



Antioxidant and antiplatelet potential of different methanol fractions and flavonols extracted from onion (*Allium cepa* L.)

Eun Young Ko¹ · Shivraj Hariram Nile² · Yi-Sook Jung³ · Young Soo Keum²

Received: 18 June 2017 / Accepted: 23 February 2018 / Published online: 1 March 2018
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Abstract

In this study, we reported the potential of various methanol fractions of onion (MFO) and its components quercetin and quercetin glucosides on platelet aggregation and antioxidant activity. The onion extracts were separated into several fractions using methanol and water. Further, these extracts were analyzed using simple high-performance liquid chromatography–diode array detector method to separate quercetin (Q), quercetin-4'-*O*-monoglucoside and quercetin-3, 4'-*O*-diglucoside from onion sample. It was observed that the bioactive compounds were accumulated in the non-polar portions rather than in the polar one. MFO and flavonol glucosides inhibited collagen-induced platelet aggregation, and the anti-aggregatory effects were comparatively studied using rat PRP (platelet-rich plasma). Among the extracted compounds, quercetin was found as a potent inhibitor of platelet aggregation compared to quercetin glucosides, whereas quercetin glucosides showed high antioxidant activities. These results suggest that MFO, quercetin and quercetin glucosides have powerful antiplatelet and antioxidant activities. These studies provide possible information leading to the intake of onions rich in these flavonols as a dietary supplement and functional food ingredient to prevent thrombosis and cardiovascular and oxidative stress-related diseases and might be utilized as a real source of valuable phytochemicals for the pharmaceutical and food industries for the development of antioxidant, anticoagulant and antiplatelet agents.

Keywords Onion · Antiplatelet · Antioxidant · Quercetin · Quercetin glucosides · HPLC

Introduction

Onion (*Allium cepa* L.), family Liliaceae, consists of over 250 genera and 3700 species (Sharma et al. 2016). Onion as a vegetable has been widely consumed and cultivated worldwide and has shown increased production by > 25% in the past few years (FAO 2009). Onion extracts and the isolated bioactive compounds have a broad range of biological effects, including antioxidant, antiplatelet, antidiabetic, anticarcinogenic, antimicrobial, anti-inflammatory and antibiotic

effects on human health (Sharma et al. 2015; Nile et al. 2016). Onion comprises two principal groups of phytochemicals; flavonoids and alk(en)yl cysteine sulfoxides, which are proved to have beneficial effects on human health (Sharma et al. 2016). Onions are rich in the bioactive flavonols such as quercetin and quercetin glucosides, which have potential biological activities and also pronounced effect to allergies, asthma, arthritis, cancer, diabetic complications, and neurodegenerative and osteoporosis effects (Kempuraj et al. 2006; Sharma et al. 2016). Quercetin, quercetin 3, 4'-*O*-diglucoside and quercetin and quercetin 4'-*O*-monoglucoside are the primary glucosides of the mature onion bulb, accounting for about 99–95% of the total flavonol content (Perez-Gregorio et al. 2011). In the past, works have focused mainly on the antiplatelet activity of onion extracts on atherosclerosis and alterations in serum lipid profiles using various in vitro and in vivo studies (Ali et al. 2000). The onion extracts and their flavonols demonstrated significant inhibition toward platelet aggregation in vitro, and also several platelet inhibitors have been isolated and characterized from onion and garlic as vegetables (Ali et al. 1999). The underlying mechanisms of

✉ Shivraj Hariram Nile
nileshivraj@gmail.com; nileshivraj@konkuk.ac.kr

¹ Department of Food Science and Biotechnology
of Animal Resources, Konkuk University, Seoul 05029,
Republic of Korea

² Department of Bioresources and Food Science, College
of Life and Environmental Sciences, Konkuk University,
Seoul 05029, Republic of Korea

³ College of Pharmacy, Ajou University, Suwon 443749,
Republic of Korea

onion's antiplatelet effects have been suggested to include the inhibition of the release of arachidonic acid (AA) from phospholipids, through inhibiting thromboxane A₂ synthase activity (Moon et al. 2000). Furthermore, onion also repressed serum thromboxane B₂ (TXB₂) level in diabetic rats, whose level was elevated compared to that in normal rats (Jang et al. 2002). Onion is known to have a potential to reduce the detrimental effect of cardiovascular risk factors, and activation of blood platelets plays a crucial role not only in homeostasis but also in the pathological development of several cardiovascular disorders, including stroke and myocardial infarction (Dutta-Roy 2002). In support of the pathophysiological role of platelets, platelet inhibitory drugs such as aspirin affects the myocardial infarction incidents, stroke, and death from cardiovascular diseases (CVD) in secondary prevention trials (Ittaman et al. 2014). Therefore, the current research was oriented toward the analysis of onion quercetin glycosides and their utilization for inhibition of platelet aggregation reversibly without any side effects. This study aimed to measure the antiplatelet and antioxidant activity of methanol extract, followed by fractionation and identification of quercetin and quercetin glucosides based on activity-guided separation using onions.

Materials and methods

Plant material

Onion (*Allium cepa* L. cv. Sunpower) bulbs were collected from a onion cultivation and processing farm (Haenam,

South Korea) in June 2016, stored at 4 °C for 3 months and used for further studies.

Chemicals

All the chemicals used in this study were of HPLC analytical grade and purchased from Duksan Chemical Co. (Gyeonggi-do, Korea). Column chromatography was carried out on an RP-18 stationary phase (Cosmosil 75C₁₈-PREP, Kyoto, Japan). Thin layer chromatography (TLC) was performed on pre-coated Silica Gel 60 F₂₅₄ (0.25 mm, Merck, Germany) and RP-18F_{254S} plates (0.25 mm, Merck).

Extraction and fractionation of onion

The peeled onion (*Allium cepa* L. cv. Sunpower) bulbs (50 kg) were homogenized using 80% methanol three times at 25 °C and passed through a 1.0 mm sieve. The extracted sample was then transferred to 500 mL glass bottles and stored in the laboratory at − 20 °C until further analysis. The extract further fractionated into 18 fractions using different methanol concentrations (M2-1 253.2 g; M2-2 676.1 g; M2-3 777.4 g; M2-4 528.1 g; M2-5 672.5 g; M2-6 40.5 g; M20-1 3.6 g; M20-2 7.0 g; M20-3 3.5 g; M20-4 3.9 g; M50-1 4.0 g; M50-2 5.3 g; M50-3 4.8 g; M50-4 6.2 g; M100-1 1.0 g; M100-2 3.2 g; M100-3 1.2 g; M100-4 2.5 g). the extraction and fractionation methods for onion extract are described in Fig. 1. Each fraction were confirmed using thin layer chromatography (TLC) using pre-coated Silica-gel 60 F₂₅₄ (0.25 mm, Merck, Germany) and characterized using RP-18 column chromatography

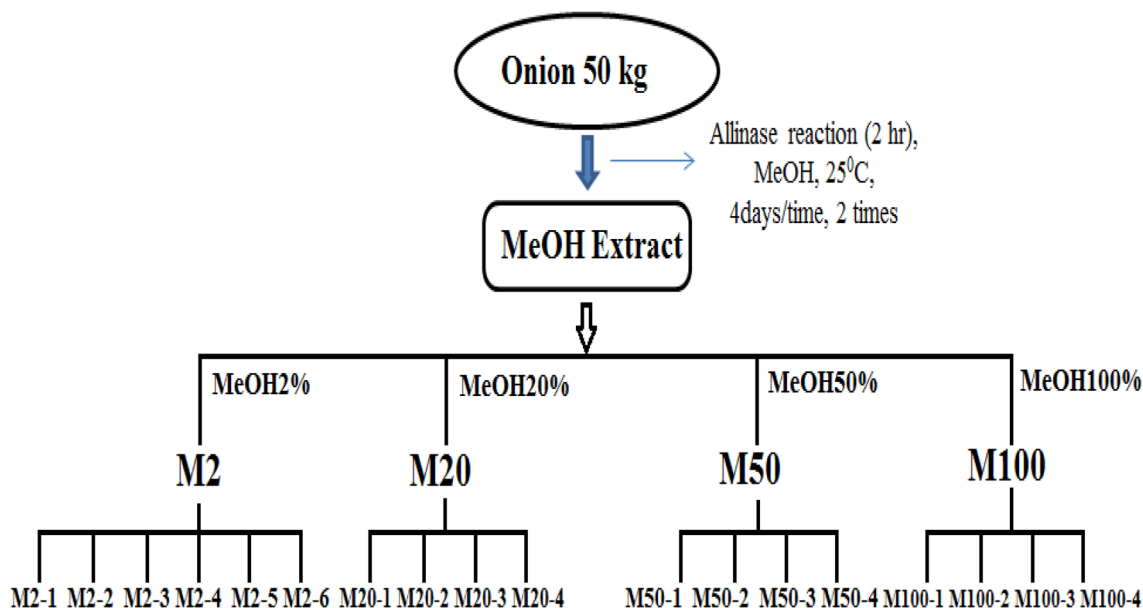


Fig. 1 Extraction and fractionation scheme for onion using methanol

(CC) with MeOH/H₂O mixture (2:98, 20:80, 50:50, 100:0; v/v) (Singh et al. 2009; Sharma et al. 2015). The extraction yield was calculated using the formula: Yield (%) = yield × 100/200, Table 1 (Nile and Park 2013).

HPLC analysis of onion fractions

The onion fractions were filtered through a 0.5 micro-filter unit (Whatman syringe filter PVDF) and collected and stored in 3 mL vial for HPLC analysis. The study was carried out on an Agilent 1100 chromatograph with a DAD detector (Agilent Technologies, USA). The column was Agilent Zorbax C18 (250 × 4.6 mm; particle size 5 mm). HPLC analytical conditions were maintained as described here. The mobile phase consisted of solvents A and B. Solvent A was 0.2% TFA in H₂O, and solvent B was methanol (100%). Each elution step was followed as: step 1 0.0–5.0 min, isocratic elution at 5% B; step 2 5.0–20.0 min, linear gradient elution from 5 to 50% B; step 3 20.0–35.0 min, isocratic elution at 50% B; step 4 35.0–38.0 min, isocratic elution at 100% B; step 5 38.0–45.0 min, isocratic elution at 5% B. Sample flow rate was maintained at 1.0 mL/min, with 20 µL sample injection and, finally, the compounds were detected using HPLC–photodiode array (PDA) detector (Sharma et al. 2014; Ko et al. 2015).

Table 1 Yields of onion extract fractions using methanol as solvent

Fractions	Weight (g)	Yield (%)
M2-1	253.2	8.5
M2-2	676.1	22.6
M2-3	777.4	26.0
M2-4	528.1	17.6
M2-5	672.5	22.5
M2-6	40.5	1.4
M20-1	3.6	0.1
M20-2	7.0	0.2
M20-3	3.5	0.1
M20-4	3.9	0.1
M50-1	4.0	0.1
M50-2	5.3	0.2
M50-3	4.8	0.2
M50-4	6.2	0.2
M100-1	1.0	0.0
M100-2	3.2	0.1
M100-3	1.2	0.0
M100-4	2.5	0.1
Total	2994.0	100

Antioxidant activity

The antioxidant activity of different methanol fractions of onion (MFO) and its extracted components, quercetin and quercetin glucosides, was evaluated for DPPH and ABTS radical scavenging, FRAP reducing power and oxygen radical absorbance capacity (ORAC) by antioxidant assays as described previously by Nile and Park (2015) and Thaipong et al. (2006). The antioxidant capacities were expressed as equivalents of gallic acid (mg GAE/g extract) and trolox (mgTE/g extract).

Preparation of platelets

Sprague–Dawley rats (Daehan Laboratory Animal Center, Korea) weighing 200–250 g were anesthetized with ethyl ether. Blood was collected in a syringe containing 3.8% sodium citrate (1:9, v/v) from the abdominal aorta and then centrifuged at 150g for 10 min at room temperature. The supernatant (platelet-rich plasma, PRP) obtained was used in the aggregation study. The platelet count in PRP was finally adjusted to about 2×10^8 cells/mL with Tyrode solution (pH 7.4, NaCl 11.9 mM, KCl 2.7 mM, MgCl₂ 2.1 mM, NaH₂PO₄ 0.4 mM, NaHCO₃ 11.9 mM, glucose 11.1 mM) containing bovine serum albumin (3.5 mg/mL) (Kim et al. 2016).

Platelet aggregation assay

The platelet aggregation turbidometric assay was performed for onion fractions and quercetin glucosides as described by Yu et al. (2011). After 5 min incubation of PRP at 37 °C in the presence or absence of onion fractions and QMG, QDG and Q, collagen (6 µg/mL) was added to trigger aggregation. Further, the extent of aggregation was measured by a Lumi-Aggregometer (Chrono-Log Co., PA, USA).

Statistical analysis

All experiments were repeated in triplicate, and resulting data were recorded as mean ± SD. In HPLC analysis, the sample was injected three times for uniform analysis and detection of compounds, and the average peak areas were used to calculate the analyte concentration. For antioxidant results, the Fisher LSD test was employed to calculate the differences between the mean values by considering the significance level of $P < 0.05$. The calculations were done using Origin-Pro 8.1 software (Origin Lab; Northampton, MA, USA).

Results and discussion

Analysis of onion fractions by HPLC

A typical HPLC chromatogram of methanol extracts of onion (*Allium cepa* L.) was detected at 254 nm. The HPLC data showed three main quantified chromatographic peaks corresponding to quercetin (Q), quercetin-4'-*O*-monoglucoside (QMG) and quercetin-3, 4'-*O*-diglucoside (QDG) depending on the retention time of each compound in the onion extract and the structural details are provided in Fig. 2. Quercetin exists in the free and conjugated form attached to the carbohydrate moiety, mainly as glucoside which represents approximately 80% of the total flavonol content of onion bulb chemical composition (Sharma et al. 2014; Ko et al. 2015). The quantified peaks were in good concordance with previous results (Bonaccorsi et al. 2008, Lee and Mitchell 2011; Sharma et al. 2015). The extracted onion fractions showed a significant amount of quercetin and quercetin glucosides, except for M2-1, M2-2, M2-3, M2-4, M2-5, M2-6 and M20-1 (Table 2). The major peaks of the M2 fraction appeared within 20 min, those of M20 fraction in 14–20 min, those of M50 fraction in 15–25 min and the peaks of M100 fraction were eluted after 25 min.

Antioxidant activity

In this study, four methods were analyzed for antioxidant activity determination of onion extracts and the extracted flavonols, viz., DPPH and ABTS radical scavenging, FRAP reducing power and oxygen radical absorbance capacity (ORAC) assay. Table 3 presents the results of antioxidant activity in which all onion fractions and extracted flavonols showed significant antioxidant activities. Flavonols such as QMG (75.2, 80.1, 72.5 and 64.2%), QDG (70.4, 75.5, 66.4 and 60.9%) and Q (62.8, 70.6, 58.9 and 52.5%) showed potential inhibition toward DPPH, FRAP, ABTS, and ORAC radical assays, respectively. For the antioxidant results, we found that M50-2, M50-3, M50-4, M100-1, M100-2, M100-3 and M100-4 out of these 18 fractions showed > 40% inhibition for DPPH, FRAP, ABTS and ORAC radical assays. The results indicate that the fractionation using methanol provided the basis for bioactivity-guided extraction of onion flavonols, quercetin-3, 4'-*O*-diglucoside (QDG), quercetin-4'-*O*-monoglucoside (QMG) and quercetin (Q), using HPLC. However, quercetin glucosides had high antioxidant activity compared to quercetin in all antioxidant assays tested. The antioxidant activity of *Allium* species mainly persisted due to the presence of significant amount of organosulfur compounds and their precursors (Sharma

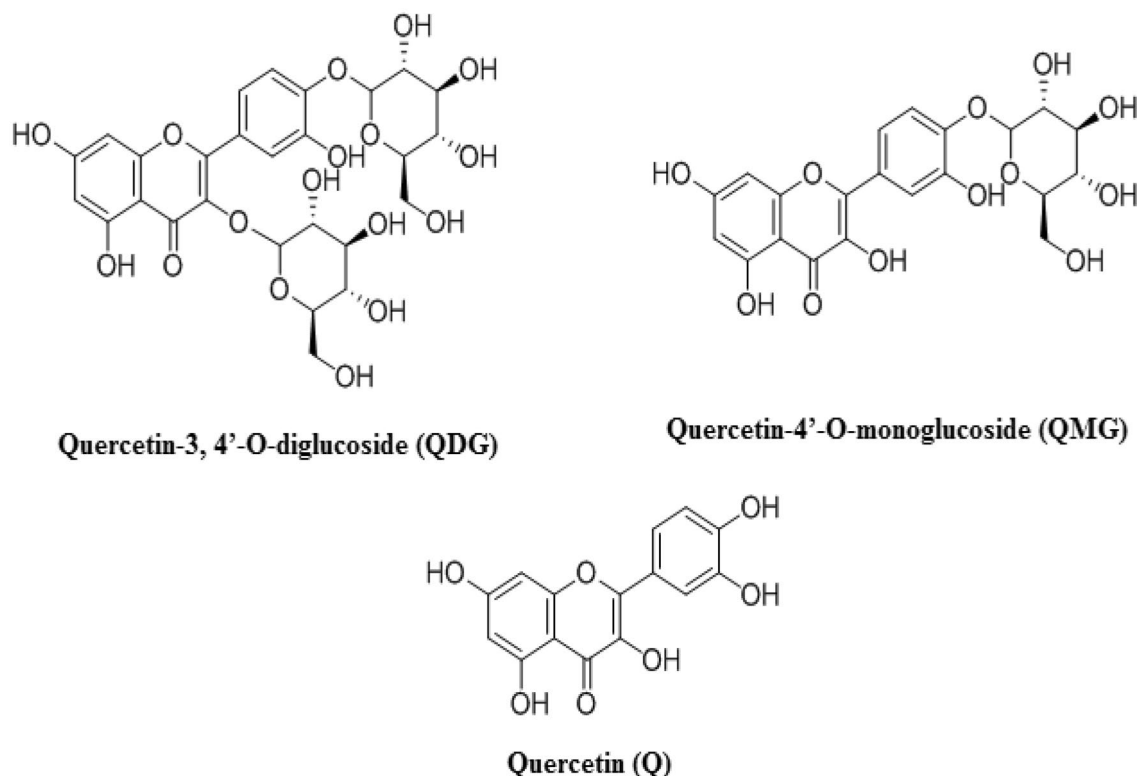


Fig. 2 Structures of quercetin-3, 4'-*O*-diglucoside (QDG), quercetin-4'-*O*-monoglucoside (QMG) and quercetin (Q) from onion

Table 2 Flavonol glucoside composition expressed as dry weight (mg/100 g) for each methanol fraction of onion extract

Samples	Concentration (mg/100 g) DW		
	(QDG)	(QMG)	(Q)
MeOH extract (MT)	155.8.34 ± 7.6	245.98 ± 8.5	64.12 ± 3.4
M2-1	LC	LC	LC
M2-2	LC	LC	LC
M2-3	LC	LC	LC
M2-4	LC	LC	LC
M2-5	54.68 ± 1.1	70.61 ± 1.4	LC
M2-6	51.61 ± 2.9	58.74 ± 2.7	LC
M20-1	65.81 ± 1.2	90.40 ± 1.7	11.78 ± 1.3
M20-2	74.89 ± 1.6	102.22 ± 3.1	14.78 ± 1.6
M20-3	68.45 ± 1.3	98.64 ± 2.7	12.92 ± 1.5
M20-4	60.22 ± 2.1	81.63 ± 1.6	10.10 ± 1.1
M50-1	96.51 ± 3.2	156.34 ± 1.4	30.42 ± 1.7
M50-2	90.42 ± 1.4	148.88 ± 2.3	26.78 ± 1.1
M50-3	85.97 ± 3.4	130.68 ± 2.7	20.12 ± 1.8
M50-4	80.16 ± 1.9	118.60 ± 1.5	16.08 ± 1.4
M100-1	145.61 ± 2.4	210.23 ± 2.4	60.54 ± 1.6
M100-2	132.33 ± 1.1	195.67 ± 1.8	54.61 ± 1.9
M100-3	100.20 ± 1.8	160.81 ± 2.3	35.44 ± 2.3
M100-4	87.63 ± 1.4	142.64 ± 3.1	20.86 ± 1.6

Values are expressed as the mean ± SD (standard deviation). Quercetin-3, 4'-*O*-diglucoside (QDG), quercetin-4'-*O*-monoglucoside (QMG) and quercetin aglycone (Q), LC low concentrations [i.e., ≤ 10 (Q), ≤ 50 (QMG and QDG) mg/100 g DW], respectively

DW Dry weight

et al. 2014). On the contrary, Lee et al. (2014) reported that flavonols present in onions were a rich source of phenolics and play key roles in the promising antioxidant activities caused by reactive oxygen species as compared to other onion bioactive compounds. The previously reported studies on onions showed that they had a higher antioxidant capacity, which was attributed to their flavonoid content and correlated using the bioactive chemical composition and biological activity of onions (Santas et al. 2010). The reduction of DPPH radical is indicative of the capacity of the onion peel extract to scavenge free radicals, independently of enzymatic activity (Lue et al. 2010). However in FRAP, ABTS and ORAC, the onion extracts and bioactive compounds showed that the antioxidant actions were ascribed to their radical scavenging activity, chelation of redox-active metal ions and reducing power (Shon et al. 2004).

Inhibitory effect of onion methanol fractions on platelet aggregation

Onion extract was prepared in 100% methanol, and the same extract was separated using column chromatography with

a Cosmosil 75C₁₈-PREP cartridge. A total of 18 fractions were collected from the methanol extract of onion with 2, 20, 50 and 100% of methanol solution used as eluents, respectively. These fractions were tested for platelet aggregation of rat PRP. It was observed that about 60% of these fractions showed significant inhibition of platelet aggregation. Figure 3 demonstrates that 11 fractions (M20-2, M20-3, M20-4, M50-1, M50-2, M50-3, M50-4, M100-1, M100-2, M100-3 and M100-4) out of these 18 fractions showed 100% inhibition of platelet aggregation. The onion fractions at different levels were used for inhibiting platelet aggregation with 6 µg/mL of collagen. The anti-aggregation effects were comparatively studied for all the fractions using rat PRP. A fraction with various concentrations showed different inhibition effects with respect to percentage inhibition. In 0.5 mg/mL, M100-1 and M100-2 were effective in platelet aggregation, whereas the others showed no effect. In 1 mg/mL, the fraction showed different inhibitory effects on platelet aggregation as compared to 3 and 5 mg/mL. In this case, only M50-3, M50-4, M100-3, M100-4, M100-3 and M100-4 had more than 80% inhibition effect, and the others, namely, M20-2, M20-3, M20-4, M50-1, and M50-2, had less than 20% inhibition effect on platelet aggregation. M100-1 and M100-2 showed 100% inhibitory effect on platelet aggregation. Similarly, it was observed that these fractions were more effective in 3 mg/mL compared to in 1 mg/mL. The fractions M20-4, M50-1, M50-2, M50-3, M50-4, M100-1, M100-2, M100-3 and M100-4 had more than 80% inhibition effect, whereas M20-1 and M20-2 were much less effective on platelet aggregation. Among all the studied onion methanol fractions, M100-1 and M100-2 were most effective on collagen-induced aggregation, whereas M2-1, M2-2, M2-3, M2-4, M2-5, M2-6, M20-1 and M20-2 showed no inhibitory effect. It has been reported that the chloroform extract of onion with the least polar fraction was more effective for platelet aggregation in both human and animal subjects (Makheja and Bailey 1990). According to Ro et al. (2015), the ethanol fractions of onion suppressed antiplatelet effects significantly in rat platelets induced by collagen in a dose-dependent manner. Hubbard et al. (2004) reported that onion with high quercetin content inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. It was confirmed that some plants of *Allium* species were effective in reducing these antiplatelet aggregations against AA and adenosine diphosphate (ADP) as platelet aggregation inducers (Lorigooini et al. 2015).

Inhibitory effect of onion fractions M100-1 and M100-2 on platelet aggregation

It was observed that fraction M100-1 and M100-2 showed significant effects on the inhibition of platelet aggregation

Table 3 Antioxidant activity of different fractions of onions extracted with methanol

Samples	Antioxidant activity (%)			
	DPPH (mg TE/g extract)	FRAP (mg TE/g extract)	ABTS (mg GAE/g extract)	ORAC (mg GAE/g extract)
MeOH extract	67.9 ± 1.6 ^{bc}	72.6 ± 1.4 ^{bc}	61.8 ± 1.1 ^c	58.9 ± 2.3 ^b
M2-1	ND	ND	ND	ND
M2-2	ND	ND	ND	ND
M2-3	ND	ND	ND	ND
M2-4	ND	ND	ND	ND
M2-5	22.1 ± 1.6 ⁿ	25.6 ± 0.9 ⁿ	15.4 ± 1.1 ⁿ	12.9 ± 1.9 ^{lm}
M2-6	18.5 ± 1.3 ^o	20.1 ± 0.6 ^o	12.8 ± 0.8 ^m	10.1 ± 0.9 ⁿ
M20-1	27.9 ± 0.4 ^m	30.5 ± 1.3 ^m	20.9 ± 1.1 ^l	15.8 ± 0.8 ^l
M20-2	38.6 ± 0.9 ^j	45.2 ± 1.1 ^j	31.9 ± 2.6 ⁱ	29.6 ± 0.6 ⁱ
M20-3	35.1 ± 1.2 ^{ik}	41.9 ± 1.6 ^k	28.4 ± 0.4 ^j	25.9 ± 0.8 ^j
M20-4	30.2 ± 1.1 ^l	36.9 ± 1.5 ^l	25.5 ± 1.2 ^k	20.7 ± 1.7 ^k
M50-1	42.4 ± 1.6 ⁱ	49.6 ± 1.1 ⁱ	35.5 ± 0.8 ^h	36.9 ± 1.6 ^h
M50-2	52.6 ± 0.6 ^{ef}	60.9 ± 2.2 ^e	51.1 ± 0.9 ^e	45.5 ± 1.4 ^f
M50-3	48.8 ± 1.2 ^g	54.1 ± 2.3 ^g	47.5 ± 1.2 ^f	41.2 ± 1.1 ^g
M50-4	45.5 ± 1.7 ^h	51.9 ± 1.9 ^h	40.9 ± 0.9 ^g	38.1 ± 1.1 ^h
M100-1	65.3 ± 1.4 ^c	70.8 ± 1.3 ^c	60.7 ± 1.8 ^c	55.7 ± 1.4 ^d
M100-2	60.9 ± 1.3 ^d	67.5 ± 1.5 ^d	55.2 ± 1.9 ^d	50.9 ± 2.6 ^e
M100-3	55.2 ± 1.9 ^e	61.3 ± 1.1 ^e	50.8 ± 1.3 ^e	46.1 ± 1.1 ^f
M100-4	50.9 ± 1.7 ^f	58.5 ± 1.1 ^f	46.8 ± 1.8 ^f	40.9 ± 1.8 ^g
QMG	75.2 ± 2.3 ^a	80.1 ± 1.9 ^a	72.5 ± 1.6 ^a	64.2 ± 1.9 ^a
QDG	70.4 ± 1.1 ^b	75.5 ± 2.3 ^b	66.4 ± 2.6 ^b	60.4 ± 1.4 ^b
Q	62.8 ± 1.3 ^d	70.6 ± 2.1 ^c	58.9 ± 1.9 ^c	52.1 ± 1.1 ^c

Gallic acid: (mg GAE/g extract), trolox: (mg TE/g extract), equivalent, (DW) dry weight, ND not detected ($\leq 10\%$), mean \pm SD, $n = 3$; values bearing different letters in the same column are significantly different ($P < 0.05$)

in a dose-dependent manner in the range of 0.5–5 mg/mL compared to the other fractions (Fig. 4a). Both the fractions inhibited 100% of platelet aggregation, but M100-2 seemed to be more potent against platelet aggregation as compared to M100-1. The M100-2 fraction was sub-divided to three fractions using preparative HPLC. The fractions were named M100-2A, M100-2B and M100-2C, respectively. These three fractions were found to have quercetin and quercetin glucosides. All the three fractions were tested with 6 μ g/mL collagen on rat blood, and the same was used for the rest of the experiments. Accordingly, M100-2B showed a 100% inhibitory effect at 0.5 mg/mL concentration on platelet aggregation, which implied that quercetin was more efficient than its glucosides against platelets aggregation. The fractions M100-2A and M100-2C were supposed to have quercetin glucosides as major compounds and were less useful than M100-2B (Fig. 4b). Goldman et al. (1996) reported that the antiplatelet activity of onion is due to the presence of organosulfur compounds. The allicin or paraffinic polysulfides from onions were found to be potent inhibiting factors toward ADP, AA and collagen-induced platelet aggregation (Makheja and Bailey 1990). Adenosine and paraffinic

polysulfides (PPS) from onions were purported to have strong antiplatelet effects (Yin and Cheng 1998). Thus, the organosulfur compounds present in *Allium* species are also considered to be major compounds with antiplatelet activity. In particular, a class of α -sulfinyl-disulfides (cepaenes) compounds has demonstrated antithrombotic activity (Block et al. 1997).

Inhibitory effect of quercetin glycosides on platelet aggregation

Inhibition of platelet aggregation was tested with different concentrations of quercetin and its glycosides. Figure 5 demonstrates the inhibitory effect of platelet with 0.5, 1 and 2 mg/mL of quercetin (Q), quercetin-4'-O-monoglucoside (QMG) and quercetin-3, 4'-O-diglucoside (QDG). It was observed that the inhibitory effect of quercetin was increased in a dose-dependent manner; 2.0 mg/mL showed 100% inhibition of platelet aggregation, whereas 0.5 mg/mL almost had no effect. Similarly, it was observed that 2.0 mg/mL of quercetin glucosides exhibited 100% inhibitory effects on platelet aggregation.

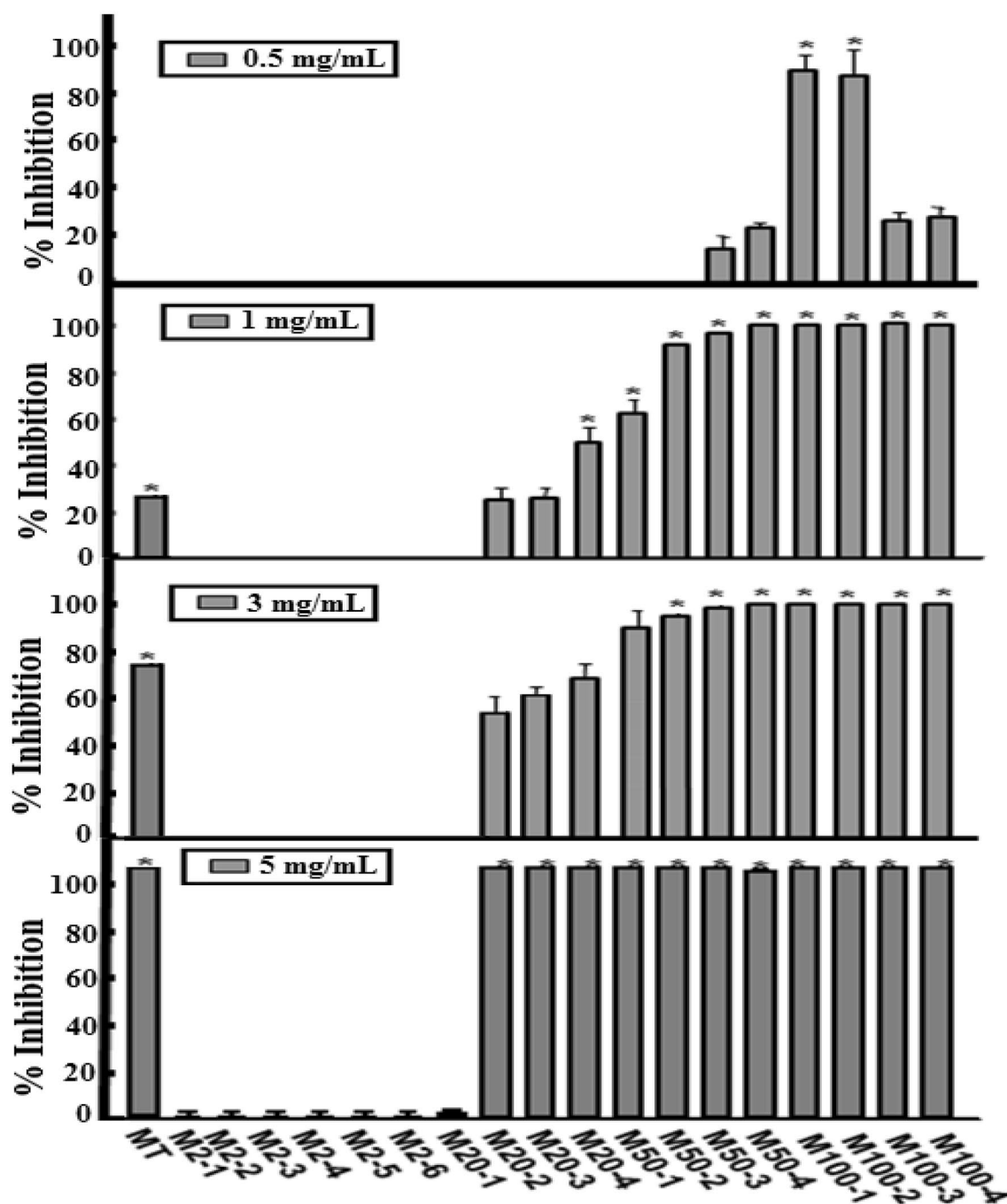
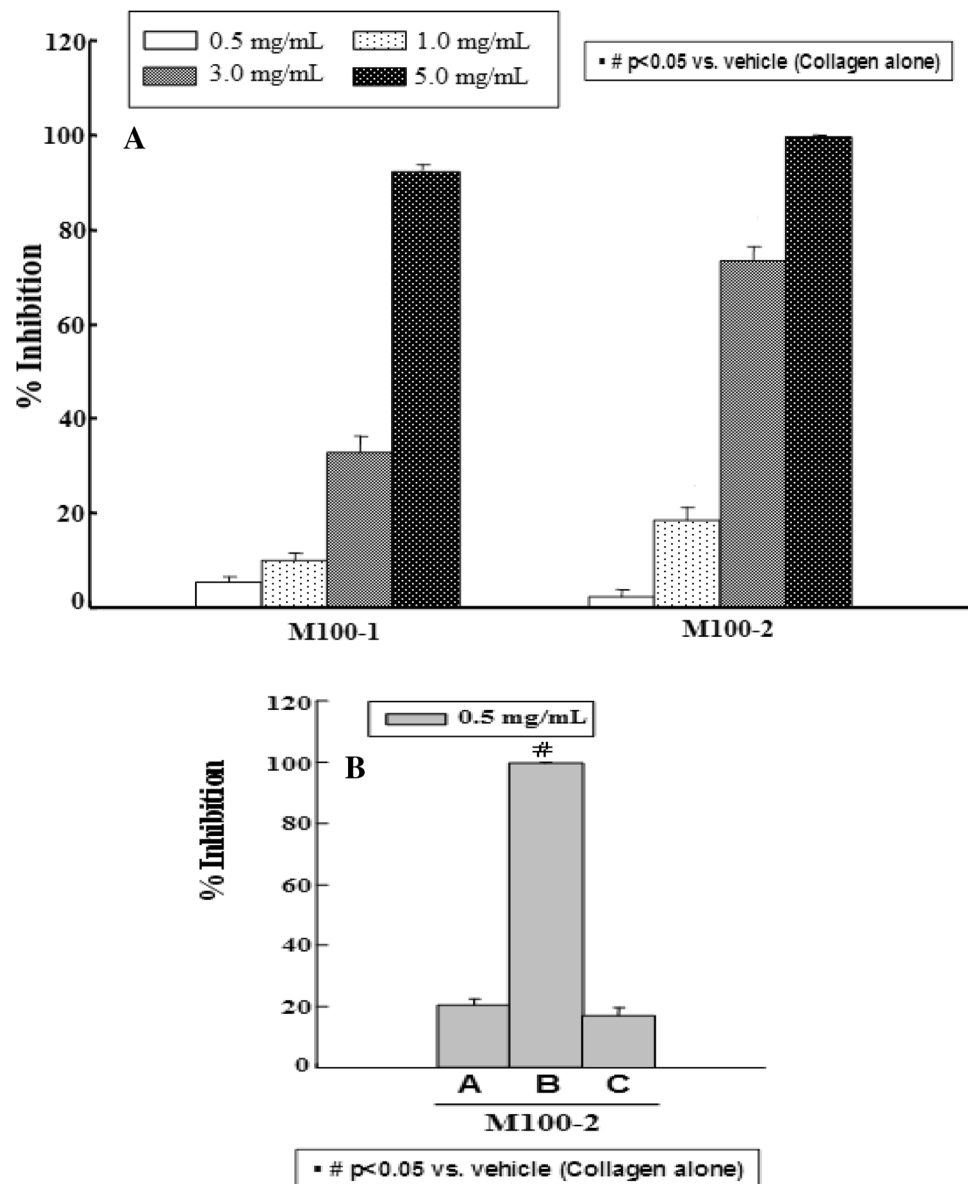


Fig. 3 Inhibitory effect of fractions on platelet aggregation. Aggregation was induced by 6 $\mu\text{g/mL}$ collagen in platelet-rich plasma from healthy rats. Data represent mean \pm SEM ($n = 3$). * $P < 0.05$ vs. vehicle

However, both quercetin glucosides showed a different effect in 1 and 0.5 mg/mL, that is, quercetin-4'-*O*-monoglucoside (QMG) was more effective than quercetin-3, 4'-*O*-diglucoside (QDG) in the inhibition of platelet aggregation. From these results, it was observed that quercetin showed significantly higher antiplatelet activity than its glucosides. The mechanism of the anti-aggregating activity of flavonoids was studied *in vitro* and revealed that

the aglycone of flavonoids in general and the flavanone derivatives that were tested did not affect platelet function. On the other hand, flavones such as chrysin, apigenin and phloretin also inhibited platelet aggregation. Myricetin and quercetin had an adamant anti-aggregating activity against AA, but these compounds were found to be almost ineffective against the collagen-induced aggregation system (Bojić et al. 2011; Wright et al. 2013).

Fig. 4 **a** Inhibitory effect of onion methanol fractions M100-1 and M100-2 on platelet aggregation. **b** Inhibitory effect of M100-2A, B and C fractions on platelet aggregation. Aggregation was induced by collagen (6 $\mu\text{g}/\text{mL}$) in platelet-rich plasma from healthy rats. Data represent mean \pm SEM ($n = 3$)



A recent work on onion has focused largely on the antiplatelet activity, atherosclerosis and alterations in serum lipid profiles (Ali et al. 2000). Studies on the action of onion extracts on platelet aggregation suggest that the release of arachidonic acid from phospholipids, which initiates eicosanoid metabolism in mammals leading to prostaglandins, thromboxanes and leukotrienes synthesis, is inhibited as is thromboxane A_2 synthase activity (Moon et al. 2000). Raw extracts of onions mostly lower systolic blood pressure and prolong bleed times in rats, possibly through inhibition of platelet function and suppression of thromboxane production (Chen et al. 2000). Antiplatelet activity is significantly affected by genotype, environment and duration of storage of onions, also considered to be a

property of the organosulfur compounds, in particular, a class of sulfinyl-disulfides (Griffiths et al. 2002).

Conclusions

In this study, onion flavonols such as quercetin and quercetin glycosides were maximum in non-polar fractions rather than in aqueous soluble fractions, and a significant amount of these flavonols was recovered from onion methanol extracts and fractions collected using HPLC analysis. Flavonol quercetin has a significant antiplatelet activity compared to quercetin glucosides, and the mixture of quercetin with its glucosides was more efficient against platelet aggregation

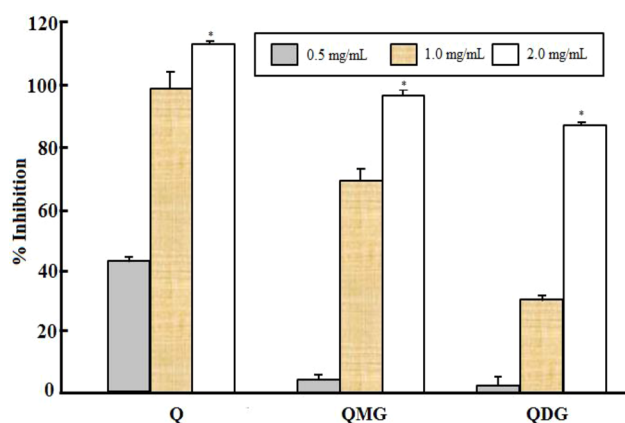


Fig. 5 Inhibitory effect of quercetin and its glycosides on platelet aggregation [aggregation was induced by collagen (6 μ g/mL) in platelet-rich plasma from healthy rats]. Data were expressed as mean \pm SEM values ($n = 7-9$). * $P < 0.05$ vs. vehicle

than quercetin, though the synergistic effects were not fully understood. All the onion methanol fractions, quercetin (Q), quercetin-4'-O-monoglucoside (QMG) and quercetin-3,4'-O-diglucoside (QDG), revealed excellent antioxidant activity. The data obtained using onion (*Allium cepa* L. cv. Sunpower) bulb extracts and their flavonol glycosides might be utilized as a real source of the valuable phytochemicals which can be used in pharmaceutical and food industries for the development of antioxidant, anticoagulant and antiplatelet agents. This study provided possible information of the intake of onions rich in quercetin and quercetin glucosides as a dietary supplement and functional food, to prevent thrombosis and cardiovascular- and oxidative stress-related diseases.

Acknowledgements This research was supported by the (KU) Research Professor Program of Konkuk University, Seoul (South Korea), 2018.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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