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NOTE

Antioxidant constituents in the dayflower (*Commelina communis* L.) and their α-glucosidase-inhibitory activity

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Abstract The dayflower, *Commelina communis* L., contains 1-deoxynojirimycin (DNJ) and (2*R*,3*R*,4*R*,5*R*)2,5-bis(hydroxymethyl)-3,4-dihydroxypyrrolidine (DMDP), potent α-glucosidase inhibitors. The extracts and powder of this herb are important food materials for prophylaxis against type 2 diabetes. Eleven flavonoid glycosides as antioxidants, isoquercitrin, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-β-D-glucoside, glucoluteolin, chrysoriol-7-*O*-β-D-glucoside, orientin, vitexin, isoorientin, isovitexin, swertisin, and flavocommelin, were identified from the aerial parts of *C. communis*. Their antioxidant activities were measured using in vitro assays employing the 1,1-diphenyl-2-picrylhydrazyl radical- and superoxide radical-scavenging assays. The results showed that glucoluteolin, orientin, isoorientin, and isoquercitrin are the predominant antioxidants in this herb. Moreover, isoquercitrin, isorhamnetine-3-*O*-rutinoside, vitexin, and swertisin inhibited the activity of α-glucosidase from rat intestine.

Keywords Dayflower, *Commelina communis* · *Commelina communis* var. *hortensis* · Antioxidant · Flavonoid glycoside · α-glucosidase inhibitor

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Introduction

Postprandial hyperglycemia is one of the most important health issues in the 21st century, because it can develop into type 2 diabetes, hypertension, and cardiovascular disease [1]. The International Diabetes Federation has estimated that approximately 500 million people worldwide experience some degree of dysglycemia [2]. This uptake of excess sugar causes an imbalance between oxidants and antioxidants in the human body. Several management strategies have been proposed for the early stage of dysglycemia with the aim of preventing further development. A key strategy is "lifestyle modification," involving changes in diet and exercise, which was shown to reduce the incidence of type 2 diabetes by 58% [3].

It is widely accepted that dietary supplements can contribute significantly to health. In particular, in postprandial hyperglycemia, herbs with α-glucosidase-inhibitory activity will become more important as food material. The dayflower, *Commelina communis* L., is distributed widely throughout the world. The whole plants have been used as a febrifuge or a diuretic in Japanese folk medicine. This herb contains 1-deoxynojirimycin (DNJ) and (2*R*,3*R*,4*R*,5*R*)2,5-bis (hydroxymethyl)-3,4-dihydroxypyrrolidine (DMDP), which are potent α-glucosidase inhibitors (Fig. 1), but no investigation of their antioxidant activity has been reported [4–6]. However, major phytochemicals, phenolic acid, flavonoids, coumarin derivatives, etc., are known to combat oxidative stress in the human body by helping to maintain a balance between oxidants and antioxidants. Moreover, the combination of α-glucosidase inhibitors and antioxidants will become more effective for the prophylaxis of type 2 diabetes with the use of dietary supplements.

In this paper, we report the isolation of 11 flavonoid glycosides, isoquercitrin, isorhamnetin-3-*O*-rutinoside,

isorhamnetin-3-*O*-*β*-D-glucoside, glucoluteolin, chrysoriol-7-*O*-*β*-D-glucoside, orientin, vitexin, isoorientin, isovitexin, swertisin, and flavocommelin (Fig. 2), as antioxidants from the aerial parts of *C. communis* and *C. communis* var. *hortensis* [7]. These compounds were also assayed to determine whether they inhibit the activity of α-glucosidase from rat intestine.

Materials and methods

General experimental procedures

The instruments used in this study were Shimadzu LC-10AT, SPD-10A, and SCL-10A LC instruments (for preparative HPLC), Varian Mercury 300 and Unity Inova-500 NMR spectrometers (for NMR spectra measured in CD_3OD or pyridine-d_5 using tetramethylsilane as an internal standard), JEOL JMS-MS700V mass spectrometer (for mass spectra), and Shimadzu UV mini 1240 and BIO-RAD Model 680XR plate readers (for radical-scavenging assays).

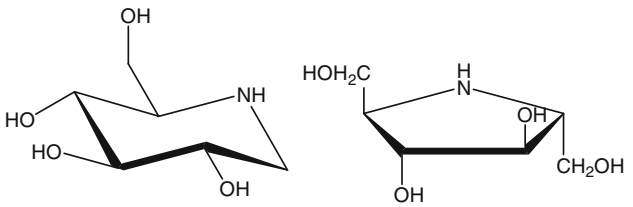


Fig. 1 Chemical structures of DNJ and DMDP

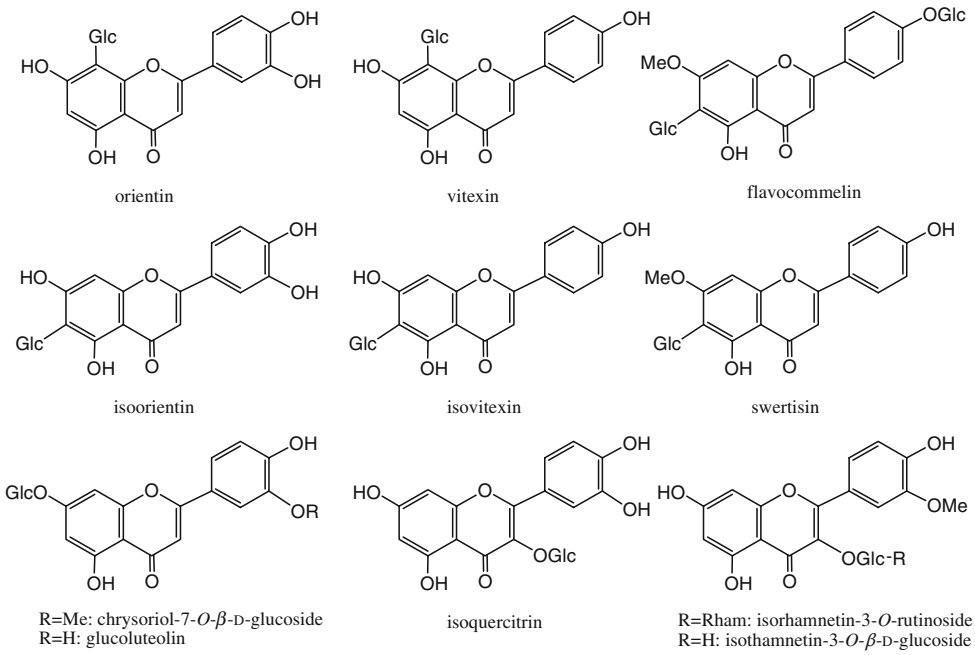
Reagents and materials

HPLC-grade methanol and acetonitrile (for preparative HPLC), reagent-grade dichloromethane, methanol, ethanol, and ethyl acetate (for extraction and column chromatography), 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium acetate, acetic acid, and epigallocatechin gallate were purchased from Nacalai Tesque Co., Ltd. (Kyoto, Japan). Wakogel C-200 (for column chromatography) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Silica gel $60F_{254}$-precoated TLC plates were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared using a Millipore Milli-Q purification system (Bedford, MA). *C. communis* L. was collected in Takatsuki ward (Osaka, Japan). *C. communis* var. *hortensis* was cultivated in Kusatsu (Shiga, Japan).

Isolation of antioxidants

After drying, the aerial parts (400 g) of the dayflowers were refluxed three times with 70% EtOH (3 L) for 3 h. The solution was concentrated under reduced pressure to give an extract (75.5 g). The extract was chromatographed on a Diaion HP-20 column (Mitsubishi Chemical Co., Ltd., Tokyo, Japan). After washing the column with water, the adsorbed material was eluted with MeOH (2 l). The eluted fraction was concentrated in vacuo to give a flavonoid fraction (51.7 g). This fraction was chromatographed on a silica gel (400 g) column (5.3 i.d. × 56 cm)

Fig. 2 Chemical structures of flavonoid glycosides



with CH_2Cl_2–MeOH. Each fraction was subjected to HPLC separation [Develosil ODS-5 10 i.d. × 250 mm (Nomura Chemical Co., Ltd., Seto, Japan) or Nucleosil C-18 AB 10 i.d. × 250 mm (Macherey-Nagel, Duren, Germany), CH_3CN-1% AcOH (12:88 or 15:85)]. Fractions 19–27 yielded isoquercitrin (22 mg), isorhamnetin-3-*O*-rutinoside (64 mg), isorhamnetin-3-*O*-*β*-D-glucoside (13 mg), glucoluteolin (461 mg), chrysoriol-7-*O*-*β*-D-glucoside (32 mg), orientin (178 mg), vitexin (69 mg), isoorientin (22 mg), isovitexin (16 mg), swertisin (11 mg), and flavocommelin (20 mg).

Determination of free radical-scavenging activity

The effects of each fraction or flavonoid glycoside on DPPH radicals were monitored according to the method of Hatano et al. [8]. The sample was dissolved in 50% ethanol (concentration of 0.015–3 mg/ml). One milliliter of 0.1 M acetate buffer (pH 5.5), 0.5 ml of 0.5 mM DPPH radical ethanolic solution, and 1 ml of each of the prepared 50% ethanol solutions were mixed. The mixture was shaken vigorously and incubated at 30°C for 30 min in the dark, and the absorbance was measured spectrophotometrically at 517 nm. The solution without DPPH solution added served as a blank. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The percentage of DPPH-scavenging activity was expressed as {1-[(test sample absorbance − blank sample absorbance)/blank sample absorbance]} × 100 (%). The purification of antioxidative compounds was done based on the antioxidative activity exhibited.

Determination of superoxide radical-scavenging activity

Various concentrations (0.05–5 mg/ml) of 20% ethanol solution of the flavonoid glycosides were prepared. The superoxide anion-scavenging activity was determined using a xanthine/xanthine oxidase reduced WST-1 system with a SOD assay kit (Dojindo Molecular Technologies, Inc., Tokyo, Japan) according to the manufacturer's protocol. The water-soluble formazan dye was monitored at 450 nm. Measurements were performed in duplicate, and the concentration required for a 50% inhibition (IC_{50}) of WST-1 formazan formation was determined graphically.

Assay of α-glucosidase inhibition

The α-glucosidase-inhibitory activity was measured using the modified method of Dahlqvist [9]. The reaction mixture consisted of the above basic extract solution (25 μl), 200 μl 50 mM phosphate buffer (pH 7.0), 175 μl 100 mM sucrose in 10 mM phosphate buffer (pH 7.0), and 100 μl α-glucosidase from rat intestine (Sigma-Aldrich Co., St. Louis, MO) solution (a stock solution of 1.0 mg/ml in 10 mM phosphate buffer, pH 7.0, was diluted 40-fold with the same buffer). The reaction mixture was incubated for 30 min at 37°C. Then, 500 μl of an aqueous solution containing 1% 3,5-dinitrosalicylic acid, 5% sodium potassium tartate, 1% NaOH, 0.2% phenol, and 0.05% sodium sulfite was added to the incubated solution, and the mixture was heated at 100°C for 10 min to stop the reaction. This solution was diluted with 2 ml of water, and the optical density at 540 nm was measured (OD test). The control sample was prepared by adding water instead of the extract and by treating in the same way as test samples to give an OD blank. The inhibition rates (%) were calculated using the formula 100 − 100 × (OD test − OD blank)/(control OD test − control OD blank).

Results and discussion

In this study, preliminary experiments were carried out using dried dayflower powder (5 g). The hot-water extracts (0.97 g) were chromatographed on Sep-Pak C18 cartridges (Waters, Milford, MA) using H_2O, H_2O–MeOH (1:1), and MeOH. The yield and antioxidant activities of each fraction are shown in Table 1. Thin-layer chromatography (TLC) of the H_2O–MeOH (1:1) fraction showed numerous spots under 254-nm UV irradiation. These results suggested that the antioxidant components of the hot-water extracts are flavonoid glycosides. In addition, the inhibitory activities of these fractions were assayed for their ability to

Table 1 Preliminary experiments on dried dayflower powder (5 g)

Fraction	Yield (g)	DPPH radical-scavenging capacity (%)[a]	α-glucosidase-inhibitory activity (%)[b]
Hot-water extracts (sep-pak C18 cartridge)	0.97	98.6	85.7
H_2O	0.53	75.2	95.0
H_2O–MeOH (1:1)	0.21	95.3	75.7
MeOH	0.09	7.6	25.8

[a] Sample concentration is 0.4 mg/ml

[b] Sample concentration is 6.0 mg/ml

Table 2 DPPH radical-scavenging effects of flavonoid glycosides and epigallocatechin gallate

Flavonoid glycoside	EC_{50}	
	(μg/ml)	(μM)
Isoquercitrin	6.2	13.4
Isorhamnetin-3-*O*-rutinoside	150>	–
Isorhamnetin-3-*O*-*β*-D-glucoside	150>	–
Glucoluteolin	5.8	12.9
Chrysoriol-7-*O*-*β*-D-glucoside	150>	–
Orientin	6.7	15.0
Vitexin	18.7	43.3
Isoorientin	6.9	15.4
Isovitexin	18.9	43.8
Swertisin	94.5	211.7
Flavocommelin	150>	–
Epigallocatechin gallate	1.2	2.7

Table 3 Superoxide radical-scavenging activity of flavonoid glycosides and epigallocatechin gallate

Flavonoid glycoside	IC_{50}	
	(μg/ml)	(μM)
Isoquercitrin	25.0	53.9
Isorhamnetin-3-*O* -rutinoside	385>	–
Isorhamnetin-3-*O*-*β*-D-glucoside	385>	–
Glucoluteolin	18.3	40.8
Chrysoriol-7-*O*-*β*-D-glucoside	385>	–
Orientin	32.6	72.8
Vitexin	385>	–
Isoorientin	39.7	88.6
Isovitexin	385>	–
Swertisin	385>	–
Flavocommelin	385>	–
Epigallocatechin gallate	3.9	8.5

inhibit the activity of α-glucosidase from rat intestine. The H_2O and H_2O–MeOH (1:1) fractions potently inhibited α-glucosidase activity. The active constituents of the H_2O fraction are DNJ and DMDP, but these polyhydroxylated alkaloids are not present in the H_2O–MeOH (1:1) fraction. Therefore, we investigated the active compounds of this fraction.

The 70% EtOH extract of *C. communis* was chromatographed on a Diaion HP-20 column. After washing the column with water, the adsorbed material was eluted with MeOH. The eluted fraction was chromatographed on silica gel using CH_2Cl_2 and MeOH to afford 32 fractions. The free radical-scavenging activity of each fraction was assayed against DPPH radicals. Fractions 19–27 exhibited stronger antioxidant activity than the others. These active fractions were further subjected to reversed-phase preparative HPLC to afford 11 flavonoid glycosides. Thus, bioassay-guided fractionation of the 70% EtOH extracts led to the isolation of the antioxidants isoquercitrin [10], isorhamnetin-3-*O*-rutinoside [10], isorhamnetin-3-*O*-*β*-D-glucoside [11], glucoluteolin [12], chrysoriol-7-*O*-*β*-D-glucoside [12], orientin [13], vitexin [14], isoorientin [13], isovitexin [15], swertisin [16], and flavocommelin [17]. These known compounds were identified by comparison of their spectral (^{1}H-NMR, ^{13}C-NMR, and MS) data with the reported values. These compounds were isolated from this herb for the first time, except for flavocommelin, which comprises the blue pigment of the dayflower. Moreover, these 11 flavonoid glycosides were also isolated from *C. communis* var. *hortensis* using the same methods.

Table 2 shows the DPPH radical-scavenging activities of the isolated flavonoid glycosides. Isoquercitrin, glucoluteolin, orientin, vitexin, isoorientin, isovitexin, and swertisin had EC_{50} values of 13.4, 12.9, 15.0, 43.3, 15.4, 43.8, and 211.7 μM, respectively, and the activity of epigallocatechin gallate was 2.7 μM. Table 3 shows the superoxide radical-scavenging activities of the isolated compounds. Glucoluteolin had the strongest activity, and isoquercitrin, orientin, and isoorientin were also relatively potent. Comparing these four flavonoid glucosides, it appears that the phenolic hydroxyl groups at the 3- and 4-positions of the flavonoid B ring play a key role in radical scavenging, although the hydroxyl group at the 7-position of the flavonoid A ring appears to have no effect. In principle, the structure–radical-scavenging relationships of these flavonoid glycosides were in agreement with data reported in the literature [18].

Table 4 α-glucosidase-inhibitory activity of flavonoid glycosides, DNJ, and DMDP

Flavonoid glycoside	IC_{50} (M)
Isoquercitrin	2.4×10^{-4}
Isorhamnetin-3-*O*-rutinoside	5.1×10^{-4}
Isorhamnetin-3-*O*-*β*-D-glucoside	$>1.0 \times 10^{-3}$
Glucoluteolin	$>1.0 \times 10^{-3}$
Chrysoriol-7-*O*-*β*-D-glucoside	$>1.0 \times 10^{-3}$
Orientin	$>1.0 \times 10^{-3}$
Vitexin	4.2×10^{-4}
Isoorientin	$>1.0 \times 10^{-3}$
Isovitexin	$>1.0 \times 10^{-3}$
Swertisin	3.7×10^{-4}
Flavocommelin	$>1.0 \times 10^{-3}$
DNJ	1.5×10^{-4}
DMDP	5.8×10^{-5}

These isolated compounds, DMDP, and DNJ were assayed to determine whether they inhibit the activity of

α-glucosidase from rat intestine (Table 4). Isoquercitrin, isorhamnetine-3-*O*-rutinoside, vitexin, and swertisin inhibited α-glucosidase from rat intestine to the same extent as DNJ, but the other constituents did not inhibit the activity of this enzyme.

We propose that these herbs (*C. communis* and *C. communis* var. *hortensis*) may be a useful food material for type 2 diabetes patients and for prophylaxis against dysglycemia.

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References

1. Hanefeld M (2007) Cardiovascular benefits and safety profile of acarbose therapy in prediabetes and established type 2 diabetes. Cardiovasc Diabetol 6:20
2. International Diabetes Federation. Diabetes atlas. [http://www.eatlas.idf.org]
3. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344:1343–1359
4. Kim HS, Kim YH, Hong YS, Peak NS, Lee HS, Kim TH, Kim KW, Lee JJ (1999) α-Glucosidase inhibitors from *Commelina communis*. Planta Med 65:437–439
5. Shibano M, Tsukamoto D, Tanaka Y, Masuda A, Orihara S, Yasuda M, Kusano G (2001) Determination of 1-deoxynojirimycin and 2,5-dihydroxymethyl 3,4-dihydroxypyrrolidine contents of *Commelina communis* var. *hortensis* and the antihyperglycemic activity. Nat Med 55:251–254
6. Shibano M, Fujimoto Y, Kushino K, Kusano G, Baba K (2004) Biosynthesis of 1-deoxynojirimycin in *Commelina communis*: a difference between the microorganisms and plants. Phytochemistry 65:2661–2665
7. This herb is cultivated in Kusatsu City (Shiga, Japan) to use the flower alone as a blue dye, with which rough sketches for *yuzen* (Japanese traditional dyeing) are drawn. The herb grows 3–5-fold higher than *C. communis*
8. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, Yoshida T, Okuda T (1989) Effects of the interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. Chem Pharm Bull 37:2016–2021
9. Dahlqvist A (1964) Method for assay of intestinal disaccharidases. Anal Biochem 7:18–25
10. Shibano M, Matsumoto Y, Kusano G, Shibata T (1996) Researches of *Glycyrrhiza* species grown at medicinal plant gardens in Japan and basic studies for selection of pharmaceutically fine races. 1. Comparative studies by HPLC patterns and constituents of aerial parts. Nat Med 50:273–283
11. Park EJ, Kim Y, Kim J (2000) Acylated flavonol glycosides from the flower of *Inula Britannica*. J Nat Prod 63:34–36
12. Sakakibara M, Difeo D, Nakatani N, Timmermann B, Mabry TJ (1976) Flavonoid methyl ethers on the external leaf surface of *Larrea tridentata* and *L. divaricata*. Phytochemistry 15:727–731
13. Kato T, Morita Y (1990) *C*-Glycosylflavones with acetyl substitution from *Rumex acetosa* L. Chem Pharm Bull 38:2277–2280
14. Palme E, Bilia AR, Feo VD, Morelli I (1994) Flavonoid glycosides from *Cotoneaster thymaefolia*. Phytochemistry 35:1381–1382
15. Lin CN, Kuo SH, Chung MI (1997) A new flavone *C*-glycoside and antiplatelet and vasorelaxing flavones from *Gentiana arisanensis*. J Nat Prod 60:851–853
16. Cheng G, Bai Y, Zhao Y, Tao J, Liu Y, Tu G, Ma L, Liao N, Xu X (2000) Flavonoids from *Ziziphus jujube* Mill var. *spinosa*. Tetrahedron 56:8915–8920
17. Oyama K, Kondo T (2004) Total synthesis of flavocommelin, a component of the blue supramolecular pigment from *Commelina communis*, on the basis of direct 6-*C*-glycosylation of flavane. J Org Chem 69:5240–5246
18. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20:933–956