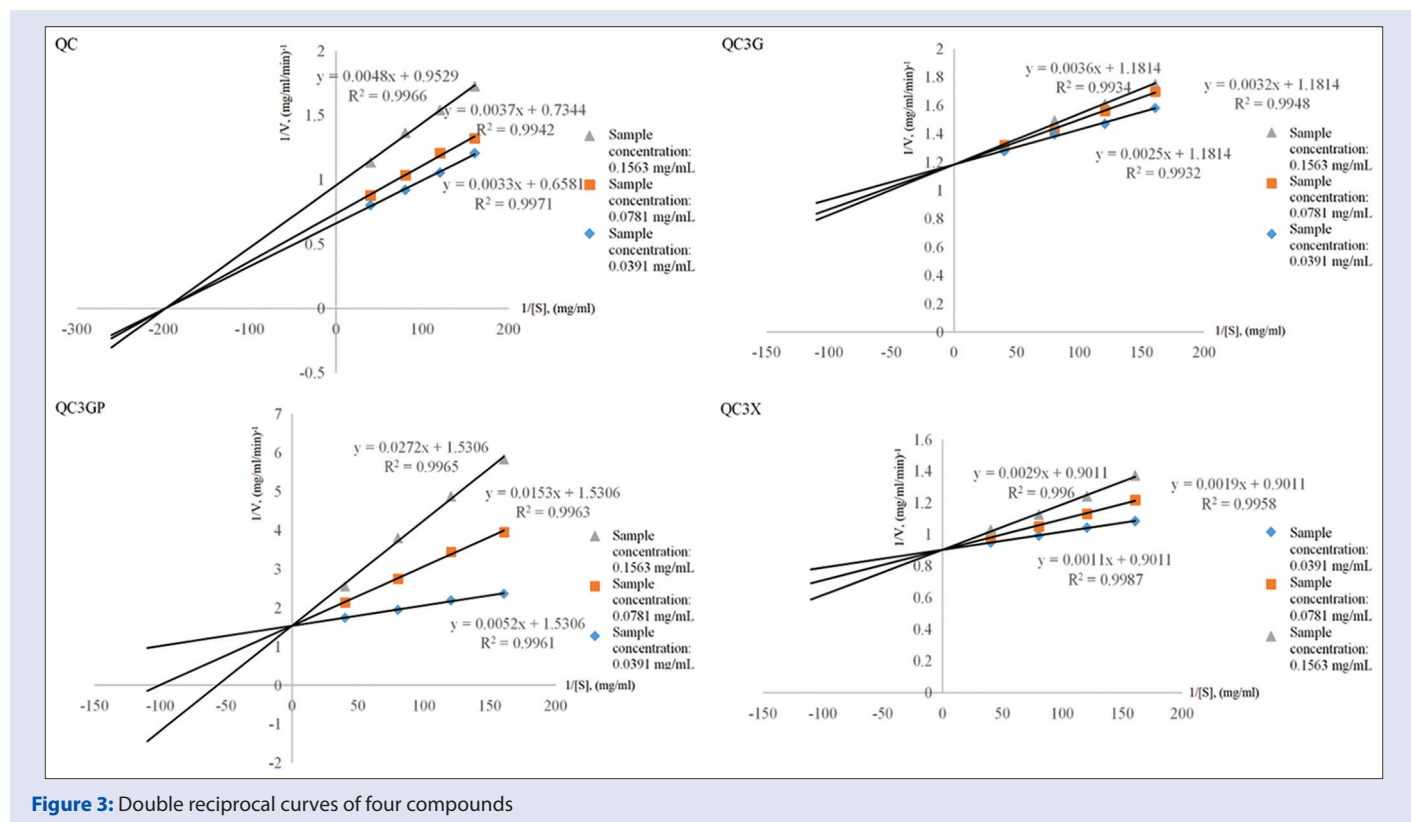


**Figure 2:** AG inhibition rate curve of each compound

**Table 2:** Enzyme inhibitor types of four compounds

Name	Sample concentration (mg·mL <sup>-1</sup> )	K <sub>m</sub> ' (mg·mL <sup>-1</sup> )	V <sub>m</sub> ' (mg·mL <sup>-1</sup> ·min <sup>-1</sup> )	Inhibitors type
QC3G	0.1563	0.0030	0.8465	Competitive
	0.0781	0.0027	0.8465	
	0.0391	0.0021	0.8465	
QC3X	0.1563	0.0032	1.1098	Competitive
	0.0781	0.0021	1.1098	
	0.0391	0.0012	1.1098	
QC3GP	0.1563	0.0178	0.6533	Competitive
	0.0781	0.0099	0.6533	
QC	0.0391	0.0034	0.6533	Non-competitive
	0.1563	0.0050	1.0494	
	0.0781	0.0050	1.3617	
	0.0391	0.0050	1.5195	

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



## CONCLUSION

Overall, the compounds from *P. bifurca* were found as potent  $\alpha$ -glucosidase inhibitors, and QC exhibited the strongest inhibitory activity on  $\alpha$ -glucosidase. This study suggested that quercetin and quercetin derivatives are the suitable lead compounds for designing new patent  $\alpha$ -glucosidase inhibitors of this kind.

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## Conflicts of interest

There are no conflicts of interest.

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