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ARTICLE

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Isolation and *Caenorhabditis elegans* Lifespan Assay of Flavonoids from Onion

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ABSTRACT: The main flavonoids were isolated from three selected onion cultivars. Three phenolic compounds were obtained by reverse-phase HPLC, and their structures were elucidated by multiple NMR measurements. There were two known compounds, quercetin and quercetin 3′-*O*-*β*-D-glucopyranoside (Q3′G), and one novel compound, quercetin 3-*O*-*β*-D-glucopyranoside-(4→1)-*β*-D-glucopyranoside (Q3M), which was identified in onion for the first time. These flavonoids were found to be more abundant in the onion peel than in the flesh or core. Their antioxidative activities were tested using the DPPH method, and their antiaging activities were evaluated using a *Caenorhabditis elegans* lifespan assay. No direct correlation was found between antioxidative activity and antiaging activity. Quercetin showed the highest antioxidative activity, whereas Q3M showed the strongest antiaging activity among these flavonoids, which might be related to its high hydrophilicity.

KEYWORDS: *Allium cepa* L., flavonoids, quercetin, antioxidative activity, lifespan assay, *Caenorhabditis elegans*

INTRODUCTION

The common onion (*Allium cepa* L.) has been part of the diet of almost every civilization in world history. In 2008, the annual production of onions was an estimated 67 million tons (http://faostat.fao.org), and the main areas of production were China, India, the United States, Pakistan, Turkey, and Russia. Numerous valuable phytonutrients have been found in the onion, including flavonoids, fructo-oligosaccharides (FOS), and thiosulfinates and other sulfur compounds.[1] In recent years, flavonoids have received continuous attention due to accumulating evidence of their beneficial properties on human health. For example, flavonoids have been linked to a reduction in the incidence of diseases such as inflammation, heart diseases, and cancer, which are closely connected to the generation of reactive oxygen species in the body.[2−4]

The free radical theory of aging is one of the most popular modern aging theories and is based on the assumption that aging is caused by lifelong accumulation of partially unrepaired oxidative damage to a critical set of biological macromolecules, such as mutations in mitochondrial DNA.[5] The nematode (*Caenorhabditis elegans*), a multicellular organism with a completely sequenced genome, is widely used as an animal model in aging research because of its relatively short lifespan, morphological simplicity, ease of genetic manipulation, and capacity for self-fertilization, which facilitates the generation of genetically homogeneous populations.[6−8] On the basis of the identification and unraveling of multiple mechanisms and corresponding genes limiting the lifespan of nematodes, it has been clarified that the insulin signaling pathway has a close relationship with lifetime determination. It has also been reported that the homologues of these genes are preserved in *Drosophila* and mice, indicating that the aging process of organisms is controlled by a universal molecular mechanism from lower to higher animals.[9−11] Moreover, some of the identified genes have been reported to affect the insulin signaling pathway and mitochondrial function, which are of particular interest with respect to the free radical theory of aging.[12,13]

The present work focuses on the isolation and characterization of the main flavonoids from a variety of onions using HPLC and NMR. We also analyzed the antioxidative and antiaging activities of the flavonoids and discuss the correlativity among these activities.

MATERIALS AND METHODS

Materials and Chemicals. Onions of different colors [a yellow cultivar, Super Kita-momiji (SK); a red cultivar, Syonan-red (SR); and a white cultivar, Aichi-shiro (AS)] were purchased from the Japan Agricultural Cooperatives. After washing with water, the onions were divided into three parts: the peel (dried protective outer scales), flesh (fleshy scales), and core. Each part was cut into small pieces, frozen in liquid nitrogen, and stored at −80 °C. All chemicals and solvents unless otherwise stated were purchased from Wako (Osaka, Japan) or Sigma-Aldrich (St. Louis, MO).

Sample Preparation. The onion pieces were chopped into smaller ones and then incubated overnight with 5 times the volume of 80% methanol at 4 °C. After homogenization, the mixture was stirred for 1 h

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at room temperature. After centrifugation for 10 min at 3500 rpm, the residue of centrifugation was re-extracted using the same steps. The two methanolic phases were mixed, filtered, and concentrated with an evaporator. After lyophilization, the powder was stored at −80 °C.

Analytical and Preparative HPLC. Analytical high-pressure liquid chromatography (HPLC) was used to identify the flavonoids from different parts of every onion cultivar. Preparative HPLC was used for the separation and purification of flavonoids from different onion peels. HPLC analyses were carried out with a Shimadzu SCL-10ADvp HPLC system (Shimadzu Corp., Kyoto, Japan). Analyses of flavonoids were performed on a DOCOSIL-B 4.6 ∅ × 250 mm Senshu Pack HPLC column (Senshu Scientific, Tokyo, Japan), whereas separations of flavonoids were performed on a DOCOSIL-B 20 ∅ × 250 mm Senshu Pack HPLC column, equipped with a DOCOSIL-B 10 ∅ × 30 mm Senshu Pack HPLC guard column (Senshu Scientific). The flow rate and the injection volume of analytical HPLC were 1 mL/min and 20 μL of a 10 mg/mL sample, respectively. For preparative HPLC, the flow rate and the injection volume were 7 mL/min and 4 mL of a 5−10 mg/mL sample, respectively. The gradient of HPLC was from 10 to 80% MeOH containing 0.3% trifluoroacetic acid, and detection was carried out at 280 and 350 nm. Different flavonoid fractions were collected, concentrated, lyophilized, and stored at −80 °C for further use.

NMR Measurements. Samples were loaded in 5 mm diameter NMR sample tubes with CD_3OD for quercetin and quercetin 3′-*O*-β-D-glucopyranoside (Q3′G) and with D_2O for quercetin 3-*O*-β-D-glucopyranoside-(4→1)-β-D-glucopyranoside (Q3M). NMR spectra were recorded on a JNM-A500 (Alpha series) 500 MHz NMR spectrometer (JEOL Ltd., Tokyo, Japan) at room temperature and 50 °C. ^1H, ^{13}C one-dimensional (1D) NMR spectra and $^1H-^{13}C$ HSQC spectra were used to identify constituents. To identify Q3M, COSY, DQF-COSY, relayed-COSY, TOCSY, and $^1H-^{13}C$ HMBC were applied.

UV−Vis Spectroscopy and LC-ESI/TOF MS Measurements. UV−vis spectra were recorded on a Shimadzu UV-2450 spectrophotometer at 25 °C in the range 250−550 nm using a quartz cell with 1 cm path length. All MS spectra were acquired in the LC-ESI/TOF mode using a JEOL AccuTOF JMS-T100LC MS instrument (JEOL Ltd., Tokyo, Japan). A 0.5 μL sample solution was applied onto the sample plate, followed by the addition of 0.5 μL of matrix solution (α-cyano-4-hydroxycinnamic acid, 5 mg/mL in a mixture of acetonitrile and 0.1% v/v TFA, 50:50 v/v), and the resultant mixture was allowed to dry at room temperature.

Total Phenolic Content Assay. The total phenolic contents of different onion parts were measured using the Folin−Ciocalteu method[14] with some modifications. Briefly, 0.125 mL of a properly diluted sample (0.1−10 mg/mL in 50% ethanol) was added to 0.5 mL of distilled water and was mixed with 0.125 mL of the Folin−Ciocalteu phenol reagent. After incubation for 6 min at room temperature, 1.25 mL of 7% Na_2CO_3 and 3 mL of distilled water were added to the solution. The reaction mixtures were incubated for 90 min at room temperature, and then their absorbances at 760 nm were measured using a Shimadzu UV-2200A spectrophotometer. A calibration curve was prepared using the standard solution of gallic acid (0.1−0.5 mg/L, $r^2 = 0.9994$). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight. All determinations were performed in triplicate ($n = 3$).

Antioxidative Activity Assay. Radical scavenging capacity was determined according to the DPPH method[15] with small modifications. A properly diluted sample (0.1−10 mg/mL for onion extracts and 0.01−0.1 mg/mL for purified flavonoid in 80% ethanol) was added to a 0.9 mL solution containing 0.4 mM DPPH, 200 mM MES (pH 6.0), and 20% ethanol. The free radical scavenging capacity was evaluated by measuring the absorbance at 520 nm after 20 min of reaction at room temperature. A calibration curve was prepared using 2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) in the concentration range 5−50 mmol/L ($r^2 = 0.997$). The antioxidative activity of onion extracts was expressed as micromolar Trolox equivalents (TE) per 100 grams of dry weight. On the other hand, the antioxidative activity of a purified flavonoid was expressed as a percentage compared with Trolox (100%). All determinations were performed in triplicate ($n = 3$).

Antiaging Activity Assay. The Bristol N2 (wild type) *C. elegans* strain was maintained at 20 °C on nematode growth medium (NGM) agar plates, supplemented with *Escherichia coli* feeding strain OP50, which is hardly affected by the tested flavonoids in S-medium (the main medium for lifespan assay).[16] The *C. elegans* strain and OP50 strain were supplied by the Tokyo Metropolitan Institute of Gerontology. Stock solutions of all onion extracts and purified flavonoids were made in 50% ethanol. For the lifespan assay, six-well plates (Nunc A/S, Roskilde, Denmark) were used with 2 mL of feeding mixture, which included OP50 ($OD_{600} = 0.9$), 20 μg/mL purified flavonoids as a final concentration.[17−19] Synchronous cultures of worms were obtained by hypochlorite treatment.[20] After hatching, 30 worms (L1) were transferred as young adults (day 2 posthatching) to new plates containing the extracts for experiments. To eliminate the influence of newborn worms, L1 worms were transferred to new plates every day for 15 days and then every 3 days thereafter. Live and dead worms were counted daily. The Kaplan−Meier survival curves and the logrank test for comparison of survival curves were performed with Graphpad Prism software. Values of $p < 0.05$ indicated statistically significant differences between tested populations. All lifespan assays were performed in triplicate ($n = 3$).

Quantitative RT-PCR. Synchronized L1 worms were transferred to plates containing 20 μg/mL Q3M or to control plates and maintained for 2 days. Three plates were prepared for each group, and approximately 100 animals were transferred to each plate. Worms were harvested and washed with ddH_2O. After total RNA isolation (TRIzol reagent, Invitrogen) and reverse transcription (SuperScript cDNA synthesis kit, Invitrogen), quantitative RT-PCR was performed for *age-1*, *daf-2*, *daf-16*, *old-1*, *osr-1*, *sek-1*, and *sir-2.1* on a LightCycler II real-time PCR System (Roche Molecular Biochemicals, Mannheim, Germany) using SYBR green (SYBR qPCR Mix, TOYOBO) and 40 cycles of amplification using the cycle 95 °C for 5 s, 55 °C for 5 s, and 72 °C for 30 s. The gene expression data were analyzed using the comparative $2^{-\Delta\Delta Ct}$ method[21] considering *act-1* as the internal control. The following gene-specific primers were used: *act-1*, CCAGGAATTGCTGATCGTATGCAGAA (F) and TGGAGAGGGAAGCGAGGATAGA (R); *age-1*, GATTATCGAATTGGACATCGACT (F) and GGCGATATTCTTCACTTTTCAGA (R); *daf-2*, AAAAGATTTGGCTGGTCAGAGA (F) and TTTCAGTACAAATGAGATTGTCAGC (R); *daf-16*, TCAGGGATAAGGGAGATTCG (F) and CAGATTGTGACGGATCGAGTT (R); *old-1*, AAGTGATGTGTGCAGTCG (F) and GAGAGCCAAATCACGATG (R); *osr-1*, CCGAACGCAGAGAGATATGG (F) and GCGATCATATTTGCAACGAG (R); *sek-1*, GGCTCGGTTTATCAATGAGATT (F) and AAAGACTTGTTCGCCATTCG (R); *sir-2.1*, TGACAATAAGTGTGTCGCGATT (F) and ATGAGCATTCGGCTCCAG (R). Samples were run in triplicate ($n = 3$).

Solubility Assay. Solubility was determined as described by Takashi and Seibi,[22] with some modifications. A 2 g portion of each sample was suspended in 20 mL of distilled water or ethanol at 30 °C. The suspension was stirred for 30 min and centrifuged at 10000 rpm for 10 min. The supernatant was drained into an evaporating dish and dried at 105 °C to constant weight. The weight of the solids recovered after drying was used to calculate the water solubility.

Statistical Analyses. All statistical analyses were carried out by independent *t* test using the Graph-Pad Prism 4.0 statistical analysis program (San Diego, CA). All results are expressed as the mean ± SD.

RESULTS

Purification and Identification of Individual Onion Extracts. The analytical HPLC chromatograms of SK (yellow),

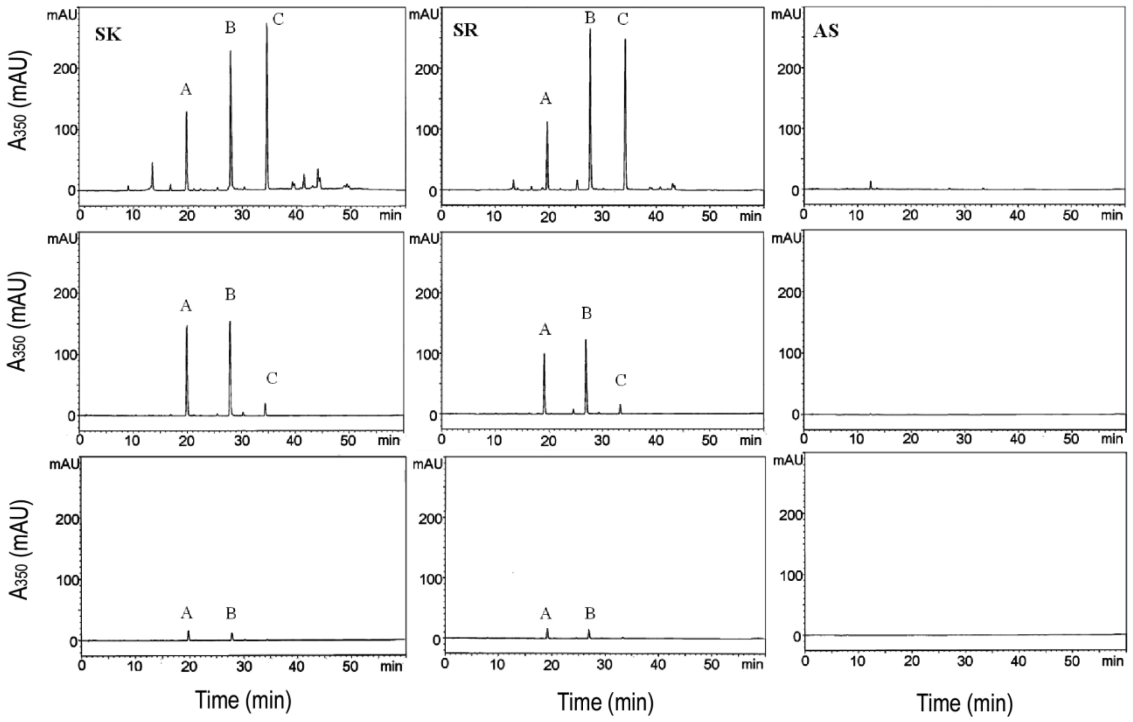

Figure 1. HPLC chromatograms of the methanol-extracted fractions from different parts (peel, top; flesh, middle; core, bottom) of SK (yellow), SR (red), and AS (white) onion cultivars at 350 nm. The following components were identified: A, quercetin 3-*O*-*β*-D-glucopyranoside-(4→1)-*β*-D-glucopyranoside (Q3M); B, quercetin 3′-*O*-*β*-D-glucopyranoside (Q3′G); C, quercetin.

SR (red), and AS (white) at 350 nm are shown in Figure 1. Three flavonoid peaks (A−C) were mainly observed in the extracts of SK and SR. On the other hand, no flavonoid peak was observed in the AS extracts. Peaks A−C were found in the outer parts of onion, and peak C was most abundant in the peel. The three main flavonoids from SK peels were used for further experiments.

^{1}H, ^{13}C 1D, and ^{1}H−^{13}C 2D HSQC NMR spectra were performed to identify peaks A−C from SK peels. ^{1}H and ^{13}C chemical shifts were compared with those from each spectrum previously reported,[23−26] and the connections between ^{1}H and ^{13}C atoms were confirmed with ^{1}H−^{13}C HSQC spectra. These analyses showed that the peaks B and C were quercetin 3′-*O*-*β*-D-glucopyranoside (Q3′G) and quercetin, respectively. However, peak A was identified only as a compound with an unidentified diglycoside attached to position 3 of the quercetin moiety. Following the application of COSY, DQF-COSY, relayed-COSY, TOCSY, and ^{1}H−^{13}C HMBC, the arrangement of the hydroxyls of glycosides was decided, and peak A was then identified as quercetin 3-*O*-*β*-D-glucopyranoside-(4→1)-*β*-D-glucopyranoside (Q3M). The chemical shift values are listed in Table 1, and the chemical structures of the three identified flavonoids are shown in Figure 2.

In the present study, quercetin aglycone and quercetin glycosides were shown to be the main flavonoids found in onion. Although the levels of the flavonoids differed among the peel, flesh, and core, the presence of Q3′G, Q3M, and quercetin in each part of the onion was confirmed by HPLC (Figure 1). This study revealed for the first time that Q3M is present in onion, especially in the peel and flesh, based on multiple NMR measurements. To further characterize Q3M, we measured the UV−vis spectrum and LC-ESI/TOF MS. Q3M had a main absorption peak at 284 nm in the UV−vis spectrum (Figure 3A), which is different from a typical spectrum of flavonoids such as quercetin.[27] On the other hand, the LC-ESI/TOF MS pattern of Q3M (Figure 3B) was composed of four peaks: quercetin moiety (m/z 303), quercetin 3-*O*-*β*-D-glucopyranoside moiety (m/z 465), Q3M (m/z 627), and its sodium-bound form (m/z 649).

Total Phenolic Content and Antioxidative Activity of Onion Extracts. The total phenolic content and antioxidative activity of the different parts of SK, SR, and AS are shown in Table 2. The largest portion of phenolic compounds was located in the peels of SK and SR, which also showed potent DPPH scavenging capacities of 11300 μmol Trolox equiv/100 g and 10400 μmol Trolox equiv/100 g, respectively, much higher values than those for other vegetables and fruits.[28−31] Moreover, a correlation was observed between the amount of phenolic compounds and the antioxidative activity of all three cultivars.

Antioxidative Activity of Onion Flavonoids. The DPPH scavenging capacities of purified flavonoids from SK were also measured and were calculated as a percentage compared with Trolox (Table 3). Quercetin showed the most potent antioxidative activity, which was 4.4-fold that of Trolox. On the other hand, Q3′G and Q3M showed decreased antioxidative activity. The antioxidative activity of flavonoids depends on the position, amount, and state of the hydroxyls located on the benzene ring, with the glycosylation of these hydroxyls resulting in a decrease of antioxidative activity.[32,33] Quercetin has a catechol structure for ring C and the hydroxyl at C-3 adjacent to the carbonyl at C-4 for ring A, which guarantees its potent antioxidative activity. On the other hand, the glycosylation at different positions in Q3′G and Q3M causes a reduction in antioxidative activity. Most of the quercetin present in onion is located in the peel (Figure 1), which suggests that this flavonoid is one of the main factors involved in the defense of the onion against outer oxidative stress.[1]

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Components A−C

component	compound	chemical shift (ppm) ^{1}H	chemical shift (ppm) ^{13}C	assignment
A	Q3M	3.45	61.0	S-12
		3.65, 3.80	61.2	S-6
		3.35	69.4	S-10
		3.40	70.1	S-8
		3.51	73.5	S-11
		3.33	74.5	S-9
		3.52	76.0	S-2
		3.15	76.3	S-5
		3.34	76.6	S-3
		3.45	76.8	S-4
		5.61	94.9	S-8
		5.70	99.5	S-6
		4.80	101.1	S-1
		4.75	103.3	S-7
			104.4	C-10
		6.67	115.4	C-2′
		7.02	116.9	C-5′
		7.05	122.7	C-1′
			124.4	C-6′
			135.0	C-3
			145.1	C-3′
			147.7	C-4′
			156.4	C-2
			156.4	C-9
			160.3	C-5
			163.6	C-7
			177.9	C-4
B	Q3′G	3.49, 3.68	60.8	S-6
		3.17, 3.23	69.7	S-4
		3.27	73.2	S-2
		3.19, 3.25	75.9	S-3
		3.19, 3.22	76.8	S-5
		6.12	92.8	C-8
		5.92	97.7	C-6
		4.68	101.8	S-1
			103.0	C-10
		7.30	114.9	C′-2
		7.05	116.0	C′-5
		7.25	119.7	C-6
			126.0	C′-1
			136.5	C-3
			145.3	C′-4
			146.3	C′-3
			146.5	C-2
			156.7	C-9
			161.0	C-5
			164.2	C-7
			175.9	C-4
C	quercetin		93.0	C-8
			97.8	C-6
			103.1	C-10
			114.5	C′-2
			114.8	C′-5
			120.2	C′-6
			122.7	C′-1
			135.8	C-3
			144.7	C′-3
			146.6	C-2
			147.3	C′-4
			156.7	C-9
			161.0	C-5
			164.1	C-7
			175.9	C-4

Antiaging Activity of Onion Flavonoids and Changes in Gene Expression Level by Q3M Treatment. Our results confirmed that the lifespan of nematodes was extended in S-medium containing 20 μg/mL purified flavonoids. The survival curves are shown in Figure 4A. In addition, the average lifespan and p values for each flavonoid treatment are summarized in Table 4. Quercetin, Q3′G, and Q3M were associated with 1.14-, 1.12-, and 1.20-fold increases in the lifespan of nematodes, respectively. Thus, all three flavonoids from onion peel showed the lifespan extension effect. On the other hand, rutin, which has a structure similar to that of Q3M, did not show any lifespan extension effect (Figure 4B).

To support the lifespan extension effects of Q3M, we verified whether Q3M influenced the expression level of multiple genes that are related to lifespan extension by polyphenols.[17,18] As shown in Figure 5, the expression levels of *daf-16*, *age-1*, and *sir-2.1* did not show significant difference by Q3M treatment. On the other hand, the expressions of *daf-2*, *old-1*, *osr-1*, and *sek-1* were enhanced by 1.38-, 15.3-, 2.85-, and 1.45-fold, respectively. This result indicates that Q3M in fact stimulates the expression of several related genes to lifespan extension of nematodes. In particular, Q3M largely elevated the expression level of *old-1*, which is a gene responsible for stress resistance and adult longevity.[34]

Solubility of Onion Flavonoids. Q3M showed the highest antiaging activity among the three flavonoids. However, the mechanism by which Q3M prolonged the lifespan of the nematodes remains unclear. On the other hand, previous studies have reported that the solubility of hesperidin was increased 10000 times by hydrolysis, which highly improved the bioavailability and inhibitory activity to the loss of thighbone tissue and the increase in blood cholesterol in mice.[35,36] In addition, it has been reported that the glycosylation of quercetin improved the bioavailability in humans.[37,38] Therefore, the solubilities of the three flavonoids were examined and are shown in Table 5. The results showed that Q3M had the best water solubility, which may play a role in its high bioavailability and lifespan extension effect. In other words, hydrophilicity might be one of the important factors affecting lifespan extension.

To examine the relationship between hydrophilicity and lifespan extension, rutin was selected for further experiments. Rutin has a structure similar to that of Q3M but with low hydrophilicity (Figure 2 and Table 5) and high antioxidative activity.[39] Rutin

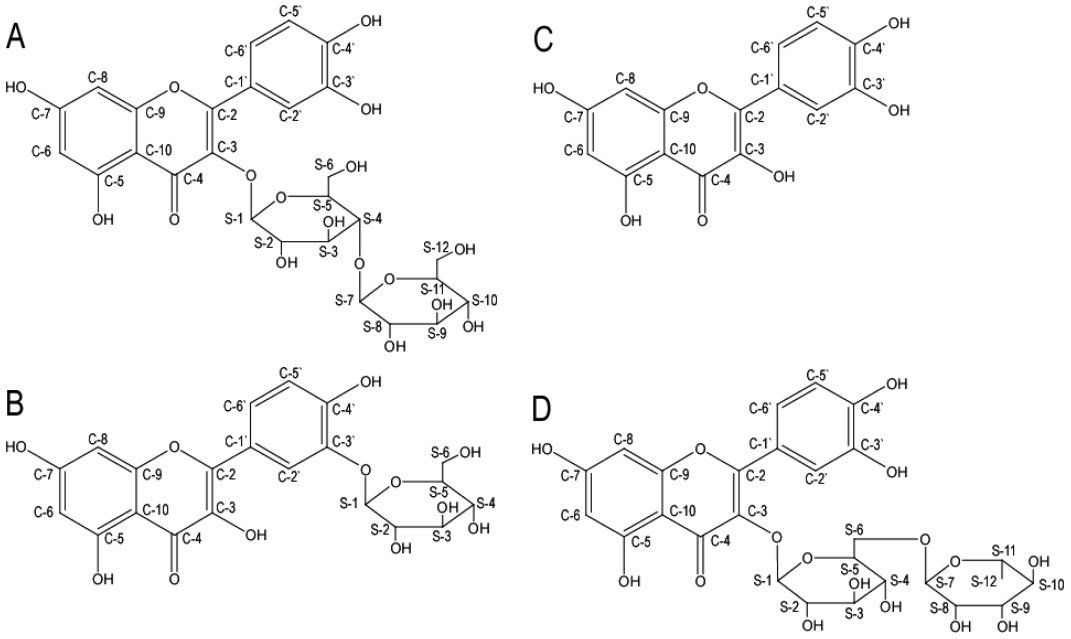

Figure 2. Chemical structures of components A, B, and C from onions and of a reference component D, rutin.

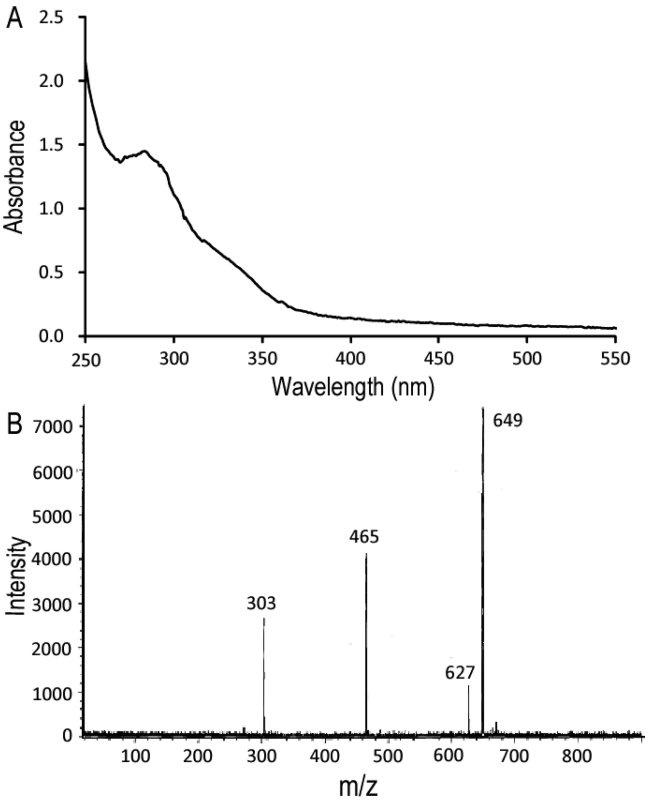

Figure 3. UV−vis spectrum (A) and LC-ESI/TOF MS spectrum (B) of Q3M. Q3M showed a main absorption peak at 284 nm, and the LC-ESI/TOF MS pattern of Q3M was composed of four peaks: quercetin moiety (m/z 303), quercetin 3-*O*-β-D-glucopyranoside moiety (m/z 465), Q3M (m/z 627), and its sodium-bound form (m/z 649).

($20\ \mu$g/mL) increased the lifespan of nematodes an average of 0.99-fold compared with the control; that is, it had no effect on lifespan (Figure 4B). This finding confirmed the close correlation between hydrophilicity and lifespan extension. In addition, it was found that rutin does not readily dissolve in ethanol, a typical amphipathic solvent, whereas Q3′G and quercetin had relatively high solubility in ethanol (Table 5). This characteristic of rutin may be one of the reasons why it had no lifespan extension effect.

Table 2. Total Phenolic Content and Antioxidative Activity of Different Parts of the SK (Yellow), SR (Red), and AS (White) Onion Cultivars

		SK	SR	AS
peel	total phenolic content[a]	53.6 ± 3.0	45.1 ± 0.6	0.47 ± 0.01
	antioxidative activity[b]	11300 ± 4970	10400 ± 480	25.1 ± 0.2
flesh	total phenolic content[a]	1.74 ± 0.01	0.85 ± 0.01	0.38 ± 0.01
	antioxidative activity[b]	61.3 ± 4.4	67.6 ± 1.7	44.9 ± 2.7
core	total phenolic content[a]	1.08 ± 0.06	0.72 ± 0.02	0.74 ± 0.07
	antioxidative activity[b]	24.3 ± 0.9	43.6 ± 0.6	119.0 ± 7.7

[a] Total phenolic content is expressed as mg gallic acid equivalents (GAE)/g dry weight. [b] Antioxidative activity is expressed as μmol Trolox equivalents (TE)/100 g dry weight.

Table 3. Antioxidative Activity of Q3M, Q3′G, and Quercetin Obtained from SK Compared with Those of a Standard Material, Trolox

	Trolox	Q3M	Q3′G	quercetin
antioxidative activity[a]	100	55.0 ± 1.3	85.6 ± 0.4	437 ± 11

[a] Antioxidative activity is expressed as the relative DPPH scavenging capacity (with the activity of the standard material Trolox taken as 100%).

DISCUSSION

Onions make a regular appearance on almost every dinner table in the world and make a major contribution to the total

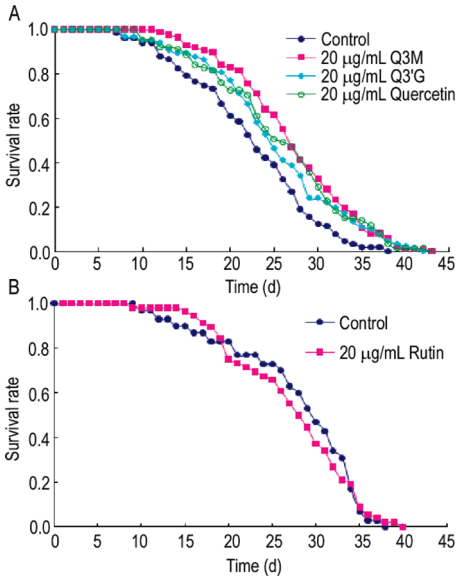

Figure 4. Survival curves of *C. elegans* following treatment with (A) 20 μg/mL Q3M, Q3′G, and quercetin (purified from SK) and (B) 20 μg/mL rutin.

Table 4. Average Lifespan of Nematodes following Treatment with Q3M, Q3′G, and Quercetin Obtained from SK

	av lifespan	rel lifespan[a]	worm count	*p*
control	22.5 ± 0.4	1.00	90	
Q3M	27.2 ± 1.3	1.20	85	<0.0001
Q3′G	25.3 ± 1.2	1.12	83	0.0274
quercetin	25.8 ± 1.9	1.14	82	0.0010

[a] Relative to that of the control, which was normalized as 1.00.

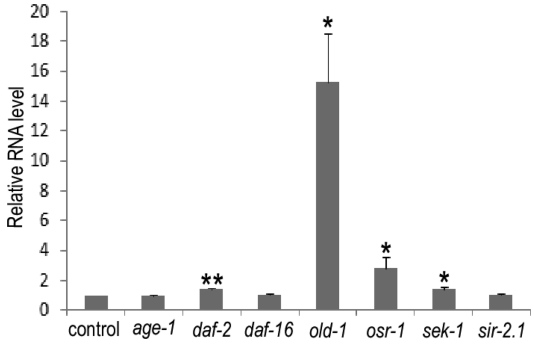

Figure 5. Relative RNA level of *daf-2*, *old-1*, *osr-1*, and *sek-1* after Q3M treatment. Compared with control, worms treated with 20 μg/mL Q3M exhibited enhanced expression of *daf-2*, *old-1*, *osr-1*, and *sek-1* (up-regulation of 1.38-, 15.3-, 2.85-, and 1.45-fold, respectively). The housekeeping gene *act-1* was used as an internal control. *, $p < 0.05$; **, $p < 0.01$.

intake of dietary flavonoids. More than 50 flavonoids have been identified so far in onion scales.[1] Among them, quercetin and quercetin glycosides are the most predominant flavonoids in all onion cultivars. In the present study, quercetin and Q3′G were identified, which is in accordance with previous results,[24,40,41] and Q3M was purified for the first time in onion bulbs and identified by multiple NMR measurements.

Table 5. Solubility (Milligrams per Milliliter) of Q3M, Q3′G, Quercetin (Obtained from SK), and a Reference Component, Rutin

	Q3M	Q3′G	quercetin	rutin
water	40.2 ± 0.3	0.20 ± 0.03	0.20 ± 0.05	0.10 ± 0.02
ethanol	8.0 ± 0.2	50.1 ± 0.5	26.2 ± 0.2	4.0 ± 0.6

Although the free radical theory of aging has been put forward for about 60 years, continuous attention is still being paid to the relationship between the antioxidative activity and the antiaging activity of antioxidants. In the present study, the antioxidative activity and the antiaging activity were evaluated using the same flavonoids to verify the correlativity between the two types of activity. Quercetin showed more potent antioxidative activity than Q3M or Q3′G, whereas Q3M exhibited the most powerful antiaging activity. This result indicates that there is no direct correlation between the antiaging activity and the antioxidative activity of onion flavonoids. A recent study similarly reported that a direct antioxidative effect was unlikely to be the main factor responsible for the modulation of nematode lifespan.[42] One of the reasons for this conclusion is the low bioavailability of flavonoids: quercetin, for example, accounts for <1% of the normal total intake due to its poor solubility, degradation caused by digestive enzymes, and rapid excretion.[43,44] In addition, the concentration of flavonoids in plasma or organs is lower than the concentrations of ascorbic acid and α-tocopherol, which are recognized as small molecular antioxidant nutrients.[44] In the present study, quercetin showed a lifespan extension effect, whereas Q3M exhibited the most potent lifespan extension effect probably due to ameliorated bioavailability caused by higher hydrophilicity. The main action of Q3M is considered to be the modulation of signal transduction and induction of stress tolerance, rather than antioxidants.

Q3M enhances several genes related to lifespan extension such as *daf-2*, *old-1*, *osr-1*, and *sek-1* (Figure 5). Resveratrol is well-known as a polyphenol with antiaging activity, and its effect on lifespan extension is dependent on *sir-2.1* but independent of *daf-16*.[45] On the other hand, the antiaging activity of quercetin is mediated by *age-1*, *daf-2*, and *sek-1* without involvement of *osr-1*, which is responsible for lifespan extension induced by blueberry polyphenols, and *sir-2.1*.[17] The genes related to the antiaging activity of Q3M are different from those of resveratrol and are not completely consistent with those of quercetin. Although many possibilities exist upon Q3M exposure, one major reason might be the improved stress resistance due to the up-regulated genes, especially *old-1* (15.3-fold).

Flavonoids do benefit health in an interesting way: the body recognizes them as xenobiotic compounds because they are good substrates and inducers of phase II enzymes, which are responsible for removing foreign compounds.[46,47] Low doses of flavonoids (<50 μM for various cell lines and 100 μM for nematodes due to the low bioavailability) act as cell-signaling modulators inside cells and may cause hormesis, a phenomenon resulting in increased stress resistance, enhanced damage repair, and sometimes lifespan extension in diverse species and cell lines in response to mild stressors.[43,48,49] However, increased concentrations (>100 μM) will activate the caspase pathway, leading to apoptosis.[50−52] Thus, the concentration of flavonoids used in the present study (20 μg/mL, comparable to 66 μM quercetin) is considered to be a functionally low dose, and Q3M, which was

purified from onion bulbs for the first time and showed the most potent lifespan extension effect among the three flavonoids examined, may have potential for use as a health care product. In addition, the peel of the onion, which contains considerable quantities of quercetin and quercetin glycosides, is usually discarded during processing and cooking. Therefore, the extraction of the flavonoids from the peel would help not only in reducing waste but also in recovering useful compounds that could greatly benefit human health.

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REFERENCES

(1) Slimestad, R.; Fossen, T.; Vagen, I. M. Onions: a source of unique dietary flavonoids. *J. Agric. Food Chem.* **2007**, *55*, 10067–10080.

(2) Middleton, E., Jr.; Kandaswami, C.; Theoharides, T. C. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **2000**, *52*, 673–751.

(3) Cook, N. C.; Samman, S. Flavonoids − chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem.* **1996**, *7*, 66–76.

(4) Wojcik, M.; Burzynska-Pedziwiatr, I.; Wozniak, L. A. A review of natural and synthetic antioxidants important for health and longetivity. *Curr. Med. Chem.* **2010**, *17*, 3262–3288.

(5) Rattan, S. I. S. Increased molecular damage and heterogeneity as the basis of aging. *Biol. Chem.* **2008**, *389*, 267–272.

(6) Klass, M. R. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mech. Ageing Dev.* **1977**, *6*, 413–429.

(7) Klass, M.; Hirsh, D. Non-ageing developmental variant of *Caenorhabditis elegans*. *Nature* **1976**, *260*, 523–525.

(8) Gershon, D. Studies on aging in nematodes. I. The nematode as a model organism for aging research. *Exp. Gerontol.* **1970**, *5*, 7–12.

(9) Bluher, M.; Kahn, B. B.; Kahn, C. R. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **2003**, *299*, 572–574.

(10) Kenyon, C.; Chang, J.; Gensch, E.; Rudner, A.; Tabtiang, R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* **1993**, *366*, 461–464.

(11) Tatar, M.; Kopelman, A.; Epstein, D.; Tu, M. P.; Yin, C. M.; Garofalo, R. S. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **2001**, *292*, 107–110.

(12) Ishii, N.; Fujii, M.; Hartman, P. S.; Tsuda, M.; Yasuda, K.; Senoo-Matsuda, N.; Yanase, S.; Ayusawa, D.; Suzuki, K. A mutation insuccinate dehydrogenase cytochrome *b* causes oxidative stress and aging in nematodes. *Nature* **1998**, *394*, 694–697.

(13) Campo, R. P.; Torres, M. L.; Cadenas, S.; Rojas, C.; Barja, G. The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. *J. Comp. Physiol. B* **1998**, *168*, 149–158.

(14) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Viticult.* **1965**, *16*, 144–158.

(15) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT−Food Sci. Technol.* **1995**, *28*, 25–30.

(16) Brenner, S. The genetics of *Caenorhabditis elegans*. *Genetics* **1974**, *77* (1), 71–94.

(17) Menzel, R.; Steinberg, C. E. W.; Sturzenbaum, S. R. Quercetin mediated lifespan extension in *Caenorhabditis elegans* is modulated by *age-1*, *daf-2*, *sek-1* and *unc-43*. *Biogerontology* **2009**, *5*, 565–578.

(18) Wood, J. G.; Rogina, B.; Lavu, S.; Howitz, K.; Helfand, S. L.; Tatar, M.; Sinclair, D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **2004**, *430*, 686–689.

(19) Gruber, J.; Tang, S. Y.; Halliwell, B. Evidence for a trade-off between survival and fitness caused by resveratrol treatment of *Caenorhabditis elegans*. *Ann. N.Y. Acad. Sci.* **2007**, *1100*, 530–542.

(20) Stiernagle, T., Ed. Maintenance of *C. elegans*. *WormBook*, The *C. elegans* Research Community, 2006; http://www.wormbook.org.

(21) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* **2001**, *25*, 402–408.

(22) Takashi, S.; Seibi, P. A. Paste and gel properties of prime corn and wheat starches with and without nitric liquids. *Cereal Chem.* **1988**, *65*, 474–483.

(23) Bonaccorsi, P.; Caristi, C.; Gargiulli, C.; Leuzzi, U. Flavonol glucoside profile of southern Italian red onion (*Allium cepa* L.). *J. Agric. Food Chem.* **2005**, *53*, 2733–2740.

(24) Ly, T. N.; Hazama, C.; Shimoyamada, M.; Ando, H.; Kato, K.; Yamauchi, R. Antioxidative compounds from the outer scales of onion. *J. Agric. Food Chem.* **2005**, *53*, 8183–8189.

(25) Agrawal, P. K. *Studies in Organic Chemistry, Carbon-13 NMR of Flavonoids*; Elsevier Science: Amsterdam, Netherlands, 1989.

(26) Spectral Database for Organic Compounds SDBS,http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng.

(27) Vicentini, F. T. M. C.; Georgetti, S. R.; Jabor, J. R.; Caris, J. A.; Bentley, M. V. L. B.; Fonseca, M. J. V. Photostability of quercetin under exposure to UV irradiation. *Lat. Am. J. Pharm.* **2007**, *26*, 119–124.

(28) Khokhar, S.; Magnusdottir, D. G. M. Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *J. Agric. Food Chem.* **2002**, *50*, 565–570.

(29) Llorach, R.; Martinez-Sanchez, A.; Tomas-Barberan, F. A.; Gil, M. I.; Ferreres, F. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem.* **2008**, *108*, 1028–1038.

(30) Chinnici, F.; Bendini, A.; Gaiani, A.; Riponi, C. Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. *J. Agric. Food Chem.* **2004**, *52*, 4684–4689.

(31) Prior, R. L.; Cao, G.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolic content and anthocyanin content, maturity, and variety of *Vaccinium* species. *J. Agric. Food Chem.* **1998**, *46*, 2686–2693.

(32) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure −antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933–956.

(33) Bors, W.; Michel, C.; Stettmaier, K. Structure−activity relationships governing antioxidant capacities of plant polyphenols. *Methods Enzymol.* **2001**, *335*, 166–180.

(34) Wang, X.; Wang, X.; Li, L.; Wang, D. Lifespan extension in *Caenorhabditis elegans* by DMSO is dependent on *sir-2.1* and *daf-16*. *Biochem. Biophys. Res. Commun.* **2010**, *400*, 613–618.

(35) Cushnie, T. P. T.; Lamb, A. J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* **2005**, *26*, 343–356.

(36) Yamada, M.; Tanabe, F.; Arai, N.; Mitsuzumi, H.; Miwa, Y.; Kubota, M.; Chaen, H.; Kibata, M. Bioavailability of glucosyl hesperidin in rats. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 1386–1394.

(37) Graefe, E. U.; Wittig, J.; Mueller, S.; Riethling, A. K.; Uehleke, B.; Drewelow, B.; Pforte, H.; Jacobasch, G.; Derendorf, H.; Veit, M. Pharmacokinetics and bioavailability of quercetin glycosides in humans. *J. Clin. Pharmacol.* **2001**, *41*, 492–499.

(38) Hollman, P. C.; de Vries, J. H.; van Leeuwen, S. D.; Mengelers, M. J.; Katan, M. B. Absorption of dietary quercetin glycosides and

quercetin in healthy ileostomy volunteers. *Am. J. Clin. Nutr.* **1995**, *62*, 1276–1282.

(39) Yang, J. X.; Guo, J.; Yuan, J. F. In vitro antioxidant properties of rutin. *LWT—Food Sci. Technol.* **2008**, *41*, 1060–1066.

(40) Yoo, K. S.; Lee, E. J.; Patil, B. S. Quantification of quercetin glycosides in 6 onion cultivars and comparisons of hydrolysis-HPLC and spectrophotometric methods in measuring total quercetin concentrations. *J. Food Sci.* **2010**, *75*, C160–C165.

(41) Tsushida, T.; Suzuki, M. Content of flavonol glucosides and some properties of enzymes metabolizing the glucosides in onion. *J. Jpn. Soc. Food Sci. Technol.* **1996**, *43*, 642–649.

(42) Pun, P. B.; Gruber, J.; Tang, S. Y.; Schaffer, S.; Ong, R. L.; Fong, S.; Ng, L. F.; Cheah, I.; Halliwell, B. Ageing in nematodes: do antioxidants extend lifespan in *Caenorhabditis elegans*? *Biogerontology* **2010**, *11*, 17–30.

(43) Gugler, R.; Leschik, M.; Dengler, H. J. Disposition of quercetin in man after single oral and intravenous doses. *Eur. J. Clin. Pharmacol.* **1975**, *9*, 229–234.

(44) Williams, R. J.; Spencer, J. P.; Rice-Evans, C. Flavonoids: antioxidants or signalling molecules? *Free Radic. Biol. Med.* **2004**, *36*, 838–849.

(45) Viswanathan, M.; Kim, S. K.; Berdichevsky, A.; Guarente, L. A role for *SIR-2.1* regulation of ER stress response genes in determining *C. elegans* life span. *Dev. Cell* **2005**, *9*, 605–615.

(46) Kong, A. N.; Owuor, E.; Yu, R.; Hebbar, V.; Chen, C.; Hu, R.; Mandlekar, S. Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (ARE/EpRE). *Drug Metab. Rev.* **2001**, *33*, 55–271.

(47) Walle, U. K.; Walle, T. Induction of human UDP-glucuronosyltransferase UGT1A1 by flavonoids — structural requirements. *Drug Metab. Dispos.* **2002**, *30*, 564–569.

(48) Chondrogianni, N.; Kapeta, S.; Chinou, I.; Vassilatou, K.; Papassideri, I.; Gonos, S. E. Anti-ageing and rejuvenating effects of quercetin. *Exp. Gerontol.* **2010**, *45*, 763–771.

(49) Chen, T. J.; Jeng, J. Y.; Lin, C. W.; Wu, C. Y.; Chen, Y. C. Quercetin inhibition of ROS-dependent and -independent apoptosis in rat glioma C6 cells. *Toxicology* **2006**, *223*, 113–126.

(50) Lee, D. H.; Szczepanski, M.; Lee, Y. J. Role of Bax in quercetin-induced apoptosis in human prostate cancer cells. *Biochem. Pharmacol.* **2008**, *75*, 2345–2355.

(51) Lee, Y. K.; Hwang, J. T.; Kwon, D. K.; Surh, Y. J.; Park, O. J. Induction of apoptosis by quercetin is mediated through AMPKα1/ASK1/p38 pathway. *Cancer Lett.* **2010**, *292*, 228–236.

(52) Shih, C. M.; Lin, H.; Liang, Y. C.; Lee, W. S.; Bi, W. F.; Juan, S. H. Concentration-dependent differential effects of quercetin on rat aortic smooth muscle cells. *Eur. J. Pharmacol.* **2004**, *496*, 41–48.