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Original article:

FLAVONOIDS AND ITS DERIVATIVES FROM *CALLISTEPHUS CHINENSIS* FLOWERS AND THEIR INHIBITORY ACTIVITIES AGAINST *A*-GLUCOSIDASE

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ABSTRACT

Inhibitors of carbohydrate-hydrolysing enzymes play an important role for the treatment of diabetes. One of the therapeutic methods for decreasing of postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate- hydrolysing enzymes, such as α-glucosidase, in the digestive organs. To investigate the therapeutic potential of compounds from natural sources, *Callistephus chinensis* flowers (CCF) were tested for inhibition of α-glucosidase, and acarboes was used as the positive control. The 70 % ethanol extract of CCF exhibited significant α-glucosidase inhibitory activities with IC_{50} value of 8.14 μg/ml. The stepwise polarity fractions of CCF were tested further for *in vitro* inhibition of α-glucosidase. The ethyl acetate (EtOAc) fraction exhibited the most significant inhibitory activity. Eight pure compounds, apigenin, apigenin-7-O-β-D- glucoside, kaempferol, hyperin, naringenin, quercetin, luteolin, and kaempferol-7-O-β-D- glucoside, were isolated (using enzyme assay-guide fractionation method) from the EtOAc fraction. Among these, quercetin was the most active one (IC_{50} values 2.04 μg/ml), and it appears that the inhibiting percentages are close to acarbose (IC_{50} values 2.24 μg/ml), the positive control, on α-glucosidase inhibition. HPLC/UV analysis indicated that the major components of CCF are kaempferol, hyperin and quercetin. The presented results revealed that CCF containing these eight flavonoids could be a useful natural source in the development of a novel α-glucosidase inhibitory agent against diabetic complications.

Keywords: *Callistephus chinensis* flowers, stepwise polarity fractions, HPLC, α-glucosidase

INTRODUCTION

Over the past several decades, the prevalence of diabetes mellitus has increased dramatically worldwide (Gorelick et al., 2011). According to some estimates, India, China, and United States will have the largest number of diabetic people by the year 2030 (Wild et al., 2004). The reasons for the rising prevalence are often linked with an increase in both obesity and ageing as well as genetic factors (Hui et al., 2010). Without proper and timely treatments, diabetes can result in many complications including hyperglycemia, diabetic ketoacidosis, cardiovascular disease and chronic renal failure (Yu et al., 2013). Accordingly,

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active and adequate treatments are essential for diabetes.

Adjustment of blood sugar is an effect approach and very important for diabetes control. The ideal final result for diabetes mellitus is to prevent the excessive rise of postprandial blood glucose. α-Glucosidas, wildly existed in the brush border surface of intestinal cells, is essential for carbohydrate digestion due to monosaccharides are only readily absorbed from the intestine. Other carbohydrates must be degraded enzymatically before they can be absorbed (Kim, 2013). The inhibitors of α-glucosidase could delay in the digestion of ingested carbohydrates and consequently lead to suppress the postprandial blood glucose (Heacock et al., 2005).Therefore, α-glucosidase inbibitors have promise as therapeutic agents for type 2 non-insulin-dependent diabetes mellitus (Baron, 1998). However, current glucosidase inhibitions such as acarbose and miglitol for diabetes management are not fully satisfactory and prolonged use often causes some serious side effects, particularly with diarrhea (Lebovitz, 1997) and corresponding intestinal pain and flatulence (Fujisawa et al., 2005). Randomized controlled trials with glucosidase inhibitions report these gastrointestinal side effects as the most common reason for noncompliance and early subject withdrawal (Neuser et al., 2005). Consequently, researchers have increasingly focused on finding more effective and safe α-glucosidase inhibitors from natural materials to develop physiological functional food to treat diabetes (Bhandari et al., 2008; Liu et al., 2011; Matsuura et al., 2002; Nishioka et al., 1998; Wang et al., 2010), such as polyphenols from fruits (Jiang et al., 2013), isoflavones from soybean (Niamnuy et al., 2011), and hydrolysate from sardine muscle (Matsui et al., 1996).

Callistephus chinensis, which belongs to the Asteraceae family, is extensively grown in both the northern and southern parts of China. Since its flowers possess high ornamental value, *C. chinensis* are now widely cultivated in many countries. Several studies have demonstrated that the Asteraceae family display strong biological activity, such as anti-diabetes, antioxidant effects and inhibitory effects against bacteria and viruses, and at the same time can also prevent indigestion, pneumonia, hepatitis and tumors (Cheng et al., 2005; Gorzalczany et al., 2013; Kim et al., 2013; Matsuda et al., 2002; Zhu et al., 2005; Yan et al., 1999). However, to our knowledge, no study has been done to determine the anti-diabetic activity of *Callistephus chinensis* flower (CCF). Thus, this present study investigates the chemical constituents of CCF and their anti-diabetic activity and aim to find new natural source for the development of α-glucosidase inhibitory agent for dealing with diabetic complications.

MATERIALS AND METHODS

Plant materials and chemicals

The flowers of *Callistephus chinensis* were collected form Inner Mongolia Autonomous Region, Republic of China, in 2012 and were kept in desiccators after they had been air dried. All the solvents for the extraction were purchased from Qingdao Haiyang Chemical Co., Ltd. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 600 NMR spectrometer (Rheinstetten, Germany). Reagents and solvents including α-glucosidase from recombinant *Saccharomyces cerevisiae* (expressed in unspecified host), α-amylase from *Bacillus licheniformis*, 4-nitrophenyl α-D-glucopyranoside, starch, potassium phosphate buffer, and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acarbose, used as a standard, was produced by the Bayer Company in Germany.

Preparation of extracts

C. chinensis flowers (CCF, 8 kg) were ground to fine a powder and then extracted with 8 L of 70 % ethanol for 2.5 h under reflux at 50 °C. After extraction, the solvent was removed by vacuum filtration, and the

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70 % ethanol extract (265 g) was collected. The aqueous solution was extracted followed by n-hexane, CH_2Cl_2, EtOAc and n-BuOH to get layer of EtOAc (73.5 g).

Isolation of active compounds from EtOAc-soluble fraction

The EtOAc fraction (70 g) was subjected to purification by silica gel (1200 g) column chromatography using CH_2Cl_2:MeOH solvent system with increased polarity (from 0:100 to 100:0, v/v). The eluent was collected into ten fractions. Fraction 4 (1.2 g) was applied to a Shephadex LH-20 column and eluted with MeOH to give compound **3** (37 mg) and **5** (14 mg) after recrystallisation with MeOH. Fraction 6 (4.7 g) was again purified by silica gel column to yield compound **1** (22 mg) and **6** (112 mg). Fraction 9 (9.2 g) was further purified by silica gel column and Sephadex LH-20 column to yield compound **2** (17.0 mg), **4** (24.0 mg) and **8** (7.8 mg).

Assay for α-glucosidase inhibitory activity

α-Glucosidase inhibitory activity of samples was performed using a previously described method (Kim et al., 2004; Yu et al., 2013), with slight modification. Thirty microliter of the *α*-glucosidase solution (2 units mL^{-1}, 0.1 mol L^{-1} potassium phosphate buffer, pH 6.8) was pre-mixed with 20 µL of the purified compound at different concentrations (in 1 % DMSO) and incubated at 37.5 °C for 5 min, 150 µL of *p*-nitrophenyl glucopyranoside (pNPG, 10 mM) and 800 µL of 0.1 M potassium phosphate buffer were added to the mixture to start the reaction. After another thirty minutes' incubation at 37.5 °C, 2 mL of 1 M Na_2CO_3 was added to per test tube for termination (Yu and Yin, 2011a; b). The absorbance of the sample at 405 nm was measured to quantify the amount of released product (p-nitro phenol) used by microplate Reader (varioskan flash-3001, Thermo Scientific, USA). Three parallel operations were done. Acarbose was used as positive control. Inhibition of *α*-glucosidase activity by each of the compound was expressed relative to control (no inhibitor).

Sample preparation and quantitative analysis of flavonoids in CCF

To quantify the amount of compounds **1-8** in CCF extract, 2 mg of each extract was dissolved in 1 mL MeOH. The resultant solutions were filtered through a Whatman 0.45 µm PVDF syringe filter (Cat No. 6779, NJ, USA) prior to HPLC. The HPLC separation of compounds **1**–**8** in CCF extracts for quantitative analysis was performed using a reverse phase column (Discovery C_{18}, 5 µm, 250 × 4.6 mm, Chuangxintongheng, China) and a mobile phase phase consisted of acetonitrile and water (v/v). The gradient solvent system was 13:87, initially, and was increased in linear gradients to 45: 65 over 65 min. The flow rate was kept constant at 1.0 mL/min, and the eluent was monitored by UV absorbance at 210 nm.

Limit of detection and quantification of flavonoids in CCF

In general, limit of detection (LOD) and limit of quantification (LOQ) were used to validate the HPLC method. Determination of LOD and LOQ values were usually based on the linear regression equation and calculated by signal-to-noise ratio of ≥ 3 and ≥ 10, respectively.

Calibration curves and statistical analysis

The calibration curves and statistical analysis were referred to a previously described method (Mok et al., 2013) with slight modification. Each of eight pure isolated compounds were prepared in MeOH (2 mg/mL) and repeatedly mixed with the same solvent. The concentration of compounds **1-8** was verified by comparing the individual peak areas of each sample to those of the corresponding standards. Calibration curves were obtained based on peak area (*Y*) as vertical coordinates and concentration (*X*, mg/mL) as horizontal axis. Data were expressed as mean ± S.D. of three re-

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plicate determinations for each sample with different concentrations (n = 5). The inhibitory activities of the samples are described as inhibitory concentration 50 % (IC_{50}) and calculated using SPSS program. Statistical significance was calculated by one-way analysis of variance (ANOVA) method and Dunnett's test.

RESULTS AND DISCUSSION

α-Glucosidase activity of the extract and fractions from CCF

The different fractions partitioned from the 70 % ethanol extract were tested for the presence of inhibitory activity against α-glucosidase, and the results are summarized in Table 1 and Figure 1. As the EtOAc fraction showed obviously higher inhibitory activities than did the 70 % ethanol extract against α-glucosidase, we supposed that the main active compositions of the 70 % ethanol extract of CCF were basically in the EtOAc fraction. Meanwhile, the data shown that the EtOAc fraction exhibited significant α-glucosidase inhibition, with an IC_{50} value of 2.97 μg/mL. In previous papers, flavonoid compounds from the leaves of fruits and grape seeds have been of increasing interest due to their anti-diabetic properties (Wang et al., 2010; Han et al., 2012; Kar et al., 2009; Montagut et al., 2010). Although extracts of the Asteraceae family plants have been reported to exhibit significant hepatic protective activity (Jeong et al., 2013), few studies have reported on the anti-diabetic activities of CCF.

Isolation of active compounds and structural determination

The EtOAc soluble portion of the 70 % ethanol extract from CCF was then subjected to repeated rounds of silica gel and Shephadex LH-20 chromatography, resulting in the isolation of compounds **1**–**8**. The structural determination of isolated compounds was confirmed by a combination of ^{1}H-, ^{13}C-NMR (Table 2). The singlet signals at δ 12.02-12.91 observed in the ^{1}H-NMR spectra of compounds **1**–**8** showed 5-OH of an A-ring in their structures. The signals of typical structures in flavonoids were shown in ^{13}C-NMR spectra and compared with values in the literature (Agrawal, 1989; Agrawal and Bansal, 1989; Andersen and Markham, 1989; Crow et al., 1986; Mok et al., 2013; Markham et al., 1978; Park et al., 2006; Harborne and Williams, 1993; Yasukawa et al., 1989). Based on the spectroscopic data obtained, the chemical structures of the purified compounds were iden-

Table 1: IC_{50} values of the fractions and compounds **1**-**8** from CCF for inhibition of α-glucosidase

Extract and Fractions	IC_{50}[a] (μg/mL)
Extract	8.14
n-Hexane	84.33
CH_2Cl_2	34.54
EtOAc	2.97*
n-BuOH	77.89

Compounds from EtOAc	IC_{50}[a] (μg/mL)
1 apigenin	25.47
2 apigenin-7-O-glucoside	29.73
3 kaempferol	16.97
4 hyperin	19.26
5 naringenin	57.40
6 quercetin	2.04*
7 luteolin	43.57
8 kaempferol-7-o-β-D-glucoside	20.68
Acarbose[b]	2.24

[a] IC_{50} value was calculated from the least-squares regression equations in the plot of the logarithm of three graded concentrations vs.% inhibition;
[b] Acarbose was used as a positive control; * Means $p < 0.05$ compared with acarbose

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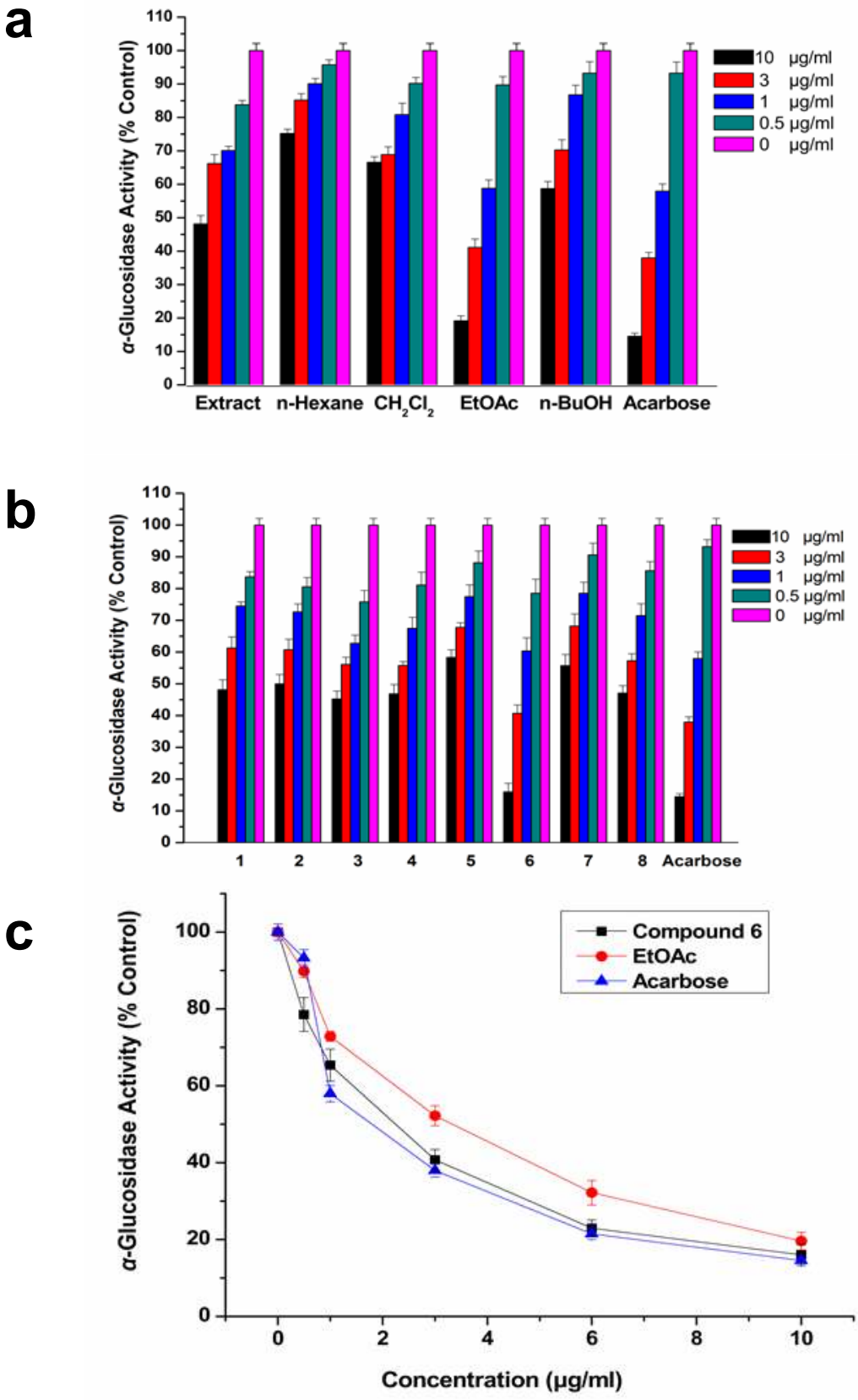

Figure 1: *α*-Glucosidase inhibition by extract, fractions, compounds **1**-**8** and acarbose:
(a) *α*-glucosidase inhibition by extract, fractions and acarbose. **(b)** *α*-glucosidase inhibition by compounds **1**-**8** and acarbose. **(c)** *α*-glucosidase inhibition by compound **6**, EtOAc fraction and acarboes

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tified as apigenin (**1**), apigenin-7-O-glucoside (**2**), kaempferol (**3**), hyperin (**4**), naringenin (**5**), quercetin (**6**), luteolin (**7**), kaempferol-7-O-*β*-D-glucoside (**8**) (Figure 2). Although common flavonoids have been isolated from various plants, to our knowledge, this is the first report on the isolation of phytochemical constituents from CCF.

Inhibition of α-glucosidase activity

Compounds **1**-**8** were tested for their inhibitory activity against *α*-glucosidase at four different concentrations. Comparing the activities of isolated compounds with those of the fractions, in particular, our results shown that the quercetin (**6**) was the main active constituent of CCF, it appears that the inhibiting percentages of isolated compounds are close to acarbose, the positive control, on *α*-glucosidase inhibition. The IC_{50} values were shown in Table 1 and the inhibiting percentages of the fractions and isolated compounds were shown in Figure 1. In addition, many flavonoids and phenol constituents with strong inhibitory activity toward *α*-glucosidase have been previously reported (Kawanishi et al., 2003; Lee and Kim, 2008; Yawadio et al., 2007). All the eight compounds isolated from CCF are the typical structures in flavonoids, but among them, only the flavonols (compounds **3**, **6** and **8**) showed higher *α*-glucosidase inhibitory activity than the flavonoid.

Table 2: ^{13}C-NMR spectral data for compounds **1**-**8** (DMSO-d_6) from CCF

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Compound	apigenin	apigenin-7-O-glucoside	kaempferol	hyperin	naringenin	quercetin	luteolin	kaempferol-7-O-β-D-glucoside
C-2	164.0	164.7	145.5	156.3	79.7	146.5	165.5	147.6
C-3	102.7	103.5	135.6	134.1	43.5	135.6	103.9	136.2
C-4	181.2	181.8	176.0	177.6	197.2	176.3	183.8	176.5
C-5	161.9	162.7	161.3	161.7	165.2	161.4	163.8	160.3
C-6	99.2	99.2	99.5	98.6	95.8	97.8	100.5	98.9
C-7	163.3	161.0	163.7	164.6	167.9	164.2	166.7	162.4
C-8	93.6	94.5	94.3	93.2	96.7	93.5	95.7	94.5
C\-9	157.0	156.8	157.1	156.7	164.5	156.7	159.5	155.9
C-10	103.3	105.2	101.6	103.9	103.2	103.5	105.6	104.3
C-1'	121.9	121.1	122.3	122.0	128.8	120.7	1207	121.2
C-2'	128.3	128.3	129.5	116.0	130.6	114.8	114.6	129.8
C-3'	115.8	116.3	115.9	145.1	116.1	146.9	146.5	115.5
C-4'	161.6	161.5	159.9	149.0	158.5	147.8	150.7	159.4
C-5'	115.8	116.6	115.9	116.3	116.1	115.7	117.2	115.5
C-6'	128.3	128.3	129.5	121.8	130.5	120.0	123.6	129.7
C-1'	-	98.5	-	101.5	-	-	-	99.8
C-2''	-	73.7	-	74.7	-	-	-	73.4
C-3''	-	76.1	-	77.3	-	-	-	77.3
C-4''	-	69.2	-	70.6	-	-	-	69.7
C-5''	-	77.6	-	77.2	-	-	-	76.5
C-6''	-	60.5	-	61.4	-	-	-	60.8

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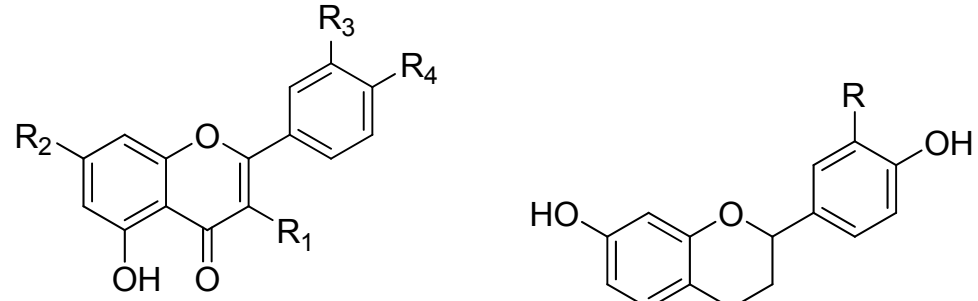
Di-hydroxy flavonoids in a B-ring (compounds **4** and **6**) showed more significant inhibitory activity against *α*-glucosidase than other mono-hydroxy flavonoids. The most interesting is that compound **6** containing di-hydroxyl groups B-rings and 3-OH in the skeleton exhibited the highest *α*-glucosidase inhibitory activity (IC_{50} values 2.04 μg/mL).

Quantitative analysis, LOD, and LOQ of flavonoids in CCF

The concentrations of compounds **1**–**8** in CCF were determined by HPLC/UV. The HPLC chromatograms of the mixture of eight pure compounds and EtOAc-soluble extract are shown in Figure 3. The calibration equations and retention times are shown in Table 3. The retention time of the expected peak for each of the compounds (**1–8**) purified from CCF was the same as that of the corresponding standard compounds. Kaempferol (**3**) and quercetin (**6**) were the major flavonols in CCF (Figure 3). The LOD and LOQ were 0.05-3.65 and 0.19-12.20 μg/mL determined at the signal-to-noise ratio S/N of 3 and 10, respectively, in CCF (Table 4). The HPLC profiles of the EtOAc fraction indicated that kaempferol (**3**) and quercetin (**6**) are the major components, and they play a crucial role in *α*-glucosidase inhibition.



1. R_1=H, R_2=OH, R_3=H, R_4=OH
2. R_1=H, R_2=-O-Gla, R_3=H, R_4=OH
3. R_1=OH, R_2=OH, R_3=H, R_4=OH
4. R_1=-O-Gla, R_2=OH, R_3=OH, R_4=OH
6. R_1=OH, R_2=OH,R_3=OH, R_4=OH
8. R_1=OH, R_2=-O-Gla, R_3=H, R_4=OH

5. R=H
7. R=OH

Figure 2: Structures of compounds **1**-**8** from CCF

Table 3: Linearity of standard curves and sensitivity of compounds **1**-**8** in CCF

Compounds	t_R	Calibration equation[a]	Correlation factor. r^{2b}	LOD (μg/mL)	LOQ (μg/mL)
1 apigenin	50.7	*Y* = 4476676.4 X – 1764378.6	0.9990	0.15 ± 0.25	0.19 ± 0.06
2 apigenin-7-O-glucoside	29.4	*Y* = 4676100.5 X – 528330.2	0.9994	0.11 ± 0.06	0.36 ± 0.14
3 kaempferol	51.3	*Y* = 5566328.6 X + 497849.6	0.9993	0.05 ± 0.10	0.19 ± 0.08
4 hyperin	22.3	*Y* = 5784705.0 X – 126647.1	0.9992	0.05 ± 0.09	0.18 ± 0.07
5 naringenin	53.3	*Y* = 23190444.8 X – 12597665.0	0.9997	0.35 ± 0.08	1.17 ± 0.13
6 quercetin	43.2	*Y* = 4117828.7 X – 3801255.7	0.9990	3.65 ± 0.22	12.20 ± 0.49
7 luteolin	41.7	*Y* = 4257791.7 X + 904356.9	0.9992	0.16 ± 0.09	0.55 ± 0.17
8 kaempferol-7-O-β-D-glucoside	28.3	*Y* = 1081875.9 X – 520795.9	0.9995	2.01 ± 0.37	15.38 ± 2.36

[a]: *Y* = peak area, X = concentration of standards (mg/ml); [b]: r^2 = correlation coefficient for three data points in the calibration curves (n = 5). Data are means ± S.D. (n = 5) in mg/ml dried sample.

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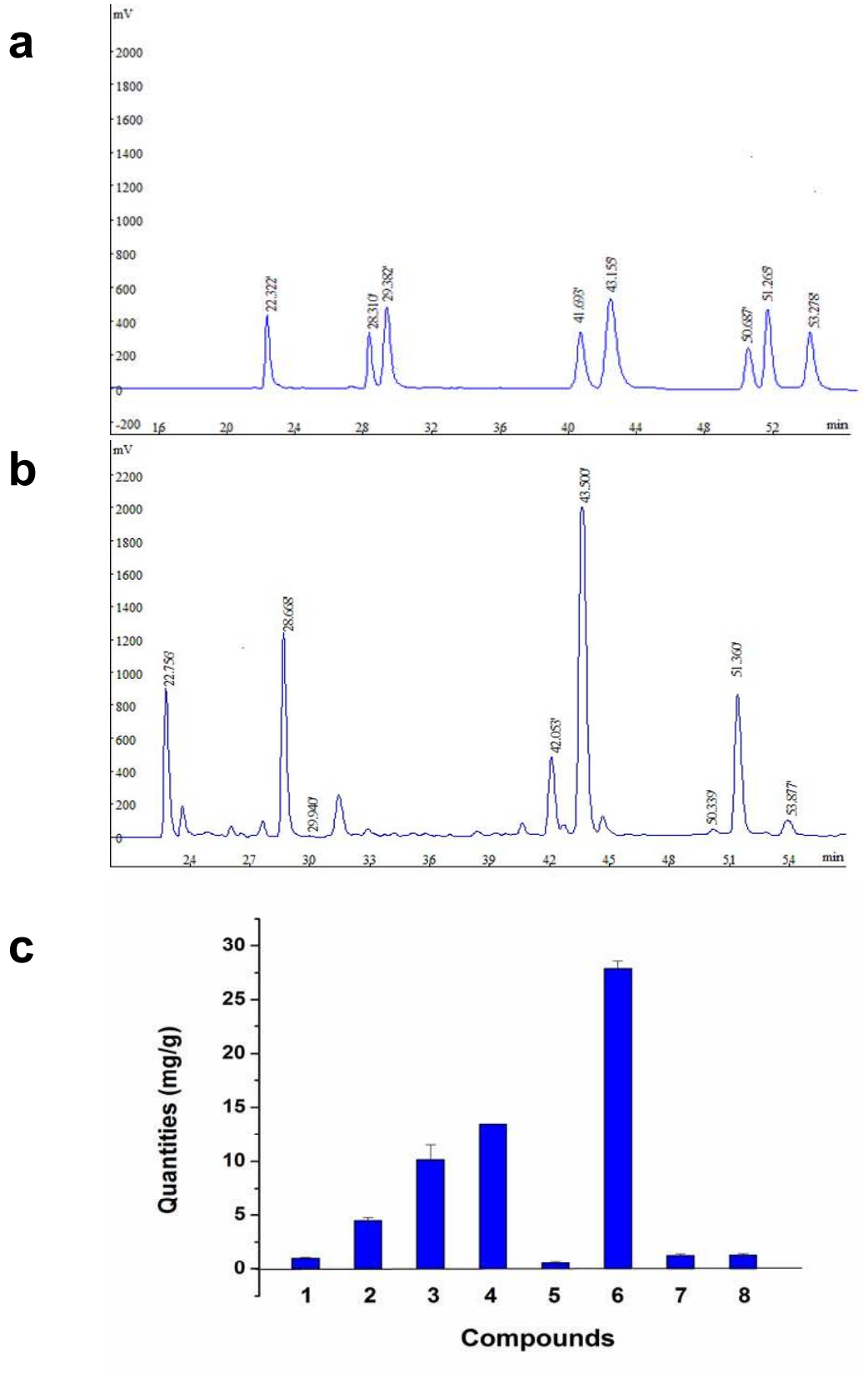

Figure 3: (a) HPLC chromatograms of the mixture of eight pure compounds. **(b)** HPLC chromatograms of EtOAc-soluble extract. **(c)** Quantities of compounds **1**-**8** in CCF

Table 4: Quantities of compounds **1**-**8** in CCF

Compounds	CCF (μg/g)
1 apigenin	1.01 ± 0.08
2 apigenin-7-O-glucoside	4.52 ± 0.28
3 kaempferol	10.15 ± 1.36
4 hyperin	13.43 ± 0.05
5 naringenin	0.54 ± 0.17
6 quercetin	27.87 ± 0.72
7 luteolin	1.26 ± 0.17
8 kaempferol-7-O-β-D-glucoside	1.30 ± 0.15

Values represent the means ± standard deviation (S.D.) of n = 5 duplicate assays.

CONCLUSIONS

This is the first study on the utilization of CCF as an anti-diabetic source. Our data suggest that CCF extract could be used as a viable alternative to pharmaceutical inhibitors for the inhibition of α-glucosidase activity, since CCF is well tolerated and relatively inexpensive. It is a good source of anti-diabetic agents, to which flavonols make an important contribution. The data also pointed out that CCF containing these flavonoids could be a useful natural source for the development of α-glucosidase inhibitory agent for dealing with diabetic complications. Further investigations will focus on clarifying the anti-diabetes activity mechanisms of the isolated compounds.

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REFERENCES

Agrawal PK (ed). Carbon-13 NMR of flavonoids (pp 150-8; 432-96). New York: Elsevier, 1989 (Studies in organic chemistry series, no. 39).

Agrawal PK, Bansal MC. Flavonoid glycosides. In: Agrawal PK (ed.): Carbon-13 NMR of flavonoids (pp 283-364). Elsevier: Amsterdam, 1989 (Studies in organic chemistry series, no. 39).

Andersen ØM, Markham KR. Flavonoids: Chemistry, biochemistry and applications (pp 38-142). London: CRC Press, 1989.

Baron AD. Postprandial hyperglycaemia and alpha-glucosidase inhibitors. Diabetes Res Clin Pr 1998;40:S51-5.

Bhandari MR, Nilubon JA, Gao H, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata* Haw.). Food Chem 2008;106:247-52.

Cheng W, Li J, You T, Hu C. Anti-inflammatory and immunomodulatory activities of the extracts from the inflorescence of *Chrysanthemum indicum* Linne. J Ethnopharmacol 2005;101:334-7.

Crow FW, Tomer KB, Looker JH, Gross ML. Fast atom bombardment and tandem mass spectrometry for structure determination of steroid and flavonoid glycosides. Anal Biochem 1986;155:286-307.

Fujisawa T, Ikegami H, Inoue K, Kawabata Y, Ogihara T. Effect of two α-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. Metabolism 2005;54:387-90.

Gorelick J, Kitron A, Pen S, Rosenzweig T, Madar Z. Anti-diabetic activity of *Chiliadenus iphionoides*. J Ethnopharmacol 2011;137:1245-9.

Gorzalczany S, Moscatelli V, Ferraro, G. Artemisia copa aqueous extract as vaso-relaxant and hypotensive agent. J Ethnopharmacol 2013;13:217-1.

Han N, Gu YH, Ye C, Cao Y, Liu ZH, Yin J. Antithrombotic activity of fraction and components obtained from raspberry leaves (*Rubus chingii*). Food Chem 2012;132: 181-5.

Harborne JB, Williams CA. Flavone and flavonol glycosides. In: Harborne JB: The flavonoids advances in research since 1986 (pp 337-87). London: Chapman and Hall, 1993.

Heacock PM, Hertzler SR, Williams JA, Wolf BW. Effects of a medical food containing an herbal α-glucosidase inhibitor on postprandial glycemia and insulinemia in healthy adults. J Am Diet Assoc 2005;105: 65-71.

Hui W, Du YJ, Song HC. α-Glucosidase and α-amylase inhibitory activities of guava leaves. Food Chem 2010;123:6-13.

Jeong SC, Kim MS, Jeong YT, Song CH. Hepatoprotective effect of water extract from *Chrysanthemum indicum* L. flower. Chinese Med 2013;8:7.

Jiang G, Lin S, Wen LR, Jiang YM, Zhao MM, Chen F. Identification of a novel phenolic compound in litchi (*Litchi chinensis Sonn.*) pericarp and bioactivity evaluation. Food Chem 2013;136:563-8.

Kar P, Laight D, Rooprai HK, Shaw KM. Cummings M. Effects of grape seed extract in type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. Diabetes Med 2009;26:526-31.

Kawanishi K, Ueda H, Moriyasu M. Aldose reductase inhibitors from the nature. Curr Med Chem 200310:1353-74.

Kim SD. α-Glucosidase inhibitor from Buthus martensi Karsch. Food Chem 2013; 136:297-300.

Kim YM, Wang MH. A novel alpha-glucosidase inhibitor from pine bark. Carbohyd Res 2004;339:715-7.

Kim SB, Kang OH, Kwon DY. Anti-inflammatory effects of tectroside on UVB-induced HaCaT cells. Int J Mol Med 2013;31: 1471-6.

Lebovitz HE. α-Glucosidase inhibitors. Endocrinol Metab Clin North Am 1997;26: 539-51.

Lee YM, Kim NH. Screening of inhibitory effect on aldose reductase of Korean herbal medicines and preventive effect of Catalpa bignonioides against xylose-induced lens opacity. Korean J Pharmacognosy 2008; 39:165-73.

Liu IM, Tzeng TF. Angelica acutiloba root alleviates advanced glycation end-product-mediated renal injury in streptozotocin-diabetic rats. J Food Sci 2011;76:H165-74.

Markham KR, Ternai B, Stanley R, Geiger H, Mabry TJ. Carbon-13 NMR studies of flavonoids-III naturally occurring flavonoid glycosides and their acylated derivatives. Tetrahedron 1978;34:1389-97.

Matsuda H, Morikawa T, Toguchida I, Harima S, Yoshikawa M. Medicinal flowers VI. Absolute stereostructures of two new flavanone glycosides and phenylbutanoid glycoside from the flowers of *Chrysanthemum indicum* L., there inhibitory activities for rat lens aldose reductase. Chem Pharm Bull 2002;50:972-5.

Matsui T, Yoshimoto C. In vitro survey of alpha-glucosidase inhibitory food components. Biosci Biotech Bioch 1996;60:2019-22.

Matsuura H, Asakawa C, Kurimoto M, Mizutani J. α-Glucosidase inhibitor from the seeds of balsam pear (Momordica charantia) and the fruit bodies of Grifola frondosa. Biosci Biotech Bioch 2002;66:1576-8.

Mok SY, Lee S. Identification of flavonoids and flavonoid rhamnosides from *Rhododendron mucronulatum* for. *albiflorum* and their inhibitory activities against aldose reductase. Food Chem 2013;136:969-74.

Montagut G, Blade C, Blay M, Fernandez-Larrea J, Ardevol A. Effects of a grapeseed procyanidin extract (GSPE) on insulin resistance. J Nutr Biochem 2010;21:961-7.

Neuser D, Benson A, Bruckner A, Goldberg RB, Hoogwerf BJ, Petzinna D. Safety and tolerability of acarbose in the treatment of type 1 and type 2 diabetes mellitus. Clin Drug Invest 2005;25:579-87.

Niamnuy C, Nachaisin M. Evaluation of bioactive compounds and bioactivities of soybean dried by different methods and conditions. Food Chem 2011;129:899-906.

Nishioka T, Kawabata J, Aoyama Y. Baicalein, α-glucosidase inhibitor from Scutellaria baicalensis. J Nat Prod 1998;61:1413-5.

Park SW, Kim SG, Kim MJ. Antioxidative activity and cytotoxicity on human KB cell of extracts from *Rhododendron mucronulatum Turcz*. flowers. Korean J Food Preserv 2006;13:501-5.

Wang H, Du YJ, Song HC. α-Glucosidase and α-amylase inhibitory activities of guava leaves. Food Chem 2010;123:6-13.

Wild S, Roglic G, King H. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.

Yan YC, Lou XE, Jiang HD. Experimental studies on the anti-oxidation effects of water extract from *Chrysanthemum indicum L*. Zhongguo xiandai yingyong yaoxue 1999;6: 16-8.

Yasukawa K, Sekine H, Takido M. Two flavonol glycosides from Lysimachia fortunei. Phytochemistry 1989;28:2215-6.

Yawadio R, Tanimori S, Morita N. Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chem 2007;101: 1616-25.

Yu Z, Yin Y. Characterization of ACE-inhibitory peptide associated with antioxidant and anticoagulation properties. J Food Science 2011a;76:C1149-55.

Yu Z, Yin Y. Novel peptides derived from egg white protein inhibiting alpha-glucosidase. Food Chem 2011b;129:1376-82.

Yu ZP, Yin YG, Zhao WZ, Yu YD, Liu BQ, Chen F. Novel peptides derived from egg white protein inhibiting alpha-glucosidase. Food Chem 2013;129:1376-82.

Zhu SY, Yang Y, Yu HD, Zou GL. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. J Ethnopharmacol 2005;96:151-8.