

## Original Research Article

# Inhibitory Effects of Various Ratios of Polysaccharides/Alkaloids from Rhizome of *Coptis chinensis* Franch on $\alpha$ -Glucosidase

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### Abstract

**Purpose:** To investigate the inhibitory effects of various ratios of polysaccharides/alkaloids from the rhizome of *Coptis chinensis* Franch (RCC) on  $\alpha$ -glucosidase.

**Methods:** The polysaccharides (PSD) and alkaloids (ALK) from RCC were prepared using the water extraction and alcohol precipitation method and Reinecke's salt precipitation method, respectively. Subsequently, the  $\alpha$ -glucosidase inhibitory effects of PSD, ALK, and PSD/ALK at various ratios were evaluated spectrophotometrically *in vitro*.

**Results:** With a half maximal inhibitory concentration ( $IC_{50}$ ) value of 171.67  $\mu$ g/mL, ALK showed higher  $\alpha$ -glucosidase inhibitory activity than PSD ( $IC_{50}$  = 296.89  $\mu$ g/mL). In addition, the polysaccharides/alkaloids (PSD/ALK) at the ratio of 3:1 exhibited stronger  $\alpha$ -glucosidase inhibitory activity ( $IC_{50}$  = 160.9  $\mu$ g/mL) than PSD, ALK and PSD/ALK at ratios of 1:3 ( $IC_{50}$  = 394.78  $\mu$ g/mL), 1:2 ( $IC_{50}$  = 185.18  $\mu$ g/mL), 1:1 ( $IC_{50}$  = 350.51  $\mu$ g/mL), and 2:1 ( $IC_{50}$  = 229.16  $\mu$ g/mL).

**Conclusion:** The results obtained suggest that PSD/ALK (3:1) possesses the strongest  $\alpha$ -glucosidase inhibitory effect and may be considered as a candidate agent in future anti-diabetes drug development.

**Keywords:** *Coptis chinensis*, Polysaccharides, Alkaloids,  $\alpha$ -Glucosidase, Antidiabetic

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## INTRODUCTION

The dried rhizome of *Coptis chinensis* Franch (RCC) (Ranunculaceae family) is one of the most widely used traditional Chinese medicines (TCMs) in China [1,2]. It has been used for thousands of years in Chinese traditional medicine to treat damp-heat, dysentery, jaundice, heart fire hyperactivity, carbuncles and sores [2]. Increasingly modern pharmacological investigations have demonstrated that the extracts/compounds isolated from RCC can be used to treat inflammation [3], infection [4], dysentery [5], hypertension [6], gastrointestinal cancer [7], and type 2 diabetes mellitus (T2DM)

[8]. In addition, recent research has also reported that RCC contains various chemical constituents including alkaloids, polysaccharides, and organic acids [8-11].

In recent years, morbidity from diabetes mellitus (DM) has increased dramatically, and DM has become one of modern medicine's most intractable diseases [12]. Importantly, T2DM makes up approximately 90 % of DM cases [13]. Although insulin and other first-line drugs for treating T2DM have been widely used in clinics, some drugs involve significant adverse effects (e.g., abdominal distention, diarrhea, and flatulence) that limit their clinical popularity [8,14].

Therefore, it is important to discover more novel drugs for treating T2DM. Previous studies have demonstrated that RCC possesses a notable anti-diabetic effect, and alkaloids were reported to be its primary bioactive constituents [8,15]. Moreover, a water-soluble polysaccharide isolated from *C. chinensis* has been proven to possess significant antidiabetic and antioxidant activity [16]. However, no investigations of the antidiabetic-like effect of these two types of active ingredients (i.e., polysaccharides and alkaloids) used in combination have been reported. Therefore, in this study, we aim to investigate the inhibitory effects of polysaccharides, alkaloids, and in particular various ratios of polysaccharides/alkaloids from the rhizome of *C. chinensis* on  $\alpha$ -glucosidase. The present study lays a solid foundation for the development and utilization of *C. chinensis*. More importantly, these results will provide information for the future development of candidate drugs for treating T2DM.

## EXPERIMENTAL

### Plant material

*Coptis chinensis* Franch was collected from Dayi County (Chengdu, China) and identified by Professor Yi Zhang (Chengdu University of Traditional Chinese Medicine), and a voucher specimen was deposited in the herbarium of the College of Ethnic Medicine, Chengdu University of Traditional Chinese Medicine (Chengdu, China).

### Chemicals

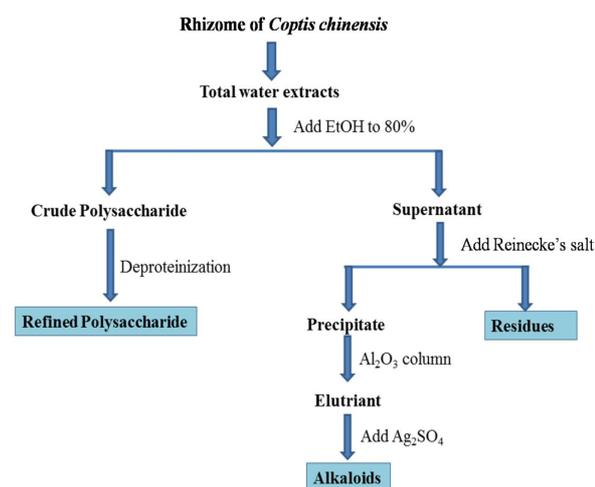
Acarbose was purchased from Chengdu Herbpurity Co., Ltd. (Chengdu, China), and its purity was  $\geq 98\%$ ;  $\alpha$ -glucosidase and 4-nitrobenzene- $\alpha$ -D-glucopyranoside (PNPG) were purchased from Sigma Co. (St., Louis, MO, USA); p-nitrophenol (PNP) was purchased from Chengdu Kelong Chemical Reagent Co. Ltd. (Chengdu, China). All of the other reagents used in the present study were of analytical grade.

### Preparation of polysaccharides and alkaloids from the rhizome of *Coptis chinensis*

The polysaccharides (PSD) and alkaloids (ALK) from the rhizome of *Coptis chinensis* were prepared as shown in the flow chart in Figure 1. The RCC was powdered and refluxed with water three times (4 h each time). Subsequently, ethanol (EtOH) was added slowly until a final concentration of 80% was obtained. After being left overnight at 4 °C, the precipitates were

collected by filtering and being washed three times with pure ethanol and acetone, and the precipitate (crude polysaccharides) (A) and supernatant (B) were collected. The crude polysaccharides were deproteinized using  $\text{CHCl}_3$ -butanol (v/v, 5:1), then the refined polysaccharides were obtained by freeze-drying.

The supernatant (B) was concentrated in vacuum and diluted  $\text{H}_2\text{SO}_4$  was added to adjust the pH value to 2–3. Subsequently, the freshly prepared Reinecke's salt was added to the supernatant and the precipitate (D) and the supernatant (C) were collected, respectively. The precipitate (D) was later redissolved in acetone, then underwent  $\text{Al}_2\text{O}_3$  column chromatography and eluted with acetone. Next, the elutriant was collected and silver sulfate solution ( $\text{Ag}_2\text{SO}_4$ ) was added. Thereafter, the supernatant was collected and freeze-dried to obtain the total alkaloids. In addition, the supernatant (C) from the previous step was freeze-dried to obtain the residues (RES).



**Figure 1:** Flow chart of preparation of polysaccharides (PSD), alkaloids (ALK) and the residues (RES) from the rhizome of *Coptis chinensis*

### Inhibition of $\alpha$ -glucosidase by extract

The  $\alpha$ -glucosidase inhibitory activity assay was performed according to the previously described method [17] with minor modifications. The assay system was performed in 96-well plates in a total volume of 250  $\mu\text{L}$ . The reaction mixture consisted of crude enzyme solution (0.2 units/mL, 10  $\mu\text{L}$ ), 0.1 M potassium phosphate buffer (100  $\mu\text{L}$ ), and 50  $\mu\text{L}$  test samples in DMSO at series final concentrations in the assay system (500, 375, 250, 187.5, 125 and 62.5  $\mu\text{g}/\text{mL}$ ). Acarbose (ACB, 500, 375, 250, 187.5, 125 and 62.5  $\mu\text{g}/\text{mL}$ ) was used as positive control. After pre-incubation at 37 °C for 15 min, 20  $\mu\text{L}$  of 2.5 mM PNPG was added. The mixture was

incubated for another 15 min at 37 °C. The reaction was terminated by adding 70 µL of 0.2 M Na<sub>2</sub>CO<sub>3</sub>. The absorbance (Abs) was then measured at 405 nm using a microplate reader (Thermo, USA). Individual blanks for test samples were prepared to correct background absorbance where the substrate was replaced with buffer. Control sample contained 50 µL DMSO instead of test samples. Thereafter, α-glucosidase inhibitory activity was calculated according to Eq 1.

$$\text{Inhibition (\%)} = \{(Ac - As)/Ac\} \dots\dots\dots (1)$$

where Ac and As are the absorbance of control and test samples, respectively.

Finally, the half maximal inhibitory concentration (IC<sub>50</sub>) value was calculated using SPSS software (SPSS for Windows 18.0, SPSS Inc., Chicago, IL, USA).

## RESULTS

### Inhibitory effect of sub-fractions of *Coptis chinensis* on α-glucosidase

As shown in Table 1, the positive reference (Acarbose) exhibited a significant inhibitory effect on α-glucosidase with IC<sub>50</sub> value of 183.15 µg/mL. For PSD and ALK test samples isolated

from RCC, a serial concentration (500, 375, 250, 187.5, 125, and 62.5 µg/mL) was determined. The results demonstrated that both PSD and ALK possess significant α-glucosidase inhibitory effects, with IC<sub>50</sub> of 296.89 and 171.67 µg/mL, respectively. Importantly, ALK showed a stronger α-glucosidase inhibitory effect than the positive drug (Acarbose) (171.67 and 183.15 µg/mL, respectively). However, the test samples of the RES had a weak effect on the α-glucosidase, with an IC<sub>50</sub> value of over 500 µg/mL. Considering its weak α-glucosidase inhibitory effect and low yield, RES was not used in the subsequent experiment.

### Inhibitory effect of various ratios of PSD/ALK on α-glucosidase

In order to obtain the best α-glucosidase inhibitory effect, the various ratios of PSD/ALK were investigated. In our study, the testing ratios of PSD/ALK were set at 1:3, 1:2, 1:1, 2:1, and 3:1. As seen in the results shown in Table 2, the IC<sub>50</sub> of these samples were 394.78, 185.18, 350.51, 229.16, and 160.9 µg/mL, respectively. Of these ratios, 3:1 showed the strongest α-glucosidase inhibitory effect, which is even stronger than the positive reference agent (Acarbose) (160.9 vs. 183.15 µg/mL) (Figure 2).

**Table 1:** Inhibitory effect of sub-fractions of *Coptis chinensis* on α-glucosidase

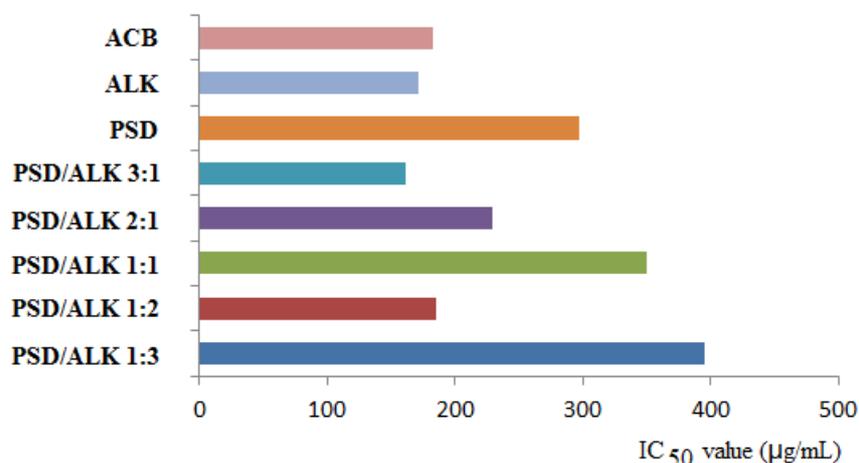
Sample	Concentration (µg/mL)	Inhibition (%)	IC <sub>50</sub> (µg/mL)
ACB	500	99.36	183.15
	375	94.51	
	250	69.72	
	187.5	53.86	
	125	33.29	
	62.5	19.29	
PSD	500	93.43	296.89
	375	68.68	
	250	39.93	
	187.5	24.66	
	125	11.27	
	62.5	1.11	
ALK	500	97.99	171.67
	375	92.27	
	250	67.51	
	187.5	55.27	
	125	41.93	
	62.5	23.55	
RES	500	12.54	> 500
	375	11.23	
	250	10.19	
	187.5	nd	
	125	nd	
	62.5	nd	

**Key:** ACB, Acarbose; PSD, Polysaccharides; ALK, Alkaloids; RES, the residues; nd means that no inhibitory effect is detected

**Table 2:** Inhibitory effects of different ratios of polysaccharides/alkaloids on  $\alpha$ -glucosidase

PSD/ALK	Concentration ( $\mu\text{g/mL}$ )	Inhibition (%)	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )
1:3	500	58.51	394.78
	375	49.48	
	250	37.01	
	187.5	30.89	
	125	22.62	
	62.5	15.8	
1:2	500	92.56	185.18
	375	92.29	
	250	66.90	
	187.5	49.88	
	125	37.19	
	62.5	20.69	
1:1	500	66.36	350.51
	375	54.58	
	250	38.81	
	187.5	31.27	
	125	21.88	
	62.5	13.94	
2:1	500	62.94	229.16
	375	56.73	
	250	53.06	
	187.5	43.02	
	125	31.48	
	62.5	17.96	
3:1	500	82.79	160.90
	375	79.69	
	250	74.66	
	187.5	59.96	
	125	39.4	
	62.5	20.55	

**Key:** PSD, Polysaccharides; ALK, Alkaloids.



**Figure 2:** Inhibitory effect of polysaccharides, alkaloids and different ratios of polysaccharides/alkaloids on  $\alpha$ -glucosidase. **Key:** ACB, acarbose; PSD, polysaccharides; ALK, alkaloids

## DISCUSSION

Increasingly, investigations have indicated that natural monomers/extracts isolated from plants or herbs are a potential resource for developing novel effective candidate drugs with low toxicity [17-19]. Here we have presented, to the best of our knowledge, the first report on the optimum ratio of polysaccharides and alkaloids (3:1)

isolated from the rhizome of *Coptis chinensis* for inhibiting  $\alpha$ -glucosidase.

It has been demonstrated that controlling blood postprandial glucose levels is an important strategy for treating DM, especially T2DM [20,21].  $\alpha$ -glucosidase is one of the most important enzymes for the hydrolysis of  $\alpha$ -glucosidic bonds in carbohydrates in order to

liberate absorbable glucose [20]. Therefore,  $\alpha$ -glucosidase inhibitors are effective drugs in treating T2DM. Acarbose, a microbial carbohydrate, is currently used in clinical practice as an  $\alpha$ -glucosidase inhibitor for attenuating T2DM [22]. Thus, we selected acarbose as a positive reference drug in this study. The  $\alpha$ -glucosidase inhibitory activity assay is commonly used for rapid *in vitro* screening of potential anti-diabetes drugs [19,20,23].

It is well known that the synergistic effect of multi-components is one of the most important features of traditional Chinese medicine [24]. In this study, the PSD/ALK at the ratio of 3:1 were found to have a stronger  $\alpha$ -glucosidase inhibitory activity ( $IC_{50} = 160.9 \mu\text{g/mL}$ ) than either the PSD or the ALK alone, indicating that the combined use of PSD and ALK can achieve a better inhibitory effect on  $\alpha$ -glucosidase than using ALK or PSD alone.

## CONCLUSION

This investigation has shown that the combined use of polysaccharides and alkaloids from the rhizome of *Coptis chinensis* at a ratio of 3:1 produced the strongest  $\alpha$ -glucosidase inhibitory effect. This combination may be considered as a candidate agent for future anti-diabetes drug development.

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