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Comparison of Functional Constituents and Biological Activity of the Seed Extracts from Two Mulberry Fruits

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Abstract

The seeds from two mulberry fruits [*Morus alba* (MA) and *Cudrania tricuspidata* (CT)] were examined for their oil content, and fatty acid, phytosterol and tocopherol compositions and contents. Moreover, polyphenolic compounds and biological activity of the two defatted seed residue extracts were also evaluated. Oil contents of MA and CT seeds were 29.36% and 16.69%, respectively, while MeOH extracts of the defatted MA and CT seed residues were 5.10% and 6.22%, respectively. The two seed oils were composed of 81.4 and 74.37% linoleic, 5.75 and 11.39% oleic, 8.40 and 10.18% palmitic acid, and 3.52 and 3.0% stearic acids, and two other minor fatty acids, such as linolenic and arachidic acids. MA seed had higher contents of phytosterols (507.59 mg/100 g of oil), tocopherols (99.64 mg/100 g of oil), and total flavonoid (106.50 mg/100 g of seed) than CT seed, whereas CT seed had higher levels of total polyphenol than MA seed. The MeOH extract of MA seed residue showed higher antioxidant, anti-diabetic, and anti-melanogenic activity than that of CT seed residue. *trans*-Resveratrol (9.62 mg/100 g), quercetin (54.83 mg/100 g), and 4-prenylmoracin (48.70 mg/100 g), were found to be the main polyphenolic components in the MeOH extract of MA seed residue. These results indicate that MA seeds are good sources of essential dietary phytochemicals with antioxidant, anti-diabetic and anti-melanogenic activity.

Key words: *Morus alba*, *Cudrania tricuspidata*, seeds, functional constituents, biological activity, phenolic compounds

INTRODUCTION

Functional foods from plant sources play important physiological roles in prevention of several pathological disorders, such as cancer, cardiovascular disease, atherosclerosis, cataracts, and aging (1,2). Particularly, plant seeds obtained as byproducts of fruit juice processing are known to be a good source of essential fatty acids and functional foods with important phytochemicals that can reduce the risk of degenerative diseases (3,4). Additionally, seed residues remaining after oil extraction are receiving a renewed interest as valuable sources of dietary antioxidants (5-7). For these reasons, functional constituents and biological activity of the plant seeds are being investigated as a source of dietary supplementation.

Two types of mulberry trees, *Morus alba* (MA) and *Cudrania tricuspidata* (CT), have been widely used in folk medicine in Korea (8). The fruits of MA (Korean name: Odui) are used for treatment of diabetes, bald head, hangover, hypertension and inflammation (9). They are rich in sugar, organic acids, and anthocyanins, which are important for palatable foods, such as beverages, jellies, and jams. They also contain numerous important phytochemicals, including 1-deoxynojirimycin (DNJ), γ-aminobutyric acid (GABA), caffeic acid, resveratrol derivatives, flavonoids, and arylbenzofurans with antidiabetic, anti-hypertentive, anticarcinogenic, antioxidative, and anti-aging activities (10-12). Meanwhile, CT (Korean name: Gguzippong) is being used as an anti-cancer, anti-inflammatory, anti-neuritis, and anti-bacterial remedy, as well as a treatment for skin irritations (13). Reports suggest that several phytochemical constituents, such as prenylated xanthones and flavonoids are mainly responsible for the important biological effects of CT (14-16). To date, many studies have been undertaken to screen biologically active compounds from different parts of the two mulberry trees. However, little information is available on the functional constituents and biological actions of mulberry seeds, which are obtained as byproducts during mulberry juice and wine production.

The objective of this study was to determine the functional constituents and biological activity of the seed extracts from two mulberry fruits, MA and CT, and to further quantify polyphenolic compounds in the defatted

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MA seed residue.

MATERIALS AND METHODS

Materials

The seeds of MA (Iksuppong) and CT fruits were obtained with large amounts of press residues during juice production. The two seed types were removed from the residues, washed with running water, and air-dried at room temperature.

Chemicals

Free fatty acids, four tocopherol isomers (α-, β-, γ-, and δ-tocopherol), three phytosterols (campesterol, stigmasterol, β-sitosterol), 1,1-diphenyl-2-picrylhydrazyl (DPPH), α-glucosidase, mushroom tyrosinase, quercetin, rutin, L-dihydroxyphenylalanine (L-DOPA), and *t*-resveratrol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Potassium hydroxide, aluminum chloride, Folin-Ciocalteau's phenol reagent, and *t*-butylated hydroxytoluene (BHT) were purchased from Wako Pure Chemical Ind. (Osaka, Japan). HPLC solvents were obtained from Merck (Darmstadt, Germany). All other reagents used for this study were analytical grade.

Preparation of oil and MeOH extracts from mulberry seeds

Each ground seed (10 g) was extracted twice with petroleum ether (100 mL) containing 0.05% BHT for 1 hr in an ultrasonic cleaner (Bransonic 5210R-DTH, USA), filtered through Whatman No. 2 filter paper (Whatman Laboratory Products, Clifton, NL, USA) and finally evaporated under reduced pressure. The oil content was expressed on a dry seed weight. Meanwhile, two seed residues obtained after oil extraction were extracted twice with 90% aqueous methanol (aq. MeOH) (200 mL) for 2 hr in an ultrasonic cleaner, filtered and evaporated under reduced pressure. The crude MeOH extract was further solubilized in the same solvent and allowed to stand overnight at refrigerator. The solution was centrifuged at 3,000 rpm for 30 min, and the upper layer was evaporated *in vacuo* to yield the MeOH extract.

Analysis of fatty acid composition

Fatty acid (FA) composition of two seeds oils was determined by gas chromatography (GC), as previously described (17). GC conditions; SupelcowaxTM-10 fused-silica capillary column (60 m × 0.25 mm i.d.), FID detector, carrier gas (He, 52.5 mL/min), injector (250°C), oven (190°C), detector (260°C) temperatures, respectively.

Analysis of tocopherols

Quantification of four tocopherol isomers in the seed oils was performed as previously described (17). The HPLC conditions were as follows: LiChrosorb DIOL column (5 μm, 3 × 100 mm, Merck Co., Chrompack, Palo Alto, CA, USA), UV detector (Younglin Absorbance, Seoul, Korea) at 295 nm, mobile phase, *n*-hexane-acetic acid (1000:1, v/v), and flow rate of 0.5 mL/min.

Analysis of phytosterols

Quantification of three phytosterols in two seed oils was conducted as previously described (17). Seed oil (0.1 g) was saponified by 2 N KOH in EtOH, purified and then injected into the GC. GC conditions were as follows: Ultra 2 fused-silica capillary column (60 m × 0.25 mm i.d.), FID detector, carrier gas (He, 25 mL/min), injector (300°C), oven (285°C), and detector (300°C).

Measurement of total polyphenolic and flavonoid contents

Total polyphenol contents of the seed MeOH extracts were determined using the Folin-Ciocalteau's phenol reagent as described by Singleton et al. (18) with modifications. Seed extract (1 mL, 90% MeOH) and Folin-Ciocalteau's reagent (0.5 mL) were placed into a 100 mL volumetric flask. After 3 min, 20% Na_2CO_3 solution (2 mL) was added, and the flask was filled with distilled water. The absorbance was measured at 760 nm with a UV-Vis spectrophotometer (Sinco, Seoul, Korea) after 1 hr. Total polyphenol contents were expressed as gallic acid equivalents (mg/100 g of seed) from a standard calibration curve.

Flavonoid content was determined with aluminum chloride according to the colorimetric method of Kim et al. (19) with some modifications. The sample solution (0.5 mL) was mixed with 0.1 mL of 10% aluminum nitrate nonahydrate, 0.1 mL of 1.0 M potassium acetate, and 4.3 mL of 80% MeOH, and then vortexed with agitation. After incubation at room temperature for 40 min, the absorbance of reaction mixture was measured at 415 nm. The same amount of distilled water substituted for the amount of 10% aluminum nitrate as the blank. Total flavonoid contents were expressed as rutin equivalents (mg/100 g of seed) from a standard calibration curve.

Assay of antioxidant activity

The antioxidant activity of the seed MeOH extracts was determined by DPPH radical, according to the modified method of Blois (20). MeOH solution (200 μL) of sample at various concentrations (0.1 ~ 10 mg/mL) was added to 0.1 mM methanolic solution of DPPH (4 mL) and shaken vigorously. The reaction mixture was allowed to stand for 10 min at room temperature and the absorbance was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the

following formula: DPPH radical scavenging activity (%)=[(A−B)/A]×100. A: optical density of control, B: optical density of sample.

Assay of anti-diabetic activity

The anti-diabetic activity of the seed MeOH extracts was determined using a α-glucosidase according to the method of Lam et al. (21) with minor modification. Sample solution [50 μL, 0.01～1.0% in MeOH (final concentration 5%)] was added to α-glucosidase (0.15 unit/mL) (50 μL) in a test tube. The reaction mixture was allowed to stand at 25°C for 10 min and 3 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG, 100 μL) in 0.2 M sodium phosphate buffer (pH 6.8) was added to start the reaction. The reaction was conducted at 37°C for 15 min and stopped by the addition of 0.1 M Na_2CO_3 (750 μL). α-Glucosidase inhibitory activity was assessed by measuring the release of *p*-nitrophenol from pNPG at 405 nm.

Assay of anti-melanogenic activity

The anti-melanogenic activity of the seed MeOH extracts was determined using a mushroom tyrosinase, as previously described by Choi et al. (22) with L-DOPA as the substrate. The reaction mixture (3 mL) using L-DOPA as substrate contained 1 mL of 1.5 mM L-DOPA solution, 0.1 mL of dimethylsulfoxide (DMSO) with or without a sample and 1.8 mL of 1/15 M phosphate buffer solution (pH 6.8) was incubated at 25°C for 10 min. A further 0.1 mL of 1,000 units/mL mushroom tyrosinase in aqueous solution was added to the mixture to immediately measure the initial rate of linear increase in optical density at 475 nm. A control reaction was conducted with DMSO. The percentage inhibition of tyrosinase was calculated as follows: Inhibition (%)=[(A−B)/A]×100, where A and B represent the absorbance of test solution and simultaneous control, respectively.

Quantification of phenolic compounds

The defatted seed residues (10 g) from the two mulberry fruits were extracted twice with 80% aqueous EtOH (200 mL) in an ultrasonicator, filtered and evaporated under reduced pressure. The EtOH extract was further redissolved in 100 mL of 80% aq. EtOH and left to stand overnight. The solution was centrifuged at 5,000 rpm for 20 min and the upper layer was filled up to 100 mL with the same solvent. The soluble extract (2 mL) was diluted two times and passed through a 0.45 μm membrane filter (Gelman, USA), and then injected in the HPLC for quantification of phenolic compounds. HPLC was performed on a Waters e2690/5 HPLC system equipped with 2998 photodiode array detector and SM7 autosampler with a 10 μL loop. HPLC analysis was carried out using a YMC-Pack Pro C_{18} column (46 mm i.d.×250 mm, YMC Inc., USA) with a Guard-Pak C_{18} precolumn insert. The separation was conducted using a linear gradient from 0.05% v/v H_3PO_4 in H_2O (solvent A) to MeOH-CH_3CN-H_2O (1:1:1, v/v/v, solvent B) for 60 min at a flow rate of 0.8 mL/min with UV detection at 250, 310, and 350 nm. The elution profile was as follows: 0～5 min, 70% A, 30% B; 10～20 min, 50% A, 50% B; 30～40 min, 30% A, 70% B; 45～50 min, 0% A, 100% B; 55～60 min, 70% A, 30% B. The column was returned to initial conditions for 10 min before the next injection. Individual phenolics were identified by a comparison of their retention time with those of the three standard phenolics (*trans*-resveratrol, quercetin, and 4-prenylmoracin) isolated from the two mulberry fruits, as previously reported (12,23). Peaks were identified by co-chromatography with authentic samples isolated previously. Linear correlation coefficients were superior to 0.999 for each phenolic. The concentration of phenolics was determined by calibration curves of three standard phenolics, and expressed as mg% of dried weight. Recovery rates of *trans*-resveratrol, 4-prenylmoracin, and quercetin were 97%, 98% and 91%, respectively.

Statistical analysis

All data are reported as a mean value±standard deviation (SD) of two samples of each seed, analyzed individually in triplicate (duplicate analysis for fatty acids, phytosterols, and tocopherols). IC_{50} values were determined by regression analysis of the results obtained at three different concentrations of the sample. Statistical analysis was performed using Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Yield of oil and MeOH extracts of two mulberry seeds

The yields (%) of oil and MeOH extracts of the two types of mulberry seeds are shown in Table 1. The yields of oil and MeOH extracts of MA seeds were 29.36 and 5.10%, respectively, while those of the oil and MeOH extracts of CT seeds were 16.69 and 6.22%, respectively. Thus, yield of the oil extract of MA seed was sig-

Table 1. Yields of oil and MeOH extracts from the seeds of two mulberry fruits

Mulberry seed	Yield (%, dry base)	
	Oil extract	MeOH extract
Morus alba (MA)	29.36±0.50	5.10±0.11
Cudrania tricuspidata (CT)	16.69±1.39	6.22±0.15

Values are mean±SD of triplicate analyses
Values in columns are significantly different at $p<0.05$.

nificantly higher than that of the oil extract of CT seed, whereas yield of the MeOH extract of MA seed residue was somewhat lower than that of the MeOH extract of CT seed residue. These data support the idea that the yields of oil and MeOH extracts of plant seeds vary with plant types, cultivars, maturity, and processing (6,7). It is noteworthy that MA seed had higher oil contents than other plant seeds, including CT (6,7,17). The yield of MA seed obtained as a byproduct in the process of mulberry juice production was about 3～5% per 100 g fresh weight of MA fruit. Although it is presently difficult for large scale production of MA seed oil due to the low amount of MA fruit cultivation in Korea, MA seed appears to be a good source of oil production. Therefore, these data suggest that the MA and CT seeds may potentially serve as natural sources for dietary phytochemicals.

Fatty acid composition

The fatty acid profiles of the mulberry seed oils are given in Table 2. MA oil consisted of 8.40% palmitic acid, 3.52% stearic acid, 5.75% oleic acid, 81.40% linoleic acid, 0.63% linolenic acid, and 0.30% arachidic acid. CT oil was comprised of 10.18% palmitic acid, 0.15% palmitoleic acid, 3.0% stearic acid, 11.39% oleic acid, 74.37% linoleic acid, 0.58% linolenic acid, and 0.33% arachidic acid. Thus, similar oil compositions were observed in the two seed oils, although their fatty acid compositions were slightly different in palmitic, oleic and linoleic acids. Among fatty acid compositions detected, linoleic acid was the predominant fatty acid, followed by palmitic and oleic acids, and other fatty acids, such as stearic, linolenic, and arachidic acids, were minor oil components. In addition, there were no considerable differences in the total contents of saturated fatty acids (SFAs) and unsaturated fatty acids (USFAs) between MA and CT seeds. It is important to note that the oil compositions of these two types of mulberry seeds, with their higher amounts of linoleic acid, are unusual compared to those of other plant seed oils, except safflower (75%) and rapeseed oil (71.5%) (24). Therefore, mulberry seed oils have the potential to be used as functional source of essential fatty acid.

Table 2. Fatty acids contents of the seed oils from two mulberry fruits

Fatty acid	Content (weight %)	
	Morus alba (MA)	*Cudrania tricuspidata* (CT)
Palmitic acid ($C_{16:0}$)	8.40±0.01	10.18±0.00
Palmitoleic acid ($C_{16:1}$)	ND[1)]	0.15±0.00
Stearic acid ($C_{18:0}$)	3.52±0.00	3.00±0.01
Oleic acid ($C_{18:1}$)	5.75±0.00	11.39±0.01
Linoleic acid ($C_{18:2}$)	81.40±0.00	74.37±0.01
Linolenic acid ($C_{18:3}$)	0.63±0.00	0.58±0.00
Arachidic acid ($C_{20:0}$)	0.30±0.00	0.33±0.02
∑SFA[2)]	12.22±0.00	13.51±0.02
∑USFA[3)]	87.78±0.00	86.49±0.02

Values are mean±SD of duplicate analyses.
[1)]Not detected.
[2)]Total saturated fatty acid.
[3)]Total unsaturated fatty acid.

Phytosterol and tocopherol composition

Contents of phytosterols and tocopherols in the two mulberry seeds are shown in Table 3 and Table 4, respectively. Three phytosterol derivatives, campesterol, stigmasterol and β-sitosterol, were found, of which β-sitosterol was the main component in the two seeds. MA seed oil had 36.64 mg% campesterol, 10.29 mg% stigmasterol, and 460.66 mg% β-sitosterol, comprising 507.59 mg% of total phytosterol contents. In contrast, CT seed oil had 24.54 mg% campesterol, 16.47 mg% stigmasterol, and 410.94 mg% β-sitosterol, with 451.95 mg% of total phytosterol contents. Thus, levels of total phytosterol of MA seed were higher than those of CT seed, and, notably, two phytosterols, campesterol and β-sitosterol contents of MA seed were higher than those of CT seed, although stigmasterol content was lower than that of CT seeds. As compared to phytosterol contents and profiles of other plant seeds, the two mulberry seed oils had intermediate total phytosterol contents, between black currant (6453 mg%) and grape seed oils (151 mg%), and comprised simliar phytosterol profiles with β-sitosterol as a principal sterol (7,17). This finding supports earlier reports that levels of phytosterols in plant seeds could be affected by cultivar, maturity and processing (5,7,25). It was reported that a daily intake of plant sterols or stanols of 1.6～2 g/day, incorporated in plant foods, could reduce cholesterol absorption from the gut by about 30%, and plasma LDL cholesterol levels by 8～10% (26). Notably, a previous study showed that a daily intake of 60～130 mg/day of β-sitosterol lowered in-

Table 3. Phytosterol contents of the seed oils from two mulberry fruits

Mulberry seed	Phytosterol (mg/100 g, seed oil)			
	Campesterol	Stigmasterol	β-Sitosterol	Total
Morus alba (MA)	36.64±7.20	10.29±1.12	460.66±8.27	507.59±5.53
Cudrania tricuspidata (CT)	24.54±0.22	16.47±2.52	410.94±9.74	451.95±4.16

Values are mean±SD of duplicate analyses.

Table 4. Tocopherols contents of the seed oils from two mulberry fruits

Mulberry seed	Tocopherol (mg/100 g, seed oil)			
	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
Morus alba (MA)	6.23±0.34	17.96±0.76	75.45±1.82	99.64±0.97
Cudrania tricuspidata (CT)	4.45±0.39	7.46±0.28	4.16±0.18	16.07±0.28

Values are mean±SD of duplicate analyses.

cidence of prostate cancer (27). Recently, phytosterols are receiving a renewed interest as valuable bioactive compounds with anti-inflammatory, and gastroprotective properties (28).

Three tocopherol isomers, α-, γ-, and δ-tocopherol, were identified in the two seeds, and their contents are shown in Table 4. MA seed oil possessed 99.64 mg% of total tocopherol content, which is comprised of 6.23 mg% α-tocopherol, 17.96 mg% γ-tocopherol, and 75.45 mg% δ-tocopherol. In contrast, CT seed oil had 16.07 mg% of total tocopherol contents, comprised of 4.45 mg% α-tocopherol, 7.46 mg% γ-tocopherol, and 4.16 mg% δ-tocopherol. Levels of three tocopherols of MA seed were considerably higher than those of CT seed. Notably, the γ- and δ-tocopherol contents of MA seed were about 2.4 and 18 times higher than those of CT seed. Thus, the MA seeds are good source of antioxidant and phytochemical vitamin E. It was found that levels of total tocopherol in plant seed oils ranged from 0.35 mg% to 116.84 mg%, of which γ- and δ-tocopherol were known to be the predominant vitamin E compound (7,17). MA seed oils had higher total tocopherol content than other plant seed oils, although its content was lower than those of corn (116.84 mg%) and soybean (114.91 mg%) oils (24,25). These results also support observations that tocopherol contents and compositions in plant seeds, together with fatty acid and phytosterol, varied with plant type, cultivar and maturation.

Table 5. Total polyphenol and flavonoid contents of the MeOH extract of two seed residues

Mulberry seed	Total polyphenol (mg/100 g, dry base)	Total flavonoid (mg/100 g, dry base)
Morus alba (MA)	367.26±7.89	106.50±3.40
Cudrania tricuspidata (CT)	500.09±11.46	74.20±1.72

Values are mean±SD of triplicate analyses.
Values in columns are significantly different at $p<0.05$.

Total phenol and flavonoid

Total phenolic and flavonoid contents in the two defatted mulberry seed residues are shown in Table 5. Total phenols of MA and CT seed residues were 367.26 and 500.09 mg/100 g, respectively, whereas total flavonoids of MA and CT seed residues were 106.50 and 74.20 mg/100 g, respectively. Thus, total phenolic content of MA residue was higher than that of CT residue, while total flavonoid content of CT residue was higher than that of MA residue. It is very interesting to note that the MA seed residue contained higher amounts of total flavonoids, although its total phenolic content was lower than CT seed residue. This fact supports previous results that there are considerable differences in antioxidant activity between the two mulberry fruits (8,15). These concentrations in the two mulberry seed residues were lower than those reported for other berry fruits, such as raspberry (2.5 g/100 g), blueberry (1.6 g/100 g), cranberry (1.5 g/100 g) (5). Additionally, total phenolic and flavonoid contents of the seed residue from these mulberry fruits were lower than those of grape seed residue (6～10 g/100 g) (17).

Biological activity of MeOH extracts

Antioxidant, anti-diabetic, and anti-aging activities of MeOH extracts of the two seed residues were determined using DPPH radical, α-glucosidase, and tyrosinase in an *in vitro* assay system, and the results are shown in Table 6. The two MeOH extracts showed high radical scavenging and inhibitory activity in a dose-dependent manner, and the 50% inhibitory concentration (IC_{50}) of each extract was calculated from the results. MA seed showed higher radical scavenging and inhibitory activity against DPPH (IC_{50}=0.15 mg/mL), α-glucosidase (IC_{50}=0.87

Table 6. Comparison of biological activity of the MeOH extract of two seed residues

Mulberry seed	Inhibitory activity (IC_{50}, mg/mL)		
	DPPH RSA[1)]	α-Glucosidase	Tyrosinase
Morus alba (MA)	0.15±0.01	0.87±0.01	1.01±0.10
Cudrania tricuspidata (CT)	0.74±0.03	2.79±0.05	2.54±0.13

Values are mean±SD of triplicate analyses.
Values in columns are significantly different at $p<0.05$.
[1)]Radical scavenging activity.

mg/mL), and tyrsoinase (IC_{50} = 1.01 mg/mL) than those of CT. Thus, the high DPPH RSA, and α-glucosidase and tyrosinase inhibitory activities of the MeOH extract of MA seed residue may be attributed to antioxidant flavonoids in MA seed residue from previous result in Table 5. These facts suggest that the MeOH extract of MA seed residues may play important roles in the prevention of radical-mediated disorders, diabetes, and skin-aging.

Phenolic compounds in MA seed

To search for the major contributor to the strong biological activity of the MA seed residue, HPLC analysis was performed on the MeOH extract of MA seed residue. Fig. 1 showed HPLC chromatograms of standard phenolic compounds (A) and MeOH extract (B) of MA seed residue. Three major phenolic components, such as *trans*-resveratrol, quercetin, and 4-prenylmoracin, were found in the MeOH extract of MA seed residue, and their contents were 9.62, 54.83, and 48.70 mg/100 g of seed residue, respectively (Table 7). Other phenolic compounds are now being isolated and identified. Thus, these results suggest that three phenolic compounds are mainly responsible for the strong biological activity of the MA seed residue. Three phenolic components have already been isolated from the fruits, leaves, and root bark of mulberry trees (12,29-31), and were known as anticancer, antihypertensive, anti-diabetic, and antioxidant agents (10,12,32). However, quantitative analysis of three phenolic compounds of the MA seed is first reported in this study. From these results, therefore, the MA seeds, byproduct from mulberry fruit processing, prove to be a potential useful source of phenolic antioxidants with health benefits as therapeutic agents. This is the first report on the functional constituents and biological actions of seed extracts from the two mulberry fruits.

The present study demonstrated that MA seeds are good sources of essential fatty acids, tocopherols and phytosterols, as well as flavonoid, *trans*-resveratrol and 4-prenylmoracin. In addition, the MeOH extract of MA seed residues showed higher antioxidant, anti-diabetic, and anti-melanogenic activity than those of CT seed residues. Particularly, it is very exciting to note that the

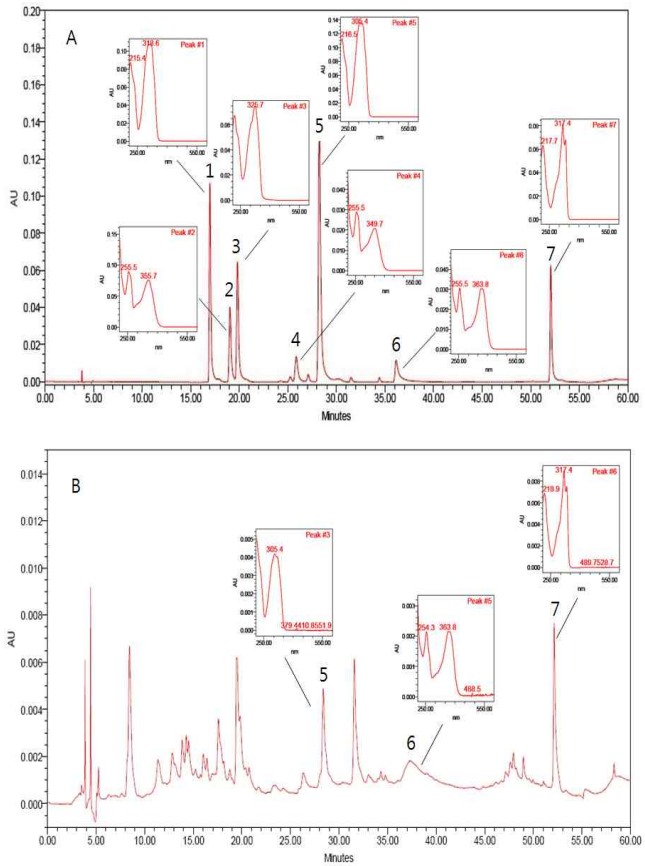

Fig. 1. HPLC chromatograms of seven standards phenolic compounds (A) and the MeOH extract (B) of the MA seed residue. 5: *trans*-resveratrol, 6: quercetin, 7: 4-prenylmoracin.

MA seed contained high concentration of *trans*-resveratrol, quercetin, and 4-prenylmoracin with anticancer, anti-hypertensive, and anti-diabetic activity. The MA seeds obtained as byproduct in mulberry processing are receiving a renewed interest as valuable phytochemical sources. Further study to isolate and elucidate the structure of other active phytochemicals in MA are in progress.

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Table 7. Levels of phenolic compounds in the MA seed residues

Mulberry seed	Phenolic compound (mg/100 g, seed residue)		
	trans-Resveratrol	Quercetin	4-Prenylmoracin
Morus alba (MA)	9.62±0.72 (6.01±0.34)[1)]	54.83±3.51 (34.27±2.84)	48.70±2.45 (30.44±2.03)

Values are mean±SD of triplicate analyses.
[1)]Contents of phenolic compounds per the dried MA seed (mg/100 g).

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