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Phenolic Profile, Antioxidant Property, and Anti-influenza Viral Activity of Chinese Quince (*Pseudocydonia sinensis* Schneid.), Quince (*Cydonia oblonga* Mill.), and Apple (*Malus domestica* Mill.) Fruits

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To evaluate the phenolic extracts of Chinese quince, quince, and apple fruits, their phenolic profiles, antioxidant properties, and anti-influenza viral activities were investigated. Chinese quince had the largest amount of phenolics consisting mainly of high polymeric procyanidins. Quince had considerable amounts of hydroxycinnamic derivatives mainly composed of 3-caffeoylquinic acid and 5-caffeoylquinic acid and polymeric procyanidins. Apple (cv. Fuji) had the lowest amount of phenolics, mainly 5-caffeoylquinic acid and monomeric and oligomeric procyanidins. The antioxidant functions of Chinese quince and quince phenolic extracts were superior to that of chlorogenic acid standard or ascorbic acid evaluated in both the linoleic acid peroxidation system and the DPPH radical scavenging system. However, those extracts were less effective than apple phenolics or (−)-epicatechin in linoleic acid peroxidation system. On the other hand, Chinese quince phenolics showed the strongest anti-influenza viral activity on the hemagglutination inhibition test.

KEYWORDS: Chinese quince; quince; apple; fruit extracts; phenolic profile; procyanidins; degree of polymerization; hydroxycinnamic acids; antioxidant activity; free radical scavenging activity; anti-influenza viral activity

INTRODUCTION

Chinese quince (*Pseudocydonia sinensis* Schneid. = *Chaenomeles sinensis* Koehne) and quince (*Cydonia oblonga* Mill.) fruits are not consumed fresh because of their strong acidity, astringency, and hard flesh. Quince fruit can be consumed cooked or processed as a jam or fruit jelly of the flesh; however, Chinese quince is much tougher than quince, and its consumption remains difficult even when cooked. Both of them are often processed as traditional fruit liquors in Japan. It is believed that the liquors can relieve a cough and clear the throat of phlegm. Moreover, extracts of the fruits are used industrially in candies or products such as glutinous starch syrup, and they are sold with a health improvement claim. Because the characteristics of the fruits and their methos of use are similar, quince and Chinese quince fruits are often confused in Japan.

Dried fruit of Chinese quince has been used as a material of traditional Chinese medicine. Osawa et al. (*1*) investigated the in vitro anti-inflammatory effect of dried Chinese quince phenolics extracted with 30% ethanol and showed that a polyphenol with a high molecular weight constituted of (+)-catechin and (−)-epicatechin (procyanidin) was the most effective compound.

Procyanidins are the most abundant of the proanthocyanidins, which are polymers constituted of a variable number of flavan-3-ol units. Proanthocyanidin oligomers and polymers are called condensed tannins (*2*).

Although proanthocyanidins are the main factor of the astringency in fruits, these compounds show beneficial properties such as potent antioxidant activity (*3*−*6*) and free radical scavenging activity (*7*, *8*). They inhibit platelet aggregation (*9*) and the oxidation of low-density lipoproteins and present antiulcerogenic activity against stomach mucosa injury (*10*). They show anti-inflammatory (*1*), antihypertensive (*11*), and antimutagenic (*12*, *13*) properties. It was also observed that proanthocyanidins from grape seeds (*Vitis vinifera*) present radioprotective effects against chromosomal damage induced by X-rays (*14*). Most of these properties seemed to be due to antioxidative activities, which are strongly related to their structural characteristics (*15*). These compounds are normally present as a mixture of different forms in nature.

A recent study showed that some phenolic extracts such as green tea, black tea, and guava leaf extract had antiviral activity against influenza virus and indicated that compositional difference of phenolics strongly affected the inactivation of the virus

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(*16*, *17*). However, anti-influenza viral activity of fruit extracts, especially from Chinese quince or quince fruits, is not reported yet.

To evaluate Chinese quince and quince fruit extracts, as a medical resource, it is necessary to know their phenolic profiles and the functions of these phenolics. In this work, the phenolic content and composition of Chinese quince and quince fruits were investigated, and apple was studied as a reference fruit. Antioxidant property and antiviral activity of flesh extracts were also measured. The relationship between the functions and phenolic profiles of the fruits will be discussed, with the hydroxycinnamic content and the mean degree of polymerization of procyanidins taken into account.

MATERIALS AND METHODS

Samples. The ripe fruits of Chinese quince (ver. Toukarin) and apple (cv. Fuji) were obtained from the Education and Research Center of Alpine Field Science of the Faculty of Agriculture, Shinshu University. The quince (cv. Smyrna) fruits were obtained from a local market in Nagano prefecture (Japan).

Standards. (−)-Epicatechin was purchased from Sigma Chemical Co. (St. Louis, MO). (+)-Catechin and 5-caffeoylquinic acid (chrologenic acid) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). 3-Caffeoylquinic acid was prepared by collection from quince fruit extract using an HPLC (Shimadzu LC-VP System) equipped with fraction collector (FRC-10A). Procyanidin B_2 was purchased from Asahi Breweries, Ltd. (Tokyo, Japan).

Solvents and Reagents. Methanol, acetone, acetonitrile, glacial acetic acid, 1-butanol, and petroleum ether were purchased from Nacalai Tesque. Folin−Ciocalteu reagent, vanillin, toluene-α-thiol, sodium dodecyl sulfate (SDS), linoleic acid, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Nacalai Tesque.

Extraction of Phenolic Compounds. Each flesh sample (5 g) was homogenized in 20 mL of aqueous acetone (80% v/v) and then filtrated under vacuum. The residue was re-extracted with 30 mL of aqueous acetone (60% v/v). The combined extracts were evaporated under reduced pressure at 40 °C until all organic solvent was removed. The concentrate was washed three times with petroleum ether to remove lipids and finally brought to a fixed volume of aqueous solution (fresh extract). The extraction and sample preparation were performed with five replications.

Colorimetric Quantification of Phenolic Compounds. The experimental procedures used to quantify the total phenolic content, vanillin-reactive compounds (flavan-3-ol derivatives) content and estimate the total procyanidin content in the quince and Chinese quince fruits were adapted from those of Hamauzu and Hanakawa (*19*). Total phenolic content was determined for the flesh extract according to the Folin−Ciocalteu method (*20*) using Folin−Ciocalteu reagent as follows. To 2 mL of solution was added 2 mL of Folin−Ciocalteu reagent. The mixture was homogenized and then a pause of 3 min was allowed for reaction, after which 2 mL of Na_2CO_3 (10 g/100 mL) was added, and the mixture was homogenized. It was then incubated for 60 min at room temperature. The absorbance was measured at 700 nm with a Shimadzu UV-1200 spectrophotometer (Tokyo, Japan) against a blank (2 mL of deionized water, plus reagents) in the reference cell. (−)-Epicatechin was used as a standard. The determination was carried out in duplicate per flesh extract. The content of flavan-3-ol derivatives (catechins and procyanidins) was determined by vanillin−sulfuric acid method (*21*, *22*) using (−)-epicatechin as a standard. The relative amount of total procyanidin was determined according to a butanol−HCl assay as follows. The 0.25 mL of aqueous extract was mixed with 0.25 mL of methanol in a screw-cap test tube, put together with 7 mL of butanol−HCl reagent prepared according to the method of Porter et al. (*23*), and heated in boiling water for 40 min. Absorbance at 550 nm was measured. The determination was carried out in duplicate per flesh extract.

Thioacidolysis Conditions. The methodology for thioacidolysis of phenolic compounds was adapted from that of Guyot et al. (*18*). Prior to the thioacidolysis of flesh extract, a solid-phase extraction with a Sep-Pak Plus C18 cartridge was conducted to purify phenolic compounds. The sugars of the sample loaded onto the cartridge (1−2 mL) were eluted with 10 mL of dilute acetic acid (2.5% v/v in water), and then the cartridge was dried with a N_2 gas stream, and the phenolic fractions were eluted with 2 mL of 0.2 M HCl in methanol. In a screw-cap test tube, 100 μL of the solution was mixed together with 100 μL of toluene-α-thiol (5% v/v in methanol). Reactions were carried out at 50 °C for 5 h (until the increase of peak area corresponding to extension units reached a plateau).

Estimation of the Mean Degree of Polymerization of Total Procyanidins. The relative degree of polymerization of total procyanidins was assessed by colorimetric assay, and the mean degree of polymerization (mDP) was determined by HPLC analysis of all the flavan-3-ol units after thioacidolysis. Because the ratio of the absorbance measured with the butanol−HCl assay to the concentration determined by the vanillin assay should increase as polymer length increases (*24*), the relative degree of polymerization (RDP) was calculated as below.

$$\mathrm{RDP} = 100 \times (A_{550} \text{ by butanol−HCl assay})/(\text{mg/100 g of FW by vanillin assay})$$

The mean degree of polymerization was determined by calculating the molar ratio of all the flavan-3-ol units (thioether adducts plus terminal units) to (−)-epicatechin and (+)-catechin corresponding to terminal units after thioacidolysis as described in Guyot et al. (*18*). The standard curve for (−)-epicatechin benzyl thioether was made by thioacidolysis of procyanidin B_2 standard.

Reversed-Phase HPLC Conditions. Chromatographic separation was carried out on a Luna 5μ C18 column (150 × 4.6 mm, Phenomenex) at 40 °C using two solvents: 0.1% aqueous phosphoric acid (A) and 0.1% phosphoric acid in acetonitrile (B); starting with 5% acetonitrile and installing a gradient to obtain 15% B at 30 min, 32% B at 35 min, 40% B at 45 min, and 75% B at 50 min and maintained to 65 min. The flow rate was 1.0 mL/min, and the injection volume was 20 μL. Detection was carried out at 280 and 325 nm on a Shimadzu SPD-M10Avp photodiode array detector.

Identification and Quantification of Phenolic Compounds. Identification was achieved by comparing retention times and UV spectra with those of standards, or those of previously purified and identified procyanidins, recorded under the same chromatographic conditions. The HPLC-MS system described below was also used.

Quantitative determinations were made using the external standard method with commercial standards. The dimers, oligomers, and the sum of highly polymerized procyanidins were quantified as (−)-epicatechin. The calibration curves were obtained by injection of different volumes of standard solutions under the same conditions as for the samples analyzed. Wavelengths used for quantification were 280 nm for flavan-3-ols and 325 nm for hydroxycinnamates.

HPLC-MS System for Qualitative Analysis. Chromatographic separation was carried out as described above using the HPLC system equipped with a UV detector and a mass detector in series (Agilent 1100 series LC-MS). It consisted of a G1322A degasser, a G1312A bin pump, a G1313A autosampler, a G1316A column heater, and a G1314A variable-wavelength detector controlled by Chem Station for LC and LC-MS System v. 9.01. The mass detector was an Agilent LC-MSD Trap equipped with an electrospray ionization (ESI) system and controlled by LC-MSD Trap software v. 4.1. Nitrogen was used as nebulizing gas at a pressure of 50 psi, and the flow was adjusted at 10 L/min. The heated capillary and voltage were maintained at 325 °C and 3 kV, respectively. The full-scan mass spectra of the phenolic compounds were measured from *m*/*z* 100 to *m*/*z* 5000. Mass spectrometry data were acquired in the positive ionization mode.

Antioxidant Activity in SDS/Linoleic Acid−AAPH System. An aliquot (50 μL) of extracts was added to the SDS micelle−linoleic acid peroxidation system (4 mL) described in Foti et al. (*25*) at different concentrations. The system (SDS/LH−AAPH system) contained 10 mM phosphate buffer (pH 7.4), 0.1 M SDS, and 2.6 mM linoleic acid. The radical reaction was started by adding 20 μL of 2% AAPH after the

addition of sample solution. The same reaction mixture without sample extract was used as a control. The absorbance at 234 nm was measured before (A_0) and after (A_{90}) incubation for 90 min at 50 °C, and ΔA ($A_{90} - A_0$) was calculated. The antioxidant activity against the SDS/LH−AAPH system expressed by inhibition of conjugated dienes formation was as follows:

$$\text{antioxidant activity (\% inhibition of conjugated dienes)} = 100 - (\Delta A \text{ for sample}/\Delta A \text{ for control}) \times 100$$

The antioxidant activities of the standard solutions of α-tocopherol, (−)-epicatechin, chlorogenic acid, gallic acid, and ascorbic acid were also measured. The IC_{50} value, defined as the amount of antioxidant necessary to inhibit the formation of conjugated diene by 50%, was calculated from the results.

Free Radical Scavenging Activity Using DPPH. The free radical scavenging activity of fruit extracts was measured using the method of Brand-Williams et al. (*26*) with some modification. A 0.1 mM solution of DPPH in methanol was prepared, and 4 mL of this solution was added to 0.2 mL of the extract at different concentrations. The decrease in absorbance at 517 nm was measured at 60 min. A control was added with 0.2 mL of distilled water instead of the extract. The EC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results. The EC_{50} for antioxidant standards was also obtained using the same method.

Anti-influenza Viral Activity by Hemagglutination Inhibition Test. Each fruit extract was diluted to 0.5 and 0.05 mg/mL (as phenolic concentration) with phosphate-buffered saline containing 0.1% bovine serum albumin (0.1% BSA−PBS). Stock solution of influenza virus (A/PR/8/34, $10^{9.2}$ EID_{50}/mL) was diluted twice. Fifty microliters of the influenza virus solution was added to 50 μL of each diluted fruit extract in a 96-well microtiter plate. After an incubation of 30 min at 37 °C, the reaction mixtures were diluted 10 times (2-fold dilution each time) with 50 μL of 0.1% BSA−PBS. Then, 50 μL of chicken erythrocyte suspension (0.5%, v/v) was added to each well, and they were mixed. One day after the reaction at room temperature, the HA titer was determined as the highest dilution number of the virus solution that agglutinated the erythrocytes by visual inspection. (−)-Epicatechin and chlorogenic acid standards were also used in this test as reference phenolics.

RESULTS AND DISCUSSION

Phenolic Content and Composition in Chinese Quince, Quince, and Apple Flesh. The total phenolic content in Chinese quince fruit measured with the Folin−Ciocalteu method was 1280 mg/100 g of FW, ~4 times higher than that in quince fruit (302.7 mg/100 g of FW) and 20 times higher than that in apple fruit (61 mg/100 g of FW) (**Figure 1**). This high phenolic content in the Chinese quince fruit was in agreement with the results of other researchers (*27*). The total phenolic content of quince fruit in this experiment was noticeably higher than previously published data. Silva et al. (*28*) showed that the total phenolic content in pulp of quince fruit collected in different places in northern and central Portugal was 13.0 mg/100 g of FW as mean value and was 26.8 mg/100 g of FW as maximum value. This difference might be due to the extraction procedure. In this experiment, the extraction was conducted with aqueous acetone that permitted the proportion of polymeric procyanidins, which may not be extracted by pressing the fruits or when only methanol or ethanol was used as extraction solvent (*18*, *19*, *29*), to be taken into account. The result of butanol−HCl degradation showed that the amount of condensed tannin (proanthocyanidins) was also highest in Chinese quince fruit and lowest in apple fruit. The vanillin−sulfuric acid method seemed to give a lower estimation of total flavan-3-ols (catechins and procyanidins) when compared to the Folin−Ciocalteu method and the HPLC method (see below), especially in the case of fruits containing highly polymerized procyanidin as a major component.

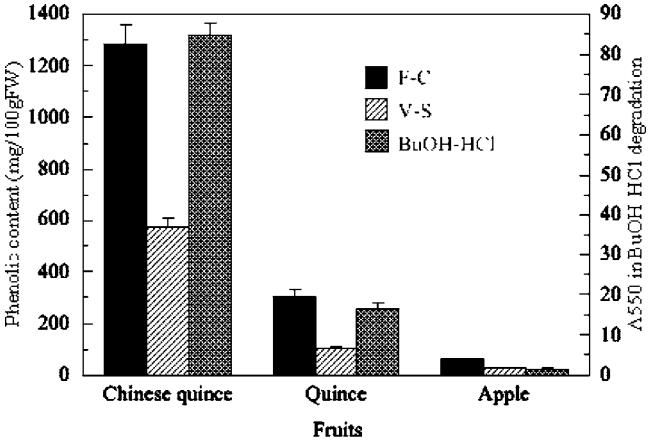

Figure 1. Total phenolics and condensed tannin content of Chinese quince, quince, and apple fruit measured by Folin–Ciocalteu method (F-C), vanillin–sulfuric acid method (V-S), and butanol–HCl degradation method (BuOH-HCl). Bars indicate SE ($n = 5$).

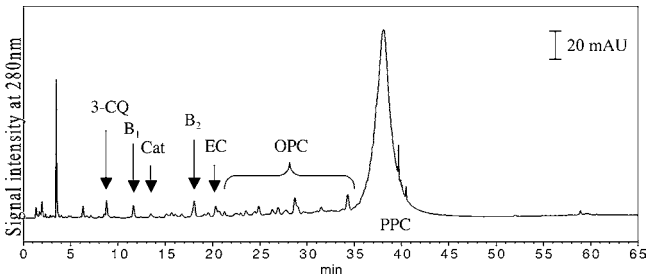

Figure 2. HPLC chromatogram of phenolic extracts from Chinese quince (var. Toukarin) fruit. Peaks have been characterized according to their UV–visible spectra, retention times, and thiolysis. Abbreviations: 3-CQ, 3-caffeoylquinic acid; B_1, procyanidin B_1; Cat, (+)-catechin; B_2, procyanidin B_2; EC, (–)-epicatechin; OPC, oligomeric procyanidins; PPC, polymeric procyanidins.

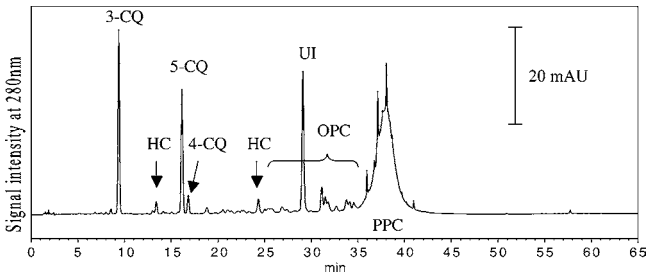

Figure 3. HPLC chromatogram of phenolic extracts from quince (cv. Smyrna) fruit. Peaks have been characterized according to their UV–visible spectra, retention times, and thiolysis. Abbreviations: HC, hydroxycinnamic derivatives; 5-CQ, 5-caffeoylquinic acid; 4-CQ, 4-caffeoylquinic acid; UI, unidentified peak; Others, see **Figure 2**.

Typical HPLC chromatograms of Chinese quince, quince, and apple phenolics are shown in **Figure 2**, **3**, and **4**, respectively. Each compound except for oligomeric and polymeric procyanidins was identified by its retention time, absorption spectra, molecular weight, and/or spiking with the standards under the same conditions. Highly polymerized procyanidins appeared as unresolved slightly sharp clumps for Chinese quince and quince extract, comparable to that found for some bitter cider apple varieties analyzed in reversed-phase condition (*18*). For convenience, the clump after 35 min was regarded as high polymeric procyanidins (PPC) because this part was difficult to extract

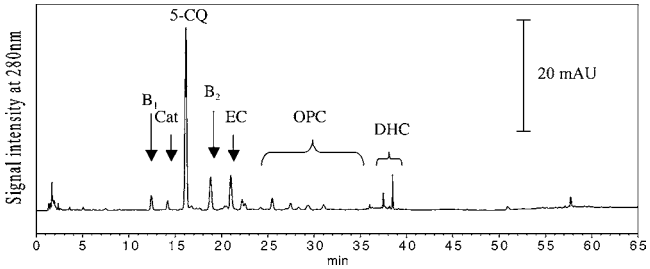

Figure 4. HPLC chromatogram of phenolic extracts from apple (cv. Fuji) fruit. Peaks have been characterized according to their UV–visible spectra and retention times. Abbreviations: DHC, dihydrochalcone derivatives; Others, see **Figures 2** and **3**.

Table 1. Hydroxycinnamic Acid Derivative and Flavan-3-ol Contents of Chinese Quince, Quince, and Apple Flesh[a]

	Chinese quince	quince	apple
HC derivatives			
3-caffeoylquinic acid	5.0 (0.4)	14.1 (0.1)	nd
5-caffeoylquinic acid	0.5 (0.2)	12.3 (0.7)	11.0 (3.8)
4-caffeoylquinic acid	1.2 (0.2)	1.5 (0.2)	nd
caffeic acid	tr	nd	tr
unidentified HC[b]	0.2 (0.1)	3.0 (0.6)	1.1 (0.1)
total HC	6.9 (0.7)	30.9 (0.4)	12.2 (3.9)
F3OL			
procyanidin B_1	9.8 (1.8)	nd	1.5 (1.4)
(+)-catechin	2.9 (0.5)	nd	1.2 (0.6)
procyanidin B_2	16.8 (3.5)	1.4 (0.3)	3.9 (2.7)
(−)-epicatechin	11.9 (2.1)	tr	4.0 (2.4)
oligomeric procyanidins	92.2 (24.4)	46.6 (10.2)	3.8 (2.5)
polymeric procyanidins	1260 (165)	200 (17.8)	13.4 (11.6)
total F3OL	1390 (197)	248 (28.3)	27.8 (2.2)
total HC + F3OL	1400 (197)	279 (28.0)	40.0 (1.7)

[a] Values are expressed in mg/100 g of FW as mean (SE) of three assays for each fruit. Abbreviations: HC, hydroxycinnamates; F3OL, flavan-3-ols; nd, not detected; tr, traces. [b] Calculated as caffeic acid.

completely with methanol only; moreover, it was strongly related to astringency. Other peaks, except for monomer and procyanidin dimer (B_1 and B_2), the spectra of which were similar to that of (−)-epicatechin, were regarded as oligomeric procyanidins (OPC) because these compounds, like PPC, were converted into monomer units (flavan-3-ols or flavan-3-ol benzylthioethers) after thiolytic depolymerization. Not only the total phenolic contents but also the phenolic profiles differed among Chinese quince, quince, and apple fruit. Whereas Chinese quince fruit contained PPC as a major component and a small amount of 3-caffeoylquinic acid, quince fruit was characterized by the presence of a significant amount of hydroxycinnamic derivatives (mainly 3-caffeoylquinic acid and 5-caffeoylquinic acid), an unidentified peak, and a large amount of PPC. Apple fruit did not contain 3-caffeoylquinic acid and a noticeable amount of PPC, but it was characterized by the presence of 5-caffeoylquinic acid, (+)-catechin, (−)-epicatechin, procyanidin dimmers (B_1 and B_2), OPC, and dihydrochalcone derivatives (probably phloretin glycoside and phloridzin).

The hydroxycinnamic acid derivative and flavan-3-ol contents of Chinese quince, quince, and apple flesh are shown in **Table 1**. In the case of Chinese quince, 99.5% of phenolics were flavan-3-ols including 90% of PPC. The amount of PPC in Chinese quince was 94 times higher than in apple and 6 times higher than in quince. On the other hand, the hydroxycinnamic derivative content in Chinese quince was only 53% of that in

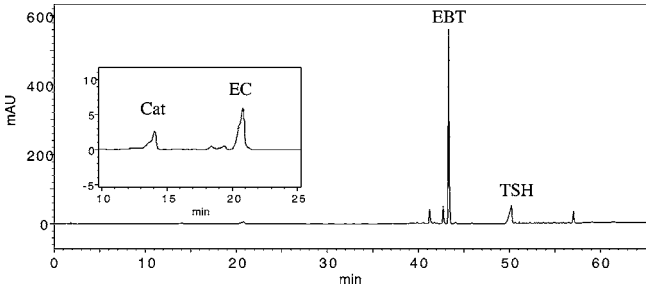

Figure 5. HPLC chromatogram of phenolic extracts from Chinese quince after thiolysis (3 times diluted from **Figure 2**). Peaks: Cat and EC, (+)-catechin and (−)-epicatechin as terminal units of procyanidins. Abbreviations: EBT, epicatechin benzyl thioether as extension unit of procyanidins; TSH, toluene-α-thiol.

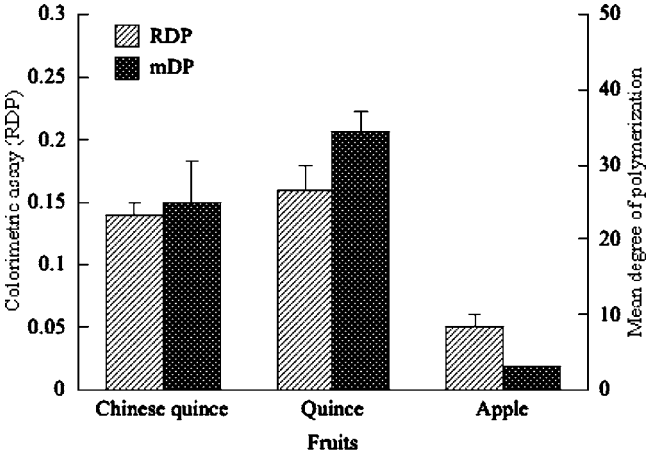

Figure 6. Relative degree of polymerization (RDP) and mean degree of polymerization (mDP) of total flavan-3-ols in Chinese quince, quince, and apple fruit. Bars indicate SE ($n = 3$).

apple and was the lowest among the fruits. Quince fruit had the highest amount of hydroxycinnamic derivatives, accounting for almost 10% of total phenolics in the fruit. Another characteristic of quince phenolics was to have quite a few amounts of monomeric and dimeric flavan-3-ols. The ratio of hydroxycinnamic derivatives to total phenolics in each fruit decreased in the following order: apple > quince > Chinese quince.

HPLC analysis of the extracts after thiolysis allowed the characterization of the procyanidins by their constitutive units and their average degree of polymerization as described by Prieur et al. (*30*). **Figure 5** shows the HPLC chromatogram of thioacidolytic products of Chinese quince phenolics, indicating that phenolics in Chinese quince practically consist of polymers of (−)-epicatechin and a small amount of (+)-catechin as terminal units. The profile of polymeric flavan-3-ols in Chinese quince was similar to that in quince and apple fruits, although the percentages of (+)-catechin as terminal units differed among the three fruits. The percentage of (+)-catechin as terminal units was the highest in Chinese quince (31.3%), the lowest in quince (3.6%), and moderate for apple (25.8%) (data not shown). The mean degrees of polymerization (mDP) of flavan-3-ols in Chinese quince, quince, and apple fruit were 25, 35, and 3, respectively (**Figure 6**). The RDP calculated from the colorimetric assay showed a similar trend, although the difference between each value was smaller than the actual mDP value. These high mDP values for Chinese quince and quince procyanidins were comparable with those of a variety of Portuguese pear (*31*) or some cider apple varieties (*32*).

Table 2. Antioxidant Activity and DPPH Radical Scavenging Activity of Some Antioxidant Standards and Phenolic Extracts from Chinese Quince, Quince, and Apple Flesh[a]

antioxidant activity (SDS/LH-AAPH system)		DPPH radical scavenging activity	
antioxidant	IC_{50}	antioxidant	EC_{50}
α-tocopherol	3.1	gallic acid	3.3
(−)-epicatechin	3.3	(−)-epicatechin	4.0
chlorogenic acid	12.4	ascorbic acid	10.1
gallic acid	14.2	chlorogenic acid	18.4
ascorbic acid	54.9	α-tocopherol	33.7
fruit extract	IC_{50}	fruit extract	EC_{50}
apple	2.0	Chinese quince	6.5
Chinese quince	9.3	quince	7.5
quince	12.1	apple	8.4

[a] Abbreviations: IC_{50}, concentration (mg/100 mL) at 50% inhibition of conjugated diene formation; EC_{50}, concentration (mg/100 mL) at 50% scavenging of DPPH.

However, these mDP values of Chinese quince and quince fruits were higher than those estimated from the average molecular weight reported by Foo and Porter (*33*), although it was consistent with their report that mDP of procyanidins in quince was higher than that in Chinese quince. The difference of mDP might be due to the difference in ripening stage of the fruits because unripe fruits have been used for their experiment. It has been thought that maturation can be accompanied by an increase in the degree of polymerization of proanthocyanidins in certain fruits, although this is not always the case (*34*). As for apple, we also observed that immature fruit had a high ratio of (−)-epicatechin monomer to procyanidins, and the ratio decreased during maturation (*35*), indicating that the immature fruit had a relatively smaller value of mDP than ripe fruits.

Antioxidant Properties of Flesh Extracts. In the SDS/LH−AAPH system, the IC_{50} values of antioxidant standards used in this experiment showed that the activity decreased in the following order: α-tocopherol > (−)-epicatechin > chlorogenic acid > gallic acid > ascorbic acid (**Table 2**). Among polyphenolics, (−)-epicatechin showed the highest activity (slightly lower than that of α-tocopherol), whereas chlorogenic acid and gallic acid had only moderate activities. In this system, Chinese quince and quince extracts showed moderate activities and were inferior to apple extract, which showed the highest activity. Chinese quince extract had higher activity than quince extract. These results might be related in the lipophilicity of compounds in addition to the actual antioxidant capacity from the structural features (*25*). Because highly polymerized procyanidins are more hydrophilic than monomeric catechins and oligomers (*36*), the lower activities in Chinese quince and quince extracts than in apple extracts seemed to be due to their phenolic profile, consisting of a large proportion of highly polymeric procyanidins. Additionally, Lotito et al. (*15*) revealed that oligomeric procyanidins consisting of a lower number of epicatechin units (which are major components of apple phenolics) have a strong protective effect against AAPH-dependent lipid oxidation. This might support our result that apple extract had the strongest activity in the SDS/LH−AAPH system.

In the DPPH-scavenging system, antioxidants and fruit extracts showed different orders of activity than that in the SDS/LH−AAPH system. The EC_{50} values of antioxidants showed that the DPPH-scavenging activities decreased in the following order: gallic acid > (−)-epicatechin > ascorbic acid > chlorogenic acid > α-tocopherol. In the case of fruit extract,

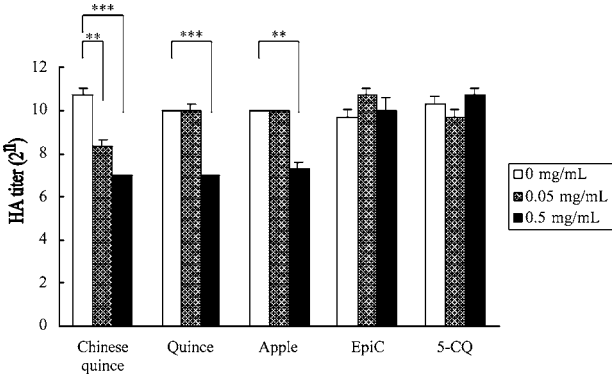

Figure 7. Hemagglutination (HA) activity of influenza virus treated with phenolic fruit extracts and phenolic standards. Abbreviations: EpiC, (−)-epicatechin; 5-CQ, 5-caffeoylquinic acid (chlorogenic acid). Bars indicate SE ($n = 3$). **, $p < 0.01$; ***, $p < 0.001$.

Chinese quince had the highest activity ($EC_{50} = 6.5$), followed by quince (7.5) and apple (8.4). The scavenging activities of these extracts were between those of (−)-epicatechin and ascorbic acid. According to Lu and Foo (*5*), the DPPH-scavenging activity of flavan-3-ols increased with the degree of polymerization from epicatechin monomer to tetramer, whereas this trend did not extend to higher molecular weight procyanidins. However, in our experiment, Chinese quince and quince, which had a high proportion of PPC, showed slightly higher activities than apple, which had mainly low polymerized procyanidins as flavan-3-ol series. This might be explained by the ratio of hydroxycinnamic derivatives to total phenolics that affect the DPPH-scavenging activity by decreasing it; thus, the apple phenolics rich in hydroxycinnamic derivatives had the lowest activity among the three fruit extracts. It was also observed that highly polymerized procyanidins had higher DPPH scavenging activities than the chlorogenic acid-rich phenolic fraction in experiments using European pear (data not shown).

Anti-influenza Viral Activity of Flesh Extracts. Influenza virus is known to interact with chicken erythrocyte to induce hemagglutination (HA). This activity reflects the ability of influenza virus to adhere to epithelial cells of the respiratory tract in a host as the first step of infection. At a concentration of 0.5 mg/mL (as phenolics), Chinese quince, quince, and apple fruit extracts all were significantly ($p < 0.001$, 0.001, and 0.01, respectively) effective in inactivating the influenza virus to 2^7 in HA titer compared to $2^{10.7}$ in HA titer of control, although apple extracts seemed to be slightly weaker than Chinese quince and quince fruit extracts (**Figure 7**). However, at a concentration of 0.05 mg/mL, only Chinese quince extracts still showed significant ($p < 0.01$) inhibition of the influenza viral activity (2^8 in HA titer). (−)-Epicatechin and chlorogenic acid standards had no effect on hemagglutination inhibition. This result indicates that phenolic extracts rich in procyanidin might have strong anti-influenza viral activity. Because the procyanidin content of Chinese quince is also very high, it seems that consumption or administration of the fruit products such as traditional fruit liquor might be effective in preventing influenza viral infection. With regard to phenolic content and anti-viral activity of phenolics, Chinese quince fruit seemed to be superior to quince fruit.

The results of this study suggest that the antioxidant activities of Chinese quince and quince fruit are superior to those of chlorogenic acid or ascorbic acid in antioxidant function measured in the SDS/LH−AAPH and DPPH-scavenging systems, and the activities of Chinese quince extracts are slightly

higher than those of quince extracts. However, those extracts seem to have less efficiency in protecting lipid oxidation in oil-in-water systems than apple extracts or (−)-epicatechin. On the other hand, Chinese quince fruit extracts have high potentiality to prevent the adherence of influenza virus, and quince fruit extracts might also have moderate effect. Thus, the difference of phenolic composition will show the different activities on antioxidant functions and inactivation of influenza virus. According to recent studies dealing with phenolics bioavailability, highly polymerized procyanidins do not seem to be absorbed directly from the small intestine (*37*), whereas lower molecular weight phenolics such as chlorogenic acid (*38*, *39*), catechins (*40*), and dimeric procyanidin (*41*) are partially absorbed. Because Chinese quince contained high molecular weight phenolics as major components and phenolics in apple were relatively low molecular weight compounds, it seems that absorption of Chinese quince phenolics would be very low but that apple phenolics might be absorbed and could act as a strong antioxidants in serum. However, our results suggest that even procyanidins which are not absorbed may play an important role as an influenza viral inhibitor. It is thought that they inactivate the influenza virus and prevent their infection on the throat. There is also a possibility that procyanidins may be absorbed and act the same as low molecular weight phenolics after decomposition by colonic microflora (*42*). Further works are in progress to compare the other functions of Chinese quince, quince, and apple phenolics relating to their health benefit and to evaluate the utility of their phenolics.

ABBREVIATIONS USED

SDS, sodium dodecyl sulfate; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; DPPH, 1,1-diphenyl-2-picrylhydrazyl; RDP, relative degree of polymerization; ESI, electrospray ionization; LH, linoleic acid; BSA, bovine serum albumin; PBS, phosphate-buffered saline; FW, fresh weight; EID, egg infectious dose; HA, hemagglutination; PPC, high polymeric procyanidins; OPC, oligomeric procyanidins; mDP, mean degree of polymerization.

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