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Alpha-Glucosidase Inhibitory Activities of Essential Oils Extracted from Three Chinese Herbal Medicines

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This paper aims to verify if the essential oils exacted from Chinese herbal medicines can inhibit alpha-glucosidase (α-glycosidase) activity. For this purpose, the essential oils were exacted from three Chinese herbal medicines collected from Taibai Mountain, China, namely Pyrola calliantha H. Andres (Pyrola calliantha), the flowers of Senecio scandens Buch.-Ham. ex D. Don (Senecio scandens) and the roots of Schisandra chinensis. The inhibitory activity of the three kinds of essential oils against α-glucosidase was contrasted with that of acarbose (positive control). The half maximal inhibitory concentrations (IC50) of each test substance in inhibiting α-glucosidase activity were calculated as: 0.02211 mg/mL (the essential oil of Pyrola calliantha), 0.1304 mg/mL (the essential oil of Senecio scandens flowers), 0.01925 mg/mL (the essential oil of Schisandra chinensis roots), and 23.48mg /mL (acarbose), indicating that the three essential oils boast a greater inhibitory activity against α-glucosidase than acarbose. The subsequent enzyme kinetic experiments reveal that the essential oils of Pyrola calliantha and Senecio scandens flowers have a competitive and irreversible inhibitory effect against α-glucosidase, while the essential oil of Schisandra chinensis roots has a non-competitive and reversible inhibitory effect against α-glucosidase. The research findings shed new light on the application of Chinese herbal medicines.

1. Introduction

Diabetes, characterized by glucose dysmetabolism, is a common endocrine and metabolic disorder that can cause systemic diseases like fat and protein dysmetabolism. The global number of diabetics (20~79) has nearly doubled from 151 million in 2000 to 425 million in 2017, and is expected to reach 629 million in 2014 (Ogurtsova et al., 2017). There are three main forms of diabetes: insulin-dependent diabetes mellitus (type 1), non-insulin-dependent diabetes mellitus (type 2), and gestational diabetes. Type 2 diabetes stands out as the most common type of the disease. One of the treatments of diabetes lies in the use of alpha-glycosidase (α-glycosidase) inhibitors. As the name suggests, these inhibitors can suppress the activity of α-glycosidase, and restore blood glucose level to the normal range (Funke and Melzig, 2006).

This paper aims to verify if the essential oils exacted from Chinese herbal medicines can inhibit α-glycosidase activity. For this purpose, the essential oils were extracted from three Chinese herbal medicines collected from Taibai Mountain, China, namely *Pyrola calliantha* H. Andres (*Pyrola calliantha*), the flowers of *Senecio scandens* Buch.-Ham. ex D. Don (*Senecio scandens*) and the roots of *Schisandra chinensis*. All of these herbal medicines have been reported to have various pharmacological effects in Chinese medicine records (Ye et al., 2010), and the essential oil, an active ingredient of Chinese herbal medicine, is known for its good curative effect (Xiang et al., 1989). Then, the author carried out several experiments to disclose the mechanism of the inhibitory activities of the essential oils against α-glycosidase.

2. Methodology

2.1 Collection of herbal medicines

The three Chinese herbal medicines (Figure 1; Table 1) were collected from Taibai Mountain, China in September 2017. The healthy plants were selected, dried in the shade, and stored for further use.

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Figure 1: The three Chinese herbal medicines (Note: A: Pyrola calliantha; B: Senecio scandens flowers; C: Schisandra chinensis roots)

Table 1. The three Chinese herbal medicines

Number	Name
1	*Pyrola calliantha H. Andres*
2	The flowers of *Senecio scandens* Buch.-Ham. ex D. Don
3	The roots of *Schisandra chinensis*

2.2 Extraction of essential oils

First, the three herbs were pulverized into dry powders. Then, 100g of each herb powder was added with 500mL methanol for ultrasonic extraction (Sokmen et al., 2004). The methanol extract was suspended in water and extracted with petroleum ether. Finally, the liquid extract was dried by a rotary vacuum evaporator and subjected to Gas chromatography–mass spectrometry (GC-MS).

The test parameters are as follows. Instrument: Agilent 7890A/5975C; column: DB-5MS UI (30 m*0.25 mm); inlet temperature: 250°C; interface temperature: 270°C; carrier gas: helium; flow rate: 20 mL/min; Split ratio: 40:1; injection volume: 1 μL. The MS conditions include: EI source, electron energy 70 eV, scan range 50~400, scan interval: 0.2 s, MSD ChemStation, and NIST Library Demo. The temperature was kept at 60°C for 3 min initially; then, the column temperature was raised at 5°C/min to 180°C and held for 1 min; after that, the temperature was increased to 270°C at 10°C/min and held for 10 min.

2.3 Experiment on inhibitory activity

The experimental method was a modified version of the approach proposed by Kazeem et al., (2013). The test system is a mixture of 20 μL phosphonolipid (0.1 m, pH 7.0), 20 μL α-glucosidase solution (0.2 U/mL α-glucosidase in 0.1 m phosphate buffer containing 0.2% of bovine serum albumin), and 20 μL acarbose or its extract solution (0.1 m phosphate buffer containing 45 % of methyl alcohol). These ingredients were added in strict accordance with the above sequence. Then, the mixture was incubated at 37°C for 15 min. Next, 20 μL α-PNPG substrate solution (2.3 mm α-glycopyranoside in 0.1 m phosphate buffer) was added to initiate the reaction. After another 15 min at 37°C, 80 μL Na_2CO_3(0.2 m) was added to terminate the reaction. Finally, the α-glucosidase activity was determined on an ELIASA by measuring PNP quantity at 405 nm. The blank group (phosphate buffer containing 45 % of methyl alcohol instead of test samples), the control blank group (phosphate buffer instead of enzyme) and the positive control test (acarbose instead of test samples) were carried out in a similar way. Moreover, the concentration of the extract required to inhibit 50 % of α-glucosidase activity under the experimental conditions was defined as the half maximal inhibitory concentration (IC_{50}).

Inhibitory rate (%)=[1-(OD_{test}-$OD_{control}$)/OD_{blank}] × 100 %.

2.4 Kinetics of Inhibition against α-glucosidase

First, the three herbs were pulverized into dry powders. Then, 100 g of each herb powder was added with 500 mL methanol for ultrasonic extrac Different concentrations of inhibitor solution were added into different concentrations of PNPG (0.5 mm/mL, 1 mm/mL, 1.5 mm/mL, 2 mm/mL, 2.5 mm/mL, 3 mm/mL, 3.5 mm/mL, 4 mm/mL) solution, aiming to disclose the α-glucosidase inhibition mechanism of each Chinese herbal medicine (Zhang, 2013). First, the microplate was placed into the ELIASA to determine the initial reaction speed (i.e. determining the kinetics model at 405 nm, 37°C, 11 times/10min). Then, the author evaluated the kinetics of α-glucosidase inhibition of Pyrola calliantha (Blank, 0.1280 mg/mL, 0.2560 mg/mL), Senecio scandens flowers (Blank, 0.5120 mg/mL, 1.024 mg/mL), and Schisandra chinensis roots (Blank, 0.0156 mg/mL, 0.3456 mg/mL). There was no other inhibitor or compound during the reactions. Finally, the double-reciprocal or Lineweaver–

Burk (LB) plots (Eisenthal and Cornishbowden, 1974) were drawn to identify the inhibition pattern of each herbal medicine. In the plots, the reciprocal of the substrate concentration is taken as the abscissa and the reciprocal of reaction speed as the ordinate.

3. Results and Discussion

3.1 Essential oil composition test

The essential oils extracted from the three Chinese herbal medicines are all semi-transparent oils. The essential oil of Schisandra chinensis roots is yellow, while that of the other two herbal medicines are brown. The preliminary GC-MS test show that the essential oils contain alcohols, ketones, acids, esters, phenols, terpenes, etc. (Table 2). Through component analysis, it was found that Senecio scandens flowers and Schisandra chinensis roots mainly consist of terpenes. The terpenoid content in the essential oil of Senecio scandens flowers was 33.62%, higher than the level reported by Y.H. Li (2006). This is because essential oil of a plant often concentrates in its flowers. For the essential oil of Schisandra chinensis roots, the terpenoid content was 23.1%, similar to the result of Z.H. Huang (2011). By contrast, the essential oil of Pyrola calliantha has much less terpenoids, but 35.13% of acids.

Table 2: Chemical compositions of the three essential oils

Number	Name	Chemical contents					
		Alcohols	Ketone	Acids	Ester	Phenols	Terpenes
1	*Pyrola calliantha H. Andres*	+	+	+	+	-	-
2	The flowers of *Senecio scandens* Buch.-Ham. ex D. Don	+	-	+	+	-	++
3	The roots of *Schisandra chinensis*	+	+	-	+	-	++

3.2 Inhibitory activity test

Alpha-glycosidase inhibitors provide first-line treatments for type 2 diabetes (Imran et al., 2016) through the control of postprandial glucose. During the development of new α-glucosidase inhibitors, the popular oral hypoglycemic drug acarbose, a reversible inhibitor of α-glucosidase activity, is often employed as the positive control (Imran et al., 2016).

The experiments on α-glucosidase inhibitory activity reveal the difference of the essential oils in inhibiting α-glucosidase activity. Figure 2 illustrates the relationship between α-glucosidase inhibitory activity and the concentration of the positive control acarbose and the three essential oils. It can be seen that the α-glucosidase inhibitory activities of the positive control and the three samples were all on the rise with the increase in concentration. The essential oil of *Pyrola calliantha* was particularly sensitive to the variation in concentration: the inhibitory rate increased 1.85 times from 18.7% to 53.3%, when the essential oil concentration grew from 0.032mg/mL to 0.064mg/mL.

As shown in Table 3, all three essential oils have strong inhibitory activity on α-glucosidase. The IC_{50} values of them were 0.02211 mg/mL (*Pyrola calliantha*), 0.13040 mg/mL (*Senecio scandens* flowers) and 0.01925 mg/mL (*Schisandra chinensis* roots), while the IC_{50} value of the positive control acarbose was 23.4800 mg/mL. This means the essential oils are 1,000 times more efficient than acarbose in suppressing α-glucosidase activity.

Table 3: 3 kinds of Taibai herbal medicine inhibition of glucosidase IC_{50}

Number	Name	IC_{50} (mg/mL)
1	Acarbose	23.4800
2	*Pyrola calliantha H. Andres*	0.02211
3	The flowers of *Senecio scandens* Buch.-Ham. ex D. Don	0.13040
4	The roots of *Schisandra chinensis*	0.01925

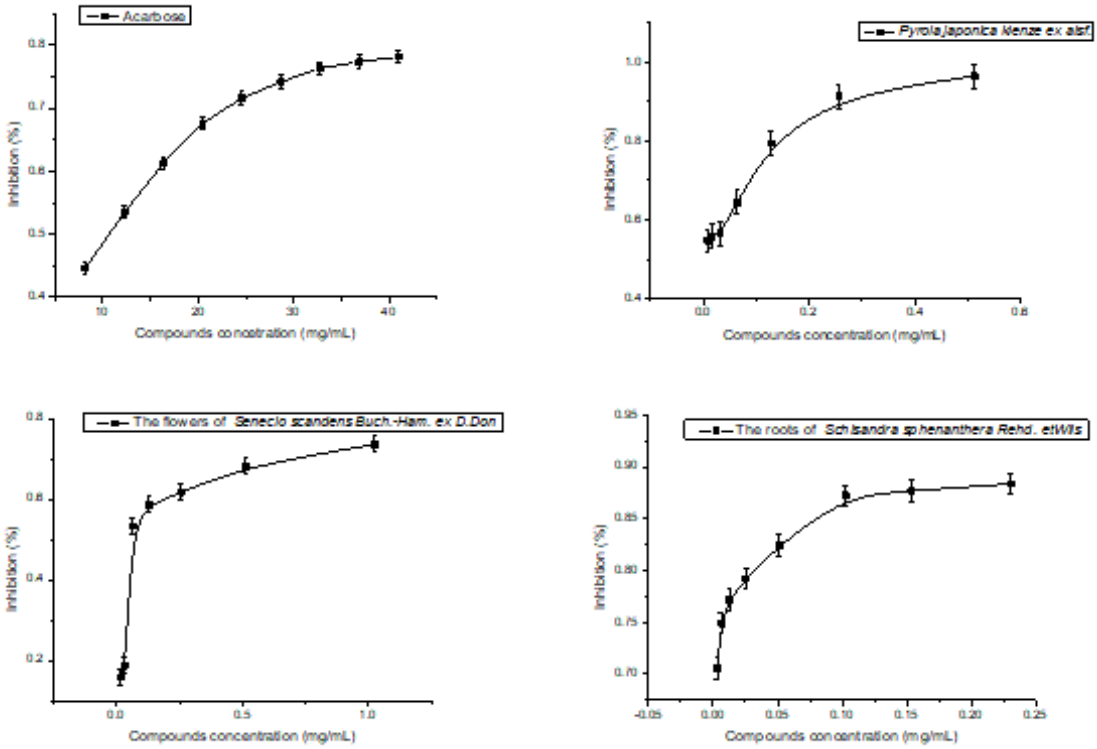

Figure 2: Inhibition rate of α-glucosidase (Note: A: Acarbose; B: Pyrola calliantha; C: Senecio scandens flowers; D: Schisandra chinensis roots)

3.3 Kinetics of inhibition against α-glucosidase

In the above sections, the inhibitory activities of the three essential oils against α-glucosidase are determined, and the data are processed by double reciprocal mapping (Figure 3; Table 4). The results show that the essential oil of *Pyrola calliantha* (blank control group and A1 group: 0.1280 mg/mL; A2 group: 0.2560 mg/mL) has similar reaction kinetics with the essential oil of *Schisandra chinensis* roots (blank control group and C1 group: 0.0156 mg/mL; C2 group: 0.3450 mg/mL). For both essential oils, the straight lines obtained after the linear regression of three datasets roughly converged at the same point on the y-axis, indicating that the maximum reaction speed (Vmax) of the reaction system did not change after adding the essential oil of *Pyrola calliantha* or the essential oil of *Schisandra chinensis* roots. Besides, the addition of each essential oil led to a substantial increase in the apparent Km of the reaction, and a significant dose-effect relationship. Hence, the two essential oils can reduce the affinity between the enzyme and the substrate after binding with the enzyme (Fa, 2005).

Nevertheless, the essential oil of *Senecio scandens* flowers has a different kinetic curve of α-glucosidase inhibition from the other two essential oils. The straight lines obtained after the linear regression of three datasets (blank control group and B1 group: 0.5120 mg/mL; B2 group: 1.0240 mg/mL) roughly converged at the same point on the x-axis, revealing the lack of variation in Km value after adding the essential oil to the reaction system. In addition, the Vmax slowed down with the increase in the essential oil concentration, indicating that the essential oil suppressed enzyme activity through binding with necessary groups other than the active centre of α-glucosidase (Sivasothy, 2016).

To sum up, the essential oils of *Pyrola calliantha* and *Schisandra chinensis* roots had no significant effect on the Vma of α-glucosidase, but suppressed the enzyme activity by lowering the affinity between the enzyme and the substrate. Therefore, the two essential oils are competitive inhibitors. In contrast, the essential oil of *Senecio scandens* flowers had no significant effect on Km, and inhibited activity of α-glucosidase by changing the shape of enzyme molecules. Hence, the essential oil belongs to the category of non-competitive inhibitors (Phan, 2013).

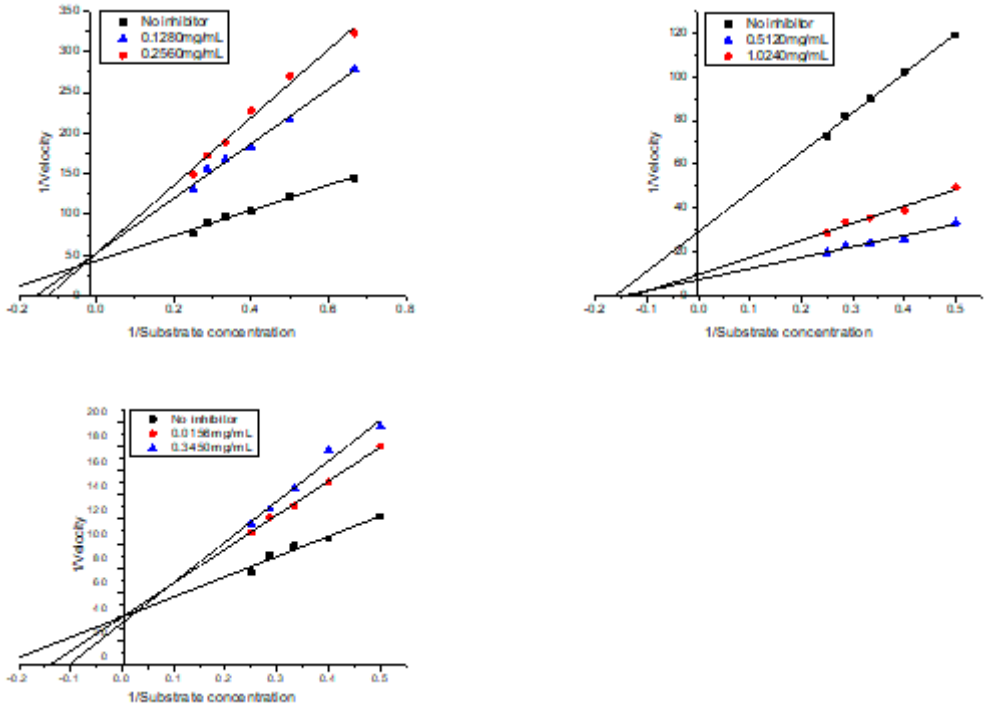

*Figure 3: The inhibitory effect of 3 essential oils on α-glucosidase by Lineweaver-Burk plots (*Note: A: *Pyrola calliantha*; B: *Senecio scandens* flowers; C: *Schisandra chinensis* roots)

4. Conclusions

All three essential oils enjoy a strong inhibitory activity against α-glucosidase. The IC_{50} of each test substance was: 0.02211 mg/mL (the essential oil of *Pyrola calliantha*), 0.1304 mg/mL (the essential oil of *Senecio scandens* flowers), 0.01925 mg/mL (the essential oil of *Schisandra chinensis* roots), and 23.48mg /mL (acarbose), indicating that the three essential oils boast a greater inhibitory activity against α-glucosidase than acarbose.

The subsequent enzyme kinetic experiments reveal that the essential oils of *Pyrola calliantha* and *Schisandra chinensis* roots suppressed the enzyme activity by lowering the affinity between the enzyme and the substrate. Therefore, the two essential oils are competitive inhibitors. By contrast, the essential oil of *Senecio scandens* flowers had no significant effect on Km, and inhibited activity of α-glucosidase by changing the shape of enzyme molecules. Hence, the essential oil belongs to the category of non-competitive inhibitors.

According to Ghosh and Rangan (2015), the key residues of diterpenes are possible reasons for the non-competitive inhibition of α-glucosidase. This may explain the non-competitive inhibition of the essential oil of *Senecio scandens* flowers against α-glucosidase. In this paper, the essential oil of *Schisandra chinensis* roots is found to be a competitive inhibitor of α-glucosidase. The finding goes against the report of Xu (2001) that the extract of *Schisandra chinensis* has α-glucosidase inhibitory activity, which bears the typical features of non-competitive inhibition. The difference in inhibition kinetics may arise because the two studies adopt different parts of *Schisandra chinensis*. In view of its rich content of terpenes, the *Schisandra chinensis* essential oil will be further investigated to determine the causes of the competitive inhibition of α-glycosidase.

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