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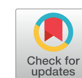
# In vitro and in vivo inhibitory activity of taxifolin on three digestive enzymes



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ABSTRACT

The inhibitory activity of taxifolin on three digestive enzymes were investigated in both vitro and vivo. Taxifolin exhibited inhibitory effect on α-glucosidase, α-amylase and pancreatic lipase with IC50 values of 0.038, 0.647 and 0.993 mg/mL, respectively. Inhibitory kinetics indicated that taxifolin was more like a competitive inhibitor of α-glucosidase and α-amylase, while it was a non-competitive inhibitor of pancreatic lipase. The binding of taxifolin caused the quenching of intrinsic fluorescence intensity of enzymes, and the binding constant ($lgK_a$) and the number of binding site (n) were further calculated through fluorescence titration. The values of $lgK_a$ were in the range of 4.93-6.65, and the values of n were all close to 1. Molecular docking indicated that taxifolin could interact with α-glucosidase and α-amylase through many kinds of secondary interaction, such as hydrogen bond, π-π stack, etc. In vivo study revealed that pre-administration with taxifolin can significantly improve the postprandial hyperglycemia in rat. Furthermore, its can also decrease triglyceride absorption through the inhibition of pancreatic lipase.

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## 1. Introduction

Today, overweight and obesity as well as their associated diseases, such as type2 diabete and cardiovascular diseases, are increasing serious around the world [1]. The excess intake of calories is one of the main cause for these health problems [1]. The reducing of carbohydrates and lipids absorption from diet may improve the situation [2,3]. α-Glucosidase, α-amylase and pancreatic lipase are the three key enzymes responsible for calories absorption. In digestive system, α-amylase first hydrolyzes polysaccharides into oligosaccharides, then α-glucosidase catalyzes the final step to release the absorbable monosaccharides [4]. Before being absorbed by the small intestine, dietary fat is first hydrolyzed by lipase into monoglycerol and free fatty acids. Pancreatic lipase is the most important enzyme that can hydrolyze 50% ~70% of food fat [5]. The inhibition of these enzymes could help in controlling postprandial hyperglycaemia and/or reducing calories intake, thereby resulting far-reaching health benefits in type2 diabete and obesity treatment [3–5]. However, long term intake of synthetic antidiabetic drugs and anti-obesity drugs such as acarbose and orlistat may cause many side effects, such as liver toxicity, fat diarrhea and gastrointestinal symptoms [6,7]. Thus, searching for multiple digestive enzymes inhibitor from edible and medical plants is attracting more and more attention [8–10]. Polyphenol, particularly flavonoids, showed the promising multiple digestive enzymes inhibitory activity [2,11].

Taxifolin, 3,5,7,3,4-pentahydroxy flavanone, also called dihydroquercetin, is a flavanonol commonly found in many plants and fruits, such as Douglas fir, onion, milk thistle, orange, grape and grapefruit [12,13]. It is also the principle component of some healthy products and drugs in American and European, such as silymarin (Legalon™) and Pycnogenol® [12]. European Food Safety Authority has confirmed the safety of Dahurian Larch extract with taxifolin content>90%. The extract is intended to be added as food ingredient in non-alcoholic beverages, yogurt and chocolate confectionery [14]. Weidmann and Sunil et al. have summarized the health-promoting effects of taxifolin in 2012 and 2019, respecively [12,15]. The modern pharmacological studies showed that taxifolin possesses multiple bioactivities in the management of inflammation, tumors, microbial infections, oxidative stress, cardiovascular, and liver disorders [15]. Particularly, taxifolin shows more prominent anti-cancer activity in both vitro and vivo models [15]. However, the study about the digestive enzymes inhibitory activity of taxifolin was few. More recently, Rehman et al. found that taxifolin significantly decreased the serum level of α-amylase in diabetic rats [16]. Molecular docking results showed that taxifolin have stronger binding affinity with α-amylase than acarbose. Thus, the authors presume that taxifolin might be a potent inhibitor of α-amylase.

In the present paper, the inhibitory activities of taxifolin as well as its rhamnose glycoside (astilbin) on three digestive enzymes, i.e. α-glucosidase, α-amylase and pancreatic lipase, were studied. The binding affinity between taxifolin and enzymes was investigated by fluorescence titration and molecular docking study. Finally, the inhibitory activities of taxifolin on glucose and fat absorption were evaluated in rat.

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## 2. Materials and methods

### 2.1. Chemicals and reagents

α-Glucosidase (EC 3.2.1.20) from *saccharomyces cerevisiae*, α-amylase from porcine pancreas, and *p*-nitrophenyl-α-D-glucopyranoside (PNPG) were purchased from Sigma Co., Ltd. (St. Louis, MO, U.S.A.). Porcine pancreatic lipase was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Taxifolin and astilbin (>98%) were purified from *Rhizoma smilacis glabrae* in our laboratory and was identified by NMR, MS and UV. Acarbose, orlistat and *p*-nitrophenyl palmitate (PNPP) were bought from Aladdin Co., Ltd (Shanghai, China). All other reagents used were of analytical grade. Milli-Q water was used throughout the study. Enzyme inhibition studies were performed by using a Thermo Microplate Spectrophotometer (Multiskan FC, USA).

### 2.2. Animals

Female Sprague-Dawley (SD) rats (~250 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd. and were breed in a controlled environment with temperature of 23 ± 2°C, humidity of 60 ± 10% and 12-h light/dark cycle. The animal studies were complied with the guidelines of Jiangxi Agricultural University on animal care.

### 2.3. Preparation of taxifolin-encapsulated zein-caseinate nanoparticles

The taxifolin-loading zein-caseinate nanoparticles used for in vivo study was prepared according to our previous study [17]. Briefly, 100 mg of zein mixed with 100 mg of taxifolin was dissolved in 10 mL of 70% ethanol solution. The mixture was dropped into 25 mL of water containing 200 mg of sodium caseinate under quick magnetic stirring. The mixture was concentrated to 10 mL and then centrifuged at 2000 g for 10 min to remove large particles and free taxifolin. The concentration of taxifolin the solution was determined by HPLC with value of 7.8 mg/mL.

### 2.4. Inhibitory activity of taxifolin and astilbin on α-glucosidase, α-amylase and pancreatic lipase

α-Glucosidase inhibitory activity was determined using PNPG as the substance, which was hydrolyzed by the enzyme to form coloured *p*-nitrophenol [18]. Briefly, 150 μL of phosphate buffer (0.1M, pH 6.8), 20 μL of enzyme (1U/mL in phosphate buffer) and 10 μL of taxifolin/astilbin (disolved in 50% ethanol with different concentration) were mixed together in a 96-well plates. The mixture was hatched at 37 °C for 20 min, then 20 μL of PNPG (10 mM) pre-incubated at 37 oC was added to start the reaction. The absorbance at 405 nm was recorded for 20 min with interval of 1 min. The absorbance growth slope (V) which represented the enzyme activity was calculated by linear regression. The percentage of inhibition was calculated by the follow equation:

$$\text{Inhibition}(\%) = \frac{V_0 - V}{V_0} \times 100\%$$

where $V_0$ and V were the enzyme activity in the absence and presence of inhibitor, respectively. Acarbose was used as the positive control. The same reaction mixture but without α-glucosidase was used as the negative control, in which no absorbance change was found.

The activity of α-amylase was determined by hydrolyzing starch to form glucose, which was measured by the reduction of 3,5-dinitrosalicylic acid (DNS) [19]. Briefly, 900 μL of gelatinized starch (2 mg/mL), 50 μL of enzyme (6 U/mL) and 50 μL of taxifolin/astilbin was reacted at 37°C for 15min. Then, 50 μL of the reaction solution was immediately mixed with 200 μL of DNS and placed in a boiling bath for 5 minutes. The absorbance of the mixture was determined at 540nm. The percentage of inhibition was calculated by follow equation

$$\text{Inhibition} = \frac{(A_1 - A_2) - (A_3 - A_4)}{A_1 - A_2} \times 100\%$$

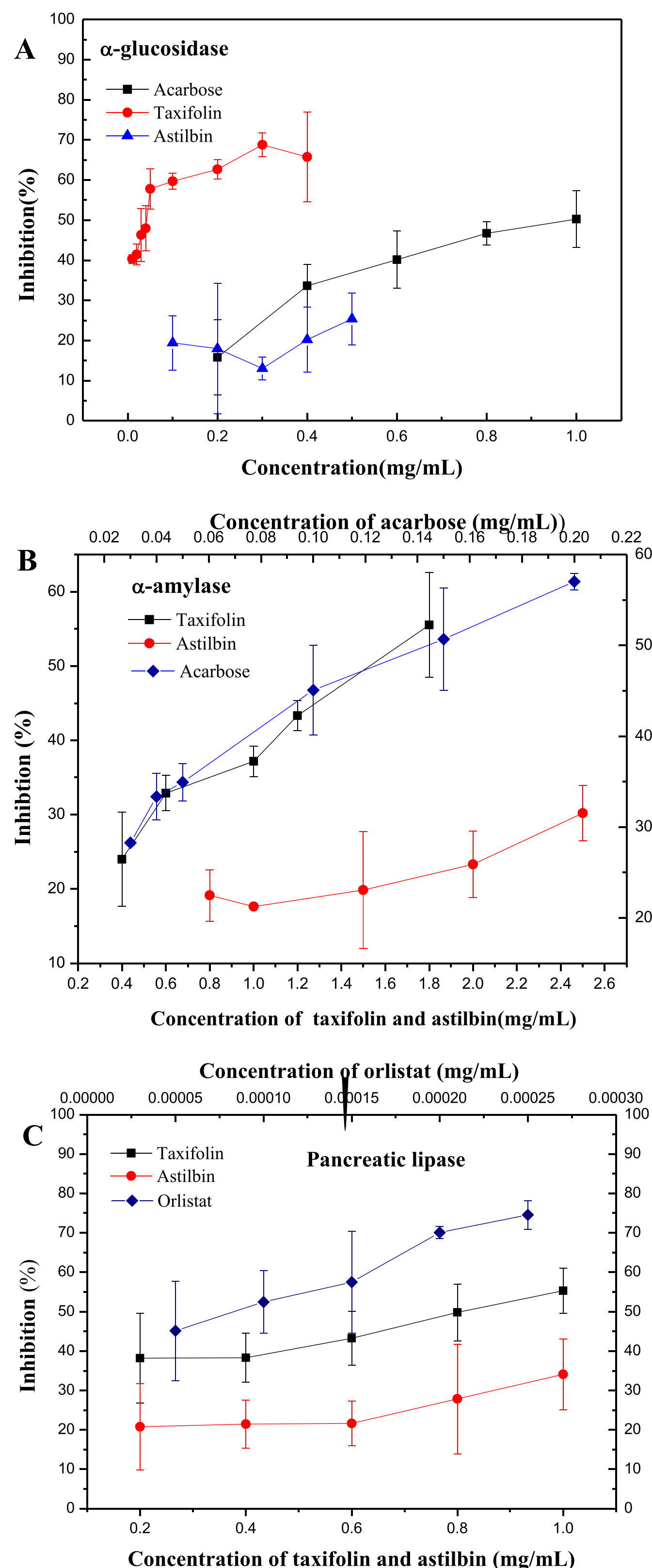


**Fig. 1.** Inhibitory activity of taxifolin and astilbin on α-glucosidase (A), α-amylase (B) and pancreatic lipase (C).

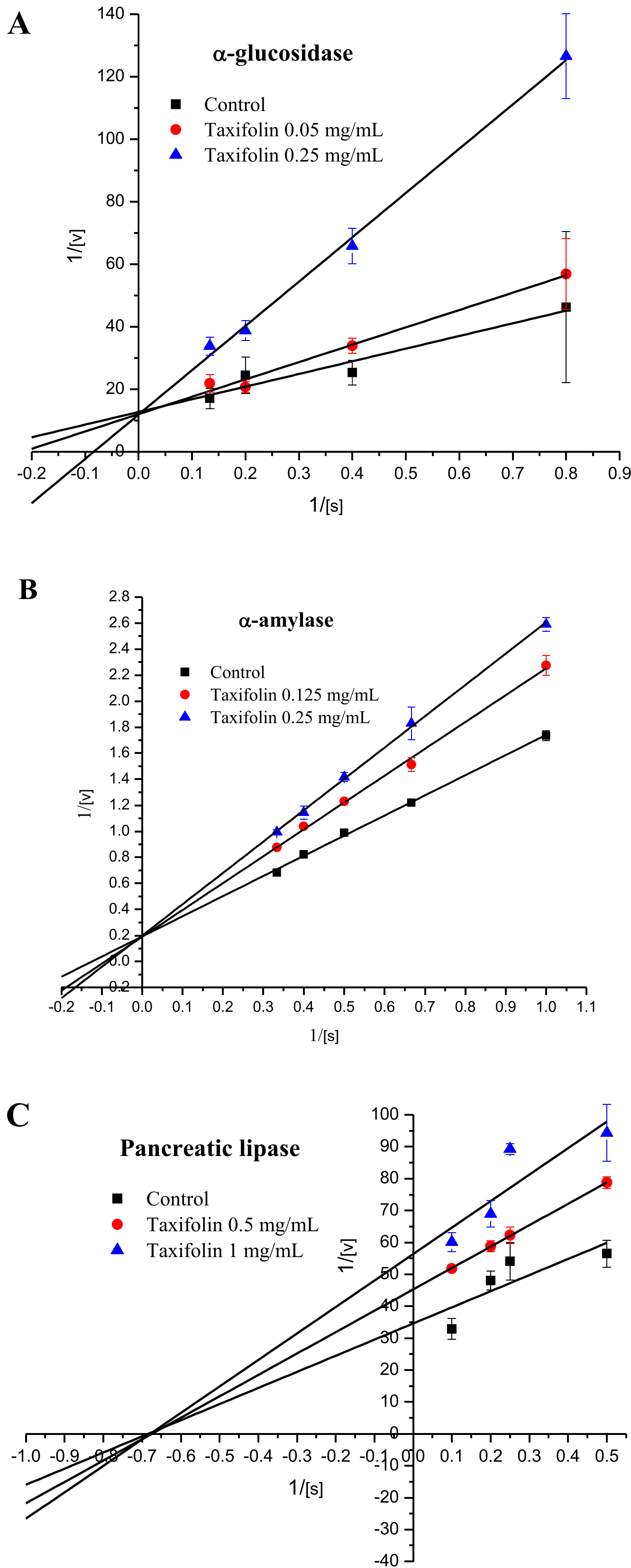


**Fig. 2.** Inhibitory kinetics of taxifolin on α-glucosidas (A), α-amylase (B) and pancreatic lipase (C).

where $A_1$ and $A_3$ were absorbance in the absence and presence of inhibitor, $A_2$ and $A_4$ were the correspond blank control without enzyme, respectively.

Pancreatic lipase inhibitory activity was determined using PNPP as the substance, which was hydrolyzed by lipase to form *p*-nitrophenol with maximum absorption around 405 nm [20]. Lipase (10 mg) was dissolved in 5 mL Tris-buffer (50 mM, pH 8, containing 0.1% gum arabic powder and 0.2% sodium deoxycholate). The mixture was stirred for 15 min and centrifuged at 1800 g for 10 min. The clear supernatant was used for the assay. Briefly, in a 96-well microplate, 30 μL Tris-buffer, 150 μL enzyme and 10 μL taxifolin/astilbin were mixed together.

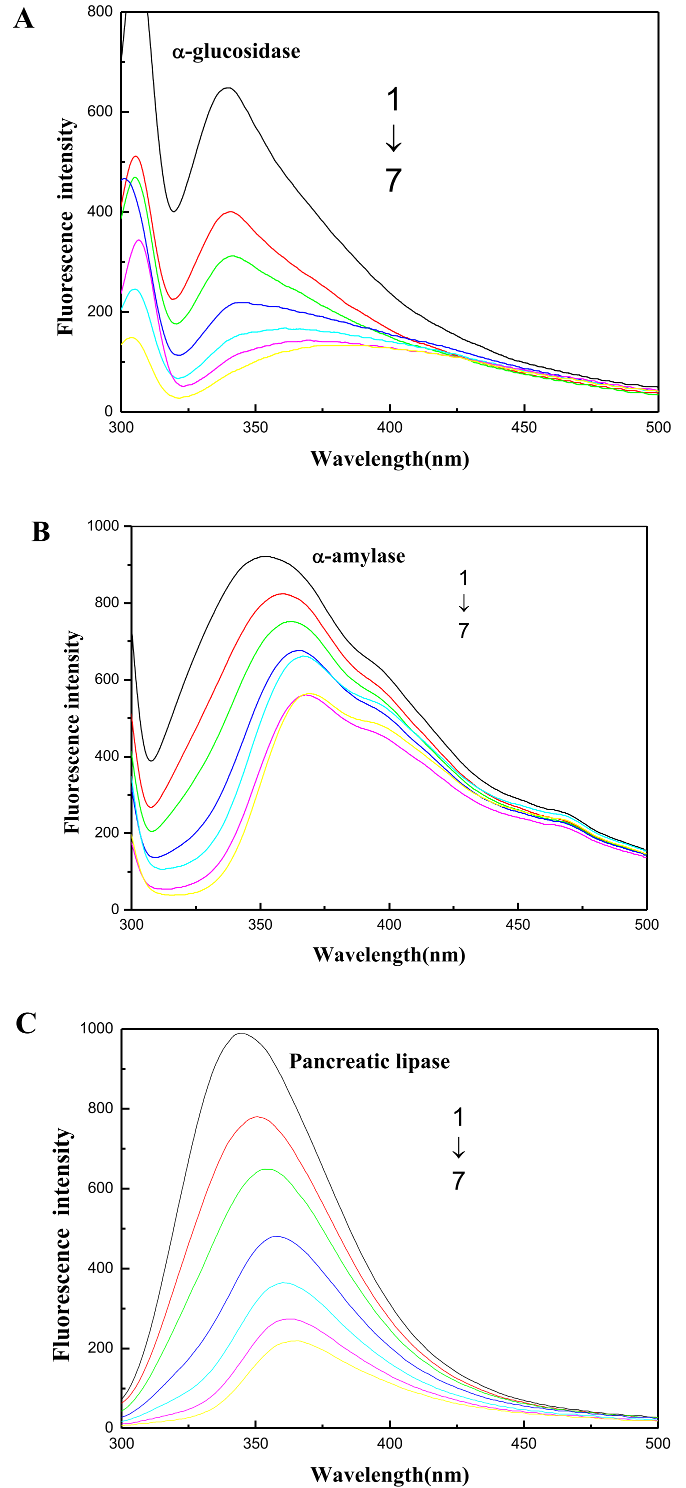


**Fig. 3.** The interaction of taxifolin with α-glucosidase (A), α-amylase (B) and pancreatic lipase (C) investigated by fluorescence quenching titration. The concentration of taxifolin from 1~7 were 0, 6.5, 13.1, 26.2, 39.4, 52.5, 65.7 μmol/L, respectively.

**Table 1**
Inhibitory activity ($IC_{50}$) of taxifolin on the three digestive enzymes and their binding parameters calculated through fluorescence titration.

| | Inhibitory activity ($IC_{50}$, mg/mL) | | | Binding parameters | | |
|---|---|---|---|---|---|---|
| | Acarbose | Taxifolin | Orlistat | $K_q$/ $10^{13}$L/(mol·s) | $lgK_a$ | n |
| α-Glucosidase | 0.917 | 0.038 | - | 1.06 | 5.34 | 1.08 |
| α-Amylase | 0.135 | 1.555 | - | 1.27 | 6.65 | 1.39 |
| Pancreatic lipase | - | 0.993 | 0.000074 | 0.206 | 4.93 | 1.0 |

The other procedures and inhibitory activity calculation were the same as α-glucosidase assay.

### 2.5. Inhibitory kinetics assay

The enzyme activity was determined under different concentrations of substrate with fixed concentration of taxifolin. The inhibition type of taxifolin on enzyme was determined by Lineweaver-Burk plot of Michaelis-Menten equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

where S is the substrate concentration, V is the initial velocity in the presence and absence of inhibitors, $K_m$ is the Michaelis-Menten constant, $V_{max}$ is the maximum enzyme speed.

### 2.6. Fluorescence quenching measurement

The three enzymes solution was mixed with different concentrations of taxifolin (from 0 to 65.7 μM), respectively. Then, the fluorescence spectra of the mixture was recorded between 300 to 450 nm. The excitation wavelengths for α-glucosidase, α-amylase and pancreatic lipase were 295, 278 and 280 nm, respectively. A 970 CRT spectrofluophotometer (Shanghai Scientific Instruments Limited Company, Shanghai, China) was used, and the excitation and emission bandwidth were set at 10 nm.

### 2.7. Molecular docking (MD) study of taxifolin with α-glucosidase and α-amylase

Molecular docking study was performed on AutoDock Vina program (http://vina.scripps.edu/). The three-dimensional structure of α-glucosidase (PDB ID 3A4A) and α-amylase (PDB ID 1B2Y) was downloaded from RCSB Protein Data Bank [18]. The three-dimensional structure of taxifolin was drawn and energy minimized by Chemdraw software (CambridgeSoft Corporation, USA). AutoDock Tools was used for hydrotreating, distributing Gasteiger particle charges, and merging non-polar hydrogen atoms. The catalytic site of α-glucosidase and α-amylase was used as the docking site. The resulting docked poses and

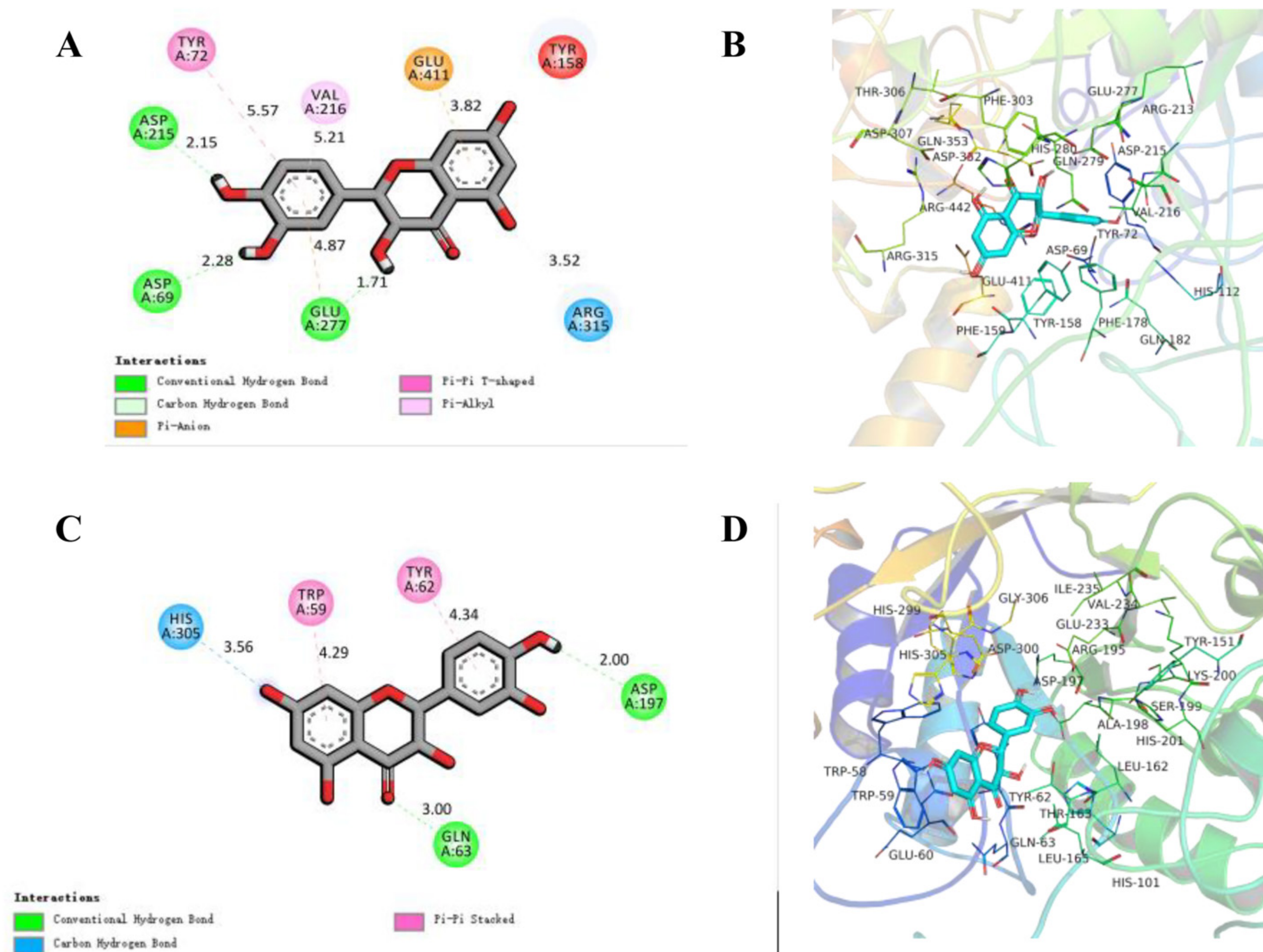


**Fig. 4.** Molecular docking study of taxifolin with α-glucosidase (A, B) and α-amylase (C, D).

receptor-ligand interactions were analyzed by using PyMOL software (Schrodinger, LLC, New York, NY, USA). Graphical visualization of all the docking complexes was conducted using Discovery Studio 2016 Client (Accelrys Inc. San Diego, CA, USA).

### 2.8. Effect of taxifolin on the reduction of glucose absorption in rat

Fifteen rats (~250g) were randomly divided into three groups (n= 5). Before the animal experiments, rats were fasted overnight with access to water. The three group of rats were intragastrically administered with 1 mL of water, acarbose (50 mg/kg bwt), taxifolin nanoparticles (50 mg/kg bwt), respectively. After 15 minutes, all rats were intragastrically administered with 3 g/kg bwt of gelatinized starch. Blood samples (~100 μL) were obtained from tail veins at 0.25, 0.5, 1 and 2 h after starch administration. After centrifugation at 2000 g for 10 min, the serum samples were used for glucose determination under the guide of commercial kit (BioSino Bio-Technology & Science Inc., China).

### 2.9. Effect of taxifolin on the reduction of fat absorption in rat

The experiment design was the same as glucose absorption test. Orlistat (10 mg/kg bwt) was used as positively control instead of acarbose, and the dose of taxifolin and corn oil were 100 mg/kg and 5 mL/kg bwt, respectively. TG content in the serum were determined with commercial kit (BioSino Bio-Technology & Science Inc., China).

### 2.10. Statistical analysis

Data were expressed as mean ± standard deviation of triplicates. Data analysis and plotting were performed with software of Origin 7.0 (Origin Lab Co., Northampton, MA, USA). One-way ANOVA was used for statistical analysis. Differences were considered significant when $P$<0.05.

## 3. Result and discussion

### 3.1. Inhibitory activity of taxifolin and astilbin on α-glucosidase, α-amylase and pancreatic lipase

α-Amylase and α-glucosidase are the two most important enzymes in carbohydrate digestion. The inhibitory effects of taxifolin and astilbin on the enzymes were shown in Fig. 1A and B. The results showed that taxifolin had a dose-dependent inhibitory effect on α-glucosidase activity, and the IC50 value was 0.038 mg/mL. The IC50 value of acarbose, the commonly used positive control of α-glucosidase, was 0.917 mg/mL. Taxifolin exhibited superior inhibitory effect than acarbose. It is also noted that the activity of taxifolin was far stronger than its rhamnoside, astilbin. At the concentration of 0.4 mg/mL, the inhibition rates of taxifolin, astilbin and acarbose on α-glucosidase were 65.75%±11%, 20.24%±8% and 33.61%±9%, respectively. Xiao et al. have summarized the structure-activity relationship of dietary polyphenols as inhibitors of α-glucosidase and concluded that the glycosylation of flavonoids lowered their inhibitory effect [21]. Similar results were found for α-amylase. With the rise of taxifolin concentration, its inhibitory effect on α-amylase gradually enhanced. The IC50 values of taxifolin and acarbose were 1.555 and 0.135mg/mL, respectively, which meaning that the inhibitory activity of taxifolin was weaker. Astilbin has no obvious inhibitory effect on α-amylase compared to taxifolin.

Orlistat is a semi-synthetic lipstatin derivative, which can covalently bind to the serine residue in the active site of pancreatic lipase and exhibit strong inhibitory effect [11,22]. Due to its strong activity in reducing fat absorption, orlistat is a FDA approved anti-obesity drug [5]. As shown in Fig. 1C, the IC50 value of orlistat on pancreatic lipase was only 74 ng/mL. Taxifolin also exhibited the inhibitory effect on pancreatic lipase, and the IC50 was 0.993 mg/mL. Although the activity of taxifolin was far weaker than orlistat, as a commonly found food flavonoid, it may also have benefit for obesity controlling due to the multiple digestive enzymes inhibitory activity.

### 3.2. Inhibitory kinetics of taxifolin on α-glucosidas, α-amylase and pancreatic lipase

Based on the Lineweaver-Burk method, the inhibitory kinetics of taxifolin on α-glucosidase, α-amylase and pancreatic lipase were constructed. It can be seen from Fig. 2A that with the addition of taxifolin, the enzyme reaction rate decreased at all tested [S], which reflected the inhibitory effect of taxifolin. However, the three Lineweaver-Burk lines closely intersected on the positive semi-axis of Y-axis, which indicated that taxilfolin has no effect on the $V_{max}$. The Michaelis constant $K_m$ revealed by the X-axis intercept became larger with the rise concentration of taxifolin. Similar results were found on the α-amylase (Fig. 2B). These results indicated that taxifolin is more like a competitive inhibitor of α-glucosidase and α-amylase, which mean that taxifolin can interact with the active center of the enzymes. The forming of reversible enzyme-inhibitor complex will interfere the binding of the enzyme with substrate, and then reduces its catalytic activity.

In term of pancreatic lipase, the three Lineweaver-Burk lines closely intersected on the negative semi-axis of X-axis (Fig. 2C). The $K_m$ was

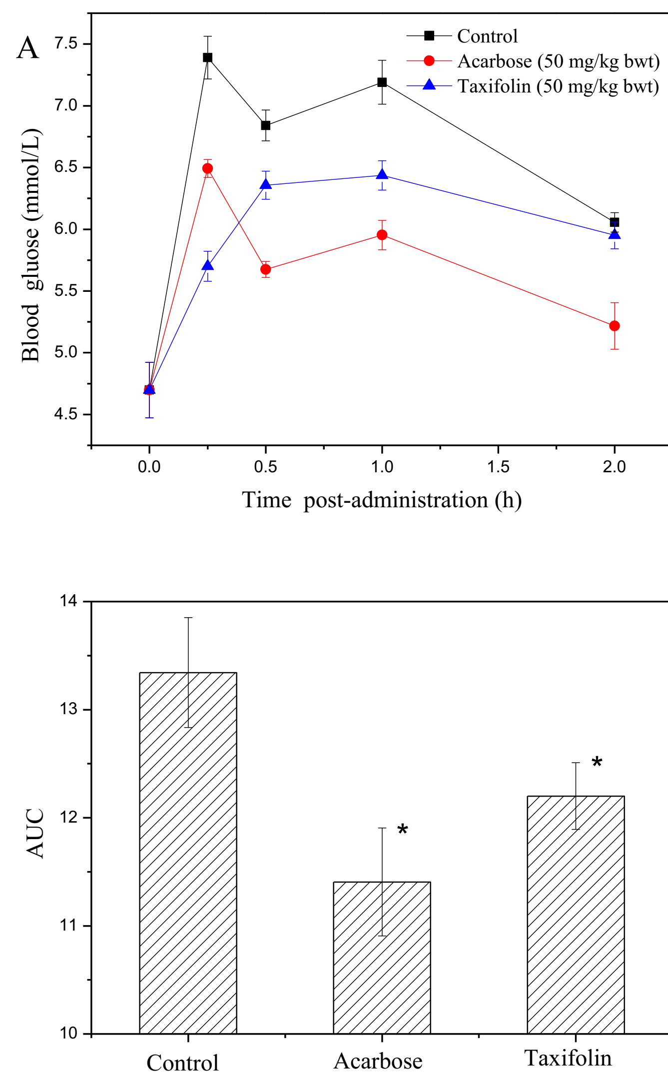


**Fig. 5.** (A) Effect of taxifolin and acarbose on blood glucose level in rat after oral administration of starch. (B) Area under the curve of each treatment. *means significantly different compared with control group ($P$ < 0.05).

almost unchanged, while $V_{max}$ decreased with the addition of taxifolin. The results indicated that taxifolin is more like a non-competitive inhibitor of pancreatic lipase. Taxifolin interacted with the enzyme on a site other than the active center.

### 3.3. The interaction of taxifolin with α-glucosidase, α-amylase and pancreatic lipase investigated by fluorescence quenching titration

Fluorescence quenching refers to the process of physical or chemical interaction between a fluorescent substance and other molecules that causes a decrease in fluorescence intensity. Protein contains aromatic amino acids (e.g. tryptophan, tyrosine and phenylalanine) and can emit fluorescence under excitation around 280 nm. Hence, fluorescence quenching titration is widely used to study the interaction between small molecules and protein [23–25]. The type of fluorescence quenching mechanism can be analyzed by Stern-Volmer equation [18]:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q]$$

where $F_0$ and F are the fluorescence intensity in the absence and presence of a quencher, [Q] is the concentration of the quencher, $K_q$ is the rate constant of the quenching process, $\tau_0$ is the average lifetime of the biomacromolecules (usually $1\times10^{-8}$ s). The slope obtained from the linear regression of experimental data with the equation was further used to calculated $K_q$. If the value of $K_q$ is larger than $2\times10^{10}$ L/(mol·S), it can be judged that the fluorescence quenching process is predominated by a static quenching mechanism rather than a dynamic mechanism [24–26]. For a static quenching process, the binding constant and the number of binding site of the interaction can be calculated with formula [18]:

$$\lg[(F_0 - F)/F] = \lg K + n\lg[Q]$$

where $K_a$ is the binding constant and n is the number of binding site.

In the present study, the quenching effects of taxifolin on the fluorescence of α-glucosidase, α-amylase and pancreatic lipase were showed in Fig. 3. Using pancreatic lipase as a example (Fig. 3C), the maximum excitation and emission wavelength were found at 280 and 341 nm, respectively. With the addition of taxifolin, its fluorescence gradually decreased. Furthermore, the maximum emission wavelength was red-shifted from 341 nm to 363 nm. Similar results were also found for α-glucosidase and α-amylase (Fig. 3A and B). These results confirmed the interaction between taxifolin and the enzymes. The quenching of intrinsic fluorescence intensity may due to the polarity change of micro-environment of fluorophore (i.e. tryptophan residue) in enzyme caused by the interaction [23–25]. The calculated $K_q$ in Table 1 were all far bigger than $2\times10^{10}$ L/(mol·S). Hence, the fluorescence quenching process of taxifolin on the three enzymes are all static quenching. The binding constant ($\lg K_a$) and the number of binding site were further calculated and listed in Table 1. The values of $\lg K_a$ were in the range of 4.93-6.65, indicating the relative strong binding affinity between taxifolin and the enzymes. The values of n were all close to 1, indicating that there were only one binding site between taxifolin and these enzymes.

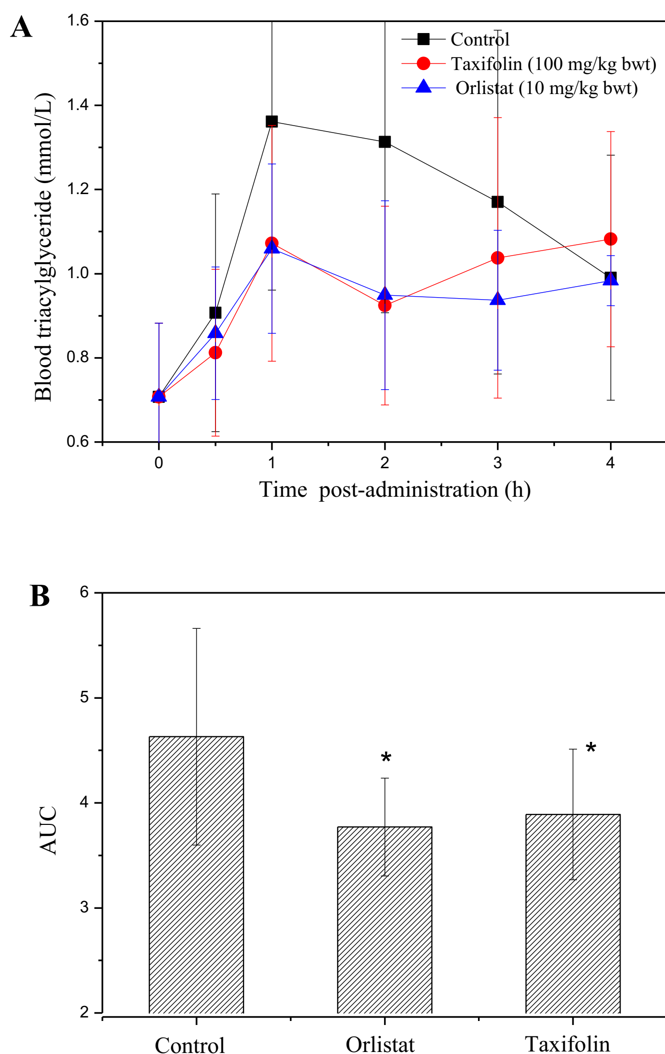


**Fig. 6.** (A) Effect of taxifolin and orlistat on blood triacylglyceride level in rat after oral administration of peanut oil. (B) Area under the curve of each treatment. *means significantly different compared with control group ($P < 0.05$).

### 3.4. Molecular docking simulation

Molecular docking was further used to simulate the interaction between taxifolin and α-glucosidase as well as α-amylase. Inhibitory kinetics revealed that taxifolin interacted with α-glucosidase and α-amylase in the activity center. Hence, a box with length, width and height of 15, 17.25 and 15 Å in the activity center was set for simulating the interaction with minimum energy. As shown in Fig. 4A, many kinds of secondary interaction between taxifolin and the residues of α-glucosidase were found. Taxifolin formed three hydrogen bonds with the residues of Asp69 (2.28 Å), Asp215 (2.15 Å) and Glu277 (1.71 Å) in the enzyme. It was reported that Asp215, Glu277 and Asp350 were three key catalytic residues responsible for the activity of α-glucosidase [26,27]. Furthermore, a π-π stack interaction at 5.57 Å distances between the aromatic ring B of taxifolin and Tyr72 was found. Additionally, two Pi-Anion electrostatic interaction and a Pi-Alkyl hydrophobic interaction were found. The ΔG of the interaction between taxifolin and α-glucosidase was -7.6 kcal/mol, which was lower than that of acarbose (-4.1 kcal/mol). The results indicated that taxifolin has a relatively higher binding affinity toward α-glucosidase than acarbose, which was in accordance with the enzyme inhibitory study.

In term of α-amylase, taxifolin formed three hydrogen bonds with the residues of Asp197 (2.00 Å), Gln63 (3.00 Å) and His305 (3.56 Å). Besides, two π-π stack interaction were found. The ΔG of the ligand–receptor interaction was -9.2 kcal/mol, which was higher than that of acarbose (-10.8 kcal/mol).

### 3.5. Effect of taxifolin on the absorption of glucose and fat in rat

Because the solubility of taxifolin in water is very poor, its zein-based nanoparticles was prepared according to our previous study [18]. The taxifolin-loading nanoparticles was well dispersed in water and its inhibitory effects on the digestion enzymes were evaluated in rat by

determining the postprandial blood glucose and triacylglycerides. As shown in Fig. 5A, after oral administration of starch with dose of 3 mg/kg bwt, the blood glucose in control group rat quickly increased from 4.7 to 7.4 mmol/L in 0.25 h, and maintained the high level in about 1 h and then decreased. In comparison, when pre-administrated with 50 mg/kg bwt of taxifolin, the postprandial blood glucose was significantly lower than that of control rat at the time point of 0.25, 0.5 and 1 h. It was also noted that the effect of taxifolin was weaker than acarbose, the positive control. Compared to control group, treating with taxifolin led to about 15% decrease of AUC (Area Under the Curve, Fig. 5B), which was statistically different ($P<0.05$). The results indicated that taxifolin can improve postprandial hyperglycemia in vivo. It is well know that before absorbed in term of glucose, starch undergo hydrolysis by α-amylase and α-glucosidase first. Hence, the effect of taxifolin was due to its inhibitory effects on intestinal α-amylase and α-glucosidase as revealed in vitro study.

Triacylglycerides, the main component of food fat, can't be absorbed directly in human small intestine. They must be hydrolyzed into monoacylglycerol and free fatty acids by lipase before absorption. Hence, the inhibition of pancreatic lipase can reduce food fat absorption and calories intake. As shown in Fig. 6A, after oral administration of peanut oil, the blood triacylglyceride levels of rat increased rapidly. In control group, the peak was found at about 1 h with value of 1.36 mmol/L. With pre-administration of taxifolin, the increase of blood triacylglyceride levels was slower than that of control rat. The peak value of blood triacylglyceride in taxifolin treated group was only 1.07 mmol/L at 1 h. The effect of taxifolin (100 mg/kg bwt) on reducing fat absorption was similar with that of orlistat (10 mg/kg bwt). The AUC values of the three groups was showed in Fig. 6B. Taxifolin caused about 16% decrease of AUC compared to control, and the difference was significant. These results indicated that taxifolin can really reduce fat absorption through the inhibition of pancreatic lipase in vivo.

## 4. Conclusion

In conclusion, the inhibitory activity of taxifolin on α-glucosidase, α-amylase and pancreatic lipase was confirmed in both vitro and vivo studies. The interaction between taxifolin and the enzymes was studied by fluorescence titration and molecular docking simulation. In vivo study showed that taxifolin has significant effect on reducing the absorption of glucose and fat in rat. Taxifolin is a natural flavonoid widely existed in plant with high safety, and is intended to be approved as a food ingredient in European. The multiple digestive enzymes inhibitory activity of taxifolin may has benefits for obesity controlling by reducing calories absorption in diet. Its anti-obestiy effect on high-fat diet feed rat was is in progress in our lab.

## CRediT authorship contribution statement

**Hang Su:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Yi-Ting Ruan:** Methodology, Investigation, Formal analysis. **Ying Li:** Methodology, Validation. **Ji-Guang Chen:** Software, Visualization, Supervision. **Zhong-Ping Yin:** Resources, Supervision, Writing - review & editing. **Qing-Feng Zhang:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

## Declaration of competing interest

The authors declare no competing financial interest.

## Acknowledgements

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