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Chemical Composition and Anti-inflammatory and Antioxidant Activities of Eight Pear Cultivars

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Supporting Information

ABSTRACT: The contents of total phenolics, total flavonoids, total anthocyanins, and total triterpenes of eight pear samples were determined, and the monomeric compounds were identified and quantitated using high-performance liquid chromatography. The in vitro antioxidant and in vivo anti-inflammatory activities of the different pear cultivars were compared. Arbutin and catechin were the dominant polyphenol compounds in the eight pear varieties, followed by chlorogenic acid, quercetin, and rutin. In addition, Xuehua pear and Nanguo pear had significantly higher total phenolics and flavonoids contents, while Dangshansu pear had the largest total triterpenes value (209.2 mg/100 g). Xuehua pear and Nanguo pear also were the highest in total anthocyanins. The pears with high total phenolics and total flavonoids contents had significantly higher antioxidant and anti-inflammatory abilities than those of other species. Anthocyanins were correlated to antioxidant capacity in pears, whereas total triterpenoids were strongly correlated to anti-inflammatory activity.

KEYWORDS: *pear, chemical compounds, antioxidant capacity, anti-inflammatory activity, relationship*

INTRODUCTION

Pyrus spp. belong to the family Rosaceae, tribe Pomaceae. More than 60 species are widely distributed in the world, including Asia, Europe, North America, and the Temperate Zone area of the Southern Hemisphere. There are 17 pear species that originated in China, including oriental pear and occidental pear, which developed separately according to geography. While thousands of pear cultivars have been introduced to China, it has now become a main pear-planting and -producing nation. The major cultivated species that originated from oriental pear include *Pyrus bretschnrideri* Reh., *Pyrus ussuriensis* Maxim., *Pyrus pyrifolia* Nakai, and *Pyrus sinkian gensis* Yu. In China, pear has been used not only as one of the most common edible fruits but also as an herbal medicine for relieving cough, embellishing lung, eliminating constipation, and relieving alcoholism for more than 2000 years.[1]

Fruits and vegetables contain a variety of useful compounds that are suggested to be important for human health. The edible part of pear contains a certain amount of antioxidant nutrients such as vitamin C, vitamin E, and β-carotene, which play a beneficial effect on the prevention of certain chronic disorders.[2] Sugars, organic acids, amino acids, and fatty acids are also established to be the major nutritional components in pear fruits.[3] These nutrient components play important roles in evaluating the quality of pear fruits including color, taste, natural value, and so on. Apart from the above nutritional components and some minerals, phenolics, flavonoids, anthocyanins, and triterpenes are also considered to be the main active components in pears, and these constituents are common in various fruits and vegetables.[4] A lot of research reveals that high fruits and vegetables consumption may protect against numerous diseases, including cancer and neurological, ocular, and cardio- and cerebrovascular diseases.[5] The natural antioxidants in fruits and vegetables might play a positive role in regulating human health by scavenging free radicals from damaging biological cells, tissues, and organs.[6]

Certain chemical components including sugars, organic acids, amino acids, and fatty acids were analyzed by high-performance liquid chromatography (HPLC) and gas chromatography (GC). Changes of the levels of sugars, organic acids, and phenolic acids in Yali pear at different storage times were investigated using HPLC.[4,7] Phenolic compounds including arbutin, chlorogenic acid, epicatechin, and quercetin glycosides in some pear cultivars distributed in Ankara and Bursa, Turkey, and southwestern Germany were determined by HPLC, and the result showed that the highest concentration is chlorogenic acid, followed by epicatechin.[8,9] Also, these authors questioned whether arbutin can be used as a characteristic marker of pear. However, there is lack of comprehensive information regarding the chemical contents and the biological activities of common pear cultivars cultivated in China. In our previous work,[10] the contents of total phenolics and flavonoids of five known pear cultivars were determined, and their antioxidant and anti-

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inflammatory activities were evaluated. The first objective of this study was to test the contents of total phenolics, total flavonoids, total anthocyanins, and triterpenes and confirm the compound compositions in different pear cultivars. In addition, the antioxidant and anti-inflammatory activities were evaluated by reducing power assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and two acute inflammation animal models. Furthermore, the relationship between biological activity and chemical composition (total phenolics, total flavonoids, total anthocyanins, total triterpenes, and the monomeric compounds) has also been considered to evaluate the contribution of the extracts and the compounds to antioxidant and anti-inflammatory capacities.

MATERIALS AND METHODS

Reagents. Gallic acid, caffeic acid, catechin, epicatechin, chlorogenic acid, arbutin, ferulic acid, rutin, quercetin, oleanolic acid, and ursolic acid (reference standard, purity ≥ 99.0% each) were purchased from the Natl. Inst. for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade acetontrile and glacial acetic acid were purchased from Concord Technology Co., Ltd. (Tianjin, China). Xylene and Folin−Ciocalteu reagent (FC reagent, puriss., ≥99.0%) were purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). The DPPH (puriss., ≥98.0%) and Evans Blue dye were purchased from the Sigma Company (St. Louis, MO), while dexamethasone was purchased from the Lisheng Pharmaceutical Company, Ltd. (Tianjin, China). All other solvents and chemicals were of analytical grade.

Materials. Eight cultivars of three species of oriental pear and one cultivar of occidental pear (*Pyrus communis*) were evaluated. Xuehua pear and Ya pear are two main cultivars of *P. bretschnrideri* Rehd., which were collected from Yantai, Shandong Province, and Zhaoxian, Heibei Province, respectively. Dangshansu pear and Yuanpingsu pear also belong to *P. bretschnrideri* Rehd. species, and both of them were collected from Jixian, Tianjin. Nanguo pear (*P. ussuriensis* Maxim.) and Huangguan pear (a hybrid variety of Xuehua pear and Xinshiji pear) were obtained from Anshan, Liaoning Province, and Cangzhou, Heibei Province, respectively. Yantai pear (*P. communis* Linn.) was also collected form Yantai, Shandong Province, while Xiang pear (*P. sp. nr.* Communis.) was purchased from Kuerle, Xinjiang Province. All of the materials were harvested at their commercial maturity (from July to September) and identified by Professor Wenyuan Gao (School of Pharmaceutical Science and Technology, Tianjin Univ., Tianjin, China). For each variety, the pear with peel was rapidly cut into thin slices, put into polyethylene bags, and treated by vacuum freeze drying. Samples were then further homogenized and stored at 4 °C until analysis.

Preparation of Pear Extracts. The frozen material of each pear cultivar (10 g) was previously homogenized in a Moulinex stirrer and then extracted with methanol:water (6:4, 3 × 100 mL) by an ultrasonic method for 30 min. The extracts were filtered and evaporated to dryness under vacuum. The dried extract was prepared at a 0.1 g/mL of the freeze-dried pear concentration in methanol. The methanol solution was stored at 4 °C until used for analysis of the content of total phenolics, total flavonoids, total anthocyanins, total triterpenes, and antioxidant capacity.

Animals. Kunming mice (about 18−22 g) were purchased from the Institute of Tianjin Laboratory Animal Center, Tianjin. Animals were kept at 25 ± 1 °C under a 12 h light/dark cycle and allowed free access to food (standard pellet diet) and water ad libitum. All experiments were performed according to the approved protocols of the Animal Ethics Committee, China Pharmaceutical University, China. Animals had free access to water and standard diet and were fasted for 10 h before each experiment. This study was carried out in accordance with the "Regulation for the Administration of Affairs Concerning Experimental Animals" (State Council of China, 1988).

Analyses of Total Phenolics, Flavonoids, Anthocyanins, and Triterpenes. *Determinations of Total Phenolics and Total Flavonoids.* The total phenolics content was determined using FC phenol reagent as described previously.[10] The absorbance of each sample was measured at 765 nm with a spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China) after incubation at 25 °C for 2.0 h. The amount of total phenolic in pears was expressed as mg gallic acid equivalents (GAE, 160−960 μg) per 100 g of dry weight.

The total flavonoids content was measured using a previously reported method.[11] The methanol extract of pear (0.5 mL, 60 mg/mL of dry weight) was mixed with 4 mL of 30% ethanol, and then, 0.3 mL of (5% w/v) $NaNO_2$ solution was added. After 5 min, 0.3 mL of (10% w/v) $AlCl_3$ was added. At 6 min, 2 mL of 1 M NaOH solution was added. The volume was made up to 10 mL by 30% ethanol. The mixture was shaken vigorously and determined at 506 nm. The content of total flavonoids was expressed as rutin equivalents (RE) per 100 g dry weight through the calibration curve, and the calibration curve ranged from 140 to 840 μg.

Determination of Total Anthocyanin. The total anthocyanins content was evaluated by the pH differential method.[12] The methanol extract of pear (0.5 mL) in 25 mM potassium chloride solution (pH 1.0, 25 mL) and 0.4 M sodium acetate buffer (pH 4.5, 25 mL) was determined simultaneously at 510 and 700 nm, respectively, after 15 min of incubation at 23 °C. The total anthocyanins content was expressed as mg cyanidin-3-glucoside equivalents per 100 g of dry pear weight.

Determination of Total Triterpenes. The quantitative analysis of total triterpenes was tested by the pH differential method. The methanol fraction was extracted with ethyl acetate five times. The dry ethyl acetate layer was diluted with 5 mL of ethyl acetate, and 2 mL was taken and dried in a boiling water bath. The dried residue was dissolved in 0.2 mL of 5% vanillin−glacial acetic acid solution and 1 mL of perchloric acid, and the mixture was heated in a water bath at a temperature of 60 °C. After 5 min, the mixture was cooled immediately in ice water and then added to 2 mL of ethyl acetate. The absorbance was measured at 550 nm after keeping for 5 min. The content of total triterpenes was expressed as oleanolic acid equivalents (oleanolic acid/mg sample) through the calibration curve, and the calibration curve ranged from 14.71 to 88.24 μg/mL.

Determination of the Main Compounds. The methanol solution was filtered through a 0.22 μm filter (Agilent Technologies, Beijing, China). Twenty microliters of the standard and sample solution was injected into a Waters 1525-2998 HPLC-PAD (photodiode array detector) system. The column used was a 250 mm × 4.6 mm i.d., 5 μm, Kromasil C18, with a 4 mm × 3.0 mm i.d. security guard column of the same material (Phenomenex). Simultaneous monitoring was performed at 280 (arbutin, gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, rutin, quercetin, and epicatechin) and 210 nm (oleanolic acid and ursolic acid). The mobile phase consisted of 1% acetic acid−water and acetonitrile with a gradient elution at a flow rate of 1.0 mL/min at 30 °C. All of the compounds in the HPLC chromatogram were identified by retention time. Retention times were 8 min for arbutin, 11.8 min for gallic acid, 37 min for catechin, 39 min for chlorogenic acid, 42 min for caffeic acid, 64 min for epicatechin, 70 min for rutin, 88 min for quercetin, 90 min for epicatechin, 109.2 min for oleanolic acid, and 109.6 min for ursolic acid under the conditions of the analysis (Figure 1).

Antioxidant Activity. *Reducing Power.* The pear extract (1 mL, 10 mg/mL), phosphate buffer (2.5 mL, 0.2 M, pH 6.6), and potassium ferricyanide (2.5 mL, 10 mg/mL) were mixed, and the solution was incubated at 50 °C for 20 min. Then, 2.5 mL of trichloroacetic acid (100 mg/mL) was added to the mixture solution and centrifuged at 1000*g* for 10 min. The distilled water (2.5 mL) and ferric chloride (0.5 mL, 1.0 mg/mL) were added to 2.5 mL of the supernatant. The absorbance was measured at 700 nm using a spectrophotometer.

DPPH Assay. The DPPH solution was prepared by dissolving 19.7 mg of DPPH with 100 mL of absolute alcohol and then was stored in a cool, dark area, until needed. One milliliter of this solution was mixed with 2 mL of different concentrations of pear extracts, ranging from 0.3 to 1.5 mg/mL. The solution in the test tubes was shaken vigorously and incubated in the dark for 30 min at 37 °C. Then, the absorbance

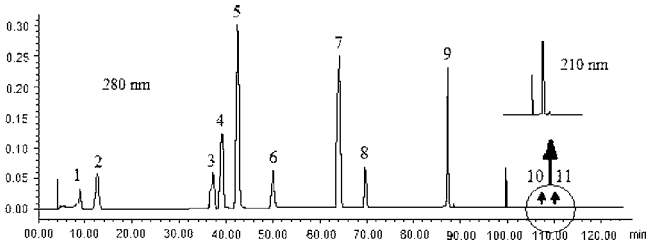

Figure 1. Representative HPLC chromatograms of mixed standards: 1, arbutin; 2, gallic acid; 3, catechin; 4, chlorogenic acid; 5, caffeic acid; 6, epicatechin; 7, ferulic acid; 8, rutin; 9, quercetin; 10, oleanolic acid; and 11, ursolic acid.

of DPPH was measured at 517 nm. The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

$$\% \text{ disappearance} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100\%$$

where A_{control} is the absorbance of the control reaction (100 μM DPPH solution) and A_{sample} is the absorbance of the mixture with the sample extract.

Anti-inflammation Capacity. *Xylene-Induced Mouse Ear Edema Assay.* Animals were randomly divided into 17 groups of eight, and each pear variety was divided into two doses. The test procedure was carried out according to our previous methods.[13] The swelling degree was calculated by the mean weight increase of each ear, while the inhibition rate was expressed as the weight reduction in comparison with the control group.

Carrageenan-Induced Paw Edema. The anti-inflammatory capacity of the pear samples was determined by carrageenan-induced edema test in the hind paws of the Kunming mice. Before any treatment, the average volume (three or four measurements) of the right paw of each animal was determined (V_0, basal volume) using a plethysmometer (UGO Basile, Italy). Mice (eight per group) were fasted for 24 h before the experiment with free access to water. The pear extract was administered intragastrically to different groups of mice. After 45 min, the acute inflammation was induced by subplantar injection (0.05 mL) of fresh carrageenan (10% w/v, in normal saline) in the plantar side of hind paw in mice. The paw volume was measured with the aid of a volume with differential meter (V_t) at 0.5, 1, 3, and 5 h. Swelling was expressed as the ratio (% control) of the volume of the hind paw before and after the carrageenan treatment, respectively.

Statistical Analysis. All experiments were carried out in three replicates and presented as means ± standard errors of mean (SEMs) using SPSS version 17.0. The data were statistically analyzed by LSD and S−N−K tests. The level of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition. The total phenolics, total flavonoids, total anthocyanins, and total triterpenes contents of the extracts from various pears are compared in Figure 2. It can be concluded that the total phenolics contents varied from 308.1 ± 4.20 to 823.3 ± 5.21 as mg gallic acid/100 g dry weight (DW), while total flavonoids ranged from 300.6 ± 6.12 to 790.8 ± 11.43 as mg rutin/100 g DW. Xuehua pear and Nanguo pear had significantly higher total phenolics and flavonoids contents than other pear cultivars. Among the eight pear cultivars studied, Dangshansu pear had the largest total triterpenes value (209.24 ± 3.25 mg/100 g DW), whereas it had a lower value of total anthocyanins (151.6 ± 3.25 mg/100 g DW). Xuehua and Nanguo were also the highest in total anthocyanins among all of the species tested.

In the present study, the different phenolic, flavonoid, and triterpene compounds present in the eight pear extracts were

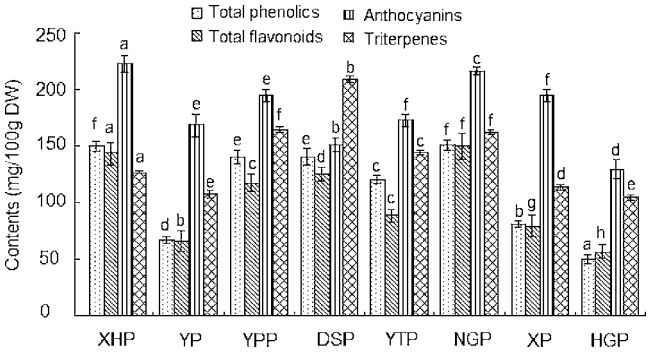

Figure 2. Contents of total flavonoids, polyphenols, anthocyanins, and triterpenes in fruit extracts from different pear cultivars. XHP, Xuehua pear; YP, Ya pear; YPP, Yuanpingsu pear; DSP, Dangshansu pear; YTP, Yantai pear; NGP, Nanguo pear; XP, Xiang pear; and HGP, Huangguan pear. Note that for letters a−h, means with the same letter are not significantly different ($P < 0.05$).

identified. Chlorogenic acid was a prominent phenolic acid compound detected in Xuehua pear (263.8 ± 1.21 μg/g DW), Nanguo pear (113.3 ± 1.87 μg/g DW), Xiang pear (142.5 ± 0.76 μg/g DW), and Yantai prar (126 ± 2.97 μg/g DW). For the flavonoid compounds, the highest amounts are exhibited in the cases of arbutin and catechin, followed by quercetin, with a trace of epicatechin and rutin in eight tests. Dangshansu pear and Nanguo pear presented the highest concentration of arbutin with values of 2298.6 ± 4.11 and 2227.5 ± 5.32 μg/g DW, respectively, while the catechin content was more pronounced in Xuehua pear with a value of 1842.2 ± 4.33 μg/g DW. Different pear cultivars represented different levels of quercetin and rutin contents. Nanguo pear possessed the highest amount of quercetin (70.8 ± 0.35 μg/g), followed by Yuanpingsu pear (43.9 ± 0.19 μg/g) and Dangshansu pear (41.2 ± 0.29 μg/g), while the lowest was found for Xiang pear (5.05 ± 0.16 μg/g). Xuehua pear had the highest rutin content with a value of 71.8 ± 0.31 μg/g; however, the values of other spices were less than 20 μg/g. For the first time, we determined the contents of two triterpene compounds in pears. Two triterpenes were isolated from Xuehua pear (*P. bretschneideri* Rehd.), and they represented a remarkable anti-inflammatory activity at a dose of 10 and 20 mg/kg in our previous work.[13] In this study, Nanguo pear had the highest content of oleanolic acid and ursolic acid as compared to the other pear cultivars, and Xuehua pear, Yuanping pear, and Xiang pear are also rich in the two triterpenes.

According to Table 1, the phenolic content varies greatly among pear varieties. It was reported that chlorogenic acid is the primary phenolic acid compound, and epicatechin is the highest flavonoid in pear juice.[14] In addition, Spanos and Wrolstad[15] found that caffeic acid also exists in pear juice as the main component of phenolic compounds. In this work, the content of chlorogenic acid is also higher than other phenolic acid compounds. However, there are differences between the findings of our study and the above reports of phenolics and flavonoids contents. In this study, the primary flavonoid in pear fruit is arbutin, and the secondary flavonoid is catechin, which is followed by quercetin and epicatechin in most of the pear cultivars examined. In the majority of fruits and vegetables, the content level of chlorogenic acid and gallic acid was much higher.[16]

Reducing Power and DPPH Assay. The results of the reducing power and DPPH bleaching ability are shown in

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Table 1. Main Chemical Compounds of the Different Pear Cultivars ($\mu g/g$, $n = 3$)[a]

samples	Xuehua	Ya	Yuanpingsu	Dangshansu	Yantai	Nanguo	Xiang	Huangguan
arbutin	1458.3 ± 3.21 b	1259.5 ± 3.22 d	1918.2 ± 3.32 g	2298.6 ± 4.11 h	498.1 ± 1.09 e	2227.5 ± 5.32 f	908.9 ± 2.31 a	224.4 ± 1.11 c
gallic acid	27.7 ± 0.32 a	15.1 ± 0.16 g	11.2 ± 0.26 e	9.68 ± 0.24 f	10.4 ± 0.34 c	31.1 ± 0.14 d	14.8 ± 0.65 g	5.39 ± 0.12 b
chlorogenic acid	263.8 ± 1.21 b	24.7 ± 0.09 g	25.2 ± 0.22 g	10.3 ± 0.19 f	126 ± 2.97 c	113.3 ± 1.87 d	142.5 ± 0.76 a	17.6 ± 0.16 e
caffeic acid	13.39 ± 0.32 b	4.58 ± 0.19 e	6.46 ± 0.31 f	9.91 ± 0.45 c	6.42 ± 0.18 f	4.75 ± 0.12 g	8.53 ± 0.12 a	5.04 ± 0.18 d
ferulic acid	22.35 ± 0.65 b	2.65 ± 0.09 d	2.31 ± 0.12 g	2.76 ± 0.09 h	1.97 ± 0.15 f	2.13 ± 0.02 e	7.68 ± 0.13 a	1.58 ± 0.05 c
catechin	1842.2 ± 4.33 b	145.9 ± 1.04 d	49.7 ± 0.91 g	217.5 ± 2.86 e	171.9 ± 2.09 f	515.6 ± 3.17 h	228.4 ± 2.31 a	70.15 ± 0.42 c
epicatechin	44.71 ± 0.28 b	15.13 ± 0.13 d	13.17 ± 0.32 g	6.37 ± 0.39 h	11.7 ± 0.13 e	14.71 ± 0.14 f	7.16 ± 0.08 a	5.81 ± 0.09 c
rutin	71.8 ± 0.31 a	2.28 ± 0.09 d	14.0 ± 0.65 g	17.2 ± 0.34 h	1.16 ± 0.09 f	16.6 ± 0.12 e	12.2 ± 0.21 b	6.77 ± 0.23 c
quercetin	31.2 ± 0.08 a	15.3 ± 0.32 h	43.9 ± 0.19 c	41.2 ± 0.29 d	22.0 ± 0.23 e	70.8 ± 0.35 f	5.05 ± 0.16 b	12.7 ± 0.54 g
oleanolic acid	285.3 ± 2.21 b	59.03 ± 0.37 d	251.8 ± 2.34 g	148.3 ± 2.43 h	43.23 ± 0.33 f	750.8 ± 4.87 e	242.4 ± 3.01 a	171.6 ± 2.12 c
ursolic acid	100.1 ± 3.99 a	70.7 ± 0.34 c	41.1 ± 1.21 e	37.8 ± 0.98 e	122.6 ± 2.03 d	168.2 ± 3.23 f	172.9 ± 4.12 f	135.4 ± 2.87 b

[a]Different letters (a–h) of each value indicate a significant difference at $P < 0.05$.

Figure 3. In terms of reducing power, Nanguo pear and Xuehua pear ranked highest, but Ya pear and Huangguan pear were significantly lower than that of other six pear cultivars (Figure 3A). In this paper, the reducing power of the eight pear cultivars was as follows: Nanguo > Xuehua > Dangshansu > Yuanpingsu > Yantai > Xiang > Huangguan > Ya. The free radical scavenging activity of eight pear samples increased regularly with an increase in the sample concentration (Figure 3B). It was observed that Xuehua pear and Nanguo pear also presented the strongest DPPH bleaching activity (IC_{50} = 0.588 and 0.701 mg/mL, respectively), followed by Yuanpingsu pear, Xiang pear, Dangshansu pear, and Yantai pear with IC_{50} values of 0.842, 0.902, 0.999, and 1.007 mg/mL, respectively. However, Huangguan pear and Ya pear did not reach the IC_{50} value when the sample solution was added to 1.5 mg/mL.

Of the eight pear samples determined in this study, the antioxidant capacity of two methods was in good agreement. Nanguo pear, Xuehua pear, Yuanpingsu pear, and Dangshansu pear with high total phenolics and total flavonoids contents exhibited significantly higher antioxidant abilities than that of Yantai pear, Xiang pear, Ya pear, and Huangguan pear assayed in reducing power and DPPH methods. Therefore, it can be deduced that flavonoid and phenolic acid compounds make a major contribution to the antioxidant capacity of pear. A number of studies have reported that polyphenols, vitamin C, and β-carotene are the main phytochemicals responsible for the antioxidant capacity of vegetables and fruits.[17,18] With respect to total phenolics content (308.1−823.3 mg/100 g DW), values obtained (Figure 1) were comparable with the contents of those of tropical fruits, such as star fruit (131 mg/100 g), guava (179 mg/100 g of fresh weight), and banana (51 mg/100 g of fresh weight).[16] The amounts of total phenolics contents in the eight pears (300.56 ± 1.12 to 790.78 ± 3.43 DW) were close to those of other fruits reported in many works.[17,19] The wide range of values among the pear samples is caused by many factors such as heredity, biology, maturation stage, and environment. In this study, anthocyanins were also correlated with DPPH bleaching activity in pears, and Xuehua pear and Nanguo pear with higher contents of total anthocyanins (233.1 ± 7.32 mg/100 g and 216.6 ± 3.56 mg/100 g, respectively) have excellent antioxidant abilities. Gracia-Alonso et al.[20] examined 28 fruits for antioxidant capacity and found that the fruit which abound in anthocyanins presented greater antioxidant activities than others, suggesting that these pigments could also be contributing to this activity.

Anti-inflammatory Activities. Xylene-induced mouse ear edema and carrageenan-induced hind paw edema models were used to evaluate the anti-inflammatory activity, and the results are reported in Figure 4 and Table 2. All of the pear methanol extracts reduced the oedematous response to a certain extent and exhibited a dose-dependent anti-inflammatory activity. The ear edema inhibition ratios of Nanguo pear, Yuanpingsu pear, and Dangshansu pear were 34.3, 31.7, and 31.1% at a doses of 2 g/kg, respectively. Dexamethasone, as a positive control drug, could induce ear edema with the inhibition ratios of 54.17% at 10 mg/kg. Huangguan pear did not show any anti-inflammatory effect on xylene-induced mouse ear edema in mice at a dose of 1 g/kg. For the carrageenan-induced hind paw edema model, Nanguo pear, Yuanpingsu pear, and Dangshansu pear could also obviously reduce the paw edema in mice in 5 h. Huangguan pear and Ya pear had no anti-inflammatory effects at any doses in this test used. The comprehensive analysis of Figure 4 and Table 2 demonstrated that the anti-inflammation

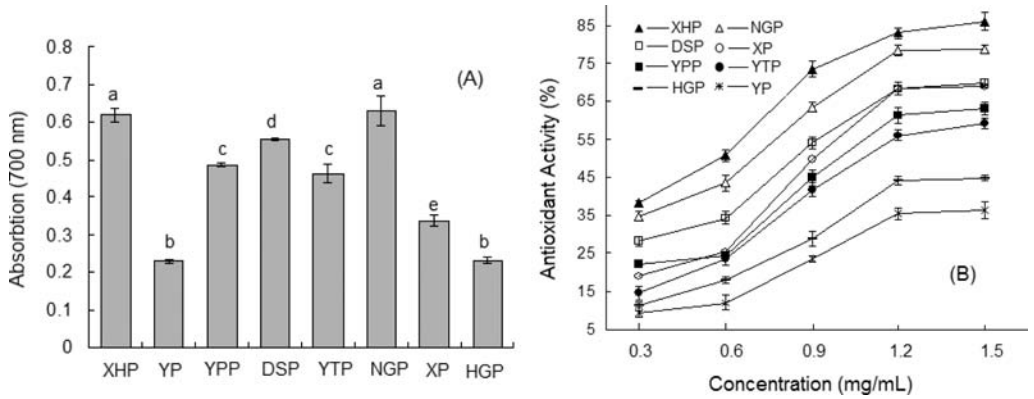

Figure 3. Antioxidant activity of eight pear cultivars. Reducing power assay (A) and −DPPH radical scavenging assay (B). XHP, Xuehua pear; YP, Ya pear; YPP, Yuanpingsu pear; DSP, Dangshansu pear; YTP, Yantai pear; NGP, Nanguo pear; XP, Xiang pear; and HGP, Huangguan pear. Note that for letters a−e, means with the same letter are not significantly different ($P < 0.05$).

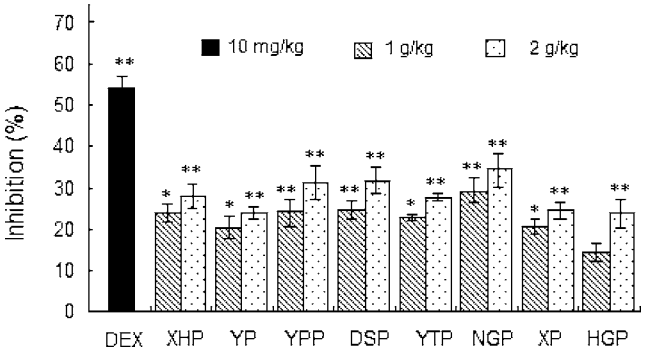

Figure 4. Effects of eight pear cultivars on the auricular edema induced by xylene [results are expressed as the mean ± SEM ($n = 8$); *$P < 0.05$ and **$P < 0.01$ compared]. DEX, dexamethasone.

activity of the eight pear cultivars decreased in the order: Nanguo > Dangshansu > Yuanpingsu > Xuehua > Yantai > Xiang > Ya > Huangguan.

Many studies have also demonstrated that flavonoids and phenolic acid compounds produced significant anti-inflammatory activities, such as arbutin, catechin, rutin, quercetin, and luteolin.[21,22] Nanguo pear, Dangshansu pear, Yuanpingsu pear, and Xuehua pear with a higher content of total flavonoids and total phenolics presented higher anti-inflammation capacities than other pear cultivars. In addition, arbutin, as an antioxidant and a depigmenting agent, was highly correlated to the two anti-inflammation animal models. Also, the content of arbutin is the highest in all of the determined phenolic and flavonoid compounds in pears (Table 1). These results suggested that arbutin plays a very important role in inflammatory processes. Arbutin is a glycosylated hydroquinone that is abundant in many fruits and the leaves of several plant species. It was reported that arbutin could be effective in postinflammatory hyperpigmentation (PIH), which demonstrated that it can regulate both radical-mediated stress and the inflammatory response.[23] Lee and Kim[24] researched the anti-inflammatory mechanism and showed that arbutin might be useful for treating the inflammatory and deleterious effects of BV2 microglial cells activation in response to LPS stimulation. Rutin and quercetin, two natural flavone derivatives, are known for their pharmacological properties, especially the antioxidant and

Table 2. Effects of the Different Pear Cultivars on Paw Edema Induced by Albumen in Mouse ($\bar{x} \pm s$)[a]

groups	dose(g/mL)	30 min	1 h	3 h	5 h
control	10 mL	62.7 ± 2.0	68.0 ± 2.8	57.5 ± 6.6	52.2 ± 4.1
dexamethasone	0.01	39.5 ± 4.5**	44.5 ± 2.9**	31.2 ± 4.3**	19.1 ± 4.2**
Xuehua-L	1	57.6 ± 1.2	62.8 ± 5.2	52.1 ± 3.6*	45.6 ± 1.2
Xuehua-H	2	52.4 ± 3.2*	57.0 ± 3*	47.3 ± 3.8**	42.6 ± 3.3*
Ya-L	1	58.0 ± 4.1	62.6 ± 3.2	54.2 ± 1.6	46.9 ± 1.1
Ya-H	2	56.9 ± 2.8	60.4 ± 1.1	56.9 ± 2.8	48.9 ± 1.5
Yuanpingsu-L	1	60.0 ± 3.0	62.0 ± 2.5	44.9 ± 1.4**	42.2 ± 2.1
Yuanpingsu-H	2	48.1 ± 3.9**	57.9 ± 3.7**	41.9 ± 1.8**	33.9 ± 3.3**
Dangshansu-L	1	56.7 ± 1.7*	58.0 ± 5.5	47.9 ± 2.4*	42.5 ± 5.4
Dangshansu-H	2	50.0 ± 2.3**	55.8 ± 3.6**	38.1 ± 3.8**	29.5 ± 3.8**
Yantai-L	1	59.5 ± 3.5	64.0 ± 2.4	55.3 ± 1.7	50.9 ± 3.4
Yantai-H	2	57.3 ± 1.8*	61.6 ± 2.9*	52.3 ± 1.8**	40.6 ± 2.5**
Nanguo-L	1	58.7 ± 7.5	64.6 ± 4.4	53.9 ± 8.6	48.4 ± 0.9
Nanguo-H	2	44.4 ± 2.7**	60.6 ± 4.1*	35.3 ± 4.6**	29.8 ± 3.9**
Xiang-L	1	58.4 ± 2.0	66.7 ± 2.6	53.6 ± 2.8	51.4 ± 3.4
Xiang-H	2	43.9 ± 2.5*	62.6 ± 3.7*	51.8 ± 2.6*	47.2 ± 1.9*
Huangguan-L	1	61.2 ± 2.5	67.5 ± 1.2	56.9 ± 1.5	51.5 ± 1.7
Huangguan-H	2	57.1 ± 3.7	63.1 ± 3.6	53.5 ± 4.0	49.8 ± 2.2

[a]Results were expressed as the mean ± SEM ($n = 8$). *P<0.05 and **P<0.01 were compared with the control group.

anti-inflammatory effects. Several reports showed that rutin and quercetin isolated from plants or fruits possess strong anti-inflammatory properties.[25,26] In this study, the anti-inflammatory capacity was not correlated to rutin and quercetin, which may be due to the lower content in pears and the relatively weaker effect than triterpene compounds.

Relationship between Chemical Composition of Pear and Biological Activities. Correlation analysis was used to explain the relationship among the two antioxidant and two anti-inflammatory methods measured for all pear cultivars. Reducing power was highly correlated with total phenol acid content (Pearson's correlation coefficient, $r = 0.972$, $p < 0.05$) and total flavonoid content (Pearson's correlation coefficient, $r = 0.971$, $p < 0.05$). However, the DPPH assay was mainly related to total flavonoid content (Pearson's correlation coefficient, $r = 0.912$, $p < 0.05$). For the antioxidant ability tested, the result between reducing power method and DPPH assay is very close. The two anti-inflammatory models were highly correlated to the total flavonoids content with values of 87.1 and 82.3%, respectively. No significant correlation was found between total anthocyanins and the three evaluation methods but DPPH. Total triterpenes, as an important chemical component in pears, were also determined, and it was not correlated to antioxidant capacity while related to the anti-inflammatory activity in our study.

To research the contribution of individual phenolic, flavone, and triterpene compounds to the antioxidant and anti-inflammatory capacities, the relationship between the biological activity and all of the compounds detected was determined. A remarkable correlation was discovered between antioxidant capacity and some phenolic acids and flavonoids, including gallic acid, caffeic acid, and rutin. The high antioxidant capacity of gallic acid has been revealed by other authors.[27] However, no obvious correlation between the rest of the phenolic and antioxidant activity was found. Moderate correlation between ursolic acid and reducing power was discovered for the first time. One flavonoid compound, arbutin, and two triterpenes, oleanolic and ursolic acids, were highly correlated to xylene-induced mouse ear edema and carrageenan-induced hind paw edema models. However, no strong correlation between the rest of compounds and the antioxidant and anti-inflammatory capacities was found. Most of naturally occurring pentacyclic triterpenoids have strong anti-inflammation and anticancer activities, including oleanolic acid, ursolic acid, a-amyrin, and betulinic acid.[28] As shown in Figures 1 and 4 and Table 2, the anti-inflammatory activity was strongly correlated to total triterpenoids, while not correlated to total anthocyanins. Oleanolic acid and ursolic acid can be effective in suppressing the inflammatory network consisting of nuclear factor-κB (NF-Kb), signal transducer and activator of transcription 3 (STAT3), and AKT.[29] They can also inhibit COX-2 transcription, inhibition of TNF-α, and IL-8 secretion.[30] In this study, oleanolic acid and ursolic acid were highly correlated to anti-inflammatory activity. Also, oleanolic acid and ursolic acid were isolated from Xuehua pear in our previous study, and they have good anti-inflammatory effects determined by in vitro animal models.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables of lineal correlation coefficients between composition and antioxidant and anti-inflammatory capacities and between chemical compounds and antioxidant and anti-inflammatory capacities. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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