SHORT COMMUNICATION

Quantification and bio-assay of α-glucosidase inhibitors from the roots of Glycyrrhiza uralensis Fisch.

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1. Introduction

Licorice is a popular Chinese herbal medicine derived from the dried roots and rhizomes of Glycyrrhiza uralensis, Glycyrrhiza inflata and Glycyrrhiza glabra (Zhang & Ye 2009). It has been used worldwide as a herbal medicine and natural sweetener for more than 4000 years. Currently, more than 300 flavonoids have been isolated from Glycyrrhiza species, licorice flavonoids are becoming popularities because of their significant biological activities (Asl & Hosseinzadeh 2008; Akram et al. 2011). It has been reported that flavonoids have role of anti-inflammatory and radicals scavenging, in addition, they were α-glucosidase, aldose reductase and glycation inhibitor (Ajish et al. 2014; Maione et al. 2015). α-Glucosidase, an
enzyme located in the epithelium of the small intestine, is the key enzyme that catalyzes cleavage of oligosaccharides and disaccharides to glucose and plays an important role in regulation of blood glucose level in the body (Kang et al. 2013). Flavonoid has glycosidic bond or multi-hydroxyl is a potent \( \alpha \)-glucosidase inhibitor (Zhang et al. 2012; Ezzat & Salama 2014; Sun et al. 2014), and licorice flavonoids have multiple glycosidic bond and/or multiple hydroxyl. Therefore, it suggested that licorice flavonoids may have a therapeutic effect on diabetes, and to confirm the main \( \alpha \)-glucosidase inhibitory active ingredients of licorice, the activity-guided isolation and simultaneous determination of the active constituents are common method that find the chemical basis of activities in traditional Chinese medicine (Wen et al. 2014).

In our previous study since 2005, we found that ethyl acetate partitioned fractions of the \textit{Glycyrrhiza} aqueous extract has the better \( \alpha \)-glucosidase inhibitory activity and more abundant flavonoids than other organic solvent partitioned fractions, and then \( \alpha \)-glucosidase inhibitory activity of \textit{Glycyrrhiza} total alkaloids, \textit{Glycyrrhiza} total flavones (GTF) and \textit{Glycyrrhiza} polysaccharides were screened separately and we found that GTF had the best \( \alpha \)-glucosidase inhibitory activity. Furthermore, \textit{in vivo}, the high level of serum glucose caused by feeding sucrose in rats was reduced by \textit{Glycyrrhiza} flavonoids, and it did not produce the pseudo-aldosteronism toxicity (Xu et al. 2005, 2006; Xu 2006; Liu et al. 2014). Further investigation is necessary to find the chemical basis of \( \alpha \)-glucosidase inhibitory activities in \textit{G. uralensis}. In the current study, we focus on \( \alpha \)-glucosidase inhibitor isolated from the roots of \textit{G. uralensis} Fisch., aiming to identify the active compounds via assessing their \( \alpha \)-glucosidase inhibitory activities \textit{in vitro} and quantify it in GTF using HPLC. The structure-activity relationship is also discussed.

2. Results and discussion

2.1. Identification of \textit{Glycyrrhiza} flavonoids compounds 1–7

The \textit{G. uralensis} Fisch. was extracted and obtained GTF accordance to \( \alpha \)-glucosidase inhibitor activity-guide, then GTF was separated by chromatography, and seven isolated compounds

![Figure 1. The structures of compounds 1–7.](image-url)
were obtained and elucidated by using spectroscopic techniques and comparison with the literature data. They were neoliquiritin (1) (Nakanishi et al. 1985), liquiritin (2) (Yang & Li 1988), liquiritigenin (3) (Ma et al. 2005), glycycomanarin (4) (Fu et al. 2013), isolicoflavonol (5) (Zheng et al. 2008), licochalcone A (6) (Wang et al. 2004) and medicarpin (7) (Hasan et al. 2012), and their structures are shown in Figure 1.

2.2. Analysis the content of seven purified flavonoids in GTF by HPLC

Total content of compound 1–7 in GTF was 61.47% (>50%) and shown in Table 1, and any of the content of compound 1, 2 and 4 was greater than 10%. Wherein the content of compound 2 was maximum.

2.3. Evaluation of the α-glucosidase inhibitory activities

The seven compounds and GTF were evaluated preliminarily for α-glucosidase (yeast) inhibitory activities with p-nitrophenyl glucopyranoside (pNPG) as the substrate and acarbose as the positive control. The results are presented in Table 1. Except compound 7 with the IC\textsubscript{50} values of 44.384 μg/mL, other six isolates had more potent α-glucosidase inhibitory activity than acarbose. Compound 5 with the IC\textsubscript{50} values of 10.854 μg/mL had a most powerful inhibitory effect on α-glucosidase and remarkably more potent approximately 3-fold than acarbose. GTF was a mixture composed by plenty of Glycyrrhiza flavonoids monomeric compounds, and it with the IC\textsubscript{50} values of 21.641 μg/mL had also more potent nearly 2-fold than acarbose. Integrated to consider two factors that was the content of seven purified flavonoids in GTF and their α-glucosidase inhibitory activities, the main α-glucosidase inhibitory activities of GTF might be attributed to compounds 1, 2 and 4.

It has been reported that some of the flavonoids compounds were also found to possess the α-glucosidase inhibitory activities, hydroxylations at C-7 positions on the A rings or C-4’ positions on the B rings is necessary for α-glucosidase inhibitory activities and hydroxylations at other positions play an assist role in the α-glucosidase inhibitory activities (Eduardo et al. 2007; Li et al. 2009; Kim et al. 2010; Phan et al. 2013; Su et al. 2015). Compound 5 has four hydroxyl at C-5 and seven positions on the A rings, C-4’ position on the B rings and C-3 position on the C rings respectively, and compound 4 has three hydroxyl at C-7 position on the A rings and C-2’ and 4’ positions on the B rings, these hydroxyl groups provided to them potent activity. In addition, the presence of chiral carbon provided certain activity for the

| Compounds | The content in GTF (%) | IC\textsubscript{50} for α-glucosidase (μg/mL)a |
|-----------|-----------------------|-----------------------------------------------|
| 1         | 15.74                 | 31.30±1.421                                   |
| 2         | 22.55                 | 30.26±1.233                                   |
| 3         | 3.33                  | 23.36±1.134                                   |
| 4         | 11.74                 | 19.52±0.963                                   |
| 5         | 2.41                  | 10.84±0.980                                   |
| 6         | 4.23                  | 26.45±1.265                                   |
| 7         | 1.52                  | 44.38±1.580                                   |
| GTF       | –                     | 21.64±0.963                                   |
| Acar       | –                     | 38.99±1.324                                   |

\textsuperscript{a}Inhibitory activity was expressed as the mean ± SD of 50% inhibitory concentration of triplicate determinations. 
\textsuperscript{b}Acarbose was used as positive control.
α-glucosidase inhibitor due to the spatial conformation made the combination of inhibitors and enzyme more stable and specific (Arcelli et al. 2005, 2007). Therefore, compound 7, 6 and 4 were decrease the inhibitory activity by reason of the inexistence of chiral carbon.

3. Conclusions

After a series of experiments, we reveal the main ingredients of the α-glucosidase inhibitor in GTF from the roots of Glycyrrhiza uralsensis Fisch. GTF and compound 1–6 had potent α-glucosidase inhibitory activities due to their specific structure. Moreover, these ingredients are derived from the medicinal and edible herbs. These results indicated that GTF and compound 1–6 may become a valid alternative of potential basis for novel hypoglycaemic and antidiabetic agents.

Disclosure statement

No potential conflict of interest was reported by the authors.

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