

# Screening for potential $\alpha$ -Glucosidase inhibitors in *Coptis chinensis* Franch extract using ultrafiltration LC-ESI-MS<sup>n</sup>

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**Abstract:** Naturally existing  $\alpha$ -glucosidase inhibitors from traditional herbal medicines have attracted considerable interest to treat type 2 diabetes mellitus (T2DM). Hundreds of herbs have been reported to have the potential to inhibit  $\alpha$ -glucosidase. However, most common methods to examine the inhibitors of  $\alpha$ -glucosidase are usually time-consuming. In the current study, the screening of  $\alpha$ -glucosidase ligands from *Coptis chinensis* Franch extract was undertaken by ultrafiltration liquid chromatography coupled to electrospray ionization tandem mass spectrometry (ultrafiltration LC-ESI-MS<sup>n</sup>). Resultantly, the enzyme inhibition studies showed that *Coptis chinensis* Franch extract carries the strongest  $\alpha$ -glucosidase inhibitory activity among the five kinds of Chinese herbal extracts. Subsequently, five compounds that could bind to  $\alpha$ -glucosidase in the *Coptis chinensis* Franch extract were found using ultrafiltration liquid chromatography, and their structures were identified by ESI-MS<sup>n</sup> to be coptisine, epiberberine, jatrorrhizine, berberine, palmatine. Cumulatively, these results were anticipated to be encouraging for applying the *Coptis chinensis* Franch extracts as efficient anti-diabetic drug candidates.

**Keywords:** Screening;  $\alpha$ -Glucosidase inhibitors; *Coptis chinensis* Franch; Ultrafiltration LC-ESI-MS<sup>n</sup>

## INTRODUCTION

Diabetes mellitus has been a common disorder due to the changes in peoples' lifestyle and dietary habits.  $\alpha$ -glucosidase inhibitors are usually used in the treatment of type 2 diabetes mellitus (T2DM) (Nirmala *et al.*, 2008). These inhibitors act by a reversible inhibition of  $\alpha$ -glucosidase by interacting with the active center of  $\alpha$ -glucosidase (Kalra, 2014). Although, some effective synthetic  $\alpha$ -glucosidase inhibitors (i.e. acarbose) are available, they usually lead to hepatic disorders and other disturbing gastrointestinal symptoms (Kihara *et al.*, 1997). Ultimately, more attention needs to be focused on natural  $\alpha$ -glucosidase inhibitors, especially those from natural resources due to its variety and low toxicity in the treatment of T2DM (Kihara *et al.*, 1997; Guo *et al.*, 2010; Wansi *et al.*, 2007; Gao *et al.*, 2008; Ichiki *et al.*, 2007; Anam *et al.*, 2009).

*Coptis chinensis* Franch is one of the most widely used botanicals worldwide, and studies have revealed it is beneficial to alleviate symptoms of diabetes mellitus. Researches in pharmacology have also proved that the main components of this herbal drug can lessen the symptoms of diabetes by different mechanisms (Yin *et al.*, 2008; Tang *et al.*, 2006; Lee *et al.*, 2006). In order to study the potential of *Coptis chinensis* Franch for the

active compounds to cure T2DM, the herb was selected to screen for candidates of  $\alpha$ -glucosidase inhibitors.

In recent years, ultrafiltration LC-ESI-MS<sup>n</sup> has been proved to be a effective approach to screen biological compounds in botanical extracts due to the high throughput screening ability, and also the sensitivity and selectivity necessary to characterize compounds present at low concentrations in complex chemical mixtures avoiding time-consuming purification (Liu *et al.*, 2013; Song *et al.*, 2014). Moreover, the reuse ability of enzymes for multiple cycles also renders ultrafiltration LC-ESI-MS<sup>n</sup> suitable for high throughput screening.

In the current work, ultrafiltration LC-ESI-MS<sup>n</sup> was developed and applied to screen the ligands of  $\alpha$ -glucosidase from the *Coptis chinensis* Franch extract. As a result, five compounds were identified to show high binding affinity to  $\alpha$ -glucosidase.

## EXPERIMENTAL

### Materials

All the Chinese herb extracts were provided by Xi'an Acetar Biology and Technology Co., Ltd. (Xi'an, China);  $\alpha$ -Glucosidase was purchased from the Fluka (Bueke, Switzerland); Acetonitrile of HPLC grade was from Fisher; Water was purified by a Milli-Q water purification

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**Table 1:** The LC/MS<sup>n</sup> data of  $\alpha$ -glucosidase ligands from *Coptis chinensis* Franch extract

Peak No.	Identification	t <sub>R</sub> (min)	UV $\lambda_{\max}$ (nm)	MS <sup>n</sup> (m/z)
1	coptisine	23.12	237, 265, 347	MS <sup>1</sup> : 320 MS <sup>2</sup> [320]: 318, 292 MS <sup>3</sup> [320→292]: 277, 264, 262, 246, 234
2	epiberberine	24.95	227, 262, 346	MS <sup>1</sup> : 336 MS <sup>2</sup> [336]: 321, 306, 292 MS <sup>3</sup> [336→321]: 320, 318, 304, 292
3	jatrorrhizine	28.33	226, 270, 343	MS <sup>1</sup> : 338 MS <sup>2</sup> [338]: 323, 322, 294 MS <sup>3</sup> [338→322]: 307, 294, 279
4	berberine	31.84	227, 262, 346	MS <sup>1</sup> : 336 MS <sup>2</sup> [336]: 321, 305, 292 MS <sup>3</sup> [336→321]: 320, 318, 304, 292 MS <sup>4</sup> [336→321→320]: 318
5	palmatine	34.15	224, 273, 343	MS <sup>1</sup> : 352 MS <sup>2</sup> [352]: 337, 308

system (Milford, MA); The standards of jatrorrhizine, coptisine, palmatine and berberine were from the Chinese Authenticating Institute of Material Medical and Biological Products (Beijing, China).

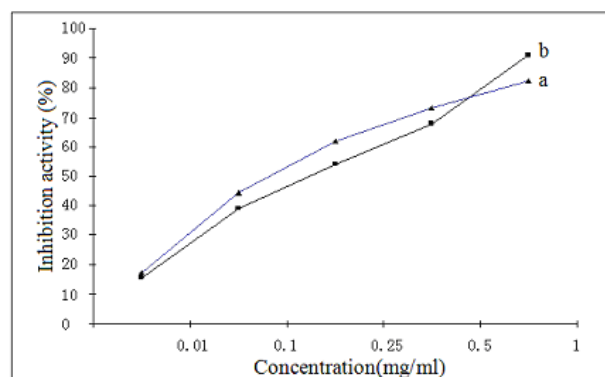
#### *In vitro* $\alpha$ -glucosidase inhibition assays

The enzyme inhibition was evaluated by the *in vitro*  $\alpha$ -glucosidase inhibition assays reported by Li (Li *et al.*, 2009). A 20 $\mu$ L volume of 100mM phosphate buffer (pH 6.8) was mixed with an equal volume of 2.5mM p-nitrophenyl  $\alpha$ -D-glucopyranoside (PNP-G). Then, 20 $\mu$ L of Chinese herb extracts were added to each well followed by 20 $\mu$ L of 10mM phosphate buffer (pH 6.8) containing 0.2U/mL  $\alpha$ -glucosidase to the mixture of treatment terminated wells. After the plate was incubated at 37°C for 15 min, 80 $\mu$ L of 0.2mol/l sodium carbonate solution was added to quench the reaction. Absorbance was measured at 405nm with a Tecan GENios multifunctional micro plate reader (Mannedorf, Switzerland). Acarbose (Bayer) was used as a positive control. The inhibition (%) was calculated as: (A1-A2)/A1 $\times$ 100%, where A1 is the absorbance of the control, and A2 is the absorbance of the sample.

#### Screening procedures of ultrafiltration LC-ESI-MS<sup>n</sup>

The screening assay in our study was performed as previously described (Li *et al.*, 2009; Zhou *et al.*, 2012a; Zhou *et al.*, 2012b). The Chinese herb extract (0.5 mg/ml, 50  $\mu$ l) was incubated with 10 $\mu$ M  $\alpha$ -glucosidase in 10mM ammonium acetate buffer (pH 6.86). After incubation at 37°C for 30 min, the mixture was filtered with a Microcon YM-10 centrifugal filter (10,000 MW cutoff) and the unbound compounds were removed by centrifugating at 10,000 rpm for 10min with 100 $\mu$ L aliquots of ammonium acetate buffer (pH 6.86). The bound ligands were released by adding 100 $\mu$ L of

methanol/water (50:50; v/v; pH 3.30) and then centrifuged at 10,000 rpm for 15 min. The procedure was repeated thrice. The control experiment without  $\alpha$ -glucosidase was also run before each screening experiment. The sample was dried in a Speedvac, and the released ligands were ready for LC-ESI-MS<sup>n</sup> analysis.

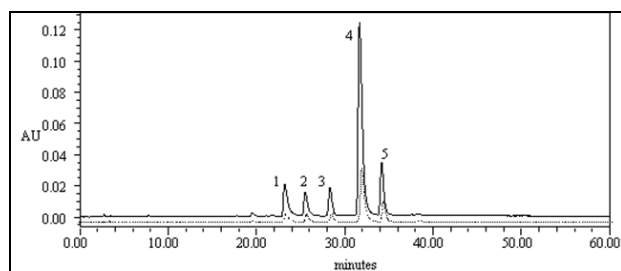


**Fig. 1:** Inhibition activity of acarbose (a) and the *Coptis chinensis* Franch extract (b) on the activity of  $\alpha$ -glucosidase.

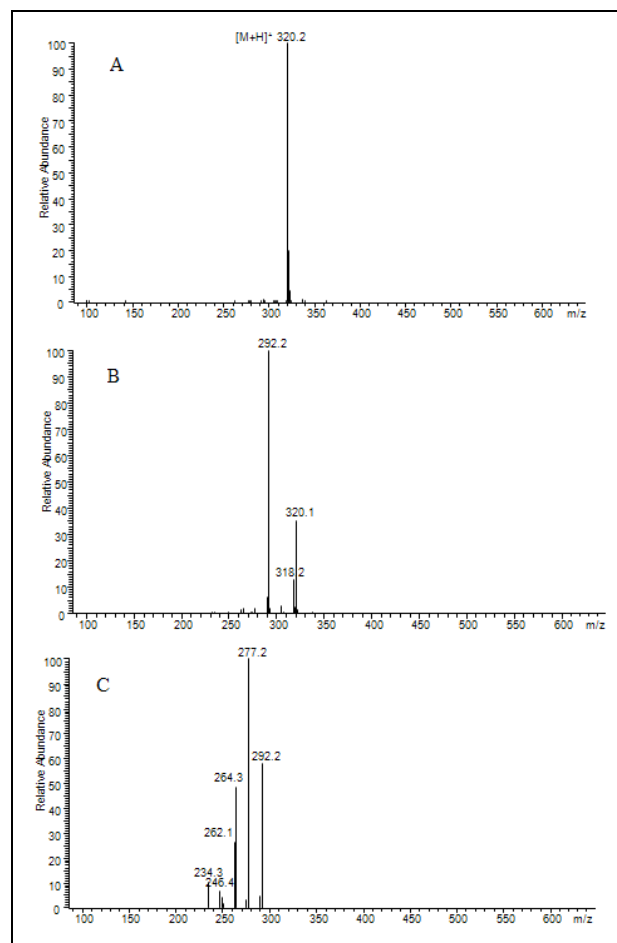
#### LC-MS/MS

The released ligands were dissolved in 80% (v/v) ACN. The ligand identification was performed with Waters (Milford, MA) 2690 HPLC system coupled to LCQ<sup>TM</sup> ion trap mass spectrometer in positive ionization mode (Finnigan, San Jose, CA). A portion of the sample was loaded onto a C<sub>18</sub> column (150mm $\times$ 4.6 mm, 5 $\mu$ m, Dikma). The flow rate was 0.5mL/min. The eluting gradient was set as follows (acetonitrile (A) and 0.5% acetic acid in water (B)): t=0-15 min, 20-25% A; t=15-40 min, 25-35% A, 40-60min, 35-75% A. The scan range was m/z 100-800 Da. The metal capillary voltage was 4.5 V and the temperature was set to 250°C, the sheath gas

(N<sub>2</sub>) flow-rate was 50 arbitrary.



**Fig. 2:** LC chromatogram of ultrafiltration screening assay of *Coptis chinensis* Franch with  $\alpha$ -glucosidase. solid line: experiment with  $\alpha$ -glucosidase; dotted line: experiment without  $\alpha$ -glucosidase.



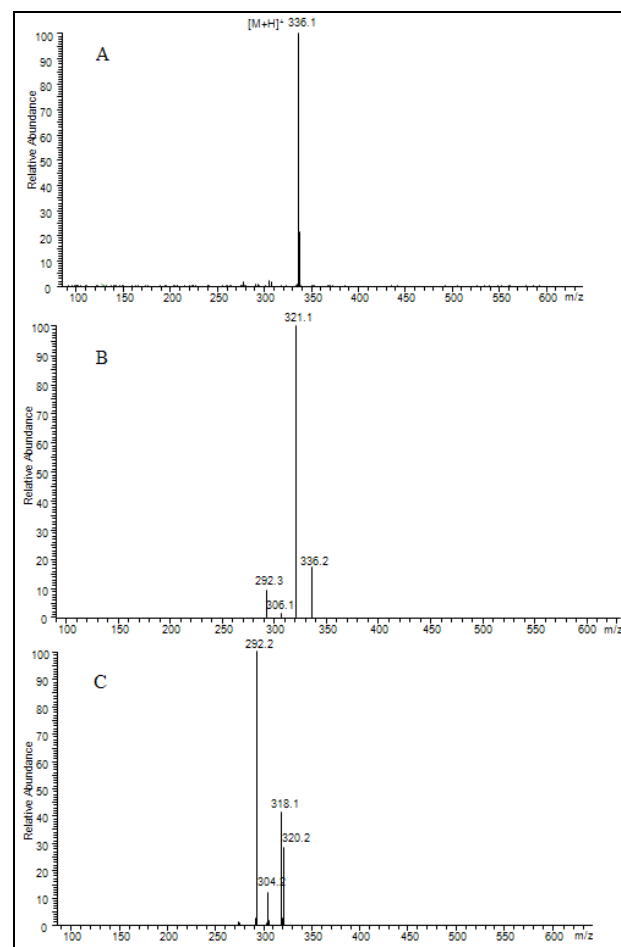
**Fig. 3:** MS<sup>n</sup> spectra of compound 1 in fig. 2. A, B and C were MS<sup>1</sup>, MS<sup>2</sup> and MS<sup>3</sup> spectra of compound 1, respectively.

## RESULTS

### $\alpha$ -Glucosidase inhibition assay

In order to prescreen Chinese herb extracts which have  $\alpha$ -glucosidase inhibitory activity, the *in vitro*  $\alpha$ -glucosidase inhibition assay was conducted to *Acanthopanax Senticosus* Harms leaves, *Pueraria lobata* (Willd.) Ohwi,

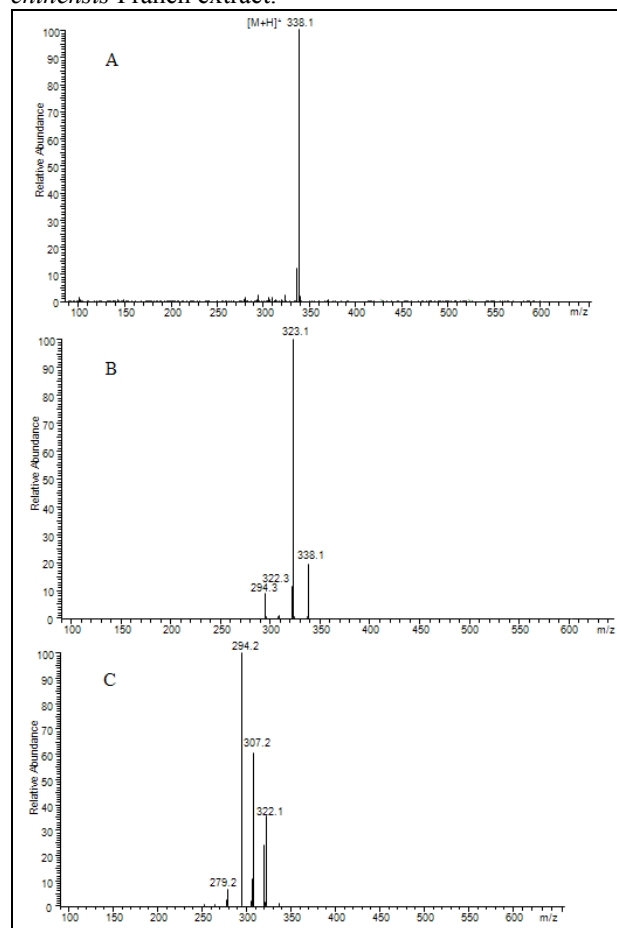
*Seutellaria baiealensis* Georgi, *Glycyrrhiza uralensis* Fisch and *Coptis chinensis* Franch, respectively. The enzyme inhibition assay showed that their IC<sub>50</sub> values of  $\alpha$ -glucosidase inhibitory activity were 0.48, 2.73, 0.54, 1.51, 0.37. The inhibitory activity decreased in the following order: *Coptis chinensis* Franch > *Acanthopanax Senticosus* Harms leaves > *Seutellaria baiealensis* Georgi > *Glycyrrhiza uralensis* Fisch > *Pueraria lobata* (Willd.) Ohwi. The results indicated that *Coptis chinensis* Franch extract carries the strongest  $\alpha$ -glucosidase inhibitory activity among the five kinds of Chinese herb extracts.



**Fig. 4:** MS<sup>n</sup> spectra of compound 4 in fig. 2. A, B and C were MS<sup>1</sup>, MS<sup>2</sup> and MS<sup>3</sup> spectra of compound 4, respectively.

Fig. 1 showed the dose titration for different concentration of *Coptis chinensis* Franch extract and acarbose. The result showed that the *Coptis chinensis* Franch extract inhibited 90% of the  $\alpha$ -glucosidase activity at 1 mg/ml, significantly higher than that of the well known synthetic  $\alpha$ -glucosidase inhibitor, acarbose (82% inhibition at 1mg/mL). Therefore, it would be valuable to

screen and identify active compounds from *Coptis chinensis* Franch extract.



**Fig. 5:** MS<sup>n</sup> spectra of compound 3 in fig. 2. A, B and C were MS<sup>1</sup>, MS<sup>2</sup> and MS<sup>3</sup> spectra of compound 3, respectively.

#### Screening of $\alpha$ -glucosidase ligands in *Coptis chinensis* Franch extract by ultrafiltration LC-ESI-MS<sup>n</sup>

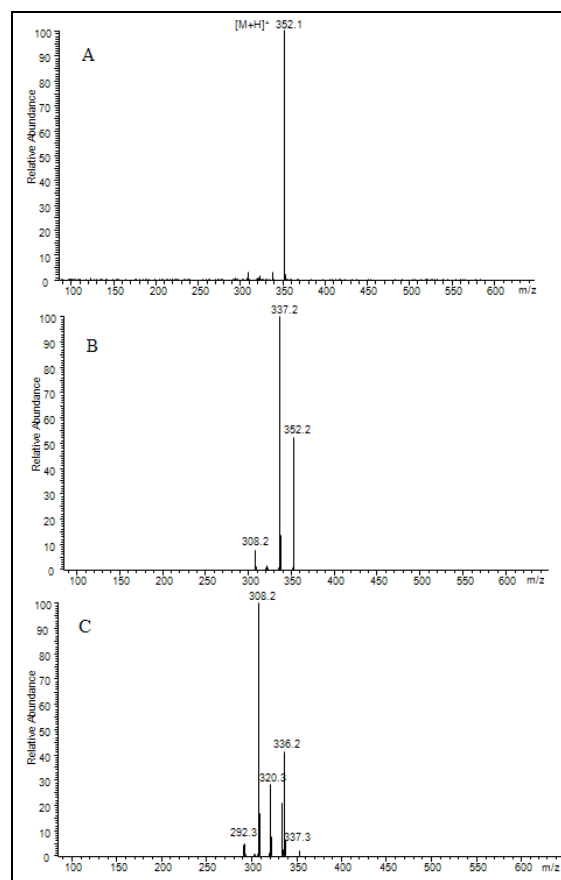
The HPLC separation of *Coptis chinensis* Franch extract was performed with C<sub>18</sub> reversed phase column. A baseline separation within 60 minutes was achieved and a total of five constituents were separated and detected. After the incubation with  $\alpha$ -glucosidase and ultrafiltration purification, the peaks showed higher intensities for the compounds incubated with  $\alpha$ -glucosidase (solid line) than those of the control sample (dotted line). As shown in fig. 2, five compounds were specifically bound to the  $\alpha$ -glucosidase. Subsequently, LC-ESI-MS<sup>n</sup> approach was performed to identify compounds binding to  $\alpha$ -glucosidase. The data of the retention time ( $t_R$ ), UV spectra and ESI-MS<sup>n</sup> data were summarized in table 1.

## DISCUSSION

Compound 1 eluted at 23.12 min (fig. 2) gave the [M+H]<sup>+</sup> precursor ion at m/z 320 as the base peak. In the ESI-MS<sup>2</sup> experiment, ions at m/z 318 and 292 were observed. In

the MS<sup>3</sup> spectrum of ion at m/z 292, ions at m/z 277, 264, 262, 246, and 234 were observed (fig. 3). Therefore, compound 1 was identified as coptisine by comparing its retention time, [M+H]<sup>+</sup> ion, and fragmentation pattern with those of the coptisine standard sample.

In the MS spectra, both compounds 2 and 4 exhibited their [M+H]<sup>+</sup> ions at m/z 336, which indicated that they were the isomers. In order to identify these two compounds, ESI-MS<sup>2</sup> analysis was employed and it was demonstrated that the ions at m/z 336 could produce three ion peaks at m/z 321, 306 and 292. When the ion at m/z 321 was selected as the precursor ion in the ESI-MS<sup>3</sup> experiment, the product ions at m/z 320, 318, 304 and 292 were detected. The retention time in LC/MS analysis and fragmentation pathway of compound 4 (fig. 4) are consistent with those of the berberine reference compound. As discussed above, compound 4 could be tentatively identified to be berberine. Interestingly, the ESI-MS<sup>n</sup> behavior of compound 2 was found to be the same as that of compound 4. Based on the reported literature, compound 2 could be identified as epiberberine (Wang *et al.*, 2004; Yu *et al.*, 2007).



**Fig. 6:** MS<sup>n</sup> spectra of compound 5 in fig. 2. A, B and C were MS<sup>1</sup>, MS<sup>2</sup> and MS<sup>3</sup> spectra of compound 5, respectively.

Compound 3 gave a  $[M+H]^+$  ion at  $m/z$  338 in the full scan MS. Moreover, two ions at  $m/z$  323 and 294 were detected in the  $MS^2$  spectrum. The ion at  $m/z$  323 was selected as the precursor ion in the ESI- $MS^3$  experiment, the product ions at  $m/z$  322, 307, 294 and 279 were detected (fig. 5). These results are in agreement with those of jatrorrhizine standard sample. Therefore, compound 3 could be identified as jatrorrhizine based on the above discussion.

As to compound 5, the  $[M+H]^+$  ion at  $m/z$  352 was observed in the full scan mass spectrum. In the  $MS^2$  spectrum, two ions at  $m/z$  337 and 308 were detected. The product ions at  $m/z$  336, 320, 308 and 292 were measured from the precursor ion at  $m/z$  337 in the ESI- $MS^3$  analysis (fig. 6). By comparing with fragmentation pattern of the palmatine standard sample and the data in literature, compound 5 could be identified as palmatine (Lu *et al.*, 2006; Deng *et al.*, 2008).

## CONCLUSIONS

The screening of  $\alpha$ -glucosidase ligands from *Coptis chinensis* Franch extract were studied by ultrafiltration LC-ESI- $MS^n$ , as a result, five compounds that could bind to  $\alpha$ -glucosidase were observed, and further identified as coptisine, epiberberine, jatrorrhizine, berberine and palmatine. Thus, the ultra filtration LC-ESI- $MS^n$  was proved to be an efficient and fast method for screening ligands of macromolecule from complex herb extract. These findings may provide valuable information for the discovery of  $\alpha$ -glucosidase inhibitors and the potential application of the *Coptis chinensis* Franch for designing efficient anti-diabetes drugs.

## ACKNOWLEDGEMENTS

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