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Identification of Major Flavonoids in Petals of Edible Chrysanthemum Flowers and Their Suppressive Effect on Carbon Tetrachloride-Induced Liver Injury in Mice

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Major flavonoids contained in petals of edible chrysanthemum flowers (*Chrysanthemum×morifolium* Ramat. forma *esculentum* Makino, c.v Kotobuki) were identified, and their chemical compounds in relation to their radical scavenging activities and preventive effects against liver injury were compared. Based on retention times and UV spectra, three peaks on the HPLC chromatogram of the polyphenol fraction of edible chrysanthemum flowers confirmed the presence of luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, and luteolin. Spectroscopic analysis determined the chemical structure of the three newly isolated compounds to be luteolin 7-*O*-(6"-*O*-malonyl)-glucoside, apigenin 7-*O*-(6"-*O*-malonyl)-glucoside, and acacetin 7-*O*-(6"-*O*-malonyl)-glucoside. Increases in plasma aspartate aminotransferase and alanine aminotransferase activities in mice (induced with liver injury by injection of carbon tetrachloride) were strongly suppressed by oral administration of luteolin and luteolin 7-*O*-(6"-*O*-malonyl)-glucoside, which have stronger radical scavenging activity than other compounds. Thus, it is suggested that compounds with chemical structures such as luteolin and luteoin 7-*O*-glucoside, which have maolonic acid on its glucosyl moiety, appear to readily available for mitigation of liver injury.

Keywords: edible chrysanthemum flower, flavonoids, liver injury, aspartate aminotransferase, alanine aminotransferase

Introduction

The edible chrysanthemum flower, *Chrysanthemum×morifolium* Ramat. forma *esculentum* Makino, is a traditional, local popular food in Yamagata Prefecture, Japan, which is usually eaten after being boiled. Kotobuki, which has a small yellow petal, is the major variety of edible chrysanthemum flowers cultivated in this region.

Petals of the edible chrysanthemum flower, *Chrysanthemum×morifolium* Hemsl., have been used as medicinal herbs in traditional Chinese medicine (Yahara *et al.*,1990). Recently, several reports have suggested that petals of edible chrysanthemum flowers, such as *Chrysanthemum×morifolium* Ramat., contain various physiologically active substances (Takahashi *et al.*, 1978; Akihisa *et al.*, 1997). Although petals of edible chrysanthemum flowers cultivated in Japan have a relatively high content of flavonoids (Tateyama *et al.*, 1997), there have been few reported cases in which flavonoids were contained.

To date, little is known about the potential use of petals of edible chrysanthemum flowers and the physiological functions of its flavonoids. As some flavonoids contained in tea and herbs have been found to be effective in preventing liver injury (Wada *et al.*, 2000; Murakami *et al.*, 2004), further investigation of major flavonoids contained in petals of the edible chrysanthemum flower (*Chrysanthemum×morifolium* Ramat. forma *esculentum* Makino, c.v Kotobuki) and their physiological functions is of great interest.

This study isolated and identified the major flavonoids found in petals of edible chrysanthemum flowers, and compared their radical scavenging activities and suppressive effects against carbon tetrachloride (CCl_4)-induced liver injury in mice.

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Materials and Methods

Preparation of polyphenol fraction Frozen petal sam-

ples (-50°C, 250 g) from edible chrysanthemum flowers, collected in farms in Yamagata City of Yamagata Prefecture in Japan in 2007, were used for 24-h extraction of polyphenols containing flavonoids with 3 L of 80% ethanol. The extracts were evaporated to remove the ethanol, and then passed through a 500-ml Diaion HP-20 resin (Mitsubishi Kasei, Co., Ltd., Tokyo, Japan) column, after which was washed with 2 L distilled water. Polyphenols adsorbed on the Diaion resin were eluted with 1.5 L of 80% ethanol. For isolation of major polyphenols, the 80% ethanol eluate was concentrated and dissolved with 50 ml methanol and fractionated by Sephadex LH-20 column chromatography (50 i.d. × 750 mm, Amersham Bioscience, Co., Ltd., Uppsala, Sweden) with MeOH as the developing solvent. The presence of polyphenols in the respective fractions was checked by the silica gel TLC using ethyl acetate-$CHCl_3$-88% HCOOH-H_2O (19:1:1:1, v/v) as the eluting solvent system. Major polyphenols in each fraction were further purified by preparative HPLC on a COSMOSIL 5C18-MS-II column (10 i.d. × 250 mm, Nacalai Tesque Co., Ltd., Kyoto Japan). The flow rate was 3.0 ml/min, and the oven temperature was 40°C. The following solvent system was used: A, 5% acetonitrile in 1% acetic acid, and B, 50% acetonitrile in 1% acetic acid. For the preparative HPLC of polyphenols the gradient conditions were: 10% B (0-5 min), 10-40% B (5-60 min), 40-80% B (60-80 min), 80% B (80-85 min). Fractions containing polyphenols in an almost purified state was left to stand at 10-20°C to crystallize. When the isolated crystalline (compounds 3, 4 and 6) were co-injected with the 80% ethanol extracts on analytical HPLC using a Mitysil RP-18 column (4.6 i.d. × 250 mm, Kanto Chemical Co., Ltd.) (Fig. 1), those compounds corresponded to peaks 3, 4 and 6 on the HPLC chromatogram, respectively.

NMR was performed on a JNM-EX 400FT-NMR spectrometer (JEOL, Tokyo, Japan) using dimethylsulfoxide-d_6 (DMSO-d_6) as the solvent. High-resolution electrospray ionization mass spectroscopy (HRESI-Tof-MS) in negative mode was performed on Synapt HDMS (Waters, Ltd., Milford, MA, USA).

Determination of flavonoid content Petals of the edible chrysanthemum flower (5 g) were extracted with 80% EtOH at 20-25°C for 24 h, followed by filtration with a DISMIC-13CP filter (0.5 μm, Advantec, Tokyo, Japan). An aliquot of the filtrate was analyzed by analytical HPLC using a Jasco HPLC system (Tokyo, Japan) equipped with a PDA detector and a Mitysil RP-18 column. The flow rate was 1.0 ml/min, the injection volume was 10 μl, and the oven temperature was 40°C. The following solvents were used for the analysis of the flavonoids: A, 5% acetonitrile in 0.05% phosphoric acid and, B, 50% acetonitrile in 0.05% phosphoric acid. For the analysis of the flavonoids the gradient conditions were: 10% B (0-5 min), 10-40% B (5-60 min), 40-80% B (60-90 min), 80% B (90-100 min). The flavonoids luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside and luteolin purchased from Funakoshi (Tokyo, Japan) were used as standard compounds for comparative quantification.

Measurement of DPPH radical scavenging activity The DPPH radical scavenging activity of each compound was measured, as described previously (Suda, 2000), with slight modification, and expressed as a Trolox equivalent. Briefly, a 50% ethanol solution containing each compound was mixed with a DPPH solution (0.5 mM in EtOH) and a 100 mM Tris-HCl buffer (pH 7.4) at a ratio of 1:4:5. The absorbance of the mixture was measured at 520 nm for 20 min after mixing. A standard curve necessary for quantification was prepared using Trolox with a known concentration.

Animal experiment Male ddY strain 8-week-old mice, each with an approximate weight of 38 g, were purchased from the Shizuoka Laboratory Animal Center (Hamamatsu, Japan), and fed a commercial diet (F-2, Funabashi Farms, Funabashi, Japan) for 2 days before the experiment. The mice were kept under controlled conditions with a 12 h light/dark cycle (lights on: 6:00-18:00), at a temperature range of 22-24°C, and a relative humidity of 40-60%.

After feeding the mice a basal diet (casein 20%, (-cornstarch:sucrose = 2:1 (w/w) 65.5%, corn oil 5%, mineral mixture (AIN-93G-MX) 3.5%, vitamin mixture (AIN-93-VX) 1.0%) for 2 days, the mice were divided into 6 groups: control group, n = 5; CCl_4-treated group (CCl_4 group), n = 7; CCl_4 plus luteolin-treated group (L group), n = 7; CCl_4 plus luteolin 7-*O*-(6”-*O*-malonyl)-glucoside-treated group (L-Ma-G group), n = 7; CCl_4 plus apigenin 7-*O*-(6”-*O*-malonyl)-glucoside-treated group (Ap-Ma-G group), n = 6; and CCl_4 plus acacetin 7-*O*-(6”-*O*-malonyl)-glucoside-treated group (Aca-Ma-G group), n = 6. All mice were deprived of the basal diet for 8 h before each treatment.

The mice from the CCl_4 and CCl_4 plus flavonoids groups were orally given a 0.1 ml 0.5% carboxymethyl cellulose (sodium salt) solution with or without flavonoids (12 mg of luteolin or 22 mg of the other flavonoids), 30 min before the intraperitoneal injection of 30 μl of CCl_4 and 60 μl CCl_4 solution, composed of olive oil and CCl_4 at a volume ratio of 1:1, respectively. The mice in the control group were treated in the same way as those in the CCl_4 group, with the exception of 30 μl olive oil being given intraperitoneally. Blood was collected *via* heat puncture under anesthesia with Nembutal (Dai Nippon Pharmaceutical Co., Ltd., Japan) at 22 h after the injection of either the CCl_4 solution or olive oil.

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in plasma, prepared from centri-

fuged blood samples (1000 × g for 15 min), were measured using kits designated for the GOT-UV test and GPT-UV test, respectively (Wako Pure Chemical, Osaka, Japan). One unit of enzyme activity was defined as the amount of enzyme to transform 1 μmol substrate per min per liter of plasma at 25°C.

Liver tissue homogenate for measurement of liver lipid hydroperoxide content was prepared as previously described (Suda, 1999), with some modifications. That is, lipid hydroperoxide was measured using a kit designated for the Determiner LPO (Kyowa Medex Co., Ltd., Osaka, Japan).

The mice were cared for according to the institutional guidelines of Yamagata University.

Statistical analysis Values were given as the means of standard errors. Differences between groups were determined by Fishers's least significant difference method, following a one-way analysis of variance (ANOVA). Significant differences between the mean values were determined at the $p < 0.05$ level.

Result and Discussion

Identification of each compound Polyphenols contained in the 80% EtOH eluate from the Diaion-absorbable fraction of petals of edible chrysanthemum flowers were examined by HPLC (Fig. 1). Compounds 1, 2, and 5 were identified as luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, and luteolin, respectively, based on their retention times on HPLC and their UV spectra measured by a photodiode array detector, compared to those of the respective authentic samples.

HRESI-Tof-MS of compounds 3, 4, and 6 showed

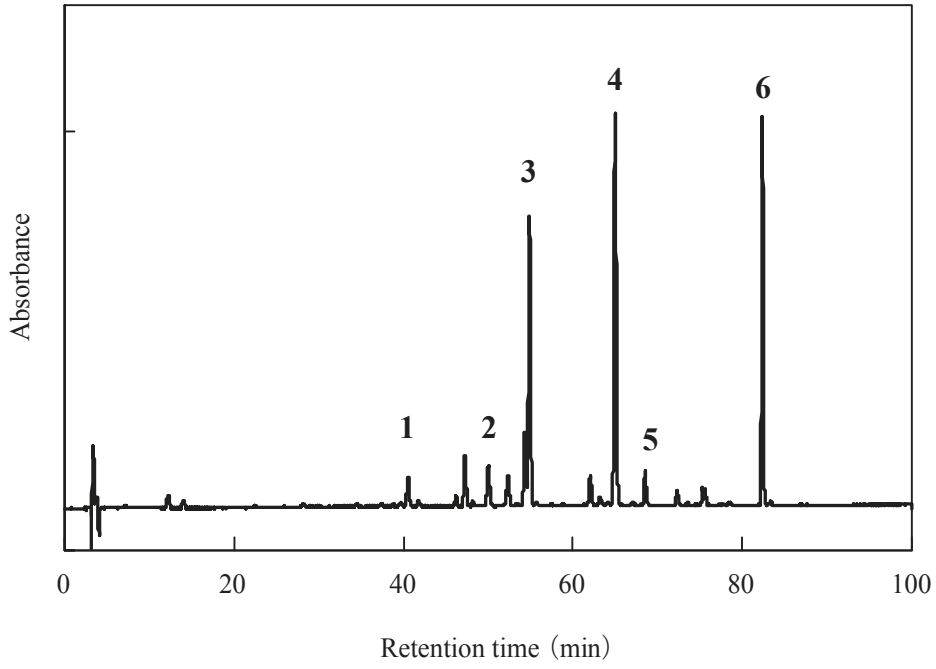

Fig. 1. HPLC chromatogram of 80% ethanol extract from the petals of edible chrysanthemum flowers.
Compounds corresponding to each peak number (1-6) were isolated and identified by spectral analysis. Peaks 1, 2, and 5 were identified as luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, and luteolin, respectively, by co-injection with authentic samples.
Column: Mitysil RP-18 (4.6 i.d. × 250 mm, Kanto Chemical Co. Ltd.). The following solvents: C, 5% acetonitrile in 0.05% phosphoric acid and, D, 50% acetonitrile in 0.05% phosphoric acid. The gradient condition: 10% D (0-5 min), 10-40% D (5-60 min), 40-80% D (60-90 min), 80% D (90-100 min). The flow rate was 1.0 ml/min. Detection, at 360 nm.
The trivial name of each compound corresponding to each peak number on the HPLC chromatogram, and its abbreviation shown in the latter parenthesis are as follows.
Peak 1 (compound 1): luteolin 7-*O*-glucoside
Peak 2 (compound 2): apigenin 7-*O*-glucoside
Peak 3 (compound 3): luteolin 7-*O*-(6"-*O*-malonyl)-glucoside
Peak 4 (compound 4): apigenin 7-*O*-(6"-*O*-malonyl)-glucoside
Peak 5 (compound 5): luteolin
Peak 6 (compound 6): acacetin 7-*O*-(6"-*O*-malonyl)-glucoside

pseudo molecular ions ([M-H]$^-$) at *m/z* 533.0930 (533.0931, calculated for $C_{24}H_{21}O_{14}$), 517.0979 (517.0982, calculated for $C_{24}H_{21}O_{13}$), and 531.1138 (531.1139, calculated for $C_{25}H_{23}O_{13}$), indicating their respective molecular formulas to be $C_{24}H_{22}O_{14}$, $C_{24}H_{22}O_{13}$, and $C_{25}H_{24}O_{13}$, respectively. Other pseudo molecular ions ([M-COOH]$^-$) at *m/z* 489.1053 (489.1033, calculated for $C_{23}H_{21}O_{12}$), 473.1086 (473.1084, calculated for $C_{23}H_{21}O_{11}$), and 487.1240 (487.1240, calculated for $C_{24}H_{23}O_{11}$) in the respective compounds 3, 4 and 6 indicated the possibility of a carboxyl group on each of them. Fragment ions at *m/z* 284.0322 (284.0321, calculated for $C_{15}H_8O_6$) in compound 3, *m/z* 269.0450 (269.0450, calculated for $C_{15}H_9O_5$) in compound 4, and *m/z* 283.0603 (283.0606, calculated for $C_{16}H_{11}O_5$) in compound 6 indicated their respective aglycone to be the luteolin, apigenin, and acacetin moieties.

Chemical shifts in the ^{1}H-NMR and ^{13}C-NMR spectra of compounds 3, 4, and 6 are shown in Table 1. For compound 3, the ^{1}H-, ^{13}C-, ^{1}H-^{1}H COSY-, ^{1}H-^{13}C COSY-, DEPT-, and HMBC-NMR spectra showed signals for glucosyl, malonyl, and luteolin moieties. In the ^{13}C-NMR spectrum, chemical shifts due to these moieties, except that for malonic acid, were very similar to those of luteolin 7-*O*-glucoside (Harborne and Mabry, 1982). In the HMBC spectrum, the anomeric proton at δ 5.10 (1H, d, J=7.3 Hz) showed a cross peak with the carbon signal at δ 162.7 (C-7), indicating that a sugar moiety with the anomeric proton at δ 5.10 was attached to the C-7 position of luteolin *via* the 1”-hydroxyl group (Fig. 2). The

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds 3, 4, and 6 in DMSO-d_6 (ppm, *J* in Hz).

C[a]	Peak 3		Peak 4		Peak 6	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		164.6		164.3		162.8
3	6.76 s	103.1	6.88 s	103.1	6.96 s	103.9
4		181.9		182.0		182.1
5		161.1		161.2		161.1
6	6.44 d (2.0)	99.6	6.44 d (2.4)	99.6	6.45 d (2.0)	99.7
7		162.7		162.7		163.9
8	6.78 d (2.0)	94.6	6.81 d (2.4)	94.7	6.84 d (2.0)	94.8
1’		121.4		121.0		122.7
2’	7.46 d (7.3)	113.6	7.96 d (8.8)	128.6	8.06 d (8.9)	128.5
3’		146.0	6.93 d (8.8)	116.0	7.12 d (9.0)	114.7
4’		150.0		161.0		162.5
5’	6.89 d (8.3)	115.9	6.93 d (8.8)	116.0	7.12 d (9.0)	114.7
6’	7.43 d (2.0)	119.0	7.96 d (8.8)	128.6	8.06 d (8.9)	128.5
OCH_3					3.86 s	55.6
1”	5.10 d (7.3)	99.7	5.16 d (7.3)	99.6	5.11 d (7.3)	99.7
2”	3.29 m	73.0	3.30 m	73.0	3.30 m	73.0
3”	3.34 m	76.1	3.37 m	76.1	3.38 m	76.2
4”	3.18 m	69.6	3.19 m	69.6	3.18 m	69.6
5”	3.74 m	73.9	3.75 m	73.8	3.75 m	73.9
6”-b	4.09 m	64.0	4.11 m	64.0	4.10 m	64.1
6”-a	4.40 m	64.0	4.39 m	64.0	4.40 m	64.1
1”’		167.2		166.9		167.0
2”’	2.51 s	41.9	2.50 s	41.5	2.50 s	41.5
3”’		167.9		167.9		167.9

[a] See the carbon number in Fig. 2.

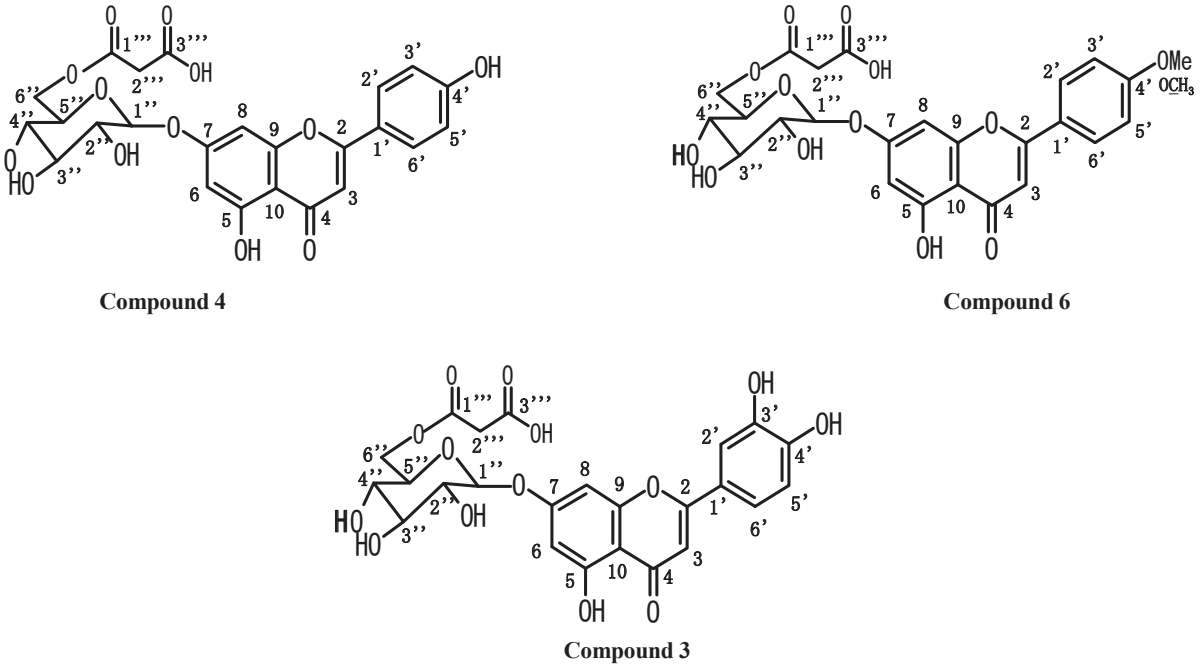

Fig. 2. Chemical structures of compounds 3, 4, and 6 isolated from petals of edible chrysanthemum flowers. The number of each compound corresponds to the peak number on the HPLC chromatogram in Fig. 1. The trivial names of each compound are shown in the legend of Fig. 1.

protons at δ 4.09 (H-6”b) and 4.40 (H-6”a) showed cross peaks with the carbon signal at δ 167.2, indicating that the carboxyl group of the malonyl moiety connected to the C-6” position of the glucose. The position of the malonyl moiety was also confirmed by referring to the chemical shifts in the ^{1}H-NMR and ^{13}C-NMR spectra of quercetin 3-*O*-(6”-*O*-malonyl)-glucoside (cited URL i). Taken together, compound 3 was identified as luteolin 7-*O*-(6”-*O*-malonyl)-glucoside ($C_{24}H_{22}O_{14}$).

For compound 4, the ^{1}H-, ^{13}C-, ^{1}H-^{1}H COSY-, ^{1}H-^{13}C COSY-, DEPT-, and HMBC-NMR spectra showed signals for glucosyl, malonyl and apigenin moieties. In the ^{13}C-NMR spectrum, chemical shifts due to these moieties, except that of malonic acid, were very similar to those of luteolin 7-*O*-glucoside (Harborne and Mabry, 1982). In the HMBC spectrum, the anomeric proton at δ 5.16 (1H, d, J=7.3 Hz) showed a cross peak with the carbon signal at δ 162.7 (C-7), indicating that a sugar moiety with the anomeric proton at δ 5.16 was attached to the C-7 position of the apigenin *via* the 1”-hydroxyl group (Fig. 2). The protons at δ 4.11 (H-6”b) and 4.39 (H-6”a) showed cross peaks with the carbon signal at δ 166.9, indicating that the carboxyl group of the malonyl moiety connected to the C-6” position of glucose. The position of a malonyl moiety was also confirmed by referring to the chemical shifts in the ^{1}H-NMR and ^{13}C-NMR spectra of luteolin 7-*O*-(6”-*O*-malonyl)-glucoside. Taken together, compound 4 was identified as apigenin 7-*O*-(6”-*O*-malonyl)-glucoside ($C_{24}H_{22}O_{13}$).

For compound 6, the ^{1}H-, ^{13}C-, ^{1}H-^{1}H COSY-, ^{1}H-^{13}C COSY-, DEPT-, and HMBC-NMR spectra showed signals for glucosyl, malonyl and acacetin moieties. In the ^{13}C-NMR spectrum, chemical shifts due to these moieties, except for that of malonic acid, were very similar to those of luteolin 7-*O*-glucoside (Harborne and Mabry, 1982). In the HMBC spectrum, the anomeric proton at δ 5.11 (1H, d, J=7.3 Hz) showed a cross peak with the carbon signal at δ 163.9 (C-7), indicating that a sugar moiety with the anomeric proton at δ 5.11 was attached to the C-7 position of the acacetin *via* the 1” -hydroxyl group (Fig. 2). The protons at δ 4.10 (H-6”b) and 4.40 (H-6”a) showed cross peaks with the carbon signal at δ 167.0, indicating that the carboxyl group of the malonyl moiety connected to the C-6” position of the glucose. The position of the malonyl moiety was also confirmed by referring to the chemical shifts in the ^{1}H-NMR and ^{13}C-NMR spectra of luteolin 7-*O*-(6”-*O*-malonyl)-glucoside. Taken together, compound 6 was identified as acacetin 7-*O*-(6”-*O*-malonyl)-glucoside ($C_{25}H_{24}O_{13}$).

Apigenin and apigenin 7-*O*-glucoside have already been

Table 2. Content of isolated flavonoids in petals of edible chrysanthemum flowers.

	Peak 1 L 7-*O*-Glc	Peak 2 Ap 7-*O*-Glc	Peak 3 L 7-*O*-(6”-*O*-Ma)-Glc	Peak 4 Ap 7-*O*-(6”-*O*-Ma)-Glc	Peak 5 L	Peak 6 Aa 7-*O*-(6”-*O*-Ma)-Glc
Petals (mg/100 g FW)	8.92	10.1	102	156	7.14	141

Data shown are the mean of three measurements.
L 7-*O*-Glc, luteolin 7-*O*-glucoside; Ap 7-*O*-Glc, apigenin 7-*O*-glucoside; L 7-*O*-(6”-*O*-Ma)-Glc, luteolin 7-*O*-(6”-*O*-malonyl)-glucoside; Ap 7-*O*-(6”-*O*-Ma)-Glc, apigenin 7-*O*-(6”-*O*-malonyl)-glucoside; L, luteolin; Aa 7-*O*-(6”-*O*-Ma)-Glc, acacetin 7-*O*-(6”-*O*-malonyl)-glucoside.

identified in petals of the edible chrysanthemum flower, *Chrysanthemum×morifolium* Hemsl. (Yahara *et al.*, 1990), while luteolin, luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, acacetin 7-*O*-glucoside, luteolin 7-*O*-(6”-*O*-acetyl)-glucoside and acacetin 7-*O*-(6”-*O*-acetyl)-glucoside have been identified in *Chrysanthemum×morifolium* Ramat. (Wang *et al.*, 2008; Ye *et al.*, 2007). However, to the best of our knowledge, this is the first report to isolate and identify flavonoids (i.e., compounds 3, 4, and 6) from petals of *Chrysanthemum×morifolium* Ramat. forma *esculentum* Makino, c.v Kotobuki.

As increasing malonylation of glycosylated flavonoids in soybeans has improved their solubility in the vacuole (Barz *et al.*, 1992), the same may be the case for malonylated flavonoids, attesting to their relatively abundant amounts, in petals of the edible chrysanthemum flower.

Flavonoid content and radical scavenging activity Table 2 shows the content of compounds isolated from the petals of the edible chrysanthemum flower *Chrysanthemum×morifolium* Ramat. forma *esculentum* Makino, c.v Kotobuki. The major flavonoids of these petals were luteolin 7-*O*-(6”-*O*-malonyl)-glucoside, apigenin 7-*O*-(6”-*O*-malonyl)-glucoside and acacetin 7-*O*-(6”-*O*-malonyl)-glucoside. Among these isolated compounds, apigenin 7-*O*-(6”-*O*-malonyl)-glucoside was the most abundant.

The DPPH radical scavenging activities of the isolated compounds were compared (Table 3). Luteolin and luteolin 7-*O*-(6”-*O*-malonyl)-glucoside showed stronger radical scavenging activity than that of apigenin, apigenin 7-*O*- (6”-*O*-malonyl)-glucoside and acacetin, acacetin 7-*O*-(6”-*O*-malonyl)-glucoside, suggesting that flavonoids with luteolin as the aglycone moiety are superior in radical scavenging activity to those with apigenin and acacetin . In addition, luteolin and its 7-*O*-(6”-*O*-malonyl)-glucoside showed almost the same level of radical scavenging activity.

Suppression of liver injury The ability of flavonoids from the petals of edible chrysanthemum flowers to suppress CCl4-induced liver injury in mice was examined (Figs. 3 and 4). The concentration of lipid hydroperoxide in liver has previously been reported to increase with CCl_4-induced liver injury (Suda *et al.*, 1997). In this study, treatment of luteolin and luteolin 7-*O*-(6”-*O*-malonyl)-glucoside significantly suppressed increases in the plasma ALT and AST activities and liver lipid hydroperoxide content induced by CCl_4. Thus, polyphenols in petals of the edible chrysanthemum flowers may be effective at mitigating liver injury. That is, the amount of trichloromethyl radical ($\cdot CCl_3$) produced from CCl_4 through the action of cytochrome P450 in the hepatocyte microsome (Basnet *et al.*, 1996) may be reduced by the radical scavenging activity of luteolin and luteolin 7-*O*-(6”-*O*-malonyl)-glucoside, thereby mitigating liver injury.

Table 3. DPPH radical scavenging activities of isolated major compounds and authentic apigenin and acacetin and their glucosides.

Compounds	Trolox equivalent (mol Trolox eq/mol sample)
Luteolin	2.2
L 7-*O*-Glc	2.2
L 7-*O*-(6”-*O*-Ma)-Glc	2.3
Apigenin	0.06
Ap 7-*O*-Glc	0.06
Ap 7-*O*-(6”-*O*-Ma)-Glc	0.06
Acacetin	0.03
Aa 7-*O*-(6”-*O*-Ma)-Glc	0.03

Isolated major compounds were luteolin 7-*O*-(6”-*O*-malonyl)-glucoside, apigenin7-*O*-(6”-*O*-malonyl)-glucoside and acacetin 7-*O*-(6”-*O*-malonyl)-glucoside.
Data shown are the mean of three measurements.

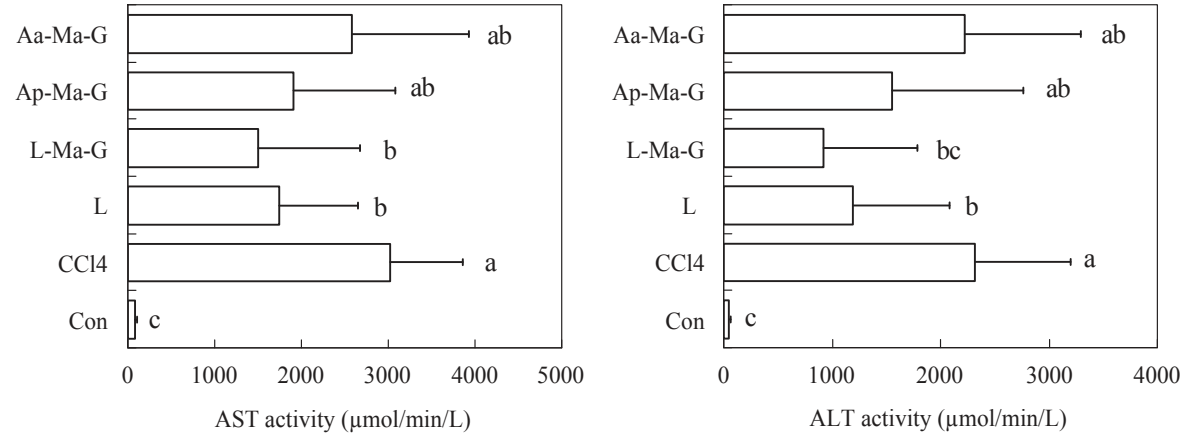

Fig. 3. Effects of flavonoids in petals of edible chrysanthemum flowers on the plasma AST and ALT activities in carbon tetrachloride-induced liver injury in mice.

Con, control group without both oral administration of flavonoids and intraperitoneal injection of CCl_4; CCl_4, group without oral administration of flavonoids but with intraperitoneal injection of CCl_4; L, L-Ma-G, Ap-Ma-G, and Aa-Ma-G, groups with both oral administration of flavonoids and intraperitoneal injection of CCl_4. Values not sharing a common letter are significantly different at $P < 0.05$.

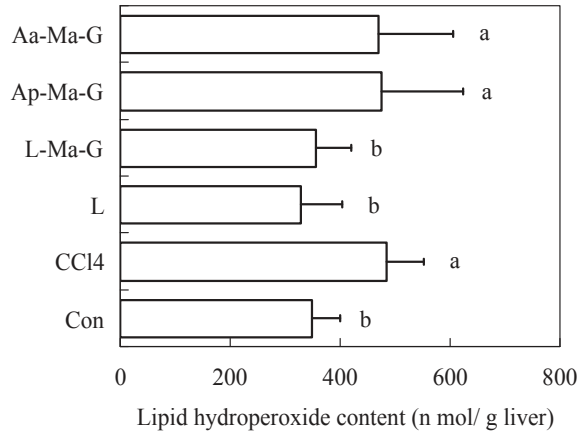

Fig. 4. Effects of flavonoids in petals of edible chrysanthemum flowers on the liver Lipid hydroperoxide content in carbon tetrachloride induced liver injury in mice.

Con, Control group without both oral administration of flavonoids and intraperitoneal injection of CCl_4; CCl_4, Group without oral administration of flavonoids but with intraperitoneal injection of CCl_4; L, L-Ma-G, Ap-Ma-G, and Aa-Ma-G, groups with both oral administration of flavonoids and intraperitoneal injection of CCl_4. Values not sharing a common letter are significantly different at $P < 0.05$.

In this study, it was suggested that compounds with chemical structures such as luteolin and luteolin 7-*O*-glucoside connected to malonic acid on their glucosyl moiety have potential to mitigate liver injury.

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