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Antioxidant activity and effective compounds of immature calamondin peel

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ABSTRACT

The antioxidant activity and the flavonoids of mature and immature calamondin (*Citrus mitis* Blanco) peel were investigated. The hot water extract of immature calamondin peel exhibited the highest oxygen radical absorbance capacity (ORAC), reducing power, and superoxide scavenging effect. 3′,5′-Di-C-β-glucopyranosylphloretin, naringin, hesperidin, nobiletin, and tangeretin are the five major flavonoids found in hot water extract with the levels of 6888 ± 522, 2333 ± 157, 1350 ± 94, 165 ± 13, and 8 ± 4 mg/100 g dry basis, respectively. The contents of nobiletin and tangeretin increased after ripening. The hot water extract of immature calamondin peel was fractionated using a semi-preparative HPLC. Fraction VI showed the highest ORAC value (28.02 ± 2.73 mmol Trolox equivalents (TE)/g fraction) and two compounds, naringin and hesperidin, were identified as the major active components attributed to the antioxidant activity. Fraction V contained 3′,5′-di-C-β-glucopyranosylphloretin, which revealed low ORAC value with 7.43 mmol TE/g fraction. However, it might also contribute to antioxidant activity in immature calamondin peel due to its greatest quantity.

1. Introduction

Calamondin bears small size citrus fruits. The hot water extract of immature fruit with green peel has been a popular beverage in Taiwan for many years due in part to its potential health beneficial properties. It is well known that dried citrus peel is a traditional Chinese medicine (Zang, 2005). Citrus peel contains a large quantity of bioactive compounds, such as flavonoids including polymethoxyflavones, phenolic acids, and limonoids (Choi et al., 2011; Li, Lo, & Ho, 2006). Good correlation between content of phenolic compounds and antioxidant activity has been reported for the citrus peel. Phenolic compounds are regarded as major bioactive compounds in citrus peel (Li et al., 2006; Ramful, Bahorun, Bourdon, Tarnus, & Aruoma, 2010; Sadek, Makris, & Kefalas, 2009). However, calamondin (*Citrus mitis* Blanco), a cultivar related to citrus, is a hybrid between *Citrus reticulate* Blanco and *Fortunella* spp (Moshonas & Shaw, 1996) and the flavonoid composition of *Fortunella* species differs from those of the *Citrus* species (Ogawa, Kawasaki, Omura, & Yoshida, 2001; Sadek et al., 2009). Therefore, the compositions of phenolic compounds of calamondin might be quite different in comparison with citrus.

Citrus contains large amounts of flavanones, with hesperidin the most abundant glycoside (Di Majo et al., 2005; Ramful et al., 2010; Ramful, Tarnus, Aruoma, Bourdon, & Bahorun, 2011). However, calamondin contains a large quantity of 3′,5′-di-C-β-glucopyranosylphloretin in its peel, juice sac and leaf (Ogawa et al., 2001). In our previous study, 3′,5′-di-C-β-glucopyranosylphloretin was identified as the major compound in hot water extract of immature calamondin peel, but naringin and hesperidin were also found in the peel (Lou, Yu, & Ho, 2012). The tyrosinase inhibitory activity of these flavonoids was evaluated. Another study demonstrated that eight polymethoxyflavones were isolated and identified from peel of calamondin (Tatum & Berry, 1978). Flavonoids, such as poncirin, dydimin, neohesperidin, hesperidin, narirutin, diosmin, and isorhoifolin, have been extracted by 80% methanol from dried calamondin pulp powder (Ramful et al., 2011).

The antioxidant activity of orange pulp was affected during ripening and maturation. A progressive decrease in the flavonoid content over ripening in Chinotto fruits and kumquat has been reported (Barreca, Bellocco, Caristi, Leuzzi, & Gattuso, 2010a, 2010b). The levels of total polyphenols, flavonoids and the DPPH (1,1-diphenyl-2-picrylhydrazine) scavenging capacity of immature calamondin peel were found to be higher than those of mature calamondin peel (Hsu, 2009).

In order to characterise the major antioxidant effective compounds in calamondin peel, extractions of calamondin peel with hot water and ethyl acetate were carried out and subjected to the analysis of antioxidant activities and phenolic compounds. Furthermore, the extracts were fractionated by a semi-preparative HPLC and the active fractions were collected. The antioxidant activities of effective compounds were evaluated and their contents in calamondin peel were also determined.

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2. Materials and methods

2.1. Materials

Calamondin (*C. mitis* Blanco) was collected from a calamondin estate in the Jao-Si region, Ilan, Taiwan during November 2009–February 2010. The collected calamondin with whole green appearance was defined as immature and its average weight was 14.53 ± 0.53 g each. The mature calamondin with whole yellow appearance was also collected and its average weight was 23.35 ± 0.59 g each. The collected calamondin was manually peeled and the peels were lyophilised for 48 h. Prior to extraction, the peels were pulverised in a blender and passed through a 40 mesh sieve. The obtained immature and mature calamondin peel powders were stored in a suitable brown bottle with screw cap at −18 °C, separately.

2.2. Chemicals

Phenazine methosulfate (PMS), β-dihydronicotinamide adenine dinucleotide (β-NADH), nitroblue tetrazolium (NBT), disodium fluorescein (FL), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 97% (Trolox), naringin, hesperidin, nobiletin, tangeretin, and standards of other flavonoids including neoeriocitrin, rutin, diosmin, neohesperidin, naringenin, hesperetin, and limettin, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Potassium ferricyanide, trichloroacetic acid, ferric chloride, and other chemicals were of analytical grade from Merck Chemical Co. (Darmstadt, Germany). 3′,5′-Di-C-β-glucopyranosylphloretin was obtained from hot water extract of immature calamondin peel. The extract was separated by a semi-preparative HPLC using C18 column and the compound was collected. After lyophilisation, the residue was redissolved in deionised water and stored at −18 °C for further use.

2.3. Extraction procedure

Three grams of dried and powdered immature and mature calamondin peels were extracted with (A) 60 mL deionised 90 °C water (1:20, w/v) for 1 h in a shaking water bath at 90 °C; or (B) 60 mL ethyl acetate (1:20, w/v) for 1 h in a shaker at room temperature (Lou et al., 2012). The shaking rate was 100 rpm. The extract was filtered with a Whatman No. 1 filter paper. The obtained residue was extracted by the same procedure two more times. Three resulting filtrates were transferred into a 250 mL flask and dried by rotary vacuum evaporator at 50 °C. The obtained hot water extracts were 1.61 ± 0.62 g and 2.22 ± 0.02 g for immature and mature calamondin peel, respectively. The ethyl acetate extracts were 0.07 ± 0.00 g for immature calamondin peel, and 0.06 ± 0.00 g for mature calamondin peel. To dissolve the filtrate, a suitable volume of deionised water was added to the flask for the hot water extracts. Instead of deionised water, methanol was used to dissolve the ethyl acetate extracts. The obtained four solutions were defined as immature calamondin peel water extract (ICW), mature calamondin peel water extract (MCW), immature calamondin peel ethyl acetate extract (ICEA), and mature calamondin peel ethyl acetate extract (MCEA). The solutions were poured into brown bottles with screw cap and stored at −18 °C, separately, until further use.

2.4. HPLC analysis of flavonoids

The extracts of ICW, MCW, ICEA, and MCEA were subjected to a RP-18 column (250 mm × 4.6 mm, 5 μm, Merck, Darmstadt, Germany) using a gradient with 2% (v/v) acetic acid in water as solvent A and 0.5% acetic acid in water/acetonitrile (1:1, v/v) as solvent B (Schieber, Keller, & Carle, 2001). The gradient was carried out as 0–10 min, 5–10% B; 10–55 min, 10–55% B; 55–60 min, 55–80% B; 60–65 min, 80–100% B; 65–70 min, 100% B; 70–75 min, 100–5% B. The flow rate was 1 mL/min. Photodiode array (PDA) detection was performed between 220 nm and 350 nm, with a resolution of 2 nm. Twelve flavonoids used as standards were available in our laboratory as follows: neoeriocitrin, rutin, diosmin, naringin, hesperidin, neohesperidin, naringenin, hesperetin, limettin, nobiletin, tangeretin, and 3′,5′-di-C-β-glucopyranosylphloretin. The flavonoids were identified by their retention time and the UV spectra of standards and quantified from peak area at 280 nm by an external standard method, using calibration curves. Their concentrations were expressed as milligram per 100 g dry weight.

2.5. Oxygen radical absorbance capacity (ORAC) assay

The reaction was carried out in 75 mM phosphate buffer (pH 7.4) in cuvettes (Ou, Hampsch-Woodill, & Prior, 2001). Fifty microlitres of plant extract solutions and 50 μL of disodium fluorescein (70 nM final concentration) were mixed in the cuvette and preincubated for 15 min at 37 °C. Twenty-five microlitres of APPH solution (221 mM final concentration) was then added, and the fluorescence was recorded for 70 min at excitation and emission wavelengths of 485 and 520 nm every 5 min. A blank sample containing phosphate buffer in the reaction mix was measured. Five calibration solutions of Trolox (10, 20, 30, 40, and 50 μM final concentration) were also tested to establish a standard curve. All samples were analysed in triplicate. The area under the curve (AUC) was calculated for each sample by integrating the relative fluorescence curve. The net AUC of the sample was calculated by subtracting the AUC of the blank. The regression equation between net AUC and Trolox concentration was determined, and ORAC values were expressed as mmol Trolox equivalents/g plant extract or fractions using the standard curve established previously.

2.6. Determination of reducing power

Two hundred and fifty microlitres of extract was mixed with 250 μL of phosphate buffer (0.2 M, pH = 6.6) and 250 μL of potassium ferricyanide solution (1% w/v) (Oyaizu, 1978; Shon, Choi, Kahng, Nam, & Sung, 2004). The mixture was incubated in a water bath for 20 min at 50 °C. After incubation, 250 μL of 10% trichloroacetic acid was added to the mixture solution, followed by centrifugation at 3000 g for 10 min at room temperature. An aliquot (100 μL) of supernatant was mixed with 100 μL of distiled water and 20 μL of 0.1% ferric chloride. After reaction for 10 min, the absorbance was measured at 700 nm against the blank. The increased absorbance of the mixture represented the increased reducing power.

2.7. Superoxide anion scavenging assay

The reaction mix in a final volume of 1.0 mL was composed of 0.1 M phosphate buffer (pH 7.4), 120 μM phenazine methosulfate (PMS), 936 μM β-dihydronicotinamide adenine dinucleotide (β-NADH), and 300 μM nitroblue tetrazolium (NBT) with or without 1.0 mL of tested extract (Robak & Gryglewski, 1988). The reaction mixture was incubated at room temperature for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The inhibition percentage of superoxide anion scavenging activity was calculated by the equation: $[(Ao - As)/Ao] \times 100\%$, where Ao is the absorbance of the control and As is the absorbance of the sample.

2.8. Isolation of flavonoids fraction by semi-preparative HPLC

The antioxidant active compounds were isolated from ICW by a semi-preparative column. The chromatographic system consisted of a Shimadzu binary pump and SPD-M10 photodiode array detector. A semi-preparative C18 column (Hibar® Pre-Packed Column, Lichrospher 100 RP-18 endcapped 5 μm, 250 × 10 mm, Merck, Darmstadt, Germany) was used for separation. Solvent A consisted of 2% (v/v) acetic acid in water, and solvent B consisted of 0.5% acetic acid in water/acetonitrile (1:1, v/v). The flow rate was 1 mL/min. The solvent gradient in volume ratio was as follows: 0–10 min, 5–10% B; 10–55 min, 10–55% B; 55–60 min, 55–80% B; 60–65 min, 80–100% B; 65–140 min, 100% B; 140–145 min, 100–5% B. Photodiode array (PDA) detection was performed between 220 nm to 350 nm. A 100 μL aliquot of the extract was subjected to the HPLC system. Nine fractions, ICW-I to ICW-IX, were isolated and collected in the flask. After concentration by rotary vacuum evaporation, the fractions were dissolved with deionised water to a proper concentration for the ORAC assay and HPLC analyses.

2.9. Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($p < 0.05$), using SAS (SAS Inst., Cary, NC, USA).

3. Results and discussion

3.1. Determination of antioxidant capacity

To demonstrate the potential health promoting properties of calamondin, we carried out a screening on the antioxidant abilities of the extracts from calamondin peel. The ORAC assay of hot water and ethyl acetate extracts from mature and immature calamondin peel is shown in Fig. 1A. The ORAC value of immature calamondin peel was higher than the mature calamondin peel in both hot water and ethyl acetate extracts. The reducing power of the calamondin peel extract showed an obvious concentration effect (Fig. 1B). The hot water extract of the immature calamondin peel had the highest reducing power, while the hot water extract of mature calamondin peel showed the lowest reducing power. However, the ethyl acetate extract of the mature calamondin peel had better reducing power than the hot water extract of immature calamondin peel. The inhibition percentage of superoxide anion scavenging activity of mature and immature calamondin peel hot water extracts also showed a concentration effect (Fig. 1C). The best inhibition percentage of immature calamondin peel was observed in hot water extract, while the ethyl acetate extract showed no significant or negative effect. The extract of ICEA might contain some nonpolar pigments, such carotenoids and chlorophylls, which could probably interfere with the absorbance at UV 560 nm. It might probably lead to the negative superoxide anion scavenging effect of ICEA.

Collectively, the hot water extract of immature calamondin peel showed the highest antioxidant activities, included ORAC, reducing power, and superoxide anion scavenging capacity. Hot water extract of immature calamondin is a popular hot drink in Taiwan and is commonly demonstrated as a healthful drink in folk medicine record. This may probably be explained by its high antioxidant capacity. It has been shown that the DPPH and anion superoxide scavenging activities of juices from immature Chinotto (*Citrus* × *myrtifolia* Raf.) fruits were higher than that from mature fruits (Barreca et al., 2010a). The flavonoids fraction of immature kumquat (*Fortunella japonica* Swingle) was found to have higher

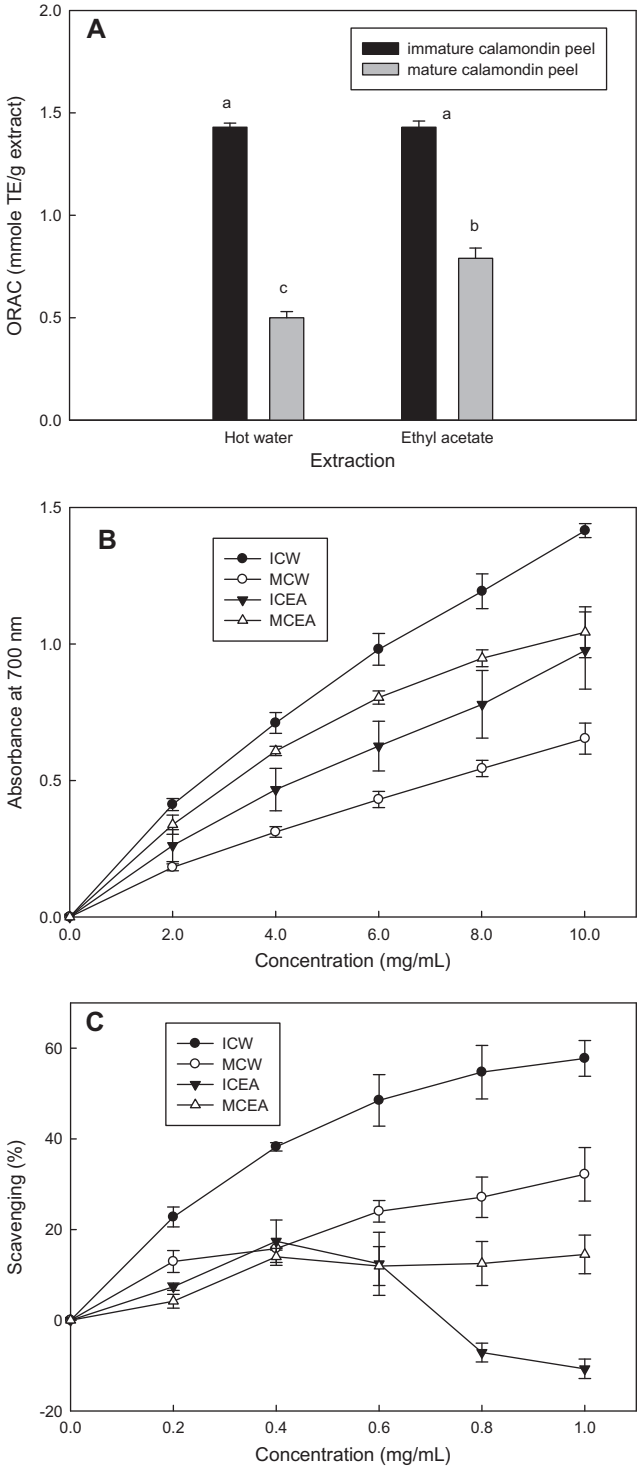

Fig. 1. Antioxidant activity of extracts from calamondin peel by hot water and ethyl acetate. (A) Oxygen radical absorbance capacity (ORAC) of different extractions. (B) Reducing power of different concentrations. (C) Superoxide scavenging activity of different concentrations. Different letters of various extracts were considered significantly different ($p < 0.05$).

DPPH and $ABTS^{+}$ (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) radical scavenging activities than the mature kumquat, except for the juice of kumquat (Barreca et al., 2010b). The free radical scavenging activities of the calamondin pulp extracts as assessed by TEAC (Trolox equivalent antioxidant capacities) assay were in the range 7.70–7.71 μmole/g fresh weight, the second highest value among the other nine citrus (Ramful et al., 2011).

3.2. Determination of phenolic compounds in extracts of calamondin peel

The HPLC chromatograms of immature calamondin peel hot water extract (ICW) and mature calamondin peel ethyl acetate extract (MCEA) are shown in Fig. 2A. Five phenolic compounds were found in ICW, as 3′,5′-di-C-β-glucopyranosylphloretin (1), naringin (2), hesperidin (3), nobiletin (4), and tangeretin (5), while only two major compounds, nobiletin, and tangeretin, were observed in MCEA (Fig. 2B). Ramful et al. (2011) showed that the pulp extract of calamondin contained flavonoids, such as poncirin, dydimin, neohesperidin, hesperidin, narirutin, diosmin, and isorhoifolin. The difference of the flavonoids composition, compared with our data, might be due to the different growth environment.

Table 1 shows the levels of flavonoids in mature and immature calamondin peel extracted by hot water and ethyl acetate. The ICW contained the highest total flavonoids content with 10746 mg/100 g dry basis, while the lowest levels of total flavonoids in MCW were observed with only 1862 mg/100 g dry basis. The content of total flavonoids in ICEA and MCEA were 2629 and 2539 mg/100 g dry basis, respectively. In ICW, the largest amount of 3′,5′-di-C-β-glucopyranosylphloretin was observed with 6888 mg/100 g dry basis, equal to 64.1% of the total flavonoids. The levels of naringin and hesperidin were 2333 and 1350 mg/100 g dry basis, respectively. Lower contents of nobiletin and tangeretin were found in ICW. The dihydrochalcone derivative, 3′,5′-di-C-β-glucopyranosylphloretin, in immature calamondin peel has been isolated and identified from hot water extract in remarkably large amounts (Lou et al., 2012; Ogawa et al., 2001). It has also been reported in the kumquat (*Fortunella margarita* and *japonica*) (Jayaprakasha, Chidambara Murthy, Etlinger, Mantur, & Patil, 2012; Sadek et al., 2009) and in Kumquat juice (Barreca et al., 2010b). The flavanones, naringin and hesperidin, existed commonly in citrus are more polar (Kawail, Tomono, Katase, Ogawa, & Yano, 1999; Kawail et al., 2000; Yoo, Lee, Park, Lee, & Hwang, 2004). Nobiletin and tangeretin are polymethoxyflavones with six and five methoxy groups, respectively. Because of the hydrophobic nature of methoxy groups, polymethoxyflavones are less polar than hydroxylated flavonoids, such as 3′,5′-di-C-β-glucopyranosylphloretin, naringin, and hesperidin (Li et al., 2006).

The content of flavonoids, including 3′,5′-di-C-β-glucopyranosylphloretin, naringin, hesperidin, nobiletin, and tangere-

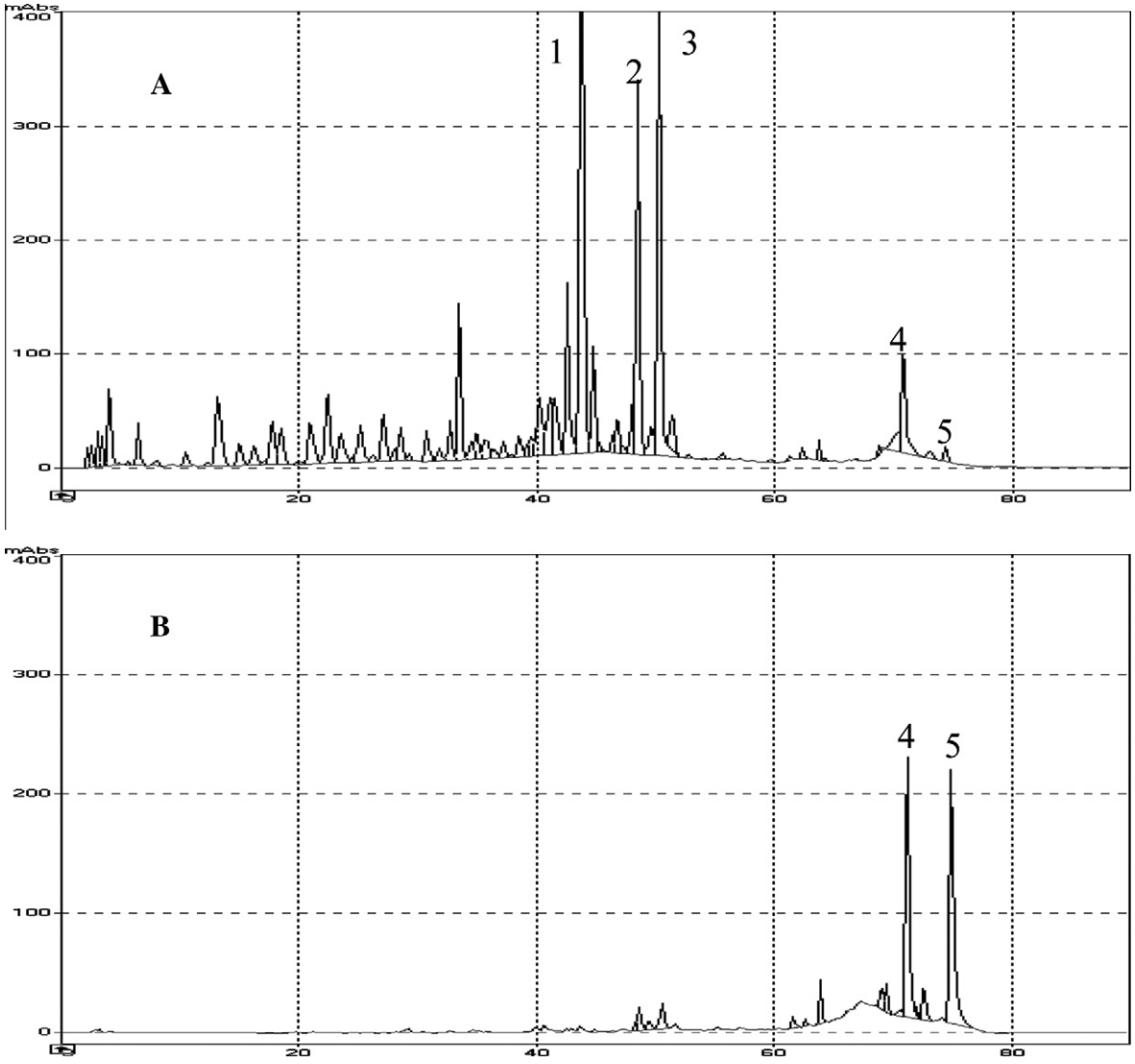

Fig. 2. HPLC profile of different extracts from calamondin peel. (A) Hot water extract of immature calamondin peel. (B) Ethyl acetate extract of mature calamondin peel. (1) 3′,5′-Di-C-β-glucopyranosylphloretin, (2) naringin, (3) hesperidin, (4) nobiletin, (5) tangeretin.

Table 1
Distribution of phenolic composition of extracts from peel of immature and mature calamondin with different solvents.

Extracts[e]	Phenolic compounds[f] (mg/100 g dry extract)					Total (mg/100 g dry extract)
	Naringin	Hesperidin	3′,5′-Di-C-β-glucopyranosylphloretin	Nobiletin	Tangeretin	
ICW	2333 ± 157[a]	1350 ± 94[a]	6888 ± 522[a]	165 ± 13[c]	8 ± 4[c]	10746 ± 554
MCW	245 ± 24[b]	101 ± 8[b]	1467 ± 111[b]	48 ± 14[d]	N.D.	1862 ± 115
ICEA	N.D.	N.D.	1026 ± 163[b]	941 ± 26[b]	661 ± 28[b]	2629 ± 168
MCEA	N.D.	N.D.	N.D.	1378 ± 31[a]	1161 ± 28[a]	2539 ± 42

[a–d] Values (Mean ± S.D., $n = 3$) in the same column with different superscripts are significantly different ($p < 0.05$).
[e] ICW: immature calamondin peel water extract; MCW: mature calamondin peel water extract; ICEA: immature calamondin peel ethyl acetate extract; MCEA: mature calamondin peel ethyl acetate extract.
[f] Neoericitrin, Rutin, Diosmin, Neohesperidin, Naringenin, Hesperetin, Limettin were not detected.

tin, was significantly lower in MCW than in ICW. A progressive decrease in the flavonoid content over ripening in Chinotto fruits and kumquat has been reported (Barreca et al., 2010a, 2010b).

In ICEA, the extractable flavonoids were 3′,5′-di-C-β-glucopyranosylphloretin, nobiletin, and tangeretin. The levels of nobiletin and tangeretin in ICEA were much higher than in ICW. Because these two polymethoxyflavones are less polar, it is more extractable in ethyl acetate than in water. The content of 3′,5′-di-C-β-glucopyranosylphloretin was only 1026 mg/100 g dry basis in ICEA, which was much lower than that in ICW. This is obviously due to its lesser solubility in ethyl acetate during extraction.

Interestingly, the content of nobiletin and tangeretin in MCEA was significantly higher than that in ICEA, which is different as compared to the polar flavonoids in water extract. This indicated that polar flavonoids, 3′,5′-di-C-β-glucopyranosylphloretin, naringin and hesperidin decreased and the less polar flavonoids, nobiletin and tangeretin, increased in calamondin peel during ripening. The growth of the fruits generally decreases the flavonoids contents per fruit weight in *Citrus* fruits (Ogawa et al., 2001). It is logical to hypothesise that the high level of bitter flavonoids in young fruit tissues may have a role in defence from herbivore, at least until seeds within the fruit are mature (Owens & McIntosh, 2011). The highest levels of these secondary metabolites were found in young developing fruits (Del Rio et al., 2004), although it has been suggested that some polymethoxyflavones might be related to the maturation phase of fruit in some *Citrus* species (Ortuno, Arcas, Benavente-Garcıa, & Del Rıo, 1999). These compounds are mainly located in the peel, the polymethoxyflavones in the flavedo and the flavanones in the albedo, suggesting that they may act in protecting the growing fruit from pathogenic attack (Ben-Aziz, 1967). Recent reviews of general roles of flavonoids in plants include many other possibilities (Hartmann, 2007; Owens & McIntosh, 2011). Some direct testing of the roles of flavonoids in citrus showed that polymethoxyflavonoids are key for defence against microbes (Del Rio et al., 2004).

Flavonoids contain phenolic groups, which serve as antioxidants due to their free radical scavenging activity or metal chelat-

Table 2
The oxygen radical absorbance capacity (ORAC) of different fractions from ICW obtained by semi-preparative reversed-phase HPLC separation.

Fractions	ORAC (mmole TE/g fraction)
I	18.39 ± 0.45[b]
II	2.50 ± 0.16[de]
III	9.01 ± 0.62[c]
IV	3.53 ± 0.12[d]
V	7.43 ± 0.35[c]
VI	28.02 ± 2.73[a]
VII	1.45 ± 0.02[e]
VIII	1.93 ± 0.17[de]
IX	0.88 ± 0.06[e]

[a–e] Values (Mean ± S.D., $n = 3$) with different superscripts are significantly different ($p < 0.05$).

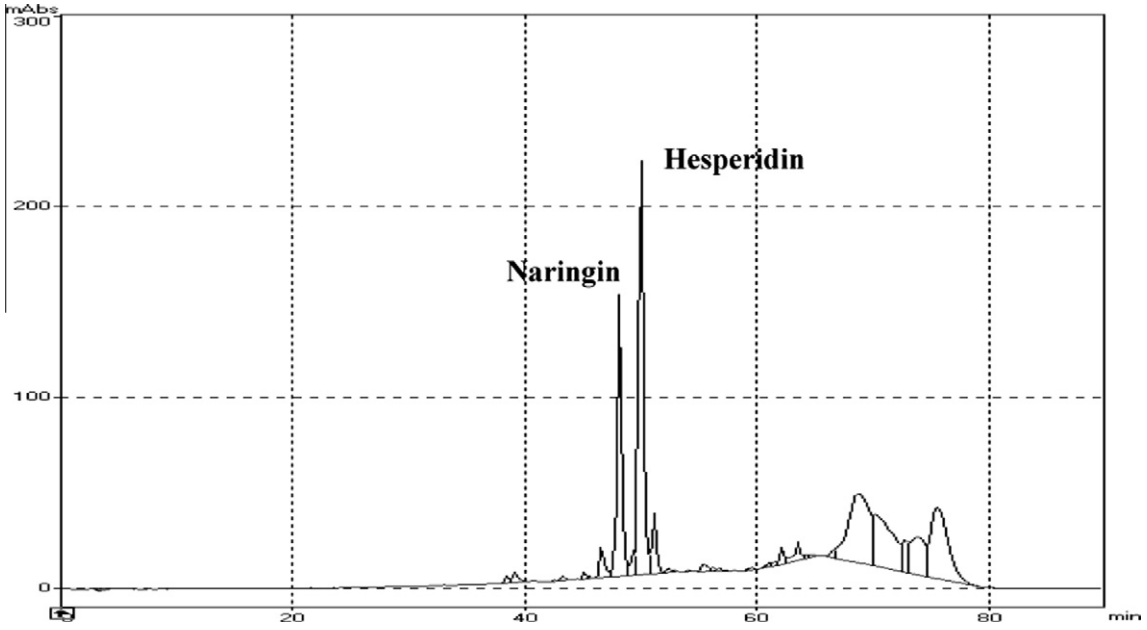

Fig. 3. Profile of fraction VI obtained from semi-preparative C18 column separation of hot water extract of immature calamondin peel.

ing ability. Therefore, the high content of flavonoids in ICW (Table 1) might result in the high antioxidant activity of ICW.

3.3. Antioxidant active components of fractions from ICW

In order to elucidate the major antioxidant active components in ICW, the ICW was first separated and collected as fractions I to IX by semi-preparative C18 column. Then, each fraction was subjected to ORAC assay. The obtained results (Table 2) indicated that fraction VI contained the highest ORAC value with 28.02 ± 2.73 mmol TE/g fraction. The ORAC values of Fractions I, III, and V were 18.39 ± 2.73, 9.01 ± 0.62, and 7.43 ± 0.35 mmol TE/g fraction, respectively. Low ORAC values of the other fractions with range of 0.88–3.53 mmol TE/g fraction were observed.

Fraction VI was subjected to analysis of phenolic compounds by HPLC. In fraction VI, two major peaks were identified as naringin and hesperidin by retention times and UV spectra as compared with standards (Fig. 3). It is therefore suggested that naringin and hesperidin might contribute to the major antioxidant activity in the immature calamondin peel. The contents of naringin and hesperidin in immature calamondin peel were 1.25 ± 0.04 g/100 g dry basis and 0.73 ± 0.03 g/100 g dry basis, respectively. The antioxidant activities of naringin and hesperidin have previously been reported (Wilmsen, Spada, & Salvador, 2005; Yu et al., 2005). Naringin and hesperidin contained 4′-OH and 3′-OH, respectively, which can probably increase the antioxidant power of flavonoids (Di Majo et al., 2005). For fraction V, the major component was identified as 3′,5′-di-C-β- glucopyranosylphloretin, which showed a low antioxidant activity with an ORAC value of 7.43 ± 0.35 mmol TE/g fraction. However, the antioxidant activity of calamondin peel might also be affected by this phloretin derivative due to its great quantity in immature calamondin peel (6888 mg/100 g dry basis in ICW). Furthermore, it has been reported that 3′,5′-di-C-β-glucopyranosylphloretin contained good tyrosinase inhibitory activity in a competitive mode (Lou et al., 2012). Nobiletin and tangeretin were found in fractions VIII at 1.93 ± 0.17 mmol TE/g fraction and IX at 0.88 ± 0.06 mmol TE/g fraction. Nobiletin and tangeretin are polymethoxyflavones, which show lower antioxidant activity due to the *O*-methylation (Di Majo et al., 2005). However, polymethoxyflavones have been of particular interest because many of these flavonoids exhibit a broad spectrum of biological activity, including anti-inflammatory, anticarcinogenic, antiviral, antithrombogenic, and antiatherogenic properties (Li et al., 2006).

4. Conclusions

The hot water extract of immature calamondin peel contained the largest quantity of flavonoids and showed the highest antioxidant activity among all extracts studied. In the hot water extract, the contents of 3′,5′-di-C-β-glucopyranosylphloretin, naringin, and hesperidin in immature calamondin peel were much higher than those in mature calamondin peel. However, the contents of polymethoxyflavones, nobiletin and tangeretin, in calamondin peel, extracted by ethyl acetate, increased after ripening. Collectively, the major antioxidant compounds in immature calamondin peel were identified as naringin and hesperidin. The compound, 3′,5′-di-C-β-glucopyranosylphloretin, might also contribute to some antioxidant activity because of its large quantity in the peel.

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