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# Inhibitory effects of *Chrysanthemum* species extracts on formation of advanced glycation end products

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ABSTRACT

The corolla of *Chrysanthemum* species (*C. morifolium* R. and *C. indicum* L.) has long been used to treat eye and inflammatory disease. However, little is known about the antiglycation properties of *Chrysanthemum* species. Our study sought to characterise their activity against the formation of advanced glycation end products (AGEs) in glycation model reactions. In BSA/glucose (fructose) systems, both *Chrysanthemum* species strongly inhibited the formation of AGEs and $N^{\varepsilon}$-(carboxymethyl)lysine (CML). *C. morifolium* R., not *C. indicum* L., also acted to inhibit the formation of fluorescent AGEs, including pentosidine. This difference correlated with the values of polyphenol and flavonoid components. We characterised the active components in these plants by liquid chromatography-diode array detector-atmospheric pressure chemical ionisation/mass spectrometry, which showed that *C. morifolium* R. contains large amounts of chlorogenic acid, flavonoid glucoside varieties, and apigenin, while *C. indicum* L. contains large amounts of caffeic acid, luteolin, and kaempferol. Our findings raise hopes for the successful treatment of pathogenesis in conditions associated with diabetic complications and aging.



## 1. Introduction

Glycation is a non-enzymatic browning reaction caused by amino-carbonyl reactions between reducing sugars and amino groups of proteins and lipids. Several reports mention the damage done by such reactions within the body, identifying them as the pathogenesis in conditions associated with diabetic complications and aging (Monnier & Cerami, 1981; Monnier, Kohn, & Cerami, 1984). The non-enzymatic reaction leads to cumulative chemical modifications of tissue proteins, called AGEs, resulting in functional disturbances in proteins such as collagen, low density lipoprotein, and lens crystallines (Garlick, Mazer, Chylack, Tung, & Bunn, 1984; Lyons, Bailie, Dyer, Dunn, & Baynes, 1991; Monnier et al., 1984; Press and Wilding, 1989). Glycation initially progresses to form the reversible Schiff base formation. Subsequently, the products of the Amadori rearrangement involving Maillard reactions give rise to a number of complex reactions (e.g., dehydration, oxidation, cyclisation, scission), all leading to the formation of AGEs, including fluorescent (e.g., pentosidine) and non-fluorescent (e.g., CML) adducts (Ahmed, Thorpe, & Baynes, 1986; Akagawa, Sasaki, Kurota, & Suyama, 2005; Sell and Monnier, 1989).

Amino acids, proteins, and sugars account for a large share of the functional constituents of living systems, these reactions play significant roles in our daily lives (Brownlee, Vlassara, Kooney, Ulrich, & Cerami, 1986; Uchida et al., 1997). Various therapeutic agents that inhibit or reverse the progress of glycation have been examined. A representative drug is aminoguanidine (AG), a hydrazine compound, which prevents AGE formation by trapping intermediates at the initial glycation stages (Brownlee et al., 1986). Recent attention has focused on the benefits of medicinal plants with both antiglycation and antioxidant properties (Booth, Khalifah, & Hudson, 1996; Kiho, Usui, Hirano, Aizawa, & Inakuma, 2004; Osawa and Kato, 2005). Cervantes-Laurean et al. (2006) report that rutin and its metabolites effectively inhibit the formation of CML but do not inhibit the formation of fluorescent adducts such as pentosidine, indicating that such inhibitory effects do not apply equally to all AGEs. Preventing the accumulation of AGE varieties in diabetic complications and in the aging process, will likely require the combination of several approaches.

The corolla of *Chrysanthemum morifolium* R. is a herb widely used in traditional medicine in China and Japan to treat eye and inflammatory disease and is used in formulas as an analgesic and antipyretic agent. The corolla of *C. indicum* L. is used as a folk medicine in China and as a traditional medicine in Japan. Several recent

*Abbreviations:* AGEs, advanced glycation end products; AG, aminoguanidine; BSA, bovine serum albumin; *C. indicum* L. and *morifolium* R., *Chrysanthemum indicum* L. and *morifolium* R.; CML, $N^{\varepsilon}$-(carboxymethyl)lysine; DPPH, 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate buffered-saline; Glu, glucoside; AGlu, acetyl glucoside; Neo, neohesperidoside; CQAs, caffeoylquinic acids.

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reports indicate that the *Chrysanthemum* species possess antimicrobial activity and vasodilating effects in coronary hemodynamics (Kato et al., 1986; Shunying, Yang, Huaidong, Yue, & Guolin, 2005). Moreover, the *Chrysanthemum* species have been demonstrated to inhibit aldose reductase (Matsuda, Morikawa, Toguchida, Harima, & Yoshikawa, 2002; Terashima, Shimizu, Horie, & Morita, 1991), suggesting that these plants have therapeutic benefits against diabetic disease. However, little is known about the antiglycation activity of these two *Chrysanthemum* species. In the present study, we verified the effects of the *Chrysanthemum* species on the formation of AGEs such as CML and pentosidine using glycation model systems. We show that these two *Chrysanthemum* species strongly inhibit the formation of various AGEs. The results reported herein should create new avenues for exploring pharmacological treatments to prevent glycation and related disease conditions.

## 2. Materials and methods

### 2.1. Materials

Acacetin, apigenin, chlorogenic acid, kaempferol, luteolin, quinic acid, and quercetin were purchased from Sigma–Aldrich (St. Louis, MO). Aminoguanidine hydrochloride and bovine serum albumin (BSA) (fraction V; fatty acid free) were purchased from Wako Pure Chemicals Industries (Osaka, Japan). Antibodies against AGE (6D12) and CML (CMS-10) was purchased from Trans Genic Inc. (Hyogo, Japan). Goat anti-mouse horseradish peroxidase was purchased from BD Biosciences (San Jose, CA). Quantification of pentosidine in BSA/fructose system was determined by a pentosidine kit (Fushimi Pharmaceutical Co., Kagawa, Japan). All other chemicals were of the highest commercially available grade.

### 2.2. Extract preparation

Samples of *C. indicum* L. and *C. morifolium* R. were prepared from the corolla of each *Chrysanthemum* species purchased commercially and pulverised in liquid nitrogen. Extracts were obtained from each species (500 g) *via* extraction with 4 l of water at room temperature over the course of 3 days. The resulting solution was centrifuged and filtered and the supernatant removed, evaporated, and freeze-dried under a vacuum. The residue (100 mg) was then dissolved in 1 ml of water.

### 2.3. Formation of AGE in the BSA/glucose and BSA/fructose systems

AGE was formed in *in vitro* systems by the method previously described (Kiho et al., 2004). In brief, BSA (10 mg/ml) in phosphate buffered-saline (PBS, pH 7.4) containing 0.02% sodium azide was incubated with glucose (500 mM) at 37 °C for 0, 7, 14, 21, and 28 days in the absence (control) and presence of each of the extracts (2.5–5.0 mg/ml) or AG (1 mM). The protein, sugar, and prospective inhibitor were simultaneously introduced into the incubation mixture. BSA (5 mg/ml) was also incubated with fructose (100 mM) in PBS (pH 7.4) containing 0.02% sodium azide at 37 °C. AG (1 mM) was used as a positive inhibitor; control reactions in the absence of each of the extracts (2.5–5.0 mg/ml) as prospective inhibitors were also established. Each solution was kept in the dark in a capped vial, and incubation was allowed to proceed in triplicate vials. For time course experiments involving fluorescent AGE formation, we measured characteristic fluorescence (excitation wavelength of 370 nm and emission wavelength of 440 nm) with a 1420 ARVO series multilabel counter (Perkin–Elmer Japan Corp., Ltd., Kanagawa, Japan). Each sample taken was immediately frozen at −80 °C to await analysis by Western blot and ELISA.

### 2.4. Western blot

Each sample was fractionated on a polyacrylamide-SDS gel, after which the proteins were transferred to nitrocellulose membranes using a semidry blotter (BIO CRAFT Co., Ltd., Tokyo, Japan) and incubated in blocking solution (5% non-fat dry milk in PBS containing 0.1% Tween-20) for 1 h to reduce non-specific binding. Membranes were then exposed to primary antibodies (overnight at 4 °C). Thereafter, the blot was washed, exposed to HRP-conjugated secondary Abs for 1 h, and finally detected using an ECL Plus Western blotting detection system (GE Healthcare Biosciences, Piscataway, NJ).

### 2.5. ELISA

We performed an ELISA assay of pentosidine according to the manufacturer's instructions. In brief, each sample was subjected to pronase digestion at 55 °C for 1.5 h. The resulting reaction mixtures were boiled at 100 °C for 15 min to inactivate the enzyme, after which an EDTA solution (0.2 M) was added. The samples were dispensed into each well of a 96-well plate and incubated with a primary antibody at 37 °C for 1 h. After the plate was washed with TPBS, the wells were incubated with goat anti-rabbit horseradish peroxidase at room temperature for 1 h. After washing, 3,3′,5,5′-tetramethylbenzidine substrate solution was added and the samples incubated at room temperature until adequate colour developed. The enzyme reaction was stopped by adding a stop solution to each well. The absorbance at 450 nm was measured with a 1420 ARVO series multilabel counter, using 630 nm as a reference wavelength. Pentosidine was quantified from a calibration curve ranging from $5.0 \times 10^{-5}$ to 5.0 (mg/ml).

### 2.6. Determination of total phenolic compounds

The concentration of total phenolic compounds in the water extracts was evaluated spectrophotometrically using Folin-Ciocalteu reagent, following a method slightly modified from that previously described (Julkunen-Tiitto, 1985). In brief, 100 μl of samples and the standard, previously dissolved in ethanol, was diluted with water to 0.45 ml, 0.5 ml of Folin-Ciocalteu phenol reagent was added, and the test tubes vigorously agitated. This was followed by the addition of 0.4 ml of 1 M sodium carbonate solution after which the mixtures were thoroughly agitated once again. The mixtures were allowed to stand at room temperature for 1 h, protected from light. The absorbance of the resulting reaction mixtures at 750 nm was measured with a GE Healthcare Biosciences Ultrospec 4300 pro UV/visible spectrophotometer. The concentration of total phenolic compounds for each extract was calculated on the basis of a standard curve obtained using gallic acid.

### 2.7. Determination of total flavonoids

The quantification of total flavonoids in each extract was performed by the method of Lamaison and Carnat modified for a microplate assay (Gálvez, Martín-Cordero, Houghton, & Ayuso, 2005). Each sample (100 μl) was added to a 96 well plate followed by 100 μl of a 2% $AlCl_3$ solution in methanol. After 10 min, absorbance at 415 nm was measured with a 1420 ARVO series multilabel counter. A standard curve was developed using quercetin.

### 2.8. HPLC-DAD

HPLC was performed with a JASCO Corp. gradient system equipped with dual Model PU-2089 plus pumps (10 ml pump heads), a Rheodyne (Cotati, CA) Model 7725i equipped with a 100 μl sample loop, and a JASCO Corp. Model MD-2010 plus mul-

ti-UV detector. Two mobile phase solvents were used. Solvent A was prepared by adding concentrated 0.1% formic acid (pH 4.0, adjusted with ammonium hydroxide) to deionised water. Solvent B was using HPLC-grade acetonitrile. The analytical method employed a reverse phase column (Develosil C18, 3 μm, 2.0 × 50 mm Nomura Chemical Corp., Ltd.) and the following mobile gradient: 0–10 min, hold 88% of solvent A; 10–60 min, linear gradient to 25% solvent B; 60–80 min, linear gradient to 60% solvent B; 80–85 min, linear gradient to 100% solvent B; 85–90 min, hold 100% of solvent B; 90–91 min, linear gradient to 88% solvent A; 91–105 min, hold 88% of solvent A. The flow rate was kept constant at 0.15 ml $min^{-1}$. The UV spectra of the flavonoids and caffeoylquinic acids (CQAs), such as acacetin, apigenin, chlorogenic acid, kaempferol, luteolin, quinic acid, and quercetin, were scanned at 190–650 nm. Identification was performed by comparing retention times and the UV spectra of the samples against standards or against figures derived from earlier publications (Lai, Lim, Su, Shen, & Ong, 2007). Absolute quantification of chlorogenic acid and caffeic acid was performed using each standard as a reference by HPLC-DAD chromatograms, with monitoring performed at 320 nm. The relative quantification of other compounds was performed with luteolin serving as a reference by HPLC-DAD chromatograms, with monitoring at 320 nm.

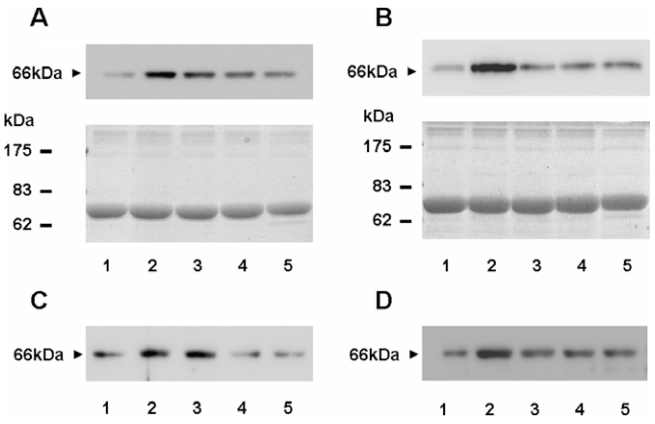


**Fig. 1.** Inhibition by the *Chrysanthemum* species of global AGEs and CML for SDS–PAGE analyses for (A and B top) formation of AGE, (A and B bottom) CBB staining of total BSA, and (C and D) formation of CML. In the BSA/glucose system, BSA (10 mg/ml) was incubated with glucose (500 mM) in PBS (pH 7.4) containing 0.02% sodium azide at 37 °C for 7 days (A and C). In the BSA/fructose system, BSA (5 mg/ml) was also incubated with fructose (100 mM) in PBS (pH 7.4) containing 0.02% sodium azide at 37 °C (B and D). The prospective inhibitor was introduced simultaneously into the reaction mixture. AG was used as a positive inhibitor, and control reactions in the absence of the prospective inhibitor were also established. Absence of glucose (fructose); 1, control; 2, AG (1 mM); 3, *C. morifolium* R. (5 mg/ml); 4, and *C. indicum* L. (5 mg/ml); 5, respectively.

### 2.9. HPLC-APCI/MS/MS

The flavonoids and CQAs in each extract were characterised by the method of Ong et al., with slight modifications (Lai et al., 2007). In brief, online HPLC separations were performed by the procedure described using an Agilent Technologies (Palo Alto, CA) 1100 HPLC series model incorporating a binary gradient pump, vacuum degas-

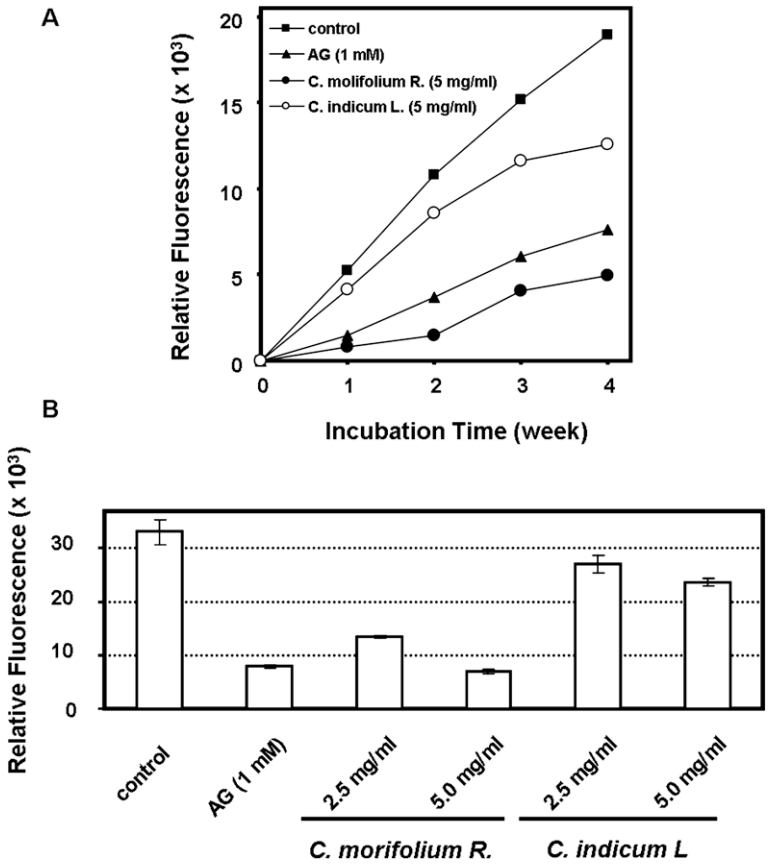


**Fig. 2.** Effects of the *Chrysanthemum* species on fluorescent modification derived from AGE formation in BSA/fructose systems. (A) Time course experiments of fluorescent modification derived from AGE formation after the indicated incubation time. (B) Fluorescent modification derived from AGE formation after 28 days of incubation at the indicated concentrations of each extract. BSA (5 mg/ml) was also incubated with fructose (100 mM) in PBS (pH 7.4) containing 0.02% sodium azide at 37 °C. AGE fluorescence was measured by characteristic fluorescence (excitation wavelength of 370 nm and emission wavelength of 440 nm) in aliquots of each reaction mixture.

ser, auto sampler, and diode array detector under analytical HPLC conditions. The column used was a Develosil C18, 3 μm, 2.0 × 250 mm using a flow rate of 0.15 ml $min^{-1}$. UV spectra were scanned at 335 nm. Mass spectrometric analyses were performed on an Applied Biosystems (Forster, CA) API 2000 triple–quadrupole mass spectrometer equipped with an APCI source operating in both positive and negative ion modes. The instrumental parameters of APCI were vaporiser temperature, 450 °C; curtain gas, 40 psi; collision activated dissociation gas, 7 psi; ion source gas 1, 60 psi; and ion source gas 2, 15 psi. The spray voltage was 6.0 kV. HPLC/MS was used in Q1 ion scan mode, while HPLC/MS/MS was used in multiple reaction monitoring (MRM) ion scan mode. The MS/MS collision energy was 51 V. Full identification was performed by comparing retention time and UV and mass spectra of samples against standards or earlier publications (Lai et al., 2007).

### 2.10. Statistical analysis

All data are expressed as mean ± SD of triplicate determination. Statistical analyses were performed by one-way ANOVA with Dunnett's multiple comparison of mean test or with Tukey's test.

## 3. Results and discussion

Endogenous AGE formation is known to contribute to the progression of pathogenesis in conditions associated with diabetic complications and aging (Monnier & Cerami, 1981, 1984). We focused on the potential benefits of exploiting *Chrysanthemum* species as medicinal plants having antiglycation properties. To confirm the potent inhibitory effects of *Chrysanthemum* species, we first evaluated their ability to suppress AGE formation in BSA/glucose and BSA/fructose systems. As shown in Fig. 1A and B, both *Chrysanthemum* species inhibited the formation of total AGEs after one week of incubation in BSA/glucose and BSA/fructose systems. The inhibitory effects of *Chrysanthemum* species at concentrations of 5.0 mg/ml were stronger than AG at concentrations of 1 mM as a positive control. In subsequent efforts to validate the inhibitory effects of *Chrysanthemum* species on several AGE formations, we verified their inhibitory effects on CML formation in these systems. A colourless and non-fluorescent compound, CML is one of the most characteristic AGE products (Ahmed et al., 1986; Dyer et al., 1993). CML also forms at higher yields than other known AGE products *via* productive pathways such as glycation and oxidation (Knecht et al., 1991). As shown in Fig. 1C and D, both of *Chrysanthemum* species also exerted stronger inhibition than AG in these systems.

We then assessed the formation of fluorescent AGEs and the inhibitory effects of *Chrysanthemum* species on the formation of fluorescent AGEs by reading the fluorescence of their reaction mixtures at Ex 370 nm and Em 440 nm. As shown in Fig. 2A and B, *C. morifolium* R. exerted stronger inhibitory effects than AG in the BSA/fructose system throughout the 28-day incubation period, with wider variations, whereas *C. indicum* L. exhibited lesser inhibitory effects. Notably, at concentrations of 5.0 mg/ml, the inhibitory effects of *C. morifolium* R. exceeded those of AG. In contrast,

**Table 1**
Extract yield, total phenolic compounds (measured by Folin-Ciocalteu assay), flavonoids (measured by $AlCl_3$ reagent) of water extracts of the *Chrysanthemum* species examined expressed as percentage values of extracts.

| Water extract | Extract yield (%) | Total phenolic compounds (%) | Flavanoids (%) |
|---|---|---|---|
| *C. morifolium* R. | 11.80 | 3.05 ± 0.06 | 6.43 ± 0.04 |
| *C. indicum* L. | 9.09 | 2 71 ± 0.03 | 2.52 ± 0.11 |

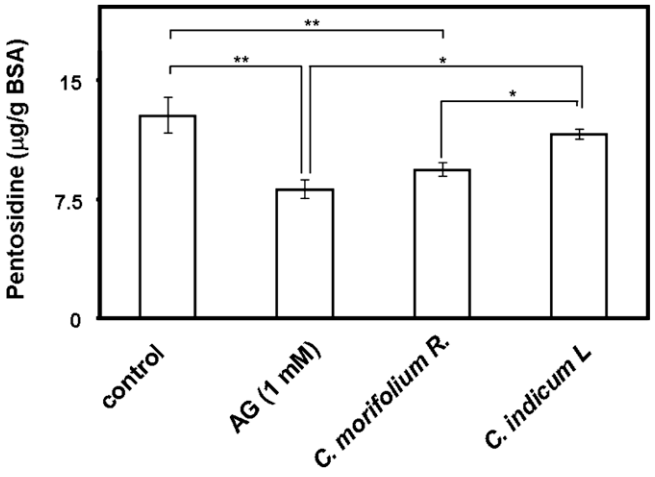


**Fig. 3.** Effects of the *Chrysanthemum* species on pentosidine formation in BSA/fructose system. Pentosidine levels were measured by an ELISA assay. The values represent mean ± SD of triplicate determinations. Where one-way ANOVA was significant, differences between series of post-four-week incubation data in the BSA/fructose system were determined using Tukey's test ($^{*}p < 0.05$, and $^{**}p < 0.01$).

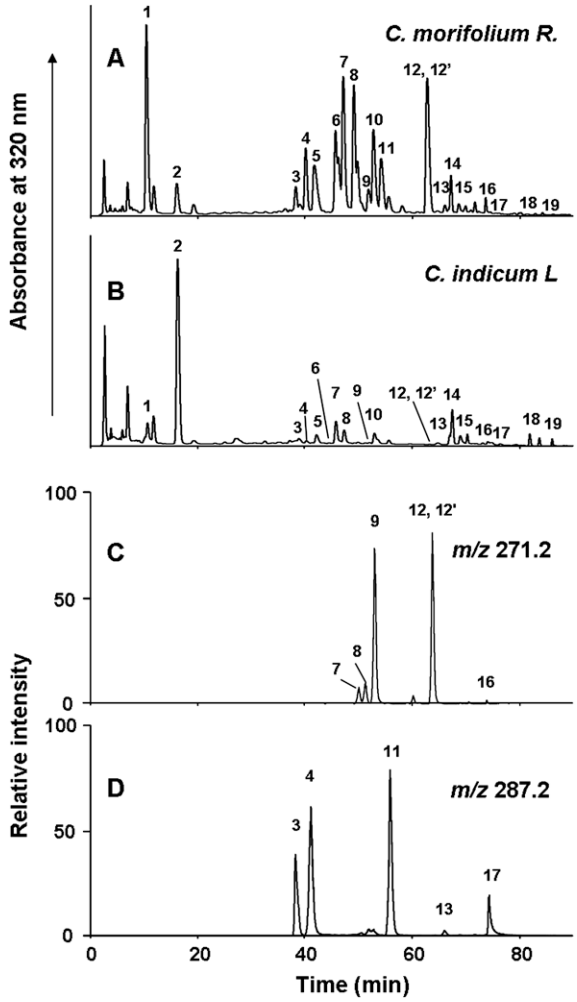


**Fig. 4.** (A) HPLC-UV–vis chromatograms at 320 nm of *C. morifolium* R.; and (B) *C. indicum* L. Selected ion monitoring HPLC–Mass spectrometry chromatograms of *C. morifolium* R. at (C) *m/z* 271.1; and (D) *m/z* 287.2.

*C. indicum* L. displayed moderate suppressive effects at all concentrations in the 28-day incubation period. Moreover, we measured levels of pentosidine formation in the reaction mixture with *C. morifolium* R. with 28 days of incubation, with the results with *C. indicum* L. and AG. Pentosidine is known to be a fluorescent and cross-linked molecule and has been much evaluated in the aging of long-lived proteins such as collagen and lens crystallines (Nagaraj, Sell, Prabhakaram, Ortwerth, & Monnier, 1991; Sell & Monnier, 1990, 1989). As shown in Fig. 3, treatment with *C. morifolium* R. at concentrations of 5.0 mg/ml for 28 days of incubation showed strong inhibitory effects (79.1% inhibition), in contrast to results with *C. indicum* L. (28.5%). At concentrations of 1 mM, AG showed the strongest inhibitory effects of the prospective inhibitors (76.1%), although the differences between AG's and *C. morifolium* R.'s inhibitory effects were not statistically significant. Furthermore, we found that *C. morifolium* R. inhibited fluorescence formation (370/440 nm) with $IC_{50}$ values of 1.3 mg/ml, thereby proving to be a more potent inhibitor than *C. indicum* L., which exhibited $IC_{50}$ values of 10.1 mg/ml. These results indicate that *C. morifolium* R. acts as a more global inhibitor of AGE varieties, in contrast to the limited inhibition of non-fluorescent types observed with *C. indicum* L.

We speculate that *C. morifolium* R. contains more flavonoids and polyphenols than *C. indicum* L., since these compounds have been known to exert antioxidant and antiglycation effects (Sichel, Corsaro, Scalia, Di Bilio, & Bonomo, 1991; Urios, Grigorova-Borsos, & Sternberg, 2007; Wu and Yen, 2005). Several researchers have demonstrated that the *Chrysanthemum* species also contains a wide variety of flavonoids and polyphenols (Chen, Li, Lu, Jiang, & Zeng, 2007; Lai et al., 2007; Matsuda et al., 2002; Miyazawa & Hisama, 2003). In fact, *C. morifolium* R. proved to have higher phenolic and flavonoid content than *C. indicum* L. (Table 1).

We also focused on the bioactive components in these two plants. In a previous study, Ong et al. described findings whereby the chromatographic fingerprinting of flavonoids and CQAs in *Compositae* plants, including *C. morifolium* R., can be developed by a liquid chromatography–diode array detector-atmospheric pressure chemical ionisation/mass spectrometry (LC/DAD-APCI/MS) method (Lai et al., 2007). Thus, to validate the characterisation of these *Chrysanthemum* species, we sought to characterise the flavonoids and CQAs in these plants under conditions virtually identical to those proposed in their study. In LC/DAD analyses, it is interesting to note that these two *Chrysanthemum* species vary significantly in the content of most of the flavonoid glucosides and in the proportions of CQAs (Fig. 4A and B).

Reports indicate that the main glucoside mass ionic fragments in the *C. morifolium* R. flowers include quercetin (*m/z* 303.1), luteolin (*m/z* 287.2), apigenin (*m/z* 271.2), acacetin (*m/z* 285.2), kaempferol (*m/z* 287.2), and kaempferide (*m/z* 301.2). In particular, the fragmental ions, including luteolin (*m/z* 287.2), apigenin (*m/z* 271.2), and kaempferol (*m/z* 287.2), are widely detected. We initially examined these fragmental ions (*m/z* 287.2 and 271.2) using the LC-APCI/MS system. As shown in Fig. 4C and D, this selection ion monitoring efficiently detected postulated ion peaks as the daughter ions of these flavonoid glucosides or as the parent ions of these flavonoid aglycones.

The flavonoid glucosides were confirmed through LC-APCI/MS/MS analyses and UV spectra. Among the detectable peaks, Leteolin-Glu (*m/z* 449.1 → 287.2), Apigenin-Neo (*m/z* 579.1 → 433.1, 271.2), Kaempferide-Glu (*m/z* 463.2 → 301.1, 287.2), Apigenin-Glu (*m/z* 433.0 → 271.2), and Apigenin-AGlu (*m/z* 475.1 → 271.2) were confirmed (Supplementary data). On the other hand, Luteolin-GluA and Kaempferol-GluA, which had been characterised as *m/z* 463.2 → 287.2, were undetectable in our prepared water-extract of the *Chrysanthemum* species. We detected the MS/MS spectra of *m/z* 491.1 → 287.2, reported as the MS/MS spectra pattern of luteolin-Glu and Kaempferol-Glu, but their retention times differed profoundly from those in the previous report of Ong et al., resulting in uncharacterised peaks of other flavonoid glucosides (Lai et al., 2007). The differing positions of hydroxyl groups in flavonoids of the same molecular weight are known to affect retention times.

To clarify differences in components between *C. morifolium* R. and *C. indicum* L., we performed relative quantitative analyses of flavonoid glucosides and absolute quantitative analyses of CQAs and flavonoid aglycones (Table 2). From the standpoint of CQAs, *C. indicum* L. possesses small amounts of CQAs but large amounts

**Table 2**
Flavonoids and monophelic acids of two *Chrysanthemum* species identified by mass spectra and UV-DAD. All UV-DAD and Mass spectra data are allocated to the characterisation of each component along with cited comparable work (Lai et al., 2007).

| Peak no. | Compounds | μg/g the corolla | | Mass spectra (parent ion/daughter ion) | UV (λmax) (nm) |
|---|---|---|---|---|---|
| | | *C. monfolium* R. | *C. indicum* L. | | |
| 1 | Chlorogenic acid | 291.0 | 18.0 | 354.9/193.1, 168.1 | 194, 218, 326 |
| 2 | Caffeic acid | 33 7 | 226.5 | 179.2 | 195, 218, 326 |
| *3* | Uncharacterised | 236.2 | 47.9 | 449.1/287.2 | 206, 268, 348 |
| *4* | Luteolin-Glu | 823.8 | 28.4 | 449.1/287.2 | 204, 266, 348 |
| 5 | Uncharacterised | –[a] | –[a] | ND[c] | 206, 268, 348 |
| 6 | Uncharacterised | –[a] | –[a] | ND[c] | 194, 218, 326 |
| 7 | Apigenin-Neo | 2121.0 | 223.3 | 579.1/433.0, 271.2 | 194, 218, 327 |
| 8 | Uncharacterised | 2455.5 | 9.8 | 463.2/301.1, 287.2 | 198, 266, 338 |
| 9 | Apigenin-Glu | 217.0 | 9.4 | 433.0/271.2 | 200, 266, 338 |
| 10 | Uncharacterised | 1213.5 | 213.0 | 491.1/287.2 | 194, 218, 327 |
| 11 | Kaempferide-Glu | 894.9 | ND[b] | 463 2/301.1, 287.2 | 206, 268, 344 |
| 12 | Apigenin-AGlu | _[d] | _[d] | 475.1/271.2 | 198, 268, 338 |
| 12' | | | | | |
| | Uncharacterised | –[d] | _[d] | 433.0/271.2 | 198, 268, 338 |
| 13 | Luteolin | 14.9 | 15.0 | 287.2 | 212, 266, 346 |
| 14 | Linanin | 407.3 | 387.2 | 593.1/447.0, 285.2 | 208, 268, 334 |
| 15 | Acacetin-AGlu | 76.4 | 2.9 | 489.0/447.0, 285.2 | 236, 268, 334 |
| 16 | Apigenin | 18.6 | 0.3 | 271.2 | 238, 268, 330 |
| 17 | Kaempferol | 1.2 | 3.6 | 287.2 | 208, 268, 334 |
| 18 | Chrysosphenol C | 4.3 | 69.1 | 361.2/331.2, 301.2 | 232, 298, 310 |
| 19 | Chrysosphenol D | 8.6 | 25.5 | 361.2/331.2, 301.2 | 230, 296, 310 |

[a] It was impossible to quantity the contents of these compounds, because of undetected of their mass spectra overlapping.
[b] ND: not detected by HPLC-DAD.
[c] ND: not detected at 271.2 or 282.2 by HPLC-APCI/MS.
[d] It was impossible to quantify the contents of these compounds due to overlapping peaks.

of caffeic acid relative to *C. morifolium* R. As a conjugated product constituted from caffeic acid and quinoic acid, chlorogenic acid has recently been reported to have more potent radical-scavenging activity than caffeic acid (Kono et al., 1997). With respect to flavonoid glucosides, numerous components (peaks 3–14, 16, and 17) can be detected in *C. morifolium* R. In contrast, the content of total flavonoid glucosides in *C. indicum* L. was less than *C. morifolium* R., based on measurements with the $AlCl_3$ reagent. The content of flavonoid aglycons, except for apigenin, in *C. indicum* L. exceeded that of *C. morifolium* R. Apigenin in *C. indicum* L. was a minor flavonoid aglycone, although apigenin in *C. morifolium* R. was the main aglycone-related component. These significant differences in main components between the two *Chrysanthemum* species suggest a possible hypothesis for their differing inhibition of fluorescent AGE formation. Our finding suggests that the distinct differences about the content of active components might contribute to the different medicinal properties.

## 4. Conclusion

Our study reports the antiglycation effects of *Chrysanthemum* species in the BSA/glucose and BSA/fructose systems. Both *Chrysanthemum* species demonstrated marked inhibition of the formation of AGEs and CML in these *in vitro* model reactions. Interestingly enough, we discovered that *C. morifolium* R. also strongly inhibits fluorescent AGE formation, including pentosidine, in contrast to the moderate suppressive effects of *C. indicum* L. The antiglycation properties of the *Chrysanthemum* species appear to underlie their potential utility as medicinal plants and offer remarkable prospects for preventive treatment with regard to pathogenesis in conditions associated with diabetic complications and aging.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2009.03.042.

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