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α-Glucosidase inhibitory effect by the flower buds of *Tussilago farfara* L.

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Abstract

Methanolic extracts from the medicinal parts of 50 traditional Chinese herbs were tested in screening experiments for rat intestinal α-glucosidase. The methanolic extract from flower buds of *Tussilago farfara* L. (Compositae) showed the highest maltase inhibitory activity, with maltose as a substrate. Enzyme assay-guided fractionation of this extract afforded 3,4-dicaffeoylquinic acid (**1**), 3,5-dicaffeoylquinic acid (**2**), 4,5-dicaffeoylquinic acid (**3**) and rutin (**4**), and the structures of these compounds were elucidated on the basis of MS and NMR data analyses. Compounds **1**, **2** and **3** showed comparative maltase inhibitory activities, and the IC_{50} values were 0.91 mM, 0.90 mM and 0.89 mM, respectively. Comparison of the activities of **1**–**3**, chlorogenic acid (**5**), quinic acid (**6**) and caffeic acid (**7**) suggested that the number of caffeoyl groups attached to a quinic acid core were important for the potency. Rutin (**4**) showed moderate activity and inhibited 41% of maltase activity at a concentration of 1 mM. This is the first report on mammalian α-glucosidase inhibition of *T. farfara* and the isolation of **1**, **2** and **3** from this herb species. These results suggest a use of the extract of *T. farfara* for antidiabetes.
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Keywords: α-Glucosidase; Maltase inhibitor; Postprandial hyperglycemia; *Tussilago farfara*

1. Introduction

There is a marked increase in cardiovascular risk in individuals with postprandial hyperglycemia. Hence, there needs to be a new focus on suppression of postprandial hyperglycemia in treatment of, not only diabetic patients, but also individuals with impaired glucose tolerance. Various targets, as well as pharmacologic agents, have been identified to manage this risk factor (Carroll, Gutierrez, Castro, Tsewang, & Schade, 2003; Johnston et al., 1988; Rendell, 2004). Mammalian α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20), located in the brush-border surface membrane of intestinal cells, is the key enzyme which catalyzes the final step in the digestive process of carbohydrates. Hence, α-glucosidase inhibitors can retard the liberation of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and delay glucose absorption, reducing postprandial plasma glucose levels and suppressing postprandial hyperglycemia (Lebovitz, 1997). Consequently, α-glucosidase inhibitors, e.g. acarbose and miglitol, effectively compensate for defective early phase insulin release by inhibiting postprandial absorption of monosaccharides, and have been examined for clinical management of type 2 diabetes (Lembcke, Diederich, Folsch, & Creutzfeldt, 1990).

Many efforts have been made to search for effective and safe α-glucosidase inhibitors from natural materials in order to develop a physiological functional food or lead compounds for use in antidiabetes. In a series of our studies on rat intestinal α-glucosidase-inhibiting principles from plant sources, we previously reported that baicalein (5,6,7-trihydroxyflavone) from *Scutellaria baicalensis* and the related 6-hydroxyflavones from *Origanum majorana* were a new class of α-glucosidase inhibitors (Kawabata et al., 2003; Nishioka, Kawabata, & Aoyama, 1998). As

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a continued study, various mechanistic and structural development studies have been reported (Gao & Kawabata, 2004; Gao & Kawabata, 2005; Gao, Nishioka, Kawabata, & Kasai, 2004).

The flower bud of *Tussilago farfara* L. (Compositae), also known as Kuan-Dong-Hua in China, is a popular folk medicine and has been studied for its pharmacological activities, namely, antioxidative activity (Kim et al., 2006), antimicrobial activity (Kokoska, Polesny, Rada, Nepovim, & Vanek, 2002), and antagonistic activity on the NK_1 receptor (Yamamoto et al., 2002). In the course of our ongoing programme for identifying α-glucosidase-inhibiting principles from natural medicines in China, we found that the aqueous methanolic extract of flower buds of *T. farfara* showed the highest maltase inhibitory activity among tested herbs, while this extract did not show sucrase inhibitory activity, in which maltase and sucrase activities designate maltose- and sucrose-hydrolyzing activities in crude rat intestinal glucosidase complexes, respectively. Since this is the first observation on the inhibitory effect of *T. farfara* on α-glucosidase activity, this finding led us to the isolation and structural elucidation of active compounds from this plant species.

2. Materials and methods

2.1. Materials

Fifty species of traditional Chinese herbs were purchased from a local herbal market in Chengdu, People's Republic of China. All voucher specimens are deposited in the department of Public Health, Hua Xi Medical Center of Sichuan University. All chemicals used were of reagent grade and were purchased from Wako Pure Chem. Co. (Osaka, Japan) unless otherwise stated. All solvents were distilled before use.

2.2. General procedure

NMR spectra were recorded with a Bruker AMX500 instrument (^{1}H, 500 MHz). Electron spray ionization (ESI) and ESI-high resolution (HR) MS were obtained with a Jeol JMS-700TZ. Fast atom bombardment (FAB) and FAB-HR MS data were obtained on a Jeol JMS-SX102A instrument. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Column chromatography was performed with Diaion HP-20 (Mitsubishi Kasei Co. Ltd.).

2.3. Intestinal α-glucosidase inhibitory activity determination

The α-glucosidase inhibitory activity was measured as described previously (Gao & Kawabata, 2005). The crude enzyme solution, prepared from rat intestinal acetone powder (Sigma Aldrich Japan Co., Tokyo, Japan), was used as the source of small intestinal α-glucosidases, sucrase, maltase and isomaltase, showing specific activities of 0.38 U/ml, 1.85 U/ml, and 1.56 U/ml, respectively. The reaction mixture consisted of crude enzyme solution (as sucrase, 0.2 ml; as maltase, 0.05 ml; as isomaltase, 0.2 ml), substrate (sucrose: 56 mM, 0.2 ml; maltose: 3.5 mM, 0.35 ml; isomaltose: 7 mM, 0.2 ml, respectively) in 0.1 M potassium phosphate buffer (pH 6.3), and the test sample in 50% aqueous dimethyl sulfoxide (DMSO, 0.1 ml). After incubation for 15 min at 37 °C, the reaction was stopped by adding 0.75 ml of 2 M Tris HCl buffer (pH 7.0). The reaction mixture was passed through a short column of basic alumina (ICN Alumina B, grade I, ICN Biomedical GmbH, Eschwege, Germany) to remove phenolic compounds, which might interfere with glucose quantification. The amount of liberated glucose was measured by the glucose oxidase method, using a commercial test kit (Glucose B test Wako, Wako Pure Chem. Co., Osaka, Japan). The concentration of inhibitors required for inhibiting 50% of the α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

2.4. Porcine pancreatic α-amylase inhibitory activity determination

The inhibitory effect of the compounds on α-amylase activity was assessed by the usual method (Gao & Kawabata, 2005). Briefly, starch azure (Sigma; 2.0 mg), used as a substrate, was suspended in 50 mM Tri-HCl buffer (pH 6.9) containing 10 mM $CaCl_2$, and boiled for 5 min at 100 °C. Then, the starch azure solution was pre-incubated for 10 min at 37 °C. The test samples in 50% DMSO, and 0.2 ml of porcine pancreatic α-amylase solution (Sigma, A-6255; 2.0 U/ml; 50 mM Tri-HCl buffer containing 10 mM $CaCl_2$, pH 6.9) were added into each assay. The reaction was carried out at 37 °C for 10 min and stopped by adding 0.5 ml of 50% acetic acid. The reaction mixture was then centrifuged at 2000 rpm for 5 min at 4 °C. The absorbance of the supernatant at 595 nm was measured.

2.5. Screening experiment

The screening experiments for rat intestinal sucrase and maltase inhibition were carried out with 50% aqueous methanol extracts from fifty species of dried herbs, then the extracts were evaporated, re-dissolved in 50% aqueous DMSO and subjected, as the test sample, to the assay for rat intestinal α-glucosidase inhibitory activity, as described in the procedure of *2.3*.

2.6. Extraction and isolation of the inhibitors from T. farfara

The dried flower buds of *T. farfara* (40 g) were extracted three times with 70% methanol (1 l in total) for 24 h with continuous stirring at room temperature and filtered. Evaporation of the solvent under reduced pressure yielded the 70% methanol extract (5.94 g). This extract was suspended in water (100 ml), and re-extracted with hexane (150 ml × 3), and then with chloroform (150 ml × 3). Each

fraction was evaporated at reduced pressure to give hexane (0.62 g), chloroform (0.48 g) and water (4.84 g) fractions. The aqueous layer (4.84 g) was applied on a Diaion HP-20 column (4.2 × 60 cm) with a water–methanol gradient to give six fractions. The eluent volume and dry weight of each fraction were as follows: fraction 1, water (2 l, 3.68 g); fraction 2, 10% methanol in water (2 l, 0.28 g); fraction 3, 20% methanol in water (3 l, 0.20 g); fraction 4, 30% methanol in water (3 l, 0.35 g); fraction 5, 50% methanol in water (3 l, 0.32 g); fraction 6, methanol (2 l, 0.3 g).

The active fractions 3, 4 and 5 were combined and subjected to reversed-phase preparative HPLC, where the conditions were as follows: column, Inertsil ODS 20 × 250 mm i.d. with a particle size of 5 μm (GL-Science); mobile phases, solvent A (0.5% formic acid in ultrapure water) and solvent B (methanol), A:B = 1:1 in 60 min; flow rate, 4.5 ml/min; detection, UV 254 nm. The eluates, at 10 min intervals, were collected to get six fraction, and tested for maltase inhibitory activity. Fractions 3 (20–30 min) and 4 (30–40 min) showed a high inhibition for this enzyme. These fractions were further purified, separately. Fraction 3 (130 mg) was purified by preparative HPLC [column, Devolosil RPAQUEOUS 4.6 × 250 mm i. d. with a particle size of 5 μm (Nomura Chemical); mobile phase, water–methanol–formic acid (40:60:0.5); flow rate, 1 ml/min; detection, UV 254 nm] to yield 32.5 mg (0.0813%) of 3,4-dicaffeoylquinic acid (**1**, t_R 22.3 min) and 15.5 mg (0.0388%) of 3,5-dicaffeoylquinic acid (**2**, t_R 26.8 min). Fraction 4 (94 mg) was purified by preparative HPLC [column, Inertsil PREP-ODS-3, 4.6 × 250 mm i. d. with a particle size of 5 μm (GL-Science); mobile phase, water–methanol–formic acid (40:60:0.5); flow rate, 1 ml/min; detection, UV 254 nm] to yield 15 mg (0.0375%) of rutin (**4**, t_R 22.5 min) and 24 mg (0.06 %) of 4,5-dicaffeoylquinic acid (**3**, t_R 27.2 min).

3. Results

3.1. General

In the screening experiments for rat intestinal sucrase and maltase inhibitors in traditional Chinese herbal medicines, 50 materials were tested (Table 1). Since the methanol extract of *T. farfara* showed potent maltase inhibitory activity, further fractionation of this extract was carried out to isolate the active principles.

3.2. Extraction and isolation of active compounds 1–4

The dried flower buds of *T. farfara* were extracted with 70% methanol and further fractionation was carried out under a guidance of maltase inhibitory activity. At a concentration of 1 mg/ml, the aqueous methanolic extracts inhibited 51% of the maltase activity. The extract was evaporated, and the residue was successively extracted with hexane and chloroform. The highest inhibitory activity was observed in the residual water fraction (55% inhibition at 1 mg/ml), and this fraction was subjected to Diaion

Table 1
Inhibitory effects of fifty species of traditional Chinese herbal materials[a] on α-glucosidase[b]

Scientific name	Parts	Sucrase[c] (%)	Maltase[d] (%)
Lespedeza virgata DC.	Fruit	16	31
Rehmannia glutinosa Steud.	Stem	1	9
Pinellia ternata Ten.	Root	2	0
Phellodendron amurense Rupr.	Bark	19	17
Sophora flavescens Ait.	Stem	6	8
Coptis japonica Makino.	Stem	16	30
Scrophularia ningpoensis Hemsl.	Stem	0	28
Glycyrrhiza glabra L.	Root	0	18
Cnidum officinale Makino	Root	39	37
Corydalis turtschaninovii Bess.	Tuber	0	9
Curcuma longa L.	Stem	8	27
Curcuma zedoaria Rosc.	Stem	0	7
Sparganium stoloniferum Buch.-Ham.	Stem	8	24
Salvia miltiorhiza Bunge	Root	17	22
Leonurus heterophyllus Sweet	Leaf	0	4
Cinnamomum cassia Blume	Burk	0	2
Perilla frutescens Britton.	Leaf	2	12
Coelopleurum multisectum Kitag.	Leaf	12	27
Zingiber officinale Roscoe	Root	12	18
Notopterygium incisum Ting ex Ho-t.Chang	Root	11	37
Angelica dahurica Maxim.	Root	0	14
Xanthium strumarium L.	Pod	0	5
Asarum sieboldii Miq.	Leaf	0	8
Anemarrhena asphodeloide Bunge	Root	7	42
Phragmites communis Trin.	Root	0	5
Bupleurum marginatum Wall.	Root	5	13
Forsythia suspensa Vahl	Root	26	43
Arctium lappa L.	Root	0	16
Schizonepeta tenuifolia Briq.	Root	6	13
Mentha arvensis L.	Root	4	15
Isatis tinctoria L.	Root	1	22
Carthamus tinctorius L.	Root	35	33
Evodia rutaecarpa Benth.	Root	12	48
Panax notoginseng F. H. Chen.	Root	0	24
Prunus armeniaca Thunb.	Root	0	14
Amomum xanthioides Wall.	Root	1	8
Rheum palmatum L.	Root	7	0
Cocculus trilobus DC.	Root	0	5
Achyranthes bidentata L.	Root	0	3
Rhamnus zizyphus L.	Root	1	8
Brucea javanica Merr.	Root	0	2
Sterculia foetida L.	Fruit	0	7
Tribulus terrestris L.	Root	0	2
Aristolochia contorta Bunge	Root	0	0
Dichroa febrifuga Lour.	Barker	0	3
Magnolia quinquepeta (Buc'hoz) Dandy	Root	0	13
Tussilago farfara L.	Bud	16	68
Prunus mume Siebold & Zucc.	Fruit	22	1
Rosa rugosa Thunb.	Root	11	45
Dalbergia odorifera T.C.Chen	Root	5	17

[a] The herbal materials were purchased from a local herbal market in Chengdu, People's Republic of China. All voucher specimens are deposited in the Department of Public Health, Hua Xi Medical Center of Sichuan University.

[b] The crude enzyme solution prepared from rat intestinal acetone powder (Sigma Aldrich Japan Co., Tokyo, Japan), was used as the source of small intestinal α-glucosidases. Sucrase and maltase, showed specific activities of 0.38 U/ml and 1.85 U/ml, respectively.

[c] 56 mM sucrose as a substrate.

[d] 3.5 mM maltose as a substrate.

HP-20 column chromatography to yield six fractions. The active fractions 3-5 were combined and were divided into six sub-fractions (based on elution time) by preparative HPLC. Sub-fractions 3 (72% inhibition at 1 mg/ml) and 4 (58% inhibition at 1 mg/ml) were then further purified by preparative HPLC to yield 3,4-dicaffeoylquinic acid (**1**), 3,5-dicaffeoylquinic acid (**2**) from the former, and 4,5-dicaffeoylquinic acid (**3**) and rutin (**4**) from the latter, as active principles.

*3.3. Identification of the chemical structures of compounds **1–4***

3.3.1. General

ESI-MS of compounds **1–3** showed an $[M-H]^-$ signal at *m/z* 515, from which a molecular formula of $C_{25}H_{24}O_{12}$ was assigned. From the negative FAB-MS analysis, three fragment ions at *m/z* 353, 191 and 179 were detected. The ion at *m/z* 353 indicated a chlorogenic acid fragment derived from the loss of a caffeoyl group. The ions at *m/z* 191 and 179 indicated fragments of quinic and caffeic acid moieties, respectively. These MS data indicated the compounds **1–3** were dicaffeoylquinic acid isomers (Iwai, Kishimoto, Kakino, Mochida, & Fujita, 2004). In the ^{1}H NMR spectra, H-3, 4 and 5 resonated as follows: **1** (δ 5.63, 5.01 and 4.34 ppm), **2** (δ 5.42, 3.96 and 5.41 ppm) and **3** (δ 4.37, 5.11 and 5.63 ppm), respectively. From these data, H-3 and 4 in **1**, H-3 and 5 in **2**, and H-4 and 5 in **3** were shifted downfield from those of quinic acid, which indicated substitution of the hydroxyl groups of quinic acid, and the NMR data were similar to those in the literature (Basnet, Katsumichi, Hase, Kadota, & Namba, 1996; Ichikawa, Sakurai, Akiyama, & Yoshioka, 1991; Iwai et al., 2004; Zhu, Zhang, & Lo, 2004). Compounds **1**, **2** and **3** were thus identified as 3,4-dicaffeoyl-, 3,5-dicaffeoyl- and 4,5-dicaffeoyl-quinic acids, respectively.

Compound **4** had a $[M-H]^-$ ion at *m/z* 609 in ESI-MS and FAB-MS analysis showed an ion at *m/z* 301, corresponding to the fragment of quercetin. The presence of the [M−H-146] fragment, but not the [M−H-162] fragment indicated **4** to be quercetin rutinoside. From these MS data and comparison of the NMR data with those in the literature, compound **4** was identified as rutin (Sang et al., 2001). The structures of compounds **1–4** are shown in Fig. 1.

*3.3.2. 3,4-Dicaffeoylquinic acid (**1**)*

Off-white powder, $[\alpha]_D^{23} - 199°$ (MeOH; *c* 0.2); ESI-HRMS (negative), *m/z* 515.1199 $[M-H]^-$ calcd. for $C_{25}H_{23}O_{12}$, 515.1190; ^{1}H NMR δ (methanol-d_4) ppm (*J* in Hz): 2.12–2.19 (4H, m, 2 and 6-H), 4.34 (1H, dt, $J = 8.4$ (t), 3.4, 5-H), 5.01 (1H, dd, $J = 8.4$, 3.2, 4-H), 5.63 (1H, m, 3-H), 6.26 (2H, dd, $J = 16.0$, 6.7, 8′ and 8″)-H, 6.73 and 6.76 (each 1H, d, $J = 8.1$, 5′ and 5″-H), 6.88 and 6.91 (each 1H, dd, $J = 8.1$, 2.1, 6′ and 6″-H), 7.02 (2H, d, $J = 2.2$, 2′ and 2″-H), 7.53 and 7.54 (each 1H, d, $J = 16.0$, 7′ and 7″-H).

*3.3.3. 3,5-Dicaffeoylquinic acid (**2**)*

Off-white powder, $[\alpha]_D^{23} - 207°$ (MeOH; *c* 0.2); ESI-HRMS (negative), *m/z* 515.1198 $[M-H]^-$ calcd for $C_{25}H_{23}O_{12}$, 515.1190; ^{1}H NMR δ (methanol-d_4) ppm (*J* in Hz): 2.13–2.22 (4H, m, 2 and 6-H), 3.96 (1H, dd, $J = 7.6$, 3.2, 4-H), 5.41 (1H, m, 5-H), 5.42 (1H, m, 3-H), 6.26 and 6.35 (each 1H, d, $J = 16.0$, 8′ and 8″-H), 6.77 and 6.88 (each 1H, d, $J = 8.1$, 5′ and 5″-H), 6.95 and 6.96 (each 1H, brd, $J = 8.1$, 6′ and 6″-H), 7.05 and 7.06 (each 1H, d, $J = 2.2$, 2′ and 2″-H), 7.57 and 7.61 (each 1H, d, $J = 16.0$, 7′ and 7″-H).

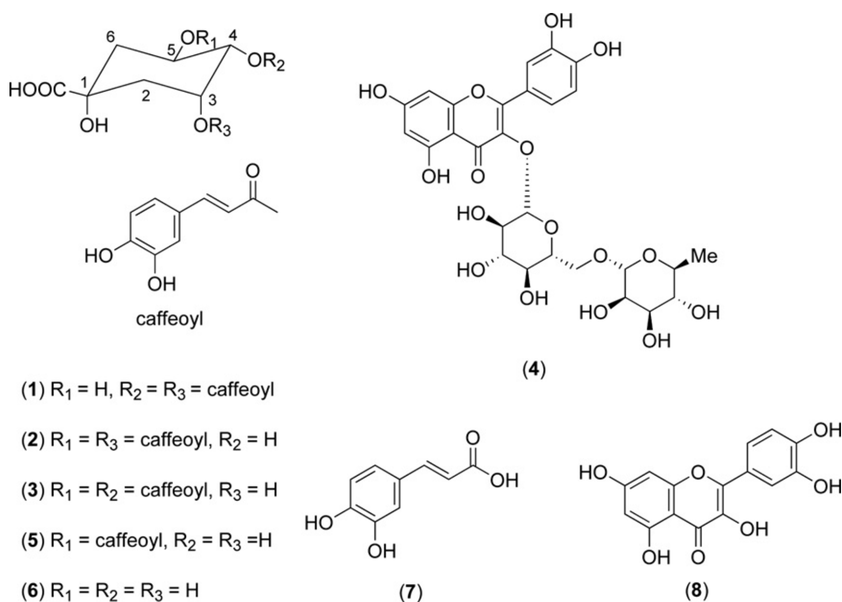

Fig. 1. Chemical structures of 3,4-dicaffeoylquinic acid (**1**), 3,5-dicaffeoylquinic acid (**2**), 4,5-dicaffeoylquinic acid (**3**), rutin (**4**), chlorogenic acid (**5**), quinic acid (**6**), caffeic acid (**7**), and quercetin (**8**).

*3.3.4. 4,5-Dicaffeoylquinic acid (**3**)*

Off-white powder, $[\alpha]_D^{23} -216°$ (MeOH; *c* 0.2); ESI-HRMS (negative), *m/z* 515.1181 $[M-H]^-$ calcd for $C_{25}H_{23}O_{12}$, 515.1190; ^1H NMR δ (methanol-d_4) ppm (*J* in Hz): 2.12-2.28 (4H, m, 2 and 6-H), 4.37 (1H, m, 3-H), 5.11 (1H, brd, $J = 8.1$, 4-H), 5.63 (1H, m, 5-H), 6.19 and 6.29 (each 1H, d, $J = 16.0$, 8′ and 8″-H), 6.72 and 6.75 (each 1H, d, $J = 8.1$, 5′ and 5″-H), 6.88 and 6.91 (each 1H, dd, $J = 8.1$, 2.1, 6′ and 6″-H), 7.02 (2H, d, $J = 2.2$, 2′ and 2″-H), 7.51 and 7.54 (each 1H, d, $J = 16.0$, 7′ and 7″-H).

*3.3.5. Rutin (**4**)*

Yellow powder; FAB-HRMS (negative), *m/z* 609.1483 $[M-H]^-$ calcd for $C_{27}H_{29}O_{16}$, 609.1455; ^1H NMR δ (methanol-d_4) ppm (*J* in Hz): 1.11 (3H, d, $J = 6.2$), 3.24–3.81 (10H, m), 4.51 (1H, s), 5.10 (1H, d, $J = 7.9$), 6.20 (1H, d, $J = 2.0$), 6.38 (1H, d, $J = 2.0$), 6.85 (1H, d, $J = 8.7$), 7.63 (1H, dd, $J = 8.7$, 2.0), 7.66 (1H, d, $J = 2.0$).

3.4. Enzyme inhibitory activity

In the screening experiment, sucrase inhibitory activity of the methanolic extracts of the selected traditional Chinese herbs ranged from 0% to 39%, and maltase activity of these extracts ranged from 0% to 40%, except *T. farfara*, and the methanolic extract of the flower buds of *T. farfara* showed the highest maltase inhibitory activity (68% inhibition) among the tested herbs (Table 1).

Compounds **1**–**3** inhibited maltase activity in a dose-dependent manner (Fig. 2). The 50% inhibitory concentration values (IC_{50}) for compounds **1**–**3** were determined to be 0.91 mM, 0.9 mM and 0.89 mM, respectively (Table 2). Rutin (**4**) showed 41% maltase inhibition at a concentration of 1 mM (Table 2).

α-Glucosidase and α-amylase inhibitory activities of compounds **1**–**3** are shown in Fig. 3. At a concentration of 1 mM, the maltase inhibitory activities of compounds **1**–**3** were 65%, 64% and 62%, respectively. However, these compounds showed weak inhibitory activity against sucrase, isomaltase and porcine pancreatic α-amylase, ranging from 13% to 20%.

4. Discussion and conclusion

The mehanolic extract from the flower buds of *T. farfara* showed the highest maltase inhibitory activity in the screening experiments of fifty species of selected traditional Chinese herbs. This is the first report in this herb species of a maltase inhibitory effect, although *T. farfara* has long been used in China for the treatment of bronchitis and asthma in traditional natural medicine (Namba, 1980). An enzyme assay-guided fractionation of the methanolic extract, using column chromatography, followed by preparative HPLC, led to the isolation of 3,4-dicaffeoylquinic acid (**1**), 3,5-dicaffeoylquinic acid (**2**), 4,5-dicaffeoylquinic acid (**3**) and rutin (**4**). Although some pure compounds,

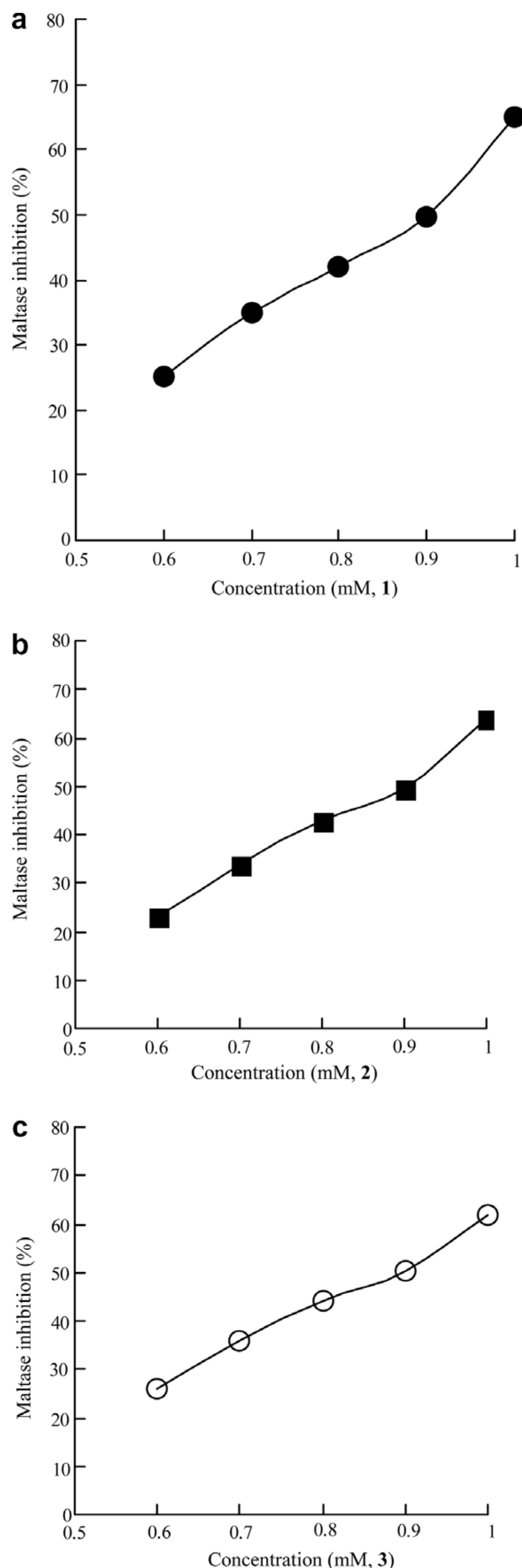

Fig. 2. Dose-dependent inhibition of maltase by compounds **1**, **2** and **3**. IC_{50} values for **1**–**3** were calculated from the dose-dependent inhibition curves (a, b and c, respectively).

Table 2
Inhibitory effects of the extract, isolates from the flower buds of *Tussilago farfara* L. and related compounds on the α-glucosidase[a] inhibition

Compounds	IC_{50} value (mM)
70% Methanol extract	1 (mg/ml)
3,4-Dicaffeoylquinic acid (**1**)	0.91
3,5-Dicaffeoylquinic acid (**2**)	0.90
4,5-Dicaffeoylquinic acid (**3**)	0.89
Rutin (**4**)	41%[b]
Chlorogenic acid (**5**)	NI[c]
Quinic acid (**6**)	NI
Caffeic acid (**7**)	NI
Quercetin (**8**)	NI
1,2,3,4,6-Penta-*O*-galloyl-β-D-glucopyranose (standard)	0.14

[a] 3.5 mM Maltose as a substrate and the specific activity is 1.85 U/ml in this assay.
[b] 41% Inhibition at a concentration of 1 mM.
[c] Less than 20% inhibition at a concentration of 1 mM.

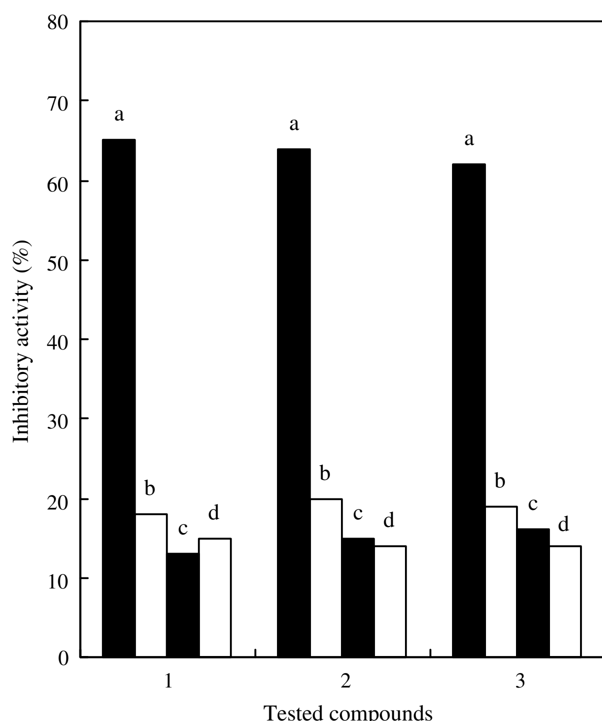

Fig. 3. Selectivity of 3,4-dicaffeoylquinic acid (**1**), 3,5-dicaffeoylquinic acid (**2**) and 4,5-dicaffeoylquinic acid (**3**) for rat intestinal α-glucosidase and porcine pancreatic α-amylase inhibition at a concentration of 1 mM. a, Maltase activity; b, Sucrase activity; c, Isomaltase activity; d, Porcine pancreatic α-amylase activity.

such as alkaloids (Roeder, Wiedenfeld, & Jost, 1981), phenolics (Kikuchi & Mori, 1992) and mucopolysaccharides (Franz, 1969) have been isolated from the flower buds of *T. farfara*, the isolation of **1**, **2** and **3** has not been previously reported.

Compound **1** has been isolated from many species and its biological activities, including antiviral (Ooi, Wang, He, & Ooi, 2006) and antioxidative (Iwai et al., 2004) activities have been reported. However, **1** has never been tested for intestinal α-glucosidase inhibitory activity. This compound inhibited maltase activity with the IC_{50} value of 0.91 mM. On the other hand, **1** had no inhibitory effects ($IC_{50} > 1$ mM) on sucrase, isomaltase or α-amylase. Hence, this result indicated that **1** was a selective maltase inhibitor.

Compounds **2** and **3** isolated from Brazilian propolis, have been previously reported as maltase inhibitors (Matsui, Ebuchi, Fujise, Abesundara, & Doi, 2004). Our finding is the first report on the isolation of **2** and **3** from the flower buds of *T. farfara*. In the present study, the IC_{50} values of **2** and **3** against intestinal maltase were 0.90 mM and 0.89 mM, respectively. On the other hand, **2** and **3** did not show inhibitory effects on intestinal sucrase, isomaltase or α-amylase. From these results, it is revealed that compounds **2** and **3**, like **1**, are selective maltase inhibitors.

At a concentration of 1 mM, chlorogenic acid (**5**), quinic acid (**6**) and caffeic acid (**7**) inhibited maltase activity by less than 10%, whereas compounds **1**, **2** and **3** inhibited 65%, 64% and 62% of maltase activity, respectively. Comparison of the structures of these compounds in relation to their activities reveals that the number of caffeoyl group attached to a quinic acid core are important for the potency as has been pointed out by Mstsui, Ogunwande, Abesundara, and Matsumoto (2006).

Rutin (**4**) is a well-known flavonol glycoside that was previously isolated from the flower buds of *T. farfara* (Kikuchi & Mori, 1992). It is interesting to note that **4** showed moderate maltase inhibitory activity and inhibited 41% of maltase activity at concentrations as high as 1 mM, whereas the aglycone, quercetin (**8**) showed only a weak inhibitory activity (less than 20% at a concentration of 1 mM) on this enzyme. So, it is suggested that the substitution of rutinoside in **4** could affect this activity.

From the results presented here, compounds **1**–**4** did not have activities comparable to the 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose, which is a known maltase inhibitor. However, the observed biological activities, in general, are in line with the traditional uses of this herb. The previous study suggested that 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose inhibited maltase in response to the numbers of the galloyl group attached to a glucose core (Toda, Kawabata, & Kasai, 2001). So, it is interesting to design and synthesize some compounds with the caffeoyl group attached to a glucose core and evaluate their α-glucosidase inhibition.

In conclusion, the enzyme-guided fractionation of the extract from the flower buds of *T. farfara* led to the isolation of 3,4-dicaffeoylquinic acid (**1**), 3,5-dicaffeoylquinic acid (**2**), 4,5-dicaffeoylquinic acid (**3**) and rutin (**4**) as intestinal maltase inhibitors. This result suggests that *T. farfara* can be physiologically useful for suppressing postprandial hyperglycemia by ingesting it or its extract in the diet.

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