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A new glucoside with a potent α-glucosidase inhibitory activity from *Lycium schweinfurthii*

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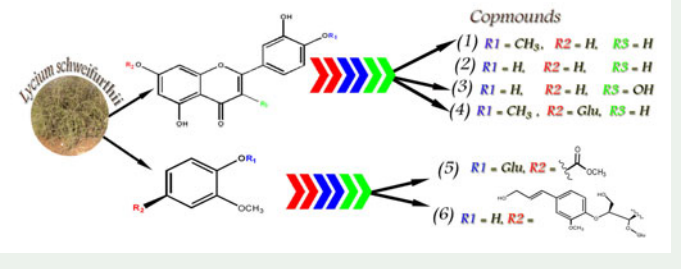
ABSTRACT

A new glucoside, 3-methoxy-4-*O*-β-D-glucopyranosyl-methyl benzoate, has been isolated from *Lycium schweinfurthii* along with five known compounds through bioactivity guided fractionation of the total plant methanolic extract towards α-glucosidase inhibitory activity. All the isolated compounds were tested for their inhibitory effect on α-glucosidase enzyme. As a result, four of them showed a potent inhibitory activity and thus constitute a therapeutic approach to decrease postprandial hyperglycemia in diabetic patients.



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1. Introduction

Diabetes is a chronic metabolic disorder characterized by abnormal high plasma glucose levels, leading to major complications, such as diabetic neuropathy, retinopathy and cardiovascular diseases (He and King 2004; Krentz et al. 2007). Hydrolysis of dietary carbohydrates such as starch is the major source of glucose in the blood. This hydrolysis is carried out by a group of hydrolytic enzymes that includes intestinal α-glucosidases and pancreatic α-amylase (Harris 1992). Inhibition of these enzymes

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could be an important strategy for management of type II diabetes (Yin et al. 2014), (Krentz and Bailey 2005), wherein α-glucosidase inhibitors could delay the rapid utilization of dietary carbohydrates and thereby suppress postprandial hyperglycemia (Watanabe et al. 1997). However, currently therapeutic drugs, such as acarbose, are shown to effectively reduce the intestinal absorption of sugars in humans. However, some have been associated with abdominal distention, flatulence, meteorism, and possibly diarrhea (Bishoff 1985).

Lycium schweifurthii is a deciduous Solanaceous shrub that is growing in temperate to the subtropical regions of North America, North Africa, South America, southern Africa and Australia (Fukuda et al. 2001). Previous phytochemical investigation of *L. schweinfurthii* reported the presence of flavonoids, glycosides and phenolic acids (Abd El-Maboud et al. 2016). Different species of *Lycium* are cultivated in the mainland of China as an important source for health foods, its polysaccharides containing fruits have proven to promote the differentiation of osteoblasts against postmenopausal metabolic disorders (Wang et al. 2018). Traditional Chinese Medicine prescriptions reported the use of some species of *Lycium* to treat Diabetes (Qian et al. 2017). Modern pharmacological studies revealed that *L. chinense* root bark extracts can decrease serum glucose levels and improve insulin resistance (Behl and Kotwani 2017), while some of its isolated glycosides exhibited potent antihyperlipidemic activities in HepG2 cells (Chen et al. 2018). In our ongoing search for α-glucosidase inhibitors, one new glucoside, 3-methoxy-4-*O*-β-D-glucopyranosyl-methyl benzoate, along with five known compounds were isolated. Their structures were elucidated using 1D and 2D NMR as well as MS analysis. Their α-glucosidase inhibitory activity was screened using acarbose as a positive control.

2. Results and discussion

2.1. Inhibition of α-glucosidase by different fractions of L. schweinfurthii methanolic extract

An *in vitro* α-glucosidase inhibitory assay was performed for the total methanolic extract showed a promising inhibitory activity, followed by screening of subsequent *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol fractions of *L. schweinfurthii* (Figure S1) All the fractions were tested at 100, 50 μg/mL. Ethyl acetate and *n*-butanol fractions showed more than 50% inhibition.

2.2. Chemical investigation of the active fractions of L. schweinfurthii

Chemical investigation of the ethyl acetate and *n*-butanol fractions afforded six compounds (Figure 1) (**1**–**6**). They were identified as diosmetin (**1**) (Park et al. 2007), luteolin (**2**) (Alwahsh et al. 2015), quercetin (**3**) (Ahmedova et al. 2012), diosmetin-7-*O*-β-D-glucoside (**4**) (Basudan et al. 2005), 3-methoxy-4-*O*-β-D-glucopyranosyl-methyl benzoate (**5**) and 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy-1-(*E*)-propenyl)-2-methoxyphenoxy]-propyl-β-D-glucopyranoside (**6**) (Takara et al. 2002). diosmetin-7-*O*-β-D-glucoside (**4**) is reported for the first time from *Solanaceae*, while diosmetin (**1**) and 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy-1-(*E*)-

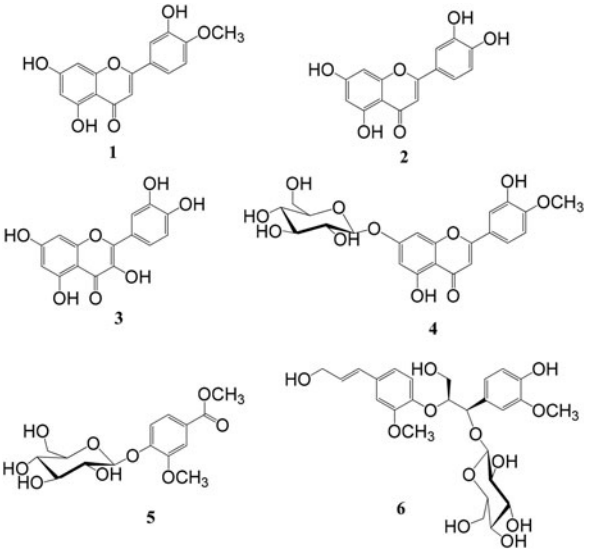

Figure 1. Structures of isolated compounds from *L. schweinfurthii*.

propenyl)-2-methoxyphenoxy]-propyl-β-D-glucopyranoside (**6**) are isolated from *L. schweifurthii* species for the first time.

Compound (**5**) was isolated as a white amorphous powder, with a molecular formula $C_{15}H_{20}O_9$ as established by the cationated molecular ion peak at *m/z* 367.0981 $[M+Na]^+$ (calculated, 367.1005) and molecular ion peak at *m/z* 389.1048 $[M+HCOO]^-$ (calculated, 389.1084) in both positive and negative HRESIMS (Figure S2). The ^{1}H NMR spectrum together with ^{1}H-^{1}H COSY correlations (Figures S3 and S4) exhibited an ABX spin system aromatic protons at δ_H 7.60 (1H, d, $J=1.8$ Hz), 7.22 (1H, d, $J=8.4$ Hz) and 7.63 (1H, dd, $J=1.8$, 8.4 Hz), two methoxy groups at δ_H 3.89 (3H, s) and 3.88 (3H, s), and one anomeric proton at δ_H 5.02 (1H, d, $J=7.8$ Hz) were observed in the upfield region. The ^{13}C NMR and HSQC spectra (Figures S5 and S6) displayed a total of 15 carbon signals; Apart from the sugar moiety signals, one carbonyl at δ_C 168.3 (C-7), two oxygenated quaternary carbons at δ_C 150.5 (C-3) and δ_C 152.5 (C-4), one quaternary at δ_C 125.0 (C-1), three aromatic methines at δ_C 125.0 (C-6), 116.5 (C-5) and 114.2 (C-2) two methoxy signals at δ_C 56.8 and 52.6. The benzoate moiety of the structure was established through the HMBC correlations (Figure S7 and S8) of H-2 [δ_H 7.60 (1H, d, $J=1.8$ Hz)] to C-4 [δ_C 152.5], C-6 [δ_C 124.6] and C-7 [δ_C 168.3], as well as H-5 [δ_H 7.22 (1H, d, $J=8.4$ Hz)] to C-1 [δ_C 125.0], and C-3 [δ_C 150.5] and H-6 [δ_H 7.63 (1H, dd, $J=1.8$, 8.4 Hz)] to C-2 [δ_C 114.2] and C-4 [δ_C 152.5]. The methoxy groups were confirmed to be at C-3 and C-7 based on HMBC correlations of the methoxy protons at δ_H 3.89 and 3.88 with C-3 and C-7, respectively. The HMBC correlation of H-1′ [δ_H 5.02 (1H, d, $J=7.5$ Hz)] with C-4 [δ_C 152.5] revealed that the sugar unit was attached to C-4. Acid Hydrolysis of the compound, revealed 4-hydroxy-3-methoxybenzoic acid (vanillic acid) and D-glucose. The aglycone was confirmed by ^{1}H-NMR and HRMS (Figures S9 and S10), while the sugar part was deduced from co-chromatography with authentic sugars as D-glucose (Baumeler et al. 2000). Furthermore, the β-configuration of D-

glucopyranose was determined by its anomeric proton coupling constant at [δ_H 5.02 (1H, d, $J = 7.8$ Hz)]. Consequently, the compound was identified as, 3-methoxy-4-*O*-β-D-glucopyranosyl-methyl benzoate. This compound is isolated for the first time from a natural source. Nonetheless, tracking the existence of this compound using ethanol as extracting solvent is necessary to eliminate the possibility of being artifact, so that LCMS-IT-TOF analysis for the re-extracted plant materials with ethanol was carried out, resulted in detection of peaks corresponding to compound **5** in both positive $[M + Na]^+$ and negative $[M + HCOO]^-$ ion modes, together with TIC chromatogram (Figure S12 and S13).

2.3. Inhibition of α-glucosidase by compounds isolated from both ethyl acetate and n-butanol fractions

The six compounds isolated from the active fractions of *L. schweinfurthii* as well as the positive control acarbose were tested for their α-glucosidase activity, the results (Table S14) showed that compounds **1**, **2**, **3** and **5** exhibited potent α-glucosidase inhibitory activity with IC_{50} equal 23.55 ± 2.65, 21.38 ± 1.82, 7.12 ± 0.84 and 22.76 ± 3.67, respectively, while compounds **4** and **6** maintained weak inhibitory activities.

The IC_{50} values of the isolated flavonoids compounds (**1**-**3**) were found to be compatible with the reported ones (Watanabe et al. 1997; Lee and Lee 2001). Hydroxylation at position 3 of flavone was found to enhance the activity (Tadera et al. 2006). Careful investigation of the active compounds from a pharmacokinetic point of view, it was assumed that 3-methoxy-4-*O*-β-D-glucopyranosyl-methyl benzoate (**5**) has the advantage of being stable *in vivo*, while the isolated flavonoids are rapidly degraded within the body into the corresponding phenylacetic acid (Manach and Jennifer 2004).

3. Experimental section

3.1. General experimental procedure

^{1}H and ^{13}C-NMR spectra were obtained on a Bruker DRX 600 NMR spectrometer (Bruker Daltonics INC., MA, USA). Chemical shifts (δ) were expressed in ppm with reference to the TMS resonance. HR-ESI-MS was determined using LC–MS–IT–TOF (Shimadzu, Tokyo, Japan). The MS instrument was operated using an ESI source in both positive and negative ionization modes with survey scans acquired from m/z 100–2000 for MS and *m/z* 50–1500 for MS/MS. The ionization parameters were as follows: probe voltage, ± 4.5 kV; nebulizer gas flow, 1.5 L/min; CDL temperature, 200 °C; heat block temperature, 200 °C. Infrared (IR) spectra were obtained by using an FTIR spectrometer 620, Jasco (Tokyo, Japan). Semi preparative Liquid Chromatography was carried out on MPLS-UV-ELSD; BUCHI Reveleris® PREP purification system. Dimethylsulfoxide (DMSO) and other organic solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan). Silica gel (75–120 mesh) and RP-C_{18} silica gel (38–63 μm) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Thin layer chromatography (TLC) silica gel 60 F_{254} was purchased from Merck (Darmstadt, Germany). The developed chromatograms were visualized under 254 nm. UV light and

the spots were made visible by spraying with vanillin/H_2SO_4 reagent before warming in an oven pre-heated to 110 °C for 5 min.

3.2. Plant material

L. schweinfurthii Dammer. (Solanaceae), whole plant, was collected from International coastal road, about fifty kilometres west Gamasa, Egypt on December 2016. The plant identity was confirmed by Dr. Ibrahim Mashaly, Professor of Ecology, Faculty of Science, Mansoura University. The fresh collected parts were air dried in shade at room temperature. A voucher specimen has been deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Mansoura University given the code: LY 01 Mansoura-3.

3.3. Extraction and isolation

The air dried powdered whole plant (1.2 kg) was extracted by maceration with 90% MeOH at room temperature. The solvent-free extract (107.2 g) was successively fractionated with *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol. The EtOAc fraction (15.7 g) was subjected to chromatographic investigation using silica gel column eluted gradient with *n*-hexane–EtOAc (100:0 → 0:100, 64 subfraction), followed by EtOAc– MeOH (100:0 → 50:50, 33 subfractions), to give 97 sub-fractions, 250 mL each. Sub-fractions 7–11 were combined and purified by reversed phase chromatography using C_{18} and MPLC gradient elution of H_2O-MeOH (50:50 → 50:100 in 40 min.), to give compound 1 (7.4 mg). Sub-fractions 17–24 was purified by reversed phase chromatography using C_{18} and MPLC gradient elution of H_2O-MeOH (50:50 → 50:100 in 40 min.), followed by PTLC-RPC_{18} (on precoated silica gel plates F_{254}) using H_2O–MeOH (30:70) to give compound 2 (11.4 mg). Compound 3 (24 mg) was purified from sub-fractions 43-50 by PTLC (on pre-coated silica gel plates F_{254}) using *n*-hexane–EtOAc (30:70).

n-Butanol fraction (20.5 g) was chromatographed on a silica gel column and eluted with EtOAc–MeOH (100:0→0:100) to yield 86 sub-fractions, 300 mL each. Compound 4 (4.8 mg) was purified from sub-fractions (9–10, 35 mg) by using MPLC eluted with H_2O-MeOH isocratically (70:30, in 20 min.). Sub-fraction (55–64, 968 mg) were subjected to MPLC eluted with H_2O-MeOH (50:50 → 50:100 in 40 min.), followed by PTLC (on precoated silica gel plates F_{254}) using H_2O–MeOH (50:50) to give compound 5 (1.6 mg). Compounds 6 (2.4 mg) was purified from sub-fractions (82-86, 470 mg) by PTLC (on pre-coated silica gel plates F_{254}) using H_2O–MeOH (50:50).

3.4. Acid hydrolysis

Compound **5** were dissolved in 5 mL MeOH, mixed with equal volume of 5% HCl (v/v), and the solution was refluxed for 2 h on boiling water bath. Methanol was distilled off and the hydrolysate was shaken with CH_2Cl_2 to separate the aglycone. The CH_2Cl_2 extract was dried over anhydrous sodium sulphate and the solvent was distilled off. The residue was dissolved in methanol and kept for identification of the aglycone

moiety. The acidic mother liquor was neutralised with silver oxide and the formed precipitate was filtered off and washed several times with few millilitres of distilled water. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in few drops of methanol and used for identification of the sugar moiety by comparison with authentic sugar samples.

3.5. LC-MS analysis

The compound and the ethanol extract were injected into LC–MS analysis with an IT-TOF instrument (Shimadzu, Kyoto, Japan). The equipment was fitted with an Agilent ZORBAX SB-C18 column (1.8 μm, 2.1 × 150 mm; USA) maintained at 40 °C. The mobile phase solvents were: solvent A, H_2O (0.1% formic acid) and solvent B, acetonitrile (0.1% formic acid). The gradient program was as follows: 0–20 min (A:B 95:5, v/v), 20–25 min (0:100, v/v). The flow rate was 0.3 mL/min and the injection volume was 2 μL. The R_t of compound 5 was around 9.82 min.

3.6. Spectral data of the new compound (5)

3-methoxy-4-*O*-*β*-D-glucopyranosyl-methyl benzoate (**5**): white amorphous powder, IR ν_{max}/cm^{-1}: 3446 cm^{-1} (OH stretching), 1700 cm^{-1} (C = O stretchinng), 1034 cm^{-1} (C-O stretching) (Figure S11); HR-ESI-MS *m/z* $[M + Na]^+$ 367.0981. ^{1}H NMR (CD_3OD, 600 MHz) δ_H 3.40 (1H, m, H-4′), 3.46 (2H, m, H-3′, 5′), 3.57 (1H, m, H-2′), 3.69 (1H, m, H-6′a), 3.84 (1H, m, H-6′b), 3.88 (3H, s, 7-OCH_3), 3.89 (3H, s, 4-OCH_3), 5.02 (1H, d, J = 7.5 Hz, H-1′), 7.22 (1H, d, J = 8.4 Hz, H-5), 7.60 (1H, d, J = 1.8 Hz, H-2), 7.63 (1H, dd, J = 8.4, 1.8 Hz, H-6). ^{13}C NMR (CD_3OD, 150 MHz) δ_C 52.6 (7-OCH_3), 56.8 (4-OCH_3), 62.5 (C-6′), 71.3 (C-4′), 74.8 (C-2′), 77.9 (C-3′), 78.4 (C-5′), 102.0 (C-1′), 114.2 (C-2), 116.5 (C-5), 124.6 (C-6), 125.0 (C-1), 150.5 (C-3), 152.5 (C-4), 168.3 (C-7).

3.7. Inhibition of α-glucosidase activity

The α-glucosidase inhibitory activity was assayed according to a previously described method (Fatmawati et al. 2011). A 0.1 mL portion of the sample solution or positive control solution dissolved in DMSO and 0.1 mL of α-glucosidase enzyme (5 units/mL) in 0.15 M HEPES buffer were added to 0.1 M sucrose solution in 0.15 M HEPES buffer and then incubated at 37 °C for 30 min. After incubation, the reaction was ended by heating at 100 °C for 10 min. The formation of glucose was determined by the glucose oxidase method, using a BF-5S Biosensor (Oji Scientific Instrument, Hyogo, Japan). IC_{50} values were determined for pure compounds isolated from *L. schweinfurthii*.

4. Conclusion

In our on-going research for finding α-glucosidase inhibitors from natural sources, *L. schweinfurthii* total methanolic extract, fractions and subsequent isolated compounds were screened for the activity. One new compound, 3-methoxy-4-*O*-*β*-D-glucopyranosyl-methyl benzoate, together with five known compounds were isolated; from which,

quercetin showed excellent inhibition on α-glucosidase with IC_{50} value of 7.1 μM. Compounds diosmetin, luteolin and 3-methoxy-4-*O*-β-D-glucopyranosyl-methyl benzoate exhibited potent inhibition against α-glucosidase with IC_{50} values of 23.6, 21.3 and 22.8 μM, respectively. 3-methoxy-4-*O*-β-D-glucopyranosyl-methyl benzoate might have possessed the advantage of being more stable after being consumed. Regarding the activity of diosmetin and its glucoside; substitution of hydroxyl group at position 7 of flavone with sugar seemed to decrease the inhibitory activity.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Abd El-Maboud MM, Elhaw MH, Haggag MI, Ewais EA. 2016. Phytochemical studies on Lycium schweinfurthii var. schweinfurthii (Solanaceae) and Isolation of five Flavonoids from leaves. J Med Plants Stud. 4:288–300.

Ahmedova A, Paradowska K, Wawer I. 2012. 1H, 13C MAS NMR and DFT GIAO study of quercetin and its complex with Al(III) in solid state. J Inorg Biochem. 110:27–35.

Alwahsh MAA, Khairuddean M, Chong WK. 2015. Supporting information chemical constituents and antioxidant activity of Teucrium barbeyanum Aschers. Rec Nat Prod. 9:159–163.

Basudan OA, Ilyas M, Parveen M, Muhisen HMH, Kumar R. 2005. Note: a new chromone from Ficus lyrata. J Asian Nat Prod Res. 7(1):81–85.

Baumeler A, Hesse M, Werner C. 2000. Benzoxazinoids–cyclic hydroxamic acids, lactams and their corresponding glucosides in the genus Aphelandra (Acanthaceae). Phytochemistry. 53(2): 213–222.

Behl T, Kotwani A. 2017. Chinese herbal drugs for the treatment of diabetic retinopathy. J Pharm Pharmacol. 69(3):223–235.

Bishoff H. 1985. Pharmacological properties of the novel glucosidase inhibitors BAY m 1099(miglitol) and BAY o 1248. Diabetes Res Clin Pr. 1:S53.

Chen H, Li Y, Sun Y, Li X, Jian-Hong G, Wu Y. 2018. Antihyperlipidemic glycosides from the root bark of Lycium chinense. :6419. doi:10.1080/14786419.2018.1466125

Fatmawati S, Shimizu K, Kondo R. 2011. Ganoderol B: a potent α-glucosidase inhibitor isolated from the fruiting body of *Ganoderma lucidum*. Phytomedicine. 18(12):1053–1055.

Fukuda T, Yokoyama J, Ohashi H. 2001. Phylogeny and biogeography of the genus Lycium (Solanaceae): inferences from chloroplast DNA sequences. Mol Phylogenet Evol. 19(2): 246–258.

Harris IM. 1992. Classification of diabetes mellitus and other categories of glucose intolerance. International Textbook of Diabetes Mellitus. New Jersey (NJ): Wiley; p. 3–18.

He Z, King GL. 2004. Microvascular complications of diabetes. Endocrinol Metab Clin North Am. 33(1):215–238.

Krentz AJ, Bailey CJ. 2005. Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs. 65(3):385–411.

Krentz AJ, Clough G, Byrne CD. 2007. Interactions between microvascular and macrovascular disease in diabetes: pathophysiology and therapeutic implications. Diabetes Obes Metab. 9(6): 781–791.

Lee D-S, Lee S-H. 2001. Genistein, a soy isoflavone, is a potent α-glucosidase inhibitor. FEBS Lett. 501(1):84–86.

Manach C, Jennifer LD. 2004. Pharmacokinetics and metabolism of dietary flavonoids in humans Agruvasc: vascular effects of flavanones from citrus View project. Free Radic Res. 38(8): 771–785.

Park Y, Moon B-H, Yang H, Lee Y, Lee E, Lim Y. 2007. Complete assignments of NMR data of 13 hydroxymethoxyflavones. Magn Reson Chem. 45(12):1072–1075.

Qian D, Zhao Y, Yang G, Huang L, Qian D, Zhao Y, Yang G, Huang L. 2017. Systematic review of chemical constituents in the genus Lycium (Solanaceae). Molecules. 22(6):911.

Tadera K, Minami Y, Takamatsu K, Matsuoka T. 2006. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. J Nutr Sci Vitaminol (Tokyo). 52(2):149–153.

Takara K, Matsui D, Wada K, Ichiba T, Nakasone Y. 2002. New antioxidative phenolic glycosides isolated from kokuto non-centrifuged cane sugar. Biosci Biotechnol Biochem. 66(1):29–35.

Wang S-Q, Liu B, Liu S, Xie S-Z, Pan L-H, Zha X-Q, Li Q-M, Luo J-P. 2018. Structural features of an acidic polysaccharide with the potential of promoting osteoblast differentiation from *Lycium ruthenicum* Murr. Nat Prod Res.1–6. doi:10.1080/14786419.2018.1466125.

Watanabe J, Kawabata J, Kurihara H, Niki R. 1997. Isolation and identification of α -glucosidase inhibitors from tochu-cha (*Eucommia ulmoides*). Biosci Biotechnol Biochem. 61(1):177–178.

Yin Z, Zhang W, Feng F, Zhang Y, Kang W. 2014. α-Glucosidase inhibitors isolated from medicinal plants. Food Sci Hum Wellness. 3(3–4):136–174.