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Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing*

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Abstract: Berries are a good source of natural antioxidants. In the present study, the total antioxidant capacity and phenolic composition of three berry fruits (blueberry, blackberry, and strawberry) cultivated in Nanjing were investigated. Blueberry, with a Trolox equivalent antioxidant capacity (TEAC) value of 14.98 mmol Trolox/100 g dry weight (DW), exhibited the strongest total antioxidant capacity using both the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. Blueberry also had the highest total phenolic content (TPC, 9.44 mg gallic acid/g DW), total flavonoid content (TFC, 36.08 mg rutin/g DW), and total anthocyanidin content (TAC, 24.38 mg catechin/g DW). A preliminary analysis using high performance liquid chromatography (HPLC) showed that the blueberry, blackberry, and strawberry samples tested contained a range of phenolic acids (including gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, and cinnamic acid) and various types of flavonoids (flavone: luteolin; flavonols: rutin, myricetin, quercetrin, and quercetin; flavanols: gallocatechin, epigallocatechin, catechin, and catechin gallate; anthocyanidins: malvidin-3-galactoside, malvidin-3-glucoside, and cyanidin). In particular, the blueberries had high levels of proanthocyanidins and anthocyanidins, which might be responsible for their strong antioxidant activities. These results indicate a potential market role for berries (especially blueberries) as a functional food ingredient or nutraceutical.

Key words: Berries, Antioxidants, Phenolics, Flavonoids, Anthocyanidins
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1 Introduction

Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals, and free radical-meditated reactions, can cause oxidative damage to cellular structures and functional molecules (e.g., DNA, proteins, and lipids) (Finkel and Holbrook, 2000). Abundant evidence suggests that oxidant stress is a major cause of many diseases, including aging, cancer, diabetes, cardiovascular disease, Alzheimer's disease, and other neurodegenerative disorders (Halliwell, 1994). Antioxidants are thought to be highly effective in the management of ROS-mediated tissue impairments. Many antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antiproliferative, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent (Liu *et al*., 2002; Ratnam *et al*., 2006). With the current upsurge of interest in the efficacy and use of naturally derived antioxidants, functional foods and nutraceuticals have received much attention in recent years.

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Berries (e.g., blueberry, blackberry, and strawberry) are well known as "super fruits" for their potential in the nutraceutical and functional food markets (Ding *et al*., 2006; Tulipani *et al*., 2008). Blueberries are flowering plants of the genus *Vaccinium* with dark-purple berries, whose anthocyanins are considered to be nature's most potent antioxidants and have demonstrated properties that extend well beyond suppressing free radicals (Srivastava *et al*., 2007). Consumption of blueberries may alleviate the cognitive decline occurring in Alzheimer's disease and other conditions of aging (Krikorian *et al*., 2010). Blueberries also help maintain healthy blood flow via several mechanisms including healthy low-density lipoprotein (LDL) oxidation, normal platelet aggregation, and maintenance of endothelial function (Kalt *et al*., 2008; Shaughnessy *et al*., 2009). Blackberries are aggregate fruits produced by several species in the genus *Rubus* (e.g., *R. fruticosus*, *R. ursinus*, and *R. argutus*). Blackberries are notable for their health benefits based on high nutritional contents of dietary fiber, vitamin C, vitamin K, folic acid, and the essential mineral, manganese (Sariburun *et al*., 2010). Blackberries also rank highly among fruits for antioxidant strength, particularly due to their high contents of phenolic compounds, such as ellagic acid, tannins, ellagitannins, quercetin, gallic acid, anthocyanins, and cyanidins (Hager *et al*., 2008). Strawberries (*Fragaria x ananassa* Duch.) are widely appreciated for their excellent taste, characteristic aroma, and bright red color. Strawberries are an excellent source of vitamin C, and are also rich in bioactive phenolic compounds including flavonoids and phenolic acids, such as hydroxycinnamic acids, ellagic acids, ellagitannins, xavan-3-ols, xavonols, and anthocyanins (Tulipani *et al*., 2008; Oszmiański and Wojdyło, 2009; Wang and Millner, 2009). Strawberries have been shown to have a remarkably high scavenging activity toward chemically generated radicals, thus making them effective in inhibiting oxidation of human LDLs (Heinonen *et al*., 1998). The antioxidant activity of strawberries could contribute to the prevention of cancer, cardiovascular and other chronic diseases (Hannum, 2004).

Because of their remarkable antioxidant capacity, berries have received increasing attention in the past two decades, especially in North America and Europe. A large number of studies on the physiological functions and chemical constituents of blueberry, blackberry, and strawberry have been reported (Tulipani *et al*., 2008; Krikorian *et al*., 2010). However, information from systematic investigations on berries in developing countries in Asia is scarce. Furthermore, no reports are available on the antioxidant capacity or detailed phenolic composition of blueberries, blackberries, or strawberries cultivated in Nanjing, China. Data on the phenolic composition of berries grown in Nanjing will reveal the potential of these fruits in regional and international markets. This study was conducted in response to recent interest in the nutritional and health benefits of berries, especially blueberries. Another driving force was research and development on functional foods from local crops aimed at the worldwide fruit market. The objectives of this study were to investigate and compare the antioxidant capacity, total contents of phenolics, flavonoids, and anthocyanins, and phenolic components in the whole fruits of blueberries, blackberries, and strawberries growing in Nanjing. The research will be helpful for seeking new effective antioxidants from these berries, and understanding their health value for Chinese consumers.

2 Materials and methods

2.1 Plant materials and sample preparation

Fresh mature fruits of *Vaccinium ashei* cv. Brightwell (rabbiteye blueberry), *Rubus laciniatus* cv. Hull (thornless blackberry), and *Fragaria x ananassa* cv. Toyonoka (strawberry) were harvested from orchards surrounding Lishui in Nanjing, China. Nanjing is located in the southeast of China, latitude 31°14′–32°36′, longitude 118°22′–119°14′, within the north subtropical monsoon climate zone. The cultivars "Brightwell" and "Hull" from California, USA and "Toyonoka" from Japan were introduced to China in 1987, and cultivated in Nanjing in 2003. The fruits were randomly picked from several plants in the orchards during the period of March (for strawberry) to July (for blueberry and blackberry) in 2010, and then the samples within each fruit type were combined for further analyses. The collected fruits were frozen immediately at −20 °C. The frozen berry fruits were kindly provided by Nanjing Xindeli Food Co., Ltd., China.

The samples of the whole fruits (including peel, pulp, and seeds) were freeze-dried with an Eyela FDU-1200 freeze dryer (Tokyo Rikakikai, Japan) and kept at 4 °C until analyzed. A dried powder sample (2.5 g) was extracted with 50 ml 80% methanol at 28 °C for 24 h in a THZ-Q shaking incubator (Huamei Biochem Inc., Taicang, Jiangsu, China) at 150 r/min. The extract was collected and centrifuged at 3500 r/min for 20 min for further extraction. The supernatant was then filtered using a medium-speed filter under a vacuum at room temperature, and stored at 4 °C until analyzed.

2.2 Chemicals and reagents

Catechin, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and all the 21 phenolic standards used in high performance liquid chromatography (HPLC) analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Acros Organics (Morris Plains, New Jersey, USA). Gallic acid was purchased from J&K Chemical Ltd. (Beijing, China), and rutin from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Other chemicals and reagents used in this study were of analytical grade and were obtained from China.

2.3 Total antioxidant capacity

Total antioxidant capacity was assayed using the improved ABTS method (Cai *et al*., 2004). The $ABTS^{\cdot+}$ radical cation was generated by reacting 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate after incubation at room temperature in darkness for 16 h. The $ABTS^{\cdot+}$ solution was diluted with 80% ethanol to an absorbance of 0.700±0.005 at 734 nm. The tested sample was diluted with 80% ethanol so as to give 20%–80% inhibition of the blank absorbance with 0.1 ml of sample. A total of 3.9 ml of $ABTS^{\cdot+}$ solution was added to 0.1 ml of the tested samples and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min and the absorbance at 734 nm was then recorded immediately. Different levels (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mmol/L) of Trolox standard solution in 80% ethanol were prepared and assayed under the same conditions. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC), i.e., mmol Trolox/100 g dry weight (DW).

2.4 DPPH radical-scavenging activity

The scavenging activity for DPPH radicals was determined using spectrophotometric analysis based on the method described by Kumaran and Karunakaran (2007). A 1-ml sample solution was added to 3 ml of 0.04 mg/ml DPPH solution (prepared using anhydrous ethanol) and mixed thoroughly at room temperature. Absorbance at 517 nm was determined after 30 min. The scavenging activity was expressed as the percentage of scavenged DPPH radicals in the above assay system, calculated as $(1-(A_i-A_j)/A_c)\times100\%$, where A_i is the absorbance of the DPPH solution mixed with the fruit sample, A_j is the absorbance of 3 ml ethanol (as a blank) mixed with the sample, and A_c is the absorbance of DPPH solution with 1 ml ethanol instead of the sample (as a control). The EC_{50} value, denoting the effective concentration of sample required to scavenge 50% of DPPH free radicals, was calculated by graphical regression analysis, and expressed as mg/ml.

2.5 Total contents of phenolics, flavonoids, and anthocyanidins

2.5.1 Total phenolic content (TPC)

The TPC was estimated using the Folin-Ciocalteu colorimetric method described by Cai *et al*. (2004). Briefly, the appropriate dilutions (the same as those used in the ABTS method) of the extracted samples (0.4 ml) were oxidized with 2 ml of 0.5 mol/L Folin-Ciocalteu reagent for 4 min at room temperature. Then the reaction was neutralized with 2 ml of 75 mg/ml saturated sodium carbonate. The absorbance was measured at 760 nm after incubation for 2 h at room temperature in the dark. Quantification was done on the basis of the standard curve of gallic acid (10, 20, 30, 40, 50, and 60 mg/L). Results were expressed as gallic acid equivalent (GAE), i.e., mg gallic acid/g DW.

2.5.2 Total flavonoid content (TFC)

The TFC was measured using a modified colorimetric method (Chun *et al*., 2003). A 1-ml sample of appropriately diluted filtered plant extract was mixed with 0.1 ml 0.05 g/ml $NaNO_2$. After 6 min,

0.1 ml 0.1 g/ml $AlCl_3 \cdot 6H_2O$ solution was added. Then 1 ml of 1 mol/L NaOH was added to the mixture after another 5 min. The reactive solution was well mixed and allowed to stand for 15 min before the absorbance at 510 nm was measured. The TFC was calculated and expressed as rutin equivalents, i.e., mg rutin/g DW.

2.5.3 Total anthocyanidin content (TAC)

The TAC was determined using the vanillin-HCl colorimetric method (Nakamura *et al*., 2003). An aliquot of 0.5 ml sample was mixed with 3 ml methanol containing 0.03 g/ml vanillin in a test tube wrapped in tinfoil. Then 1.5 ml hydrochloric acid was added to the reaction solution and mixed thoroughly at room temperature. The mixture was put in the dark for 15 h and the absorbance at 500 nm was measured with ethanol solution containing 0.02 g/ml hydrochloric acid as a blank. The standard curve of catechin was obtained under the same conditions. Results were expressed as catechin equivalent, i.e., mg catechin/g DW.

2.6 Phenolic composition by reversed phase (RP)-HPLC

RP-HPLC analysis was performed using a Shimadzu HPLC system (LC-10A series, Tokyo, Japan), consisting of a binary pump and a diode-array detector (DAD), and equipped with a Shim-pack RP-C18 column (5 μm, 250 mm×4.6 mm) (Shimadzu Co., Japan). Sample preparation for HPLC analysis was as follows: the berry extract was centrifuged at 10000 r/min for 15 min, filtered using a Millipore filter (0.22 μm nylon membrane) at room temperature, and then injected into HPLC for analysis. Phenolic compounds in the samples were analyzed at 35 °C with the following gradient elution program (solution A, 0.1% formic acid, and solution B, 100% methanol): 0–10 min, 0%–10% B; 10–25 min, 10%–20% B; 25–35 min, 20%–23% B; 35–45 min, 23%–28% B; 45–60 min, 28%–35% B; 60–75 min, 35%–50% B; 75–80 min, 50%–55% B; 80–85 min, 55%–75% B; 85–90 min, 75% B. The flow rate was 0.8 ml/min and the injection volume was 10 μl. Detection was monitored at 280 nm.

2.7 Statistical analysis

All tests were performed in triplicate and the results are presented as mean±standard deviation (SD). Coefficients of determination (R^2) were calculated using Microsoft Excel 2003. Differences between mean values were compared by least significant difference (LSD) in a one-way analysis of variance (ANOVA) using the PASW Statistics 18 software. Differences with P values of <0.05 were considered significant.

3 Results

3.1 Total antioxidant capacity and DPPH radical-scavenging activity

The berry fruits tested in Nanjing showed good antioxidant capacity. Among them, blueberry was the best, followed by blackberry and strawberry. Their TEAC values were 14.98, 11.48, and 4.44 mmol Trolox/100 g DW, respectively, and their EC_{50} values for DPPH radicals were 0.42, 0.44, and 0.81 mg/ml, respectively (Table 1). The DPPH radical-scavenging curves clearly indicated that DPPH radical-scavenging activity for blackberry and blueberry increased significantly with extract concentrations over 0.4 mg/ml, while for strawberry it increased more gradually. The three berries scavenged from 26.71% to 30.90% of DPPH radicals at low concentration (0.08 mg/ml). At 2.0 mg/ml, blueberry and blackberry extracts could scavenge nearly all DPPH radicals (96.96% and 95.37%, respectively), and strawberry extract showed 58.32% DPPH radical-scavenging activity (Fig. 1).

Table 1 Total antioxidant capacity, DPPH radical-scavenging activity, and total contents of phenolics, flavonoids, and anthocyanins of the three berries in Nanjing*

Berry fruit sample	TEAC (mmol Trolox/100 g DW)	EC_{50} of DPPH (mg/ml)	TPC (mg gallic acid/g DW)	TFC (mg rutin/g DW)	TAC (mg catechin/g DW)
Blueberry	14.98 ± 0.49^{A}	0.42^{A}	9.44 ± 0.22^{A}	36.08 ± 0.56^{A}	24.38 ± 0.75^{A}
Blackberry	11.48 ± 1.32^{B}	0.44^{A}	5.58 ± 0.18^{B}	11.83 ± 0.24^{B}	3.99 ± 0.08^{B}
Strawberry	4.44 ± 0.45^{C}	0.81^{B}	2.72 ± 0.18^{C}	7.04 ± 0.59^{C}	1.16 ± 0.12^{C}
Mean	10.30	0.56	5.91	18.32	9.84
LSD	1.890		0.903	1.431	1.359

* Data are shown as mean±SD except EC_{50}. EC_{50} was calculated by graphical regression analysis using Fig. 1. Different letters in the same column indicate significant differences (P<0.05, using Fisher's least significance difference (LSD) test) among these berry fruits. 80% methanol was used as the negative control for all the assays, and all the assayed values were zero

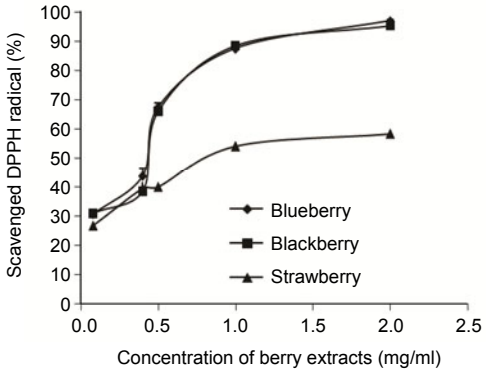

Fig. 1 DPPH radical-scavenging activities of the three berry extracts determined at different concentrations
Data represent mean values±SD of three separate experiments

3.2 Total contents of phenolics, flavonoids, and anthocyanidins

In the present study, the TPC, TFC, and TAC of methanolic extracts of three berry fruits growing in Nanjing were investigated. The TPC values of blueberry, blackberry, and strawberry were 9.44, 5.58, and 2.72 mg gallic acid/g DW, respectively (Table 1). The corresponding TFC values were 36.08, 11.83, and 7.04 mg rutin/g DW, and the TAC values were 24.38, 3.99, and 1.16 mg catechin/g DW, respectively. Anthocyanidins (a class of flavonoids), as plant pigments, are responsible for the intense color of fruits and vegetables. The blueberries, which were reddish-purple, and finally indigo when ripe, had the highest TAC value in our test. The blackberries, which were black or dark purple, also had a relatively high anthocyanidin content. The strawberries were bright red, and had a relatively low TAC value. Our results were consistent with previous findings (Hosseinian and Beta, 2007).

The blueberries were found to have the strongest total antioxidant capacity, and the highest TPC, TFC, and TAC values. A highly significant correlation (R^2=0.941) was found between TEAC and TPC. However, the correlations between TEAC and TFC (R^2=0.7607) and between TEAC and TAC (R^2=0.7955) were lower (all including the other six co-assayed samples; data not shown). This suggests that the phenolic compounds (including flavonoids and anthocyanidins) in the sampled fruits contributed significantly to their antioxidant activities. However, these compounds were not the only antioxidants in the fruits since some other phenolics (e.g., phenolic acids) also contributed to the total antioxidant capacity.

3.3 Primary identification of phenolic constituents

Different phenolic compounds normally have specific chromatographic behavior (retention time, t_R) and UV-visible spectral characteristics (λ_{max} and spectral shapes). Because of the diversity and complexity of natural mixtures of phenolic compounds in the three berries, it is difficult to characterize every compound and elucidate its structure. Therefore, only a preliminary identification of the major phenolic compounds was carried out in the present study. We analyzed the major phenolic compounds of the three berries using RP-HPLC with DAD by comparison with authentic phenolic standards and related published data (Sakakibara *et al*., 2003; Cai *et al*., 2004; Li *et al*., 2009). The 21 phenolic standards used in this study were eluted with t_R from 15.236 to 88.134 min in the following order: gallic acid, protocatechuic acid, catechin, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, malvidin-3-galactoside, malvidin-3-glucoside, *p*-coumaric acid, ferulic acid, sinapic acid, rutin, naringin, 3,4-dimethoxycinnamic acid, myricetin, dihydrofisetin, cinnamic acid, quercetin, and luteolin.

Fig. 2 shows a typical HPLC chromatogram of parts of phenolic standards used in this study. The preliminary results showed that the major types of phenolic compounds in the three berries included phenolic acids, flavonoids (including flavones, flavonols, flavanols, as well as anthocyanidins) and their derivatives, tannins (including hydrolysable and mainly condensed tannins, i.e., proanthocyanidins),

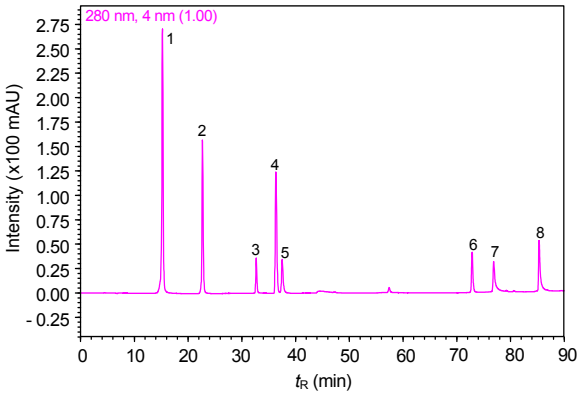

Fig. 2 Typical HPLC chromatogram of parts of phenolic standards at 280 nm
Peaks: 1, gallic acid; 2, protocatechuic acid; 3, catechin; 4, chlorogenic acid; 5, *p*-hydroxybenzoic acid; 6, rutin; 7, myricetin; 8, quercetin

and some volatile and aliphatic constituents. HPLC chromatographs of major phenolic compounds in the tested berry samples are shown in Fig. 3 and structures of some typical detected phenolic compounds are shown in Fig. 4.

The blueberries had high levels of *p*-hydroxybenzoic acid and vanillic acid (peaks 7 and 8 in Fig. 3a). Anthocyanidins and their derivatives (e.g., malvidin-3-galactoside and malvidin-3-glucoside) or proanthocyanidins (condensed tannins, peak 6 in

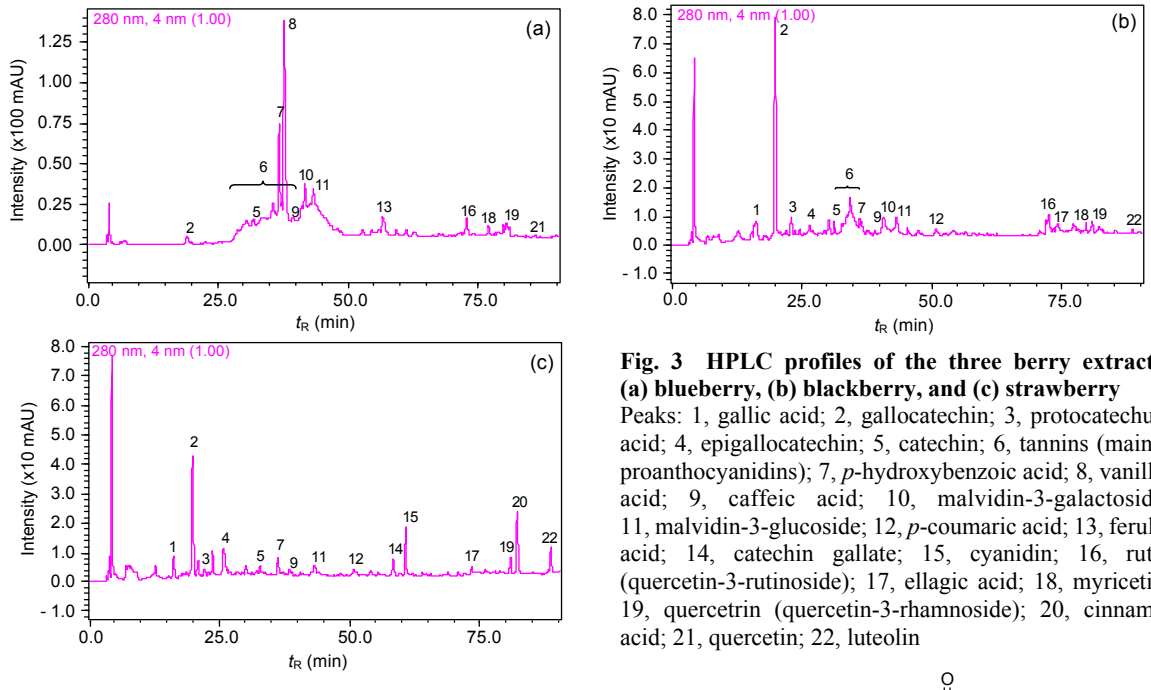

Fig. 3 HPLC profiles of the three berry extracts: (a) blueberry, (b) blackberry, and (c) strawberry
Peaks: 1, gallic acid; 2, gallocatechin; 3, protocatechuic acid; 4, epigallocatechin; 5, catechin; 6, tannins (mainly proanthocyanidins); 7, *p*-hydroxybenzoic acid; 8, vanillic acid; 9, caffeic acid; 10, malvidin-3-galactoside; 11, malvidin-3-glucoside; 12, *p*-coumaric acid; 13, ferulic acid; 14, catechin gallate; 15, cyanidin; 16, rutin (quercetin-3-rutinoside); 17, ellagic acid; 18, myricetin; 19, quercetrin (quercetin-3-rhamnoside); 20, cinnamic acid; 21, quercetin; 22, luteolin

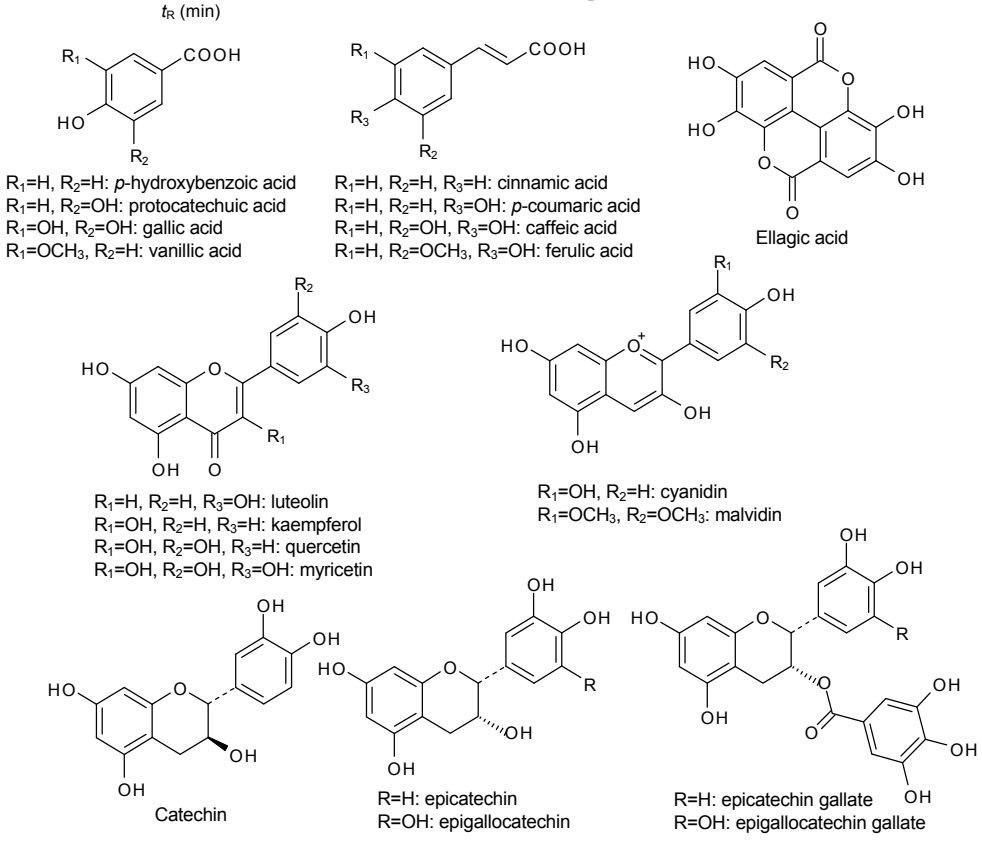

Fig. 4 Structures of some typical phenolic compounds detected in the berry fruit samples

Fig. 3a) were identified as the dominant phenolic compounds in blueberry. Catechin, caffeic acid, ferulic acid, rutin (quercetin-3-rutinoside), myricetin, quercetrin (quercetin-3-rhamnoside), and quercitin were also detected in the blueberry samples (Fig. 3a). The blackberries and strawberries both contained a series of phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ellagic acid), epigallocatechin, catechin, malvidin-3-glucoside, quercetrin, luteolin, and a high level of gallocatechin (peak 2 in Figs. 3b and 3c). In addition, malvidin-3-galactoside, rutin, myricetin, and some proanthocyanidins in the blackberries and catechin gallate, cyanidin, and cinnamic acid in the strawberries, were detected in this test (Figs. 3b and 3c). Most phenolic compounds detected in this study were consistent with previous reports on blueberries, blackberries, and strawberries from North and South America (Juranić and Žižak, 2005; Hosseinian *et al*., 2007; Hager *et al*., 2008; Li *et al*., 2009; Céspedes *et al*., 2010).

4 Discussion

The improved ABTS method has been widely used to evaluate the total antioxidant capacities of both aqueous and lipophilic systems in vitro, while the DPPH method has been used for evaluating the scavenging activities of antioxidants in lipophilic systems (Singh and Singh, 2008). In the present study, these two methods were valid, easy, accurate, sensitive, and economic for evaluating the antioxidant capacities of the three berryfruit extracts. The tested blueberries, blackberries, and strawberries growing in Nanjing all showed a good antioxidant capacity with both high inhibitory activity against $ABTS^{\cdot+}$ radical cations and strong scavenging activity against DPPH radicals, especially the blueberries. The blueberries and blackberries both exhibited a much higher total antioxidant capacity than the strawberries and our other previously tested common fruits, vegetables and spices (orange, broccoli, carrot, spring onion, and ginger; mean TEAC value=3.39 mmol Trolox/100 g DW) (Huang *et al*., 2008). This confirmed that berries cultivated in Nanjing are also a potential antioxidant resource that could be used like Manitoba berries (Hosseinian and Beta, 2007) for nutraceutical and functional food purposes. Previous studies revealed that phenolic compounds are major antioxidant constituents in medicinal plants, vegetables, fruits, and spices (Cai *et al*., 2004). Berries are rich in phenolic compounds (including flavonoids and anthocyanidins). In particular, blueberries are considered to contain the highest content of anthocyanidins in common fruits and vegetables (Juranić and Žižak, 2005). However, many factors such as genes, soil type, light, temperature, and agronomic conditions affect anthocyanin composition in plants (Hosseinian *et al*., 2007). Normally, the effect of antioxidant capacity depends on the solubility of the antioxidants (e.g., whether they are water-soluble or lipid-soluble) (Hosseinian *et al*., 2007). The blueberries had a similar strong scavenging activity against DPPH radicals to, but a higher total antioxidant capacity than the blackberries, which may be attributable to blueberries containing more hydrophilic antioxidants, e.g., anthocyanidins.

Moreover, the basic structural orientation of the compounds determines the antioxidant activity of phenolics, such as how easily a hydrogen atom from a hydroxyl group can be donated to a free radical, and the ability of the compounds to support an unpaired electron (Roginsky, 2003). The position of hydroxyl groups seems more important than their number for the antioxidant capacity of phenolics; for example, hydroxyl groups in the ortho position of the B ring can greatly enhance the antioxidant capacity, such as in catechins (Rice-Evans *et al*., 1996). The berries exhibit good antioxidant capacity mostly because they possess these special phenolic compounds. The dominant phenolic compounds identified in this study were phenolic acids, catechins (flavanols), and proanthocyanidins (condensed tannins). Proanthocyanidins contain the oligomers and polymers of catechins, but further confirmation is needed using additional analytical tools and methods. Tannins and flavanols contain a variety of phenolic hydroxyl groups and show the strongest antioxidant capacity and free radical-scavenging activity among around a hundred phenolic compounds (Rice-Evans *et al*., 1996; Chun *et al*., 2003; Cai *et al*., 2004). Phenolic acids also have a high antioxidant capacity, which decreases in the order: protocatechuic acid>caffeic acid>*p*-hydroxybenzoic acid>ferulic acid>vanillic acid>*p*-coumaric acid (Rice-Evans *et al*., 1996; Li *et al*., 2009). These three major groups of phenolic compounds are likely to be

the most significant contributors to the total antioxidant capacity of the berry samples tested in this study. Higher levels of anthocyanidins (peaks 10 and 11 in Fig. 3) were detected in the blueberries than in the blackberries and strawberries, which was consistent with the total anthocyanidin content assay. Comparing the height and area of the peaks in Fig. 3, the highest concentrations of proanthocyanidins (peak 6) were detected in the blueberries, which had the highest TEAC value (14.98 mmol Trolox/100 g DW). Although the strawberries contained phenolic acid constituents similar to those in the blackberries, few proanthocyanidins were detected in the strawberry sample, which might explain its lower antioxidant activity.

Although there have been some previous studies on the phenolic compounds in berryfruits, none have included the identification of phenolic compounds in blueberries, blackberries, or strawberries cultivated in Nanjing, China. Our study indicated that berries grown in Nanjing had phenolic constituents similar to those from other sources. Hosseinian and Beta (2007) reported that the Saskatoon berry and wild blueberry in Canada contained higher amounts of delphinidin 3-glucoside, malvidin-3-glucoside, and malvidin-3-galactoside. We also found blueberries with high levels of malvidin-3-glucoside and malvidin-3-galactoside in our samples from Nanjing. Céspedes *et al*. (2010) detected gentisic acid, ferulic acid, gallic acid, *p*-coumaric acid, sinapic acid, 4-hydroxybenzoic acid, delphinidin, cyanidin, vanillic acid, delphinidin gallate, gallocatechin gallate, quercetin, rutin, myricetin, catechin, epicatechin, and several glycosides of anthocyanidins in Chilean wild blackberry. We found most of these compounds in the cultivated blackberries from Nanjing. Delphinidin, cyanidin, ellagic acid, myricetin, quercetin, and their glycoside derivatives were the most abundant components of strawberries from Spain (Pallauf *et al.*, 2008). In our strawberry samples from Nanjing, we found all these compounds except delphinidin. Though qualitative and quantitative variabilities of phytochemicals were confirmed in berries of different genotypes and cultivation conditions (Tulipani *et al*., 2008), the main phenolic categories found in the same species from different places were generally consistent.

5 Conclusions

Blueberries, blackberries, and strawberries cultivated in Nanjing exhibited potent antioxidant capacity and contained a variety of phenolic compounds. The results showed that the blueberries had the strongest total antioxidant capacity, and also had the highest TPC, TFC, and TAC. Preliminary HPLC analysis of berry samples detected a series of phenolic acids, various types of flavonoids, and tannins (mainly proanthocyanidins). The blueberries had particularly high levels of anthocyanidins and proanthocyanidins, which may be responsible for their very strong antioxidant activity. However, further structural identification of proanthocyanidins (containing the oligomers and polymers of catechins) is required. Generally, high levels of the phenolic compounds responsible for high total antioxidant capacity are found in these berries. Consumption of the berries can provide a good source of antioxidants and nutrients, and therefore they may have potential for use in the development of nutraceuticals or as functional food ingredients of benefit to human health.

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