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Polyphenols and Triterpenes from *Chaenomeles* Fruits: Chemical Analysis and Antioxidant Activities Assessment

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Abstract

Mugua, fruit of the genus *Chaenomeles*, is a valuable source of health food and Chinese medicine. To elucidate the bioactive compounds of five wild *Chaenomeles* species, extracts from fresh fruits were investigated by HPLC-DAD/ESI-MS/MS. Among the 24 polyphenol compounds obtained, 20 were flavan-3-ols (including catechin, epicatechin and procyanidin oligomers). The mean polymerization degree (mDP) of procyanidins was examined by two acid-catelyzed cleavage reactions; the mDP value was the highest in *C. sinensis* and the lowest in *C. japonica*. Total polyphenol content (TPC) reached 46.92 and 46.28 mg/g gallic acid equivalents (GAE) in *C. speciosa* and *C. thibetica*, respectively, although their main bioactive compounds were different (being epicatechin and procyanidin B2 in the former, and catechin and procyanidin B1 in the latter). These two species also exhibited equally strong free radical scavenging activities. Our results further showed that the antioxidant ability of *Chaenomeles* fruits was significantly correlated to their total polyphenol contents. Two triterpenes (oleanolic acid and ursolic acid) were the highest quantity in *C. cathayensis* and *C. thibetica*, respectively.

Key words

Chaenomeles, *Mugua*, polyphenol, procyanidin, antioxidant activity, triterpenes

1 Introduction

Fruits of the genus *Chaenomeles* (Rosaceae), commonly known as *Mugua* in China, have multiple uses. They are used in food industry (for liquors and candies) (Hamauzu, Yasui, Inno, Kume & Omanyuda, 2005) and in Chinese medicine. *Mugua* has been used for thousands of years in the treatment of rheumatoid arthritis, hepatitis, asthma and common cold (Yang, Fen, Lei, Xiao & Sun, 2009; Zhang, Cheng, Liu,

Wang, Wang & Du, 2010). There are five wild *Chaenomeles* species in China (*Flora of China*, 1974), which are *C. speciosa*, *C. thibetica*, *C. cathayensis*, *C. sinensis*, and *C. japonica* (Fig. 1). In the *Pharmacopoeia of the People's Republic of China* (*PPRC*, 2010), *Fructus Chaenomelis speciosa* is designated as the medicinal herb source for *Mugua*, but fruits from other species were frequently used as substitutes. Further information is needed to validate/invalidate this practice.

The chemical compositions information of these materials is limited, especially for *C. speciosa*, *C. thibetica*, and *C. cathayensis*. Based on previous studies about *C. sinensis* (Lee, Son & Han, 2002) and *C. japonica* (Hamauzu et al., 2005; Strek et al., 2007), it is proposed that polyphenols and two triterpenes (oleanolic acid and ursolic acid) are the bioactive compounds. Polyphenols, such as flavonoids (flavanols, flavones, flavonols, anthocyanins, and proanthocyanidins, etc.) and phenolic acids have received enormous attention in the recent years. These compounds protect against cardiovascular diseases and exhibit anti-tumoral, antimicrobial, anti-adhesive, and anti-inflammatory effects (Kylli et al., 2011; Rasmussen, Frederiksen, Krogholm & Poulsen, 2005). Proanthocyanidins (PAs) are condensed flavan-3-ols, also known as condensed tannins, including oligomers and polymers of flavan-3ols. PAs are widespread natural polyphenols. In fact, PAs were the main polyphenol compounds found in grape (skin and seed) (Spranger, Sun, Mateus, de Freitas & Ricardo-Da-Silva, 2008), hawthorn (Liu, Kallio, Lu, Zhou & Yang, 2011), cranberry (Tarascou et al., 2011), and lingonberry (Kylli et al., 2011). PAs present in these plants are largely responsible for their antioxidant activities. Besides PAs, the two triterpenes, oleanolic acid (OA) and ursolic acid (UA), also have anti-inflammation, anti-tumor promotion, and anti-hyperlipidemia effects (Fang, Wang, Yu, Zhang & Zhao, 2010). In addition,

69 because OA and UA are two active ingredients designated in *PPRC*, their contents
70 were used for quality control purposes.

71 Our knowledge of polyphenols from *Chaenomeles* is fragmented, as summarized
72 below.

73 *C. speciosa*: Extracts displayed anti-inflammatory effects, and included
74 chlorogenic acid, quercetin, OA and UA (Zhang et al., 2010). In addition, vitamin
75 C and phenols were examined (Ros, Laencina, Hellin, Jordan, Vila & Rumpunen,
76 2004).

77 *C. thibetica*: Essential oils were analyzed by GC-MS, and OA and UA were
78 examined (Yang et al., 2009).

79 *C. cathayensis*: OA and UA were examined (Yang et al., 2009), as well as
80 Vitamin C and phenols (Ros et al., 2004).

81 *C. sinensis*: Quercetin, luteolin, and genistein derivates were identified, and five
82 genistein derivates were active components (Lee, Son & Han, 2002). Catechin,
83 epicatechin, procyanidin B1 & B2 were identified (Hamauzu et al., 2005). OA
84 and UA were examined (Yang et al., 2009).

85 *C. japonica*: Chemical and functional analyses were conducted for PAs, OA, UA,
86 pectins, polysaccharides, vitamin C and phenols. For PAs, TLC and HPLC
87 profiling were performed for their monomers and dimers (Ros et al., 2004; Strek
88 et al., 2007).

89 In the present study, we identified and quantified a number of polyphenols from
90 five *Chaenomeles* species using HPLC-DAD-MS/MS. We compared their total
91 polyphenol (TPC) and total flavan-3ol content (TFC) as well as their antioxidant
92 properties (using three assays). This work would establish a foundation for the

93 investigation of bioactive compounds in *Chaenomeles* species and cultivars, and
94 facilitate further development and utilization of *Mugua*.

95 **2 Materials and methods**

96 *2.1 Chemicals*

97 (–)-epicatechin, (+)-catechin and chlorogenic acid (CGA) were purchased from
98 the National Institute for the Control of Pharmaceutical and Biological Products
99 (Beijing, China). Procyanidin B1, procyanidin B2, gallic acid (GA), oleanolic acid
100 (OA) and ursolic acid (UA) were obtained from Shanghai Tauto Biotech (Shanghai,
101 China). 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
102 (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox),
103 2,4,6-tripyridyl-*S*-triazine (TPTZ), 1,1-Diphenyl-2- picrylhydrazyl (DPPH$^{\bullet}$), ascorbic
104 acid, butylated hydroxytoluene (BHT), and Folin–Ciocalteu's phenol reagent were
105 bought from Sigma-Aldrich (St. Louis, MO). HPLC grade methanol and acetonitrile
106 were provided by Promptar (ElK Grove, CA). All other regents used were of
107 analytical grade.

108 *2.2 Sample preparation*

109 All five *Chaenomeles* species were grown at Beijing Botanical Garden, Beijing,
110 China. These plants can be identified according to their features of leaves, thorns and
111 fruits (Fig. 1). Fruit mature period of *C. japonica* was from August to October, which
112 of the other four species was from September to October. The mature fruit color of *C.*
113 *speciosa* is red-brown, and that of the other four species is green-yellow. We collected
114 ripe fruits in mid-October, 2010. Three trees of each species were used for fruit
115 collection. Five fruits were collected randomly from each tree and kept at –40□
116 before use. Each freezing fruit was cut vertically with radial symmetry to 4 or 6 slices

(depending on the fruit size of each species) with the skin attached. Seeds and endocarp were removed. One slice of each fruit was collected, five slices in total were combined and triturated using a blender, about 10 g of which was extracted as a sample. A sample was extracted with 30 mL 80% (v/v) acetone in a flask (Hamauzu et al., 2005). After adding the solvent, the flasks were shaken 30 s followed by sonication at 20□ for 20 min. Then the mixtures were filtered through qualitative filter paper. The filtrate was collected, and the sediment was further extracted with 15 mL 80% acetone for 2 h. The two filtrates were combined and the solvent was removed by evaporating at 30□ under reduced pressure. The concentrate was defatted by washing three times with petroleum ether, then was fixed to 20 mL aqueous solution and then purified by Supelclean ENVI-18 cartridge (Supelco Park, Bellefonte, PA), which had been previously activated with methanol and water. The cartridge loaded with 2 mL extract was washed with 5× column volume of water, and then eluted with methanol (Li et al., 2009). Finally, all the extracts were brought to 5 mL methanol, and filtered through a 0.22 μm membrane (Shanghai ANPEL Scientific Instrument, Shanghai, China). The extraction was performed one time for the combined slices of each tree. Three extractions for three trees of each species were conducted independently.

2.3 Identification of polyphenols by HPLC-DAD/ESI-MS/MS

The samples were analyzed by a Dionex HPLC system (Sunnyvale, CA), equipped with a P680 HPLC pump, an UltiMate 3000 autosampler, a TCC-100 thermostated column compartment and a Dionex PDA100 photodiode array detector. The analytical column was C_{18} column of ODS 80Ts QA (150 mm × 4.6 mm, 5 μm i.d., Tosoh, Tokyo) protected with a C_{18} guard cartridge (Shanghai ANPEL Scientific

Instrument, Shanghai). The following solvent and gradient were used: A, 0.1% aqueous formic acid; B, 0.1% formic acid in acetonitrile; constant gradient from 5 to 23% B within 55 min and back to 5% B in 5 min; the flow rate was 0.8 mL/min; Column temperature was maintained at 35□; 10 μL of analyte was injected. Chromatograms were obtained at 280 nm for flavanol derivates and 350 nm for other flavonoids, and photodiode array spectra were recorded from 200 to 800 nm.

HPLC-ESI-MS/MS analysis was carried out in an Agilent-1100 HPLC system equipped with a UV detector coupled to an LC-MSD Trap VL ion-trap mass spectrometer via an ESI source (Agilent Technologies, Palo Alto, CA). The HPLC separation condition was as described above. The MS conditions were as follows: negative-ion (NI) mode; capillary voltage of 3.5 kV; a nebulization pressure of 241.3 kPa; and a gas (N_2) temperature of 350□ with flow rate of 6.0 L/min. Capillary offset voltage was 77.2 V. MS and MS^2 spectra were recorded over the range from *m/z* 50 to 1000.

2.4 Quantitative analysis of polyphenol compounds

Catechin and CGA were used as standards to semi-quantify flavanols and CGA, respectively, by linear regression. The flavanols were expressed as milligrams of catechin equivalents (CE) per g fresh weight (FW), using the calibration curve obtained: polyphenol [mAU] = 178.60 [CE (mg/mL)] – 0.47 (r^2 = 0.999). CGA was measured under standard control (expressed as milligrams of per g FW), with the calibration curve: [mAU] = 420.90 [CGA (mg/mL)] – 2.81 (r^2 = 0.999). The flavonols were expressed as milligrams of rutin: [mAU] = 413.70 [rutin (mg/mL)] – 2.33 (r^2 = 0.999). All samples were analyzed in triplicate.

2.5 Estimation of the mean degrees of polymerization (mDPs) of proanthocyanidins

The mDPs of proanthocyanidins were analyzed by HPLC-DAD after acid catalyzed cleavage in the presence of either benzyl mercaptan or phloroglucinol. Thiolysis of the proanthocyanidins was carried out according to Gu et al. (2002). The analysis was performed by Dionex HPLC system as above. The solvent system was the same and the constant gradient was from 15% to 80% B in 45 min, then back to 15% in 5 min. mDP = [(total area of catechin and epicatechin benzyl mercaptan adducts) / (total area of catechin and epicatechin)] + 1. The procedure with phloroglucinol was performed according to Kennedy & Jones (2001). Catechin phloroglucinol adduct was prepared as in the literature (Kennedy et al., 2001); grape seed was processed with the same procedure to assist the identification of products. mDP = [(total area of catechin and epicatechin phloroglucinol adducts) / (total area of catechin and epicatechin)] + 1.

2.6 Determination of OA and UA

OA and UA were analyzed by the same HPLC-DAD system as above. The liquid chromatography was equipped with a 5 μm Kromasil C_{18} column (250 × 4.6 mm i.d., AKZO NOBEL, Anpu, Shanghai), which was protected with a C_{18} guard cartridge (Shanghai ANPEL Scientific Instrument, Shanghai). The mobile phase consisted of methanol / 0.05% phosphoric acid (91:9, v/v), with isocratic elution for 30 min. The flow rate was 0.6 mL/min. Column temperature was kept at 25□, and the absorption was recorded at 215 nm (Fang et al., 2010). OA and UA were identified against standards (0.01 – 1.00 mg/mL), and quantified by the calibration curves: [mAU] = 115.30 [mg/mL] – 0.66 (r^2 = 0.999) and [mAU] = 89.74 [mg/mL] – 0.31 (r^2 = 0.999), respectively.

2.7 Folin–Ciocalteu test and vanillin assay

The total polyphenol content (TPC) in the extracts was determined according to the Folin–Ciocalteu method, using GA as a standard (Li et al., 2009). The TPC was calculated with: [TPC (mg/mL)] = $0.73Abs_{750\ nm} - 0.03$ ($r^2 = 0.999$), and the result was expressed as mg of GA equivalent (mg GAE) per g FW. The extraction and sample preparation were performed with three replications. The absorbance was measured with a UNICO UV-4802 spectrophotometer (UNICO Instrument Co. Ltd., Shanghai).

The vanillin assay was used to measure total flavan-3-ol content (TFC) (monomers and PAs), carried out with vanillin and sulfuric acid (Oki et al., 2002). Specifically, 500 μL of 1.0% (w/v) vanillin and 500 μL of 9.0 M H_2SO_4 (both in methanol) was mixed with 200 μL of sample solution (in methanol). The mixture was kept at 30□ for 30 min, and the absorbance was read at 500 nm. As a blank control, vanillin was omitted. Catechin was used as a reference. TFC was calculated using: [TFC (mg/mL)] = $2.56Abs_{500\ nm} - 0.07$ ($r^2 = 0.998$), and the result was expressed as mg of catechin equivalent (mg CE) per g FW.

2.8 Evaluation of antioxidant capacity

2.8.1 ABTS assay

The ABTS assay was performed according to the references (Muller, Frohlich & Bohm, 2011), on the basis of scavenging the synthetic radical $ABTS^{\bullet+}$, which was produced by reacting 10 mL of 7 mM $ABTS^{\bullet+}$ solution with 178 μL of 140 mM potassium persulfate ($K_2S_2O_8$) in the dark at room temperature for 13 h. The $ABTS^{\bullet+}$ solution was diluted with phosphate buffered saline (PBS) to an absorbance at 0.70 ± 0.05 at 734 nm. An aliquot of 0.1 mL of diluted sample, standard or blank (methanol)

was added to 3.9 mL of diluted $ABTS^{\bullet+}$ to react in the dark at room temperature for 5 min, and absorbance at 734 nm was recorded. Trolox was used as a standard with its final concentrations ranging from 0 to 16.5 μM. Results were expressed as Trolox equivalent antioxidant capacity (TEAC, μmol of Trolox/g of FW). All samples were analyzed in triplicate.

2.8.2 FRAP assay

The ferric reducing ability of plasma (FRAP) assay was carried out according to the method of Benzie & Strain (1996). Briefly, 900 μL of freshly prepared FRAP reagent was mixed with 90 μL of distilled water and 30 μL of test sample (or methanol, for the blank), and then warmed at 37□ for 5 min. The absorbance was taken at 595 nm. Trolox was used as a standard with its final concentrations ranging from 0 to 16.5 μM. Results were expressed as TEAC (μmol of Trolox/g of FW). All samples were performed with three replications.

2.8.3 DPPH assay

The antioxidant capacities were evaluated using $DPPH^{\bullet}$ as a free radical, ascorbic acid, BHT and Trolox as references (Brand-Williams, Cuvelier & Berset, 1995). Antioxidant solution in methanol (0.1 mL) was added to 3.9 mL of 6×10^{-5} M $DPPH^{\bullet}$ in methanol, the absorbances were determined at 515 nm until the reaction reached a plateau. The concentration of the remaining $DPPH^{\bullet}$ (mM) was calculated by the calibration curve: $[Abs_{515\ nm}] = 11.268\ [DPPH^{\bullet}\ (mM)] - 0.002$ ($r^2 = 0.999$). The percentage of the remaining $DPPH^{\bullet}$ $[\%DPPH^{\bullet}_{REM}] = [DPPH^{\bullet}\ (mM)]_t / [DPPH^{\bullet}\ (mM)]_0$, where $_t$ and $_0$ are the time needed to reach the reaction steady state and the initial, respectively. For each antioxidant, different concentrations were tested, to make $\%DPPH^{\bullet}_{REM}$ between 20% and 80%. The ratio of [TPC (μM GAE)] / $[DPPH^{\bullet}$ (μM)]

was plotted against $\%DPPH^{\bullet}_{REM}$ to calculate the efficient concentration (EC_{50}), the amount of sample necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50%. The antiradical power (ARP) is calculated as $ARP = 1/EC_{50}$ (Brand-Williams et al., 1995). All samples were examined with three replications.

2.9 Statistics

Statistical analysis was performed with one-way ANOVA. Bivariate correlate analysis was performed by SPSS 11.5 (SPSS Inc., Chicago, IL). Values of $p < 0.05$ and $p < 0.01$ were considered statistically significant and extremely significant, respectively.

3 Results and discussion

3.1 Composition and content of polyphenol compound

A total of 24 polyphenol compounds were found in the fruits of the five *Chaenomeles* species by using HPLC-DAD/ESI-MS/MS (Fig. 2; Table 1). In the chromatogram profiles obtained at 280 nm, the labeled Peaks 1 to 24 followed an elution order. Among these compounds, 20 were flavan-3-ols (catechin, epicatechin, and procyanidin), one was CGA, a quinic ester of a phenolic acid (caffeic acid), one was a CGA isomer, and two were quercetin glucosides.

There are two types of PAs: those linked by bonds from C4 on the upper unit to C8 and/or C6 on the lower unit are of the B-type, and those linked through double linkages with an additional bond from C2 of the upper unit to the oxygen at C7 of the lower unit are of the A-type (Fig. S1). The *m/z* values of PA ions in negative ESI-MS mode were: $[M–H]^-$ 289 for monomer of catechin or epicatechin, $[M–H]^-$ 577 for B-type procyanidin dimer, $[M–H]^-$ 865 for procyanidin trimer, $[M–H]^{2-}$ 720 for procyanidin pentamer, and $[M–H]^{2-}$ 864 for procyanidin hexamer (Foo, Newman,

Waghorn, McNabb & Ulyatt, 1996; Sivakumaran et al., 2006; Tarascou et al., 2011). The proanthocyanidins in *Mugua* were composed of catechin and epicatechin, i.e. procyanidins, which agreed with the earlier reports for *C. sinensis* and *C. japonica* (Hamauzu et al., 2005; Strek et al., 2007). The main peaks 6 and 12 were identified as catechin and epicatechin by comparing with the standards, respectively. Peaks 3, 4 and 10 have the same mother molecular ion 577$[M–H]^-$, and the types of ion fragments from 577$[M–H]^-$ by MS^2 are identical (Table S5), although the abundance of each fragment varies as presented below, suggesting that they are B-type procyanidin dimers. The comparison analysis with standards confirmed that Peaks 4 and 10 were procyanidin B1 [epicatechin-(4β→8)-catechin] and B2 [epicatechin-(4β→8)-epicatechin], respectively. The fragment ion *m/z* 451 is specific to the interflavan linkage linking the C-4 position with a catechin unit (Sun & Miller, 2003). *m/z* 451 is most abundant at Peak 3, suggesting that it is rich in catechin. The elution order of procyanidin monomers and dimers was: procyanidin B3 < procyanidin B1 < catechin < procyanidin B2 < epicatechin, obtained using RP-HPLC (Pekic, Kovac, Alonso & Revilla, 1998). After considering all the references, Peak 3 was identified as procyanidin B3 [catechin-(4α→8)-catechin]. It is worth noting that detection of procyanidin B3 in *Mugua* is the first report. Additionally, seven procyanidin trimers, five pentamers and one hexamer were separated in this work. Peaks 2 and 8 both had ions $[M+Na–H]^-$ 375 and $[M–H]^-$ 353; peak 8 was identified as CGA (5-caffeoylquinic acid) by coelution with the standard, and peak 2 was deduced as its isomer 3-caffeoylquinic acid according to elution order in the literature (Hamauzu et al., 2005).

The content of each polyphenol compound was calculated using HPLC-DAD

analysis. The total flavan-3-ol content (TFC) including catechin, epicatechin and procyanidins oligomers, accounts for 94 to 99% of the total polyphenol content, indicating that PAs were the main polyphenol compounds in *Mugua*. Overall, there were five representative compounds (chlorogenic acid, catechin, procyanidin B1, epicatechin, and procyanidin B2), their content and distribution being different among the five species (Fig. 3). CGA was abundant in *C. speciosa* (1.82 ± 0.15 mg/g FW), *C. thibetica* (1.17 ± 0.06 mg/g FW) and *C. cathayensis* (1.19 ± 0.11 mg/g FW), and low in *C. sinensis* (0.09 ± 0.00 mg/g FW) and *C. japonica* (0.10 ± 0.00 mg/g FW). Catechin and procyanidin B1 were abundant in *C. thibetica* (1.56 ± 0.13 and 2.22 ± 0.17 mg/g FW, respectively) and *C. cathayensis* (1.13 ± 0.03 and 1.45 ± 0.02 mg/g FW, respectively), and moderate in *C. speciosa* (0.54 ± 0.06 and 0.83 ± 0.04 mg/g FW, respectively). On the contrary, epicatechin and procyanidin B2 predominated in *C. speciosa* (2.35 ± 0.26 and 2.96 ± 0.26 mg/g FW, respectively), *C. sinensis* (0.54 ± 0.03 and 0.40 ± 0.02 mg/g FW, respectively) and *C. japonica* (1.02 ± 0.09 and 0.98 ± 0.12 mg/g FW, respectively). Flavonol quercetin glucosides were found in this work, in agreement with earlier studies (Kylli et al., 2002; Zhang et al., 2010).

The present work provided polyphenol profiles of fruits from five *Chaenomeles* species using RP-HPLC. Twenty flavan-3-ols were identified, including monomers, procyanidin dimers, trimers and pentamers. In particularly, CGA, catechin, epicatechin, procyanidin B1 and procyanidin B2 were confirmed with standards, and were well separated and quantified. In this study, the contents of CGA (0.09 ± 0.00 mg/g FW), epicatechin (0.54 ± 0.03 mg/g FW), procyanidin B2 (0.40 ± 0.02 mg/g FW), catechin (0.05 ± 0.00 mg/g FW) and procyanidin B1 (0.13 ± 0.01 mg/g FW) in *C. sinensis* were higher than those found in previous a study, which were CGA (0.05

mg/g), epicatechin (0.12 mg/g), procyanidin B2 (0.17 mg/g), catechin (0.03 mg/g) and procyanidin B1 (0.10 mg/g) in fresh fruit (Hamauzu, et al., 2005). Both studies showed a similar chemical makeup, with more epicatechin and procyanidin B2, and less catechin and procyanidin B1. To the best of our knowledge, reports on individual compound contents for the other *Mugua* species are limited. This method allowed for the identification and characterization of polyphenols in the five species, showing the diversity in chemical constituents.

Judged from results with the Folin–Ciocalteu assay for total polyphenol content (TPC), the five species were very different, with the lowest in *C. japonica* (19.35 ± 0.59 GAE mg/g FW) and the highest in *C. speciosa* (46.92 ± 2.76 GAE mg/g FW) and. *C. thibetica* (46.28 ± 0.59 GAE mg/g FW) (Fig. 4A), but all had higher values than for other sources (*Lycium ruthenicum*, 6.98 – 13.11 GAE mg/g FW (Zheng et al., 2011); black raspberries, 4.95 – 9.8 mg/g FW (Wada & Ou, 2002)). TFC of *Mugua* extracts by using both vanillin assay and HPLC-DAD followed the same order as TPC.

The highest contents, estimated by the vanillin assay (Fig. 4), were found in *C. speciosa* and *C. thibetica* (20.97 ± 0.41 and 20.09 ± 1.08 CE mg/g FW, respectively), and the lowest in *C. japonica* (3.64 ± 0.71 mg/g). The similar content of 13.50 ± 0.46 mg/g in *C. sinensis* was obtained with the former report of 13.90 ± 1.97 mg/g FW in *C. sinensis* (Hamauzu et al., 2005). Here are a few examples of reported TFC values: grape seed, 18.57 ± 1.15 mg/g (the highest among six seed sources) (Wang, Wang, Geng & Li, 2008); cocoa bean 163.5 ± 17.87 mg/g (fresh) and 49.53 ± 5.14 mg/g (powder) (Ortega et al., 2008). *Mugua* second only to coca in TFC. However, since *Mugua* had large fruit size, high yield, and a wide distribution, it would make an

excellent source of procyanidin oligomers.

It was noticed that the TFC from vanillin assay was higher than that from HPLC for all samples but *C. japonica* (Fig. 4). This difference is likely due to the following reasons. TFC obtained from a vanillin assay is a unitary value; meanwhile, TFC using an HPLC analysis is a summation of each compound separated by a C_{18} column. The principle and calculation method between them were different. The TFC value of fruits from *C. sinensis* (13.50 ± 0.46 mg/g FW) using a vanillin assay was conformed to the former report of 13.9 ± 1.97 mg/g FW (Hamauzu et al. 2005). No doubt that an HPLC assay is suitable for absolute quantification of individual compound which are well separated. However, PA oligomers and polymers are difficult to separate completely in an HPLC analysis, leading to the ascending baseline of the HPLC profile (Fig. 2), which affects the accuracy of quantification for individual peaks. That is, the quantification of individual peaks tends to be underestimated. For this study, the HPLC analysis was better suitable for identification of PAs than quantification of them. A vanillin assay was more suitable for the *Mugua* TFC quantification. Moreover, the results from the two methods were parallel among the five *Mugua* species, suggesting that the distribution pattern of PAs among the five species is reliable.

3.2 The mDPs of PAs in Mugua

Based on the mass of molecular ions (Table 1), all procyanidins from *Mugua* extracts were recognized as the B-type. In the HPLC profiles generated from the acid-catalysis cleavage reactions (Fig. 5), Peaks 1 and 2 were catechin and epicatechin, respectively, based on comparisons with standards. Peak 3 and 4 each had fragment ions of $[M–H]^-$ 287 and $[M–H]^-$ 411 obtained from MS analysis. Compared to

356 cleavage products from grape seed (unpublished data) and published results (Kennedy
357 et al., 2001), Peaks 3 and 4 were designated as catechin and epicatechin benzyl
358 mercaptan adducts, respectively. Peak 3' and 4' each had fragment ions of $[M–H]^-$
359 287 and $[M–H]^-$ 413. They were identified as catechin and epicatechin phloroglucinol
360 adducts, respectively, by comparison with authentic catechin phloroglucinol prepared
361 as described by Kennedy et al. (2001).

362 The mDP values of PAs, were high for *C. sinensis* and low for *C. japonica,* with
363 those for the other three species in between (Table 2). This result was in accordance
364 with TFC data produced from vanillin assay and HPLC. The mDP data for
365 procyanidins from *C. thibetica*, *C. cathayensis* and *C. speciosa* represented the first
366 report. Correlation between the two sets of mDP values was extremely significant,
367 confirming a high confidence of the results. We noticed that our mDP results for *C.*
368 *sinensis* and *C. japonica* were lower than reported previously (25 (Hamauzu et al.,
369 2005) and 5.3 (Strek et al., 2007), respectively). The low level of mDPs shown in the
370 current work is apparently due to the higher content of monomers (catechin or
371 epicatechin) than in the published reports. The monomer / procyanidins ratios
372 decreased during fruit ripening in some species, contributing to mDP changes
373 (Kennedy, Matthews & Waterhouse, 2000).

374 The PA cleavage pattern consisted of one starter unit and n extension units ($n \geq$
375 1; Fig. 5A). The extension units of *Mugua* procyanidins were high in epicatechin
376 (Peaks 4 and 4'), with a small amount of catechin (Peak 3 and 3') (Fig. 5B,C). Even in
377 *C. thibetica* and *C. cathayensis* that were rich in catechin monomers, the amount of
378 epicatechin was higher than that of catechin. We attempted to predict the starter unit
379 by the catechin / epicatechin ratio before and after the cleavage reactions, but we only

found that the changes were not significant in all samples except *C. thibetica*. Estimation of starter unit cannot be accurate due to the inevitable asymmetric epimerization between catechin and epicatechin (Gu et al., 2002). The proportion of catechin as starter unit tends to be overestimated because of its lower epimerization rate than that of epicatechin. It is speculated that most starter units of *Mugua* procyanidins are comprised of both catechin and epicatechin.

3.3 Separation and quantification of OA and UA

To estimate the total content of OA and UA in *Mugua*, the RP-HPLC method was developed and validated. Three independent calibration curves were generated to determine the range of linearity, with six standard solutions of OA and UA at concentrations of 0.01 to 1.00 mg/mL prepared for each case. In our study, the LOD and LOQ were, respectively, 2.4 and 2.8 μg/mL for OA, and 8.2 and 9.6 μg/mL for UA, suggesting that the RP-HPLC method was sensitive enough for a quantitative measurement. In order to evaluate the precision of RP-HPLC, relative standard deviations (RSDs) with retention time (RT) and peak area were calculated from five injects of one low standard concentration. RSDs with RT and peak area were < 0.1% and < 3%, respectively (Table S1), indicating that the RP-HPLC method was sensitive and precise for OA and UA.

The concentration of OA ranged from 14.7 ± 3.2 μg/g to 338.7 ± 78.2 μg/g FW, and that of UA from 47.0 ± 25.6 μg/g to 272.7 ± 19.7 μg/g FW (Fig. S2. and Table 3). The highest OA and UA contents were found in *C. cathayensis* and *C. thibetica*, respectively. *C. speciosa* was not significant different from the other species in total OA and UA contents. Fang et al. (2010) examined dry *Mugua* fruits using RP-HPLC-PAD, and showed that OA 0.05 to 0.3 mg/g, and UA 0.4 to 2.0 mg/g in

404 species. Our results are similar to these. Interestingly, the highest total OA and UA
405 content in *Mugua* cultivars reached 1.57 mg/g DW (Wang et al., 2008), confirming
406 that cultivars abundant in OA and UA could be developed from breeding. *C. thibetica*
407 and *C. cathayensis* that had higher OA and UA content than other species make them
408 excellent choices for this purpose.

409 *3.4 Antioxidant activity of Mugua*

410 The antioxidant activity of *Mugua* extracts were investigated by ABTS, FRAP
411 and DPPH assays (Fig. 4B). The free radical scavenging abilities shown with ABTS
412 and FRAP were both expressed as TEAC, and an extremely significant correlation
413 between the two assays was observed ($R^2 = 0.947$, $p < 0.01$), suggesting that the free
414 radical scavenging activity of *Mugua* extracts was stable and effective. The maximal
415 TEAC was achieved from *C. speciosa*, with 310.55 ± 6.83 and 96.84 ± 10.40 μmol
416 Trolox/g FW with $ABTS^{\bullet+}$ and FRAP, respectively. The *C. thibetica* extract was less
417 effective, exhibiting TEAC at 253.88 ± 9.08 μmol and 84.43 ± 1.22 Trolox/g FW with
418 $ABTS^{\bullet+}$ and FRAP, respectively. Both species possess stronger antioxidant activities
419 than other fruits, such as *Lycium ruthenicum* ($ABTS^{\bullet+}$, 26.84 μmol Trolox/g FW;
420 FRAP 32.44 μmol Trolox/g FW) (Zheng et al., 2011), guava (18.03–32.25 μmol
421 Trolox/g FW) (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos & Byrne, 2006).
422 Among our samples, *C. japonica* had the lowest TEAC (118.15 ± 4.10 and 19.10 ±
423 0.76 μmol Trolox/g FW with ABTS and FRAP, respectively).

424 The $DPPH^{\bullet}$ scavenging capacity was evaluated by ARP ($1/EC_{50}$). *C. sinensis* had
425 the highest ARP, *C. speciosa* followed, and *C. japonica* had the lowest (Fig. 4B).
426 Among the five extracts, ARP values of four samples, namely *C. speciosa* (5.63 ±
427 0.17), *C. thibetica* (4.89 ± 0.21), *C. cathayensis* (4.88 ± 0.25) and *C. sinensis* (6.48 ±

0.23), fell between those of the standard antioxidants ascorbic acid (4.68 ± 0.02) and BHT (8.94 ± 0.02), but were higher than Trolox (3.79 ± 0.07). *C. japonica* had an ARP of 3.22 ± 0.13, which is slightly lower than that of Trolox. The kinetic behavior of *C. thibetica* extract at different dilutions was presented in Fig. S3, showing calculation approach of EC_{50}.

3.5 Correlation coefficients of TPC, TFC, ABTS, FRAP, and DPPH assays

Pearson correlation coefficients (*r*) between the antioxidant activity assays and the different parameters (TFC, TPC, CGA, OA, and UA) were obtained using SPSS 11.5 (Table S4). The correlations between TFC and TEAC (from ABTS and FRAP assays) were dramatically high ($r = 0.923$ and $r = 0.925$), which confirmed that PAs and monomers had strong antioxidant and radical scavenging activities as previously reported (Rasmussen et al., 2005). TPC had lower correlation coefficients with ABTS and FRAP assays ($r = 0.836$ and 0.794, respectively) than TFC. One reason for the divergence maybe that vanillin assay is generally more specific than Folin–Ciocalteu assay. Another reason was speculated that polyphenols except for flavan-3-ols provided with less contribution for antioxidant activity. CGA also contributed to the antioxidant capacity of *Mugua* ($r = 0.712$ and 0.562 with ABTS and FRAP, respectively), which agreed with the published result in *C. speciosa* (Li et al., 2009b). OA and UA have no significant contribution to the antioxidant activity in the present study.

DPPH assay had a negative correlation with TFC ($r = –0.604$), and no significant correlation with other parameters. Negative correlations were reported in earlier studies (Zheng et al., 2011); an explanation has been lacking. The DPPH assay is technically simple, but it has some disadvantages; these include: the reaction kinetics

between DPPH$^{\bullet}$ and antioxidants is not linear to DPPH$^{\bullet}$ concentrations, the reaction time varies (from less than five min to hours), the reaction can be influenced dramatically by adventitious acids or bases in the solvent, and some reactions between chemical molecules with DPPH$^{\bullet}$ were reversible, such as eugenol (Brand-Williams et al., 1995).

4 Conclusions

For the polyphenol compounds from *Chaenomeles* fruits, we established a HPLC method, allowing simultaneous separation and quantification of phenolic acids, catechin, epicatechin, and procyanidin oligomers. We also developed a sensitive and reliable analyzing method for OA and UA. The present methods are important for the study of bioactive compounds in multiple cultivars for classification and quality.

The chemical compositions and antioxidant activities of five *Chaenomeles* species were investigated in detail. Five representative polyphenol compounds exist in *Chaenomeles* fruits at different abundance. CGA, Catechin and procyanidin B1 were abundant in *C. thibetica* and *C. cathayensis*; epicatechin and procyanidin B2 dominated in *C. sinensis* and *C. japonica*. *C. speciosa* was intermediate in the distribution pattern of the five compounds. The mDP was high in *C. sinensis*, and low in *C. japonica*. TPC and antioxidant activities were equally high in *C. thibetica* and *C. speciosa*, although their main compounds were different. No significant difference between *C. speciosa* and the other species was observed in total OA and UA content. Thus, our results expanded *Mugua* sources for medicinal applications.

With the developed analytical methods and composition data presented here, species rich in procyanidins can be selected for medicinal sources and nutritional/health supplements. This work can also provide useful information about

polyphenol compounds for hybridization or molecular assisted breeding.

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Appendix A. Supplementary data

Fig. S1 Chemical structures of flavan-3-ol monomers, chlorogenic acid, OA and UA.

Fig. S2 HPLC chromatogram of OA and UA in *Mugua* at 210 nm.

Fig. S3 Disappearances of $DPPH^{\bullet}$ as a function of μM TP/μM $DPPH^{\bullet}$ in *C. thibetica*.

Table S1 Mean degree of polymerization (mDP) from acid-catalysis in the presence of benzyl mercaptan and phloroglucinol.

Table S2 Precision evaluation of the RP-HPLC method for OA and UA.

Table S3 Oleanolic acid (OA) and ursolic acid (UA) contents in *Chaenomeles* fruits.

Table S4 Correlation coefficients of total polyphenols, total flavan-3-ols, and CGA, with the three assays (ABTS, FRAP and DPPH).

Table S5 Daughter ions from mother ion of *m/z* 577^{-} by MS^2.

References

Benzie, I. F. F., & Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76.

500 Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free-radical
501 method to evaluate antioxidant activity. *Food Science and*
502 *Technology-Lebensmittel-Wissenschaft & Technologie*, *28*(1), 25-30.
503 Fang, X., Wang, J., Yu, X., Zhang, G., & Zhao, J. (2010). Optimization of
504 microwave-assisted extraction followed by RP-HPLC for the simultaneous
505 determination of oleanolic acid and ursolic acid in the fruits of *Chaenomeles*
506 *sinensis*. *Journal of Separation Science*, *33*(8), 1147-1155.
507 Foo, L. Y., Newman, R., Waghorn, G., McNabb, W. C., & Ulyatt, M. J. (1996).
508 Proanthocyanidins from *Lotus corniculatus*. *Phytochemistry*, *41*(2), 617-624.
509 Gu, L., Kelm, M., Hammerstone, J. F., Beecher, G., Cunningham, D., Vannozzi, S., &
510 Prior, R. L. (2002). Fractionation of polymeric procyanidins from lowbush
511 blueberry and quantification of procyanidins in selected foods with an
512 optimized normal-phase HPLC-MS fluorescent detection method. *Journal of*
513 *Agricultural and Food Chemistry*, *50*(17), 4852-4860.
514 Hamauzu, Y., Yasui, H., Inno, T., Kume, C., & Omanyuda, M. (2005). Phenolic
515 profile, antioxidant property, and anti-influenza viral activity of Chinese
516 quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.),
517 and apple (*Malus domestica* Mill.) fruits. *Journal of Agricultural and Food*
518 *Chemistry*, *53*(4), 928-934.
519 Kennedy, J. A., & Jones, G. P. (2001). Analysis of proanthocyanidin cleavage
520 products following acid-catalysis in the presence of excess phloroglucinol.
521 *Journal of Agricultural and Food Chemistry*, *49*(4), 1740-1746.
522 Kennedy, J. A., Matthews, M. A., & Waterhouse, A. L. (2000). Changes in grape seed
523 polyphenols during fruit ripening. *Phytochemistry*, *55*(1), 77-85.

524 Kylli, P., Nohynek, L., Puupponen-Pimia, R., Westerlund-Wikstrom, B., Leppanen,
525 T., Welling, J., Moilanen, E., & Heinonen, M. (2011). Lingonberry
526 (*Vaccinium vitis-idaea*) and European cranberry (*Vaccinium microcarpon*)
527 proanthocyanidins: Isolation, identification, and bioactivities. *Journal of*
528 *Agricultural and Food Chemistry*, *59*(7), 3373-3384.
529 Lee, M. H., Son, Y. K., & Han, Y. N. (2002). Tissue factor inhibitory flavonoids from
530 the fruits of *Chaenomeles sinensis*. *Archives of Pharmacal Research*, *25*(6),
531 842-850.
532 Li, C., Du, H., Wang, L., Shu, Q., Zheng, Y., Xu, Y., Zhang, J., Yang, R., & Ge, Y.
533 (2009). Flavonoid composition and antioxidant activity of tree peony (*Paeonia*
534 section *moutan*) yellow flowers. *Journal of Agricultural and Food Chemistry*,
535 *57*(18), 8496-8503.
536 Liu, P. Z., Kallio, H., Lu, D. G., Zhou, C. S., & Yang, B. R. (2011). Quantitative
537 analysis of phenolic compounds in Chinese hawthorn (*Crataegus* spp.) fruits
538 by high performance liquid chromatography-electrospray ionisation mass
539 spectrometry. *Food Chemistry*, *127*(3), 1370-1377.
540 Muller, L., Frohlich, K., & Bohm, V. (2011). Comparative antioxidant activities of
541 carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS
542 bleaching assay (alpha TEAC), DPPH assay and peroxyl radical scavenging
543 assay. *Food Chemistry*, *129*(1), 139-148.
544 Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y., Furuta, S., Suda, I., & Sato, T.
545 (2002). Polymeric procyanidins as radical-scavenging components in
546 red-hulled rice. *Journal of Agricultural and Food Chemistry*, *50*(26),
547 7524-7529.

548 Ortega, N., Romero, M. P., Macia, A., Reguant, J., Angles, N., Morello, J. R., &
549 Motilva, M. J. (2008). Obtention and characterization of phenolic extracts
550 from different cocoa sources. *Journal of Agricultural and Food Chemistry*,
551 *56*(20), 9621-9627.
552 Pekic, B., Kovac, V., Alonso, E., & Revilla, E. (1998). Study of the extraction of
553 proanthocyanidins from grape seeds. *Food Chemistry*, *61*(1-2), 201-206.
554 Rasmussen, S. E., Frederiksen, H., Krogholm, K. S., & Poulsen, L. (2005). Dietary
555 proanthocyanidins: Occurrence, dietary intake, bioavailability, and protection
556 against cardiovascular disease. *Molecular Nutrition & Food Research*, *49*(2),
557 159-174.
558 Ros, J. M., Laencina, J., Hellin, P., Jordan, M. J., Vila, R., & Rumpunen, K. (2004).
559 Characterization of juice in fruits of different *Chaenomeles* species.
560 *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*,
561 *37*(3), 301-307.
562 Sivakumaran, S., Rumball, W., Lane, G. A., Fraser, K., Foo, L. Y., Yu, M., &
563 Meagher, L. P. (2006). Variation of proanthocyanidins in *Lotus* species.
564 *Journal of Chemical Ecology*, *32*(8), 1797-1816.
565 Spranger, I., Sun, B., Mateus, A. M., de Freitas, V., & Ricardo-Da-Silva, J. M. (2008).
566 Chemical characterization and antioxidant activities of oligomeric and
567 polymeric procyanidin fractions from grape seeds. *Food Chemistry*, *108*(2),
568 519-532.
569 Strek, M., Gorlach, S., Podsedek, A., Sosnowska, D., Koziolkiewicz, M., Hrabec, Z.,
570 & Hrabec, E. (2007). Procyanidin oligomers from Japanese quince
571 (*Chaenomeles japonica*) fruit inhibit activity of MMP-2 and MMP-9

572 metalloproteinases. *Journal of Agricultural and Food Chemistry*, *55*(16),
573 6447-6452.
574 Sun, B. S., Ricardo-da-Silva, J. M., & Spranger, I. (1998). Critical factors of vanillin
575 assay for catechins and proanthocyanidins. *Journal of Agricultural and Food*
576 *Chemistry*, 46(10), 4267-4274.
577 Sun, W. X., & Miller, J. M. (2003). Tandem mass spectrometry of the B-type
578 procyanidins in wine and B-type dehydrodicatechins in an autoxidation
579 mixture of (+)-catechin and (–)-epicatechin. *Journal of Mass Spectrometry*,
580 *38*(4), 438-446.
581 Tarascou, I., Mazauric, J. P., Meudec, E., Souquet, J. M., Cunningham, D., Nojeim, S.,
582 Cheynier, V., & Fulcrand, H. (2011). Characterisation of genuine and derived
583 cranberry proanthocyanidins by LC-ESI-MS. *Food Chemistry*, *128*(3),
584 802-810.
585 Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H.
586 (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating
587 antioxidant activity from guava fruit extracts. *Journal of Food Composition*
588 *and Analysis*, *19*(6-7), 669-675.
589 Wada, L., & Ou, B. X. (2002). Antioxidant activity and phenolic content of oregon
590 caneberries. *Journal of Agricultural and Food Chemistry*, *50*(12), 3495-3500.
591 Wang, D. J., Wang, X., Geng, Y. L., & Li, S. B. (2008). Determination of oleanolic
592 acid and ursolic acid in different species of *Fructus chaenomeles* by RP-HPLC
593 [J]. *Food Science*, *10*.
594 Yang, G., Fen, W., Lei, C., Xiao, W., & Sun, H. (2009). Study on determination of
595 pentacyclic triterpenoids in *Chaenomeles* by HPLC-ELSD. *Journal of*

Chromatographic Science, *47*(8), 718-722.

Zhang, L., Cheng, Y. X., Liu, A. L., Wang, H. D., Wang, Y. L., & Du, G. H. (2010). Antioxidant, Anti-Inflammatory and Anti-Influenza Properties of Components from *Chaenomeles speciosa*. *Molecules*, *15*(11), 8507-8517.

Zheng, J., Ding, C. X., Wang, L. S., Li, G. L., Shi, J. Y., Li, H., Wang, H. L., & Suo, Y. R. (2011). Anthocyanins composition and antioxidant activity of wild *Lycium ruthenicum* Murr. from Qinghai-Tibet Plateau. *Food Chemistry*, *126*(3), 859-865.

Figure Captions

Fig. 1. The leaves and fruits of five *Chaenomeles* L. species.

Fig. 2. HPLC chromatogram of *C. thibetica* (A) and *C. japonica* (B) at 280 nm. Peaks: 1, Procyanidin trimer; 3, Procyanidin dimer B3; 4, Procyanidin B1; 5, Procyanidin trimer; 6, catechin; 7, Procyanidin trimer; 8, 5-caffeoylquinic acid (CGA); 9, Procyanidin trimer; 10, Procyanidin B2; 11, Procyanidin pentamer; 12, Epicatechin; 13, Procyanidin pentamer; 14, Procyanidin pentamer; 15, Procyanidin trimer; 16, Procyanidin pentamer; 17, Procyanidin pentamer; 18, Procyanidin hexamer; 19, Procyanidin trimer; 20, Procyanidin trimer; 21, Procyanidin oligomer; 22, Procyanidin oligomer; 23, Quercetin-3-*O*-rutinoside; 24, Quercetin-3-*O*-hexose.

Fig. 3. Contents of five representative bioactive compounds. The contents of each compound in five species were compared statistically. Bars with no common letter indicating significant differences ($p < 0.05$). Results are mean ± SE (n = 3).

Fig. 4. A. Total polyphenol content (TPC) and total flavan-3-ol content (TFC). TPC was measured using the Folin-Ciocalteu assay, and TFC was obtained by using the vanillin and HPLC assays, expressed by gallic acid equivalents (GAE) and catechin equivalents (CE) mg/g FW, respectively. B. Antioxidant capacities (TEAC with ABTS and FRAP assay) and antiradical power (ARP with DPPH assay) of each *Mugua*. ARP was compared based on TPC (μM GAE). Bars with no common letter indicating significant differences ($p < 0.01$). Results are mean ± SE (n = 3). Values in each column indicated by the same letter are not significantly different ($P < 0.01$).

Fig. 5. Procyanidin stucture and its hypothetical acid-catelyzed cleavage reactions with two nuleophilic reagents (A). HPLC profile of procyanidin cleavage products from *C. thibetica* extract with benzyl mercaptan (B) and phloroglucinol (C). Peaks: 1,

644 catechin; 2, epicatechin; 3 and 3', catechin adduct; 4 and 4', epicatechin adduct with

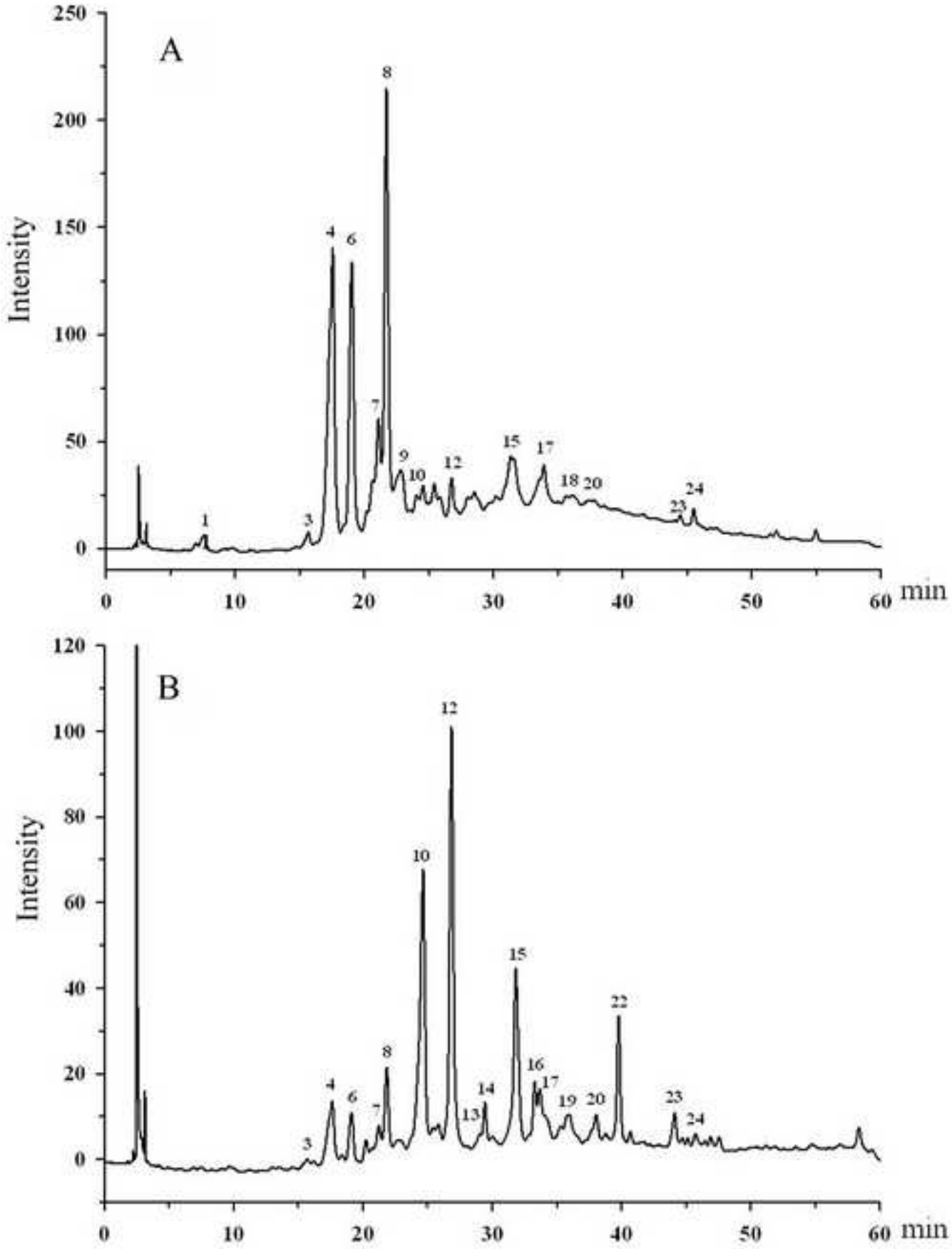
645 benzyl mercaptan and phloroglucinol.

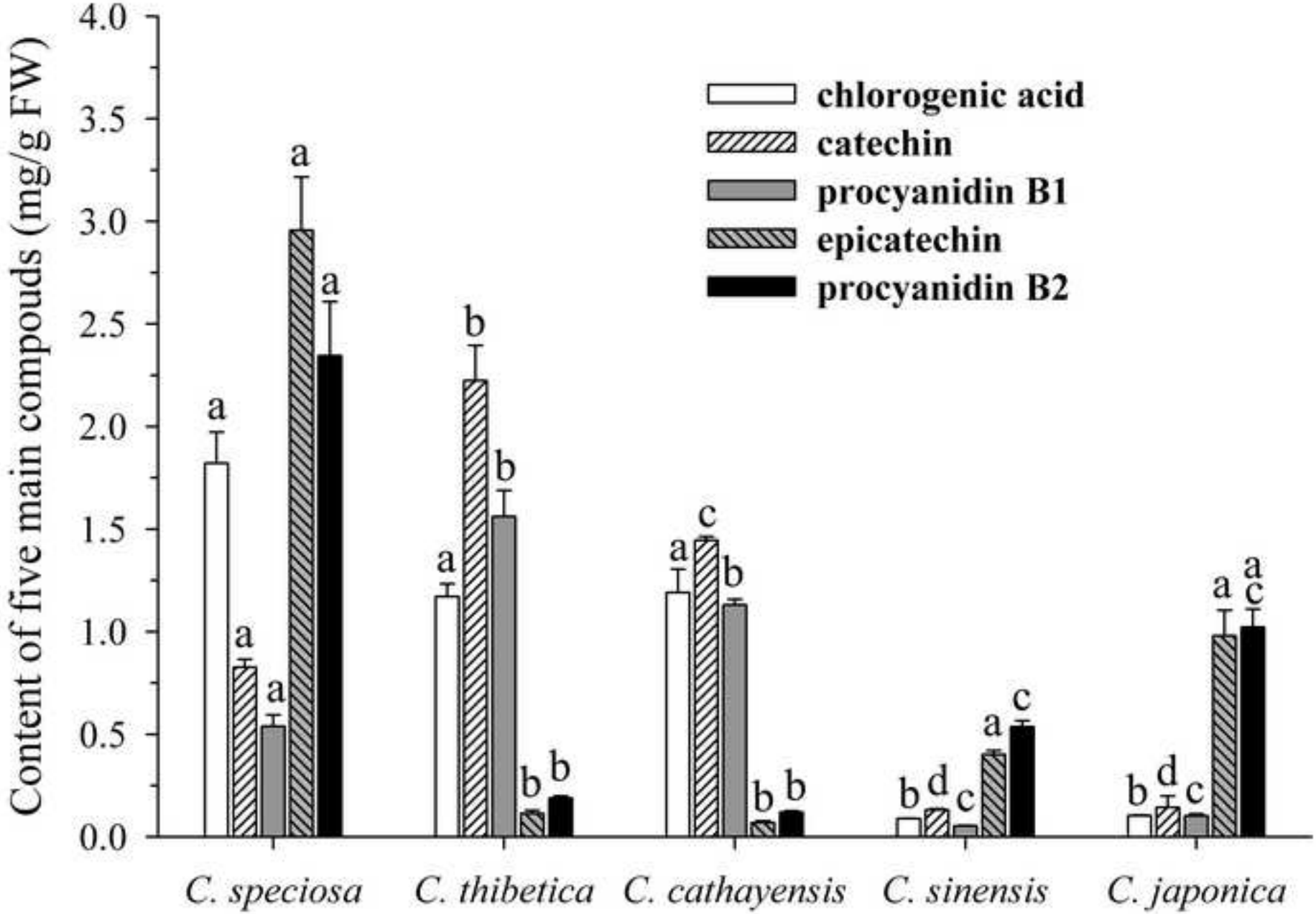
Table 1 Polyphenol compounds from *Chaenomeles* fruits.

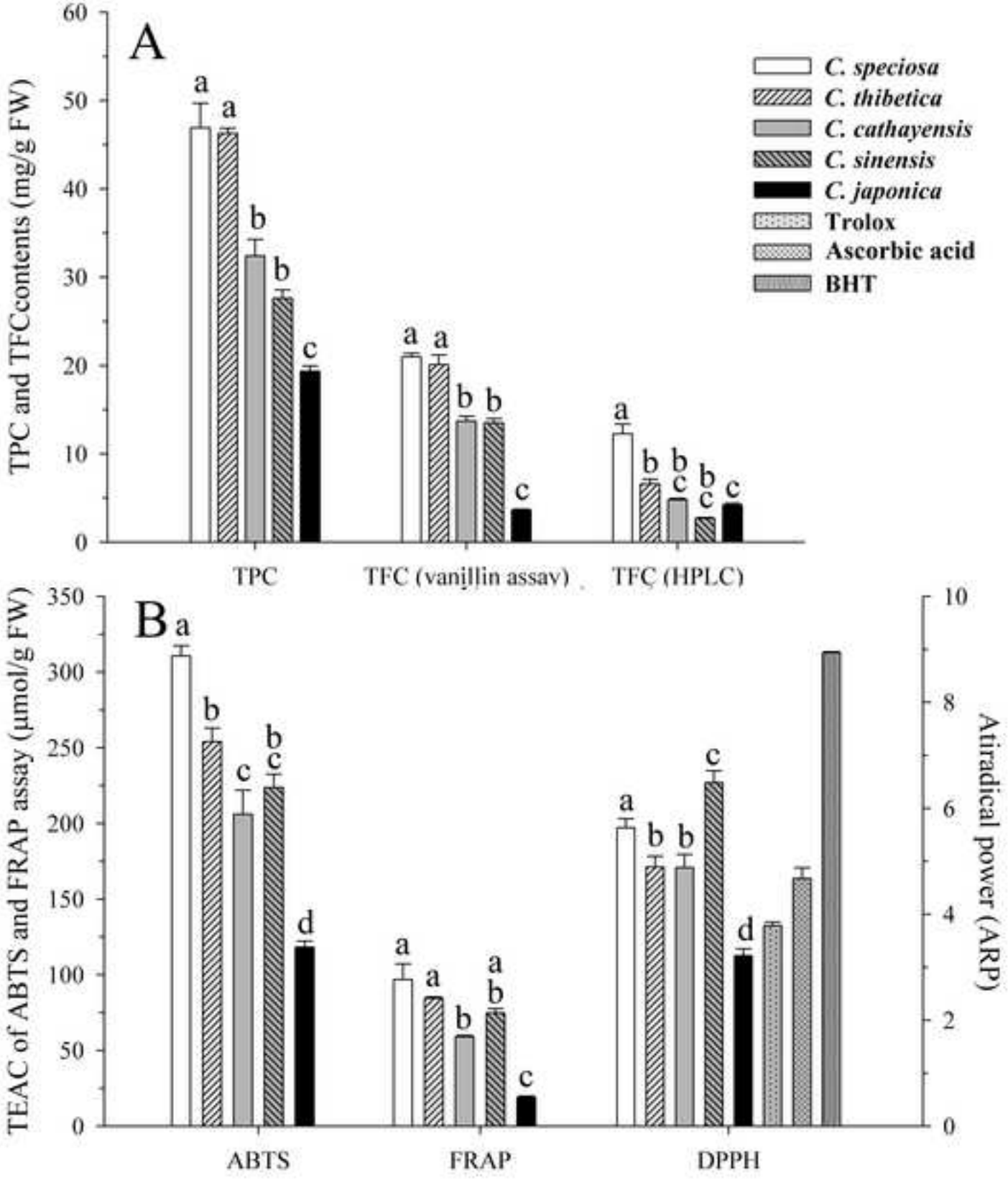
Peak No.	Identification*	RT[a] (min)	UV λ_{max} (nm)	*m/z* Value $[M–H]^-$	ESI-NI MS^2 (*m/z*)	Species[b]	References
1	Procyanidin trimer*	6.44	280.4	865(100)	575(8.25), 441(5.20)	1,2,3	Foo, 1996
2	3-caffeoylquinic acid*	12.43	325.9	353(100)	--	4[c]	Hamauzu, 2005
3	Procyanidin B3	15.67	281.0	577(65.66)	451(44.34), 425(4.99), 289(100)	1,2,3,5	Ricardo da Silva, 1991
4	Procyanidin B1	17.57	280.1	577(100)	451(6.90), 425(16.21), 289(6.51)	1,2,3,4,5	standard
5	Procyanidin trimer*	18.34	286.0	865(10.98)	577(20.66), 487(50.84), 465(100), 289(15.54),	4[c]	Foo, 1996
6	Catechin	19.04	279.8	289(100)	--	1,2,3,4,5	standard
7	Procyanidin trimer*	21.33	280.4	865(100)	577(9.44), 289(2.70)	1,2,3,4,5	Foo, 1996
8	5-caffeoylquinic acid(CGA)	21.75	325.8	353(100)	--	1,2,3,4,5	standard
9	Procyanidin trimer*	22.85	281.9	865 (37.45)	577(100),425(14.25), 289(4.71)	1,2,3,5	Foo, 1996
10	Procyanidin B2	24.59	280.3	577(100)	425(14.61), 289(1.61)	1,2,3,4,5	standard
11	Procyanidin pentamer*	25.47	280.1	720(21.25) $[M–H]^{2–}$	865(14.06), 577(100), 720.6(12.68), 289(23.42)	1,2,3,4,5	Sivakumaran, 2006
12	Epicatechin	26.80	279.8	289(100)	--	1,2,3,4,5	standard
13	Procyanidin pentamer*	28.03	311.6	720(44.79) $[M–H]^{2–}$	865(60.88), 720.7(19.34), 577(68.61), 289(36.03)	1,2,3,4,5	Sivakumaran, 2006
14	Procyanidin pentamer*	28.60	280.6	720(100) $[M–H]^{2–}$	865 (45.86), 720.6(65.87), 577(66.88), 289(29.80)	1,2,3,4,5	Sivakumaran, 2006
15	Procyanidin trimer*	30.53	280.5	865(100)	577(3.10), 289(0.31)	1,2,3,4,5	Foo, 1996
16	Procyanidin pentamer*	32.30	280.5	720(20.67) $[M–H]^{2–}$	863(21.30), 720.6(9.32), 576(100), 289(44.54)	1,2,3,4,5	Sivakumaran, 2006
17	Procyanidin pentamer*	32.84	280.5	720(13.75) $[M–H]^{2–}$	865(7.86), 577(100), 720.6(21.08), 289(11.31)	1,2,3,4,5	Sivakumaran, 2006

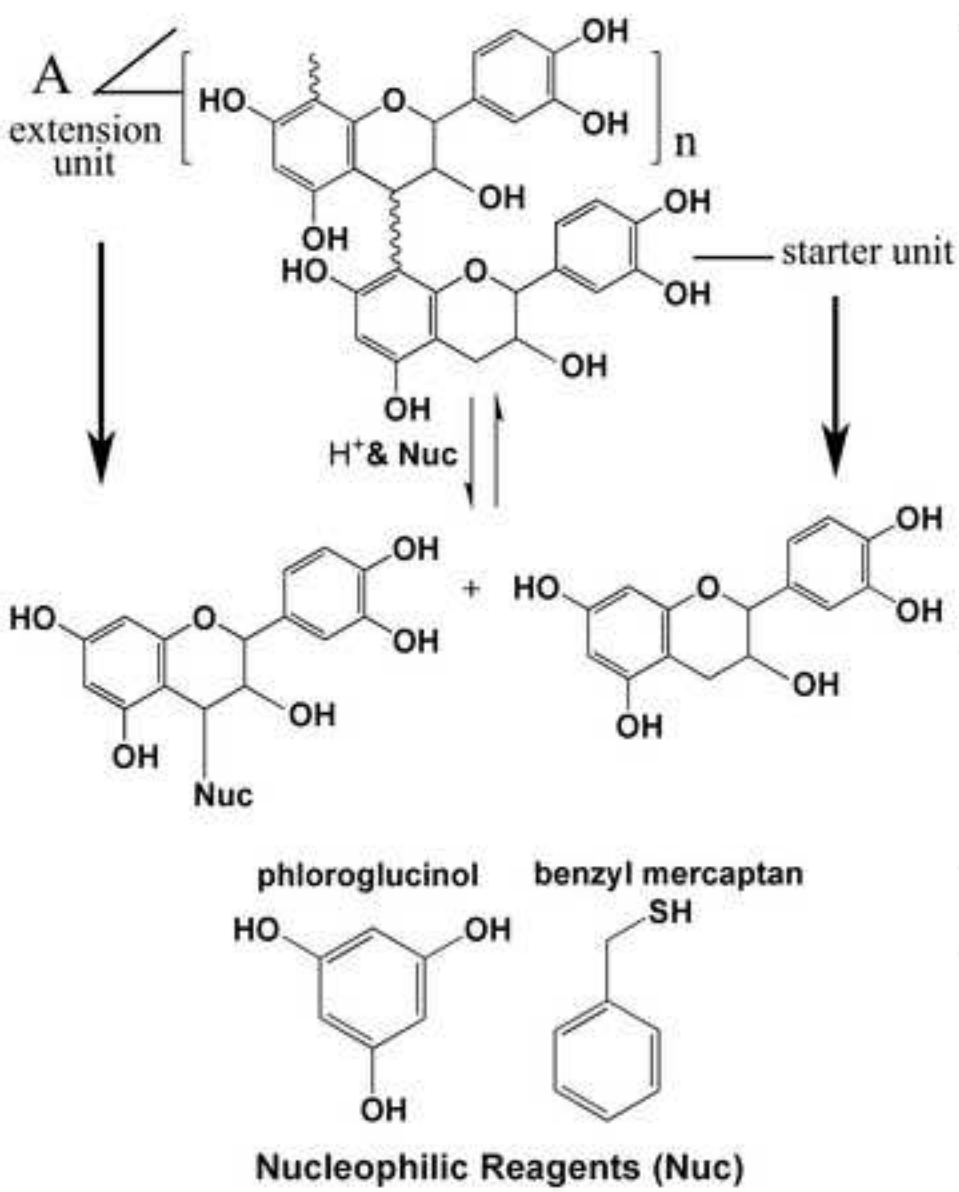
18	Procyanidin hexamer*	37.7	280.6	864(48.56) $[M–H]^{2–}$	865(31.89), 289(7.65), 577(29.30)	1,2,3,4,5	Sivakumaran, 2006
19	Procyanidin trimer*	38.40	282.0	864(45.50)	577(100), 575(80.93), 289(29.48)	1,3,4,5	Foo, 1996
20	Procyanidin trimer*	39.53	279.0	865(58.01)	575(100), 289(21.79)	1,2,4,5	Foo, 1996
21	Procyanidin oligomer	41.03	281.0	unkown	960.3(13.01), 865(14.32), 864(39.46) 720(21.85), 575(30.9), 535(100), 287(13.3)	1,2,3,4	
22	Procyanidin oligomer	43.88	280.5	unkown	960.4(1.0), 865(9.89), 887(3.42), 579(100) 289(0.91), 285(4.46), 271(2.48)	1,4,5	
23	Quercetin-3-*O*-rutinoside*	44.57	257,352	609(100)	463(10.75), 301(0.49), 300(2.54)	1,2,3,4,5	
24	Quercetin-3-*O*-hexose*	45.60	266,358	463(100)	301(5.34), 300(7.91)	1,2,3,4,5	

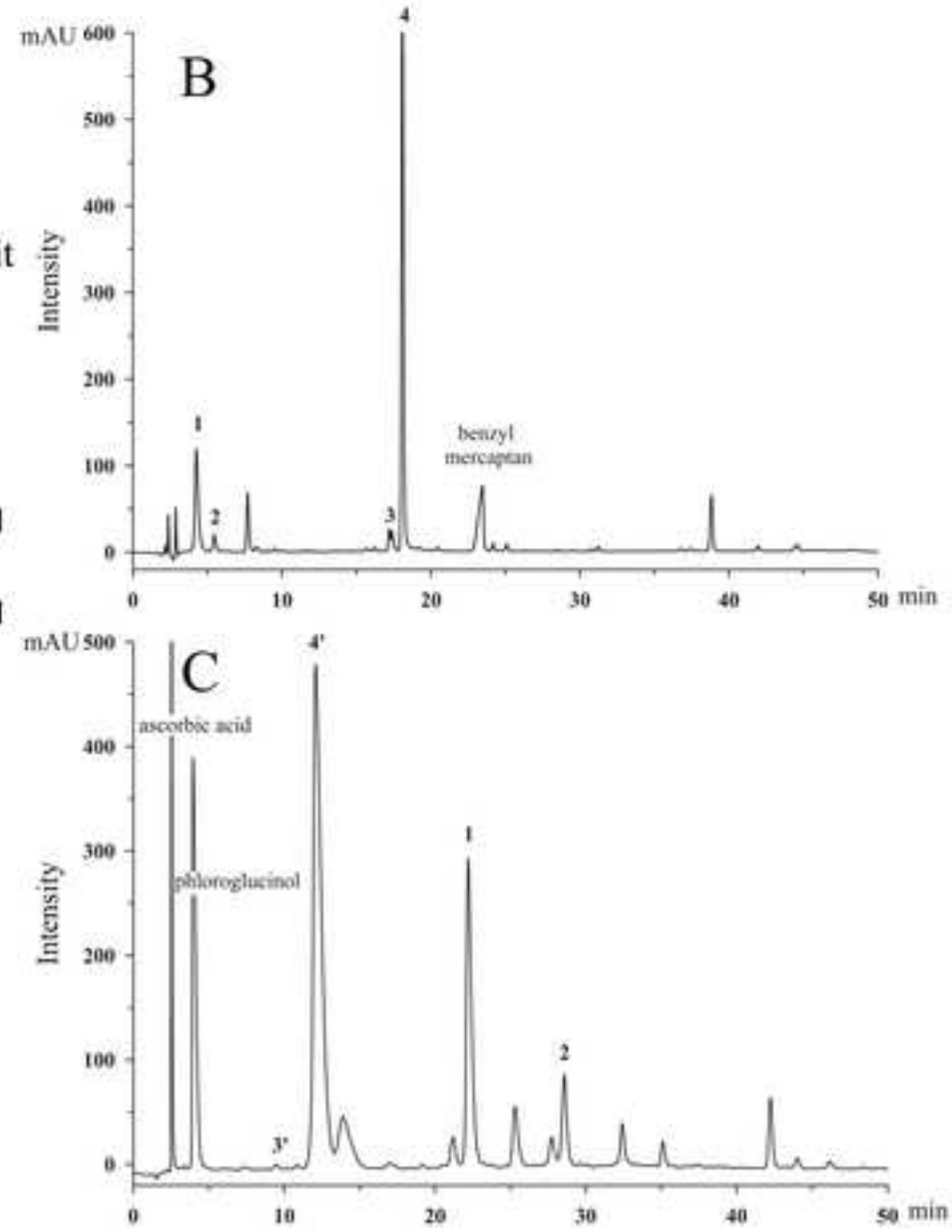
[a] RT, retention time on HPLC. [b] 1, *C. speciosa*; 2, *C. thibetica*; 3, *C. cathayensis*; 4, *C. sinensis*; 5, *C. japonica*. [c] None or peak area less than 0.3 mAU×min. * Tentative identification.











649 Highlights

650 - Polyphenols from five species in genus *Chaenomeles* were analyzed and compared.

651 - 20 flavanols (monomers and procyanidin oligomers) were separated by RP-HPLC.

652 - *C. speciosa* and *C. thibetica* exhibited equally strong antioxidant activities.

653 - Total flavanol content is markedly correlated with antioxidant activity.

654 - Procyanidin B3 was firstly detected in four *Chaenomeles* species.

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