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Characterization of Triterpenic Acids in Fruits of *Ziziphus* Species by HPLC-ELSD-MS

Sheng Guo, Jin-Ao Duan,* Yu-Ping Tang, Nian-Yun Yang, Da-Wei Qian, Shu-Lan Su, and Er-Xin Shang

Jiangsu Key Laboratory for TCM Formulae Research, Nanjing University of Chinese Medicine, Nanjing 210046, People's Republic of China

The fruits of *Ziziphus* species have been utilized as food as well as crude drugs for their health benefits in China for thousands of years. This paper reported a reversed-phase high-performance liquid chromatography (HPLC) method for the simultaneous characterization and quantitation of 11 triterpenic acids in chloroform extracts of jujube fruits by using an evaporative light scattering detector (ELSD) and electrospray ionization−mass spectrometry (ESI-MS). The results showed that the contents of triterpenic acids in the fruits of *Ziziphus jujuba* var. *spinosa* were higher than those in the fruits of *Z. jujuba*, especially for the compound pomonic acid. Differences were also found among the different parts of *Z. jujuba* var. *spinosa* fruits with the sarcocarp having a higher amount of triterpenic acids than the seed and hard core.

KEYWORDS: *Ziziphus jujuba* var. *spinosa*; *Ziziphus jujuba*; HPLC-ELSD; triterpenic acid; comparative analysis

INTRODUCTION

Triterpenic acids are widespread in plants in the form of free acids or aglycones for triterpenoid saponins (*1*), which have been reported to have multiple biological effects such as anti-inflammatory (*2*, *3*), antimicrobial (*4*, *5*), hepatoprotective (*6*), and antioxidant (*7*, *8*) activities. In recent years, triterpenic acids held attraction in the scientific field because of their anticarcinogenic activity (*9*−*11*), which makes them effective in cosmetics and healthcare products as functional compounds (*1*).

The fruits of the *Ziziphus* species (Rhamnaceae family), which are commonly used in folklore medicine for the treatment of various diseases, have been reported to have many kinds of triterpenic acids (*5*, *12*). The two species *Ziziphus jujuba* Mill. and *Z. jujuba* var. *spinosa* (Bunge) Hu ex H. F. Chow are indigenous to China with a history of over 4000 years and are widely distributed in northern China. The fruits of these two *Ziziphus* species are much admired for their high nutritional value. They have been commonly used as crude drugs in traditional Chinese medicine for analeptic, palliative, and antitussive purposes and also commonly utilized as foods, food additives, and flavoring for thousands of years (*13*). Previous phytochemical studies have been done on the fruits of *Z. jujuba* (known as dazao in China), and as a result, several triterpenic acids have been isolated and identified (*14*−*16*). As for the fruits of *Z. jujuba* var. *spinosa* (known as suanzao in China), the chemical studies mainly focused on its seed, which is a famous Chinese medicine (known as suanzaoren in China) for treating insomnia, and led to the isolation of flavonoids, triterpenic acids, and their saponins (*17*, *18*). Quantitative analyses of the flavonoids in the fruits of *Z. jujuba* (*19*) and the seeds of *Z. jujuba* var. *spinosa* (*20*−*22*) have also been reported. To our knowledge, there are few data from chemical and quantitative studies on the sarcocarp and hard core of *Z. jujuba* var. *spinosa* until now. Because of the growing interest in the triterpenic acids of *Ziziphus*s species (*5*, *15*, *16*), a deeper understanding of the differences between the fruits of *Z. jujuba* and *Z. jujuba* var. *spinosa* and their contents in different parts of *Z. jujuba* var. *spinosa* fruits is required.

Therefore, the objectives of this work were to compare and evaluate the triterpenic acid profiles of these edible fruits as well as their sarcocarps, hard cores, and seeds, in order to draw attention to these species and to improve their potential value as foods. For these purposes, we present a reversed-phase high-performance liquid chromatography (HPLC) coupled with evaporative light scattering detection (ELSD) method. In the ELSD, the signal response does not depend on the samples' optical characteristics, so it is capable of detecting most nonvolatile compounds such as triterpenoids and their saponins (*22*). Therefore, in this paper, the HPLC-ELSD method was utilized for quantification of the triterpenic acids in these two edible fruits. Additionally, an HPLC coupled with an electrospray ionization tandem mass spectrometer (ESI-MS/MS) method was also used for further confirming the target compounds by comparing their MS data with those of reference standards.

MATERIALS AND METHODS

Chemicals and Reagents. Methanol (MeOH) was of HPLC grade from Merck (Darmstadt, Germany), and deionized water (H_2O) was purified by an EPED superpurification system (Eped, Nanjing, China). Other reagent solutions, such as acetic acid, triethylamine, and chloroform, were of analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Chemical standards of ceanothic acid, pomonic acid, alphitolic acid, maslinic acid, epiceanothic acid, betulinic acid, oleanolic

*Corresponding author (telephone +86 25 8581 1116; fax +86 25 8581 1116; e-mail dja@njutcm.edu.cn).

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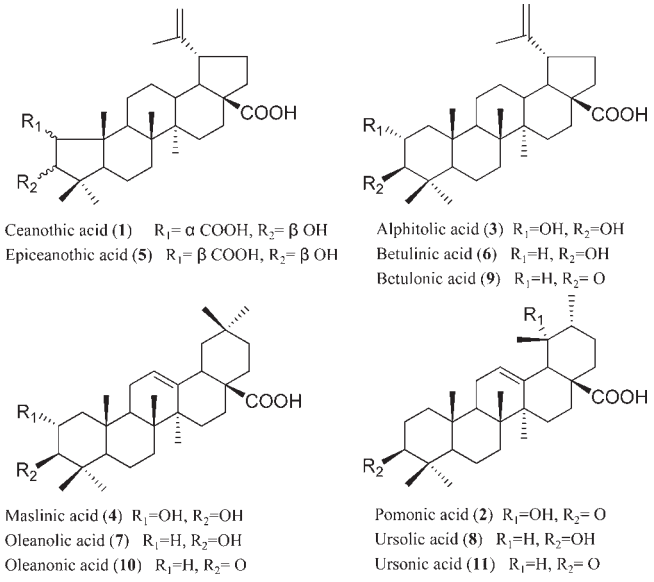

Figure 1. Chemical structures of the identified triterpenic acids.

acid, ursolic acid, betulonic acid, oleanonic acid, and ursonic acid were isolated from the fruits of *Z. jujuba* or the fruits of *Z. jujuba* var. *spinosa* in our laboratory, and their purities (> 98%) and structures were determined by HPLC, MS, and NMR. Their structures are presented in **Figure 1**.

Chromatographic Conditions and Instrumentation. Analysis was performed on a Waters 2695 Alliance HPLC system (Waters Corp., Milford, MA), consisting of a quaternary pump solvent management system, an online degasser, and an autosampler. The raw data were detected by a Waters 2424 ELSD, acquired and processed with Empower software. A Hypersil C_{18} column (250 mm × 4.6 mm, 5 μm) preceded by a Waters Symmetry Shield RP C_{18} guard column (20 mm × 3.9 mm, 5 μm) was applied for all analyses. The mobile phase was composed of A (MeOH) and B (0.3% acetic acid and 0.15% triethylamine in H_2O, v/v) with a linear gradient elution: 0−15 min, 75−87% A; 15−45 min, 87% A; 45−50 min, 87−100% A. Re-equilibration duration was 15 min between individual runs. The flow rate of the mobile phase was 0.5 mL min^{-1}, and the column temperature was maintained at 25 °C. The analytes were monitored with ELSD. The drift tube temperature of the ELSD was set at 80 °C, and the nitrogen flow rate was 2.7 L min^{-1}. MS analysis was performed on a Micromass Q/TOF mass spectrometer connected to the Waters Alliance 2695 HPLC instrument via an electrospray ionization interface (ESI). High-purity nitrogen was used as the nebulizer and auxiliary gas; argon was utilized as the collision gas. The Q/TOF mass spectrometer was operated in negative ion mode with a capillary voltage of 3 kV, a sampling cone voltage of 10 V, a cone gas flow of 50 L h^{-1}, a desolvation gas flow of 700 L h^{-1}, a desolvation temperature of 350 °C, a source temperature of 120 °C, a collision energy of 45 V, and the full scan spectra from 100 to 1000 Da.

Plant Materials. The fruits of *Z. jujuba* var. *spinosa* and *Z. jujuba* were collected from Ningxia and Xinjiang provinces, China, in September 2008. Their botanical origins were identified by the corresponding author, and voucher specimens were deposited at the Herbarium in Nanjing University of Chinese Medicine, China. After collection, the fruits were dried at 45 °C for 6 days.

Preparation of Sample Solutions. The dried fruits of *Z. jujuba* var. *spinosa* were divided into sarcocarp, hard core, and seed parts, which were further pulverized to homogeneous powders (40 mesh), respectively. Likewise, the sarcocarp powder of *Z. jujuba* was obtained according to the same method. Five grams of thee above powders was weighed accurately into a 100 mL conical flask with stopper and then extracted with 100 mL of chloroform by sonication for 60 min. The extract was filtered through analytical filter paper, and 50 mL of filtrate was evaporated to dryness by using a rotary evaporator in vacuum. The residue was dissolved with methanol in a 10 mL volumetric flask and then filtered through a 0.22 μm membrane filter before injection into the HPLC system for analysis.

Preparation of Standard Solutions. A mixed standard stock solution containing ceanothic acid (**1**), pomonic acid (**2**), alphitolic acid (**3**), maslinic acid (**4**), epiceanothic acid (**5**), betulinic acid (**6**), oleanolic acid (**7**), ursolic acid (**8**), betulonic acid (**9**), oleanonic acid (**10**), and ursonic acid (**11**) was prepared in methanol. Working standard solutions for calibration curves were prepared by diluting the mixed standard stock solution with methanol at different concentrations within the following ranges: **1**, 4.10−328.00 μg mL^{-1}; **2**, 22.00−1760.00 μg mL^{-1}; **3**, 13.10−1048.00 μg mL^{-1}; **4**, 12.80−1024.00 μg mL^{-1}; **5**, 8.00−640.00 μg mL^{-1}; **6**, 13.70−1096.00 μg mL^{-1}; **7**, 12.70−1016.00 μg mL^{-1}; **8**, 9.15−732.00 μg mL^{-1}; **9**, 14.05−1124.00 μg mL^{-1}; **10**, 17.00−1360.00 μg mL^{-1}; and **11**, 16.00−1280.00 μg mL^{-1}. The standard solutions were filtered through a 0.22 μm membrane prior to injection. All solutions were stored in a refrigerator at 4 °C before analysis. For calibration, the log−log plots for the peak area versus concentration were drawn to obtain linearity.

HPLC Method Validation. Stock solution containing 11 reference compounds was diluted to a series of appropriate concentrations with methanol. The limits of detection (LOD) and quantification (LOQ) were determined at signal-to-noise (S/N) ratios of 3 and 10, respectively. The intraday and interday precisions were investigated by determining the 11 analytes in six replicates during a single day and by duplicating the experiments on three consecutive days. To confirm the reproducibility, six different working solutions prepared from the sarcocarp of suanzao (collected from Ningxia) were analyzed, and one of them was injected into the apparatus at 0, 2, 4, 8, 12, and 24 h, respectively, to evaluate the stability of the solution. Variations were evaluated using relative standard deviations (RSD). A recovery test was used to evaluate the accuracy of this method. Accurate amounts of reference compounds were added to the sarcocarp of suanzao (collected from Ningxia) separately and then extracted and analyzed in accordance with the methods mentioned above. The average recovery percentage was calculated using the ratio of contents detected (actual) to those added (theoretical).

Identification and Quantification. Identification of the triterpenic acid compounds was carried out by comparing the HPLC retention times and mass/charge ratios of target peaks with those of the standards. To further confirm the structures of the constituents, standards and samples were analyzed by LC-ESI-MS/MS in negative ion mode. Quantification was performed on the basis of linear calibration plots of the logarithm of peak areas versus the logarithm of concentration.

RESULTS AND DISCUSSION

Optimization of Extraction Procedure. In China, these *Ziziphus* fruits are mainly used after drying, especially for those used as medicines. The drying method in cultivation locations is to roast the fruits at 40−50 °C. Therefore, in this study, the fruits were dried at 45 °C after collection, and at this temperature, the fruits could be dried for 6 days. To achieve optimal extraction conditions, extraction variables such as extraction method (refluxing and sonication), extraction solvent (methanol, ethanol, ethyl acetate, and chloroform), solvent volume (50, 100, 150, and 200 mL), and extraction time (15, 30, 60, and 90 min) were investigated on the sarcocarps of suanzao collected from Ningxia, China. When one of the parameters was determined, the others were set at the default (solvent, chloroform; solvent volume, 200 mL; extraction time, 60 min).The results (**Figure 2**) revealed that an ultrasonic bath extraction was more effective than refluxing for the 11 triterpenic acids analyzed. As for the extraction solvent, chloroform was found to be more suitable for the samples because it could enable less interfering peaks and provide the highest values in the contents of the 11 markers. Furthermore, the volume of chloroform and the duration of extraction were also investigated to optimize the extraction procedure. The results demonstrated that the established extraction method (each sample was extracted by sonication with 100 mL of chloroform for 60 min) was adequate and appropriate for the analysis.

Optimization of the HPLC Chromatographic Conditions. To obtain good separation in the experiment with short analysis

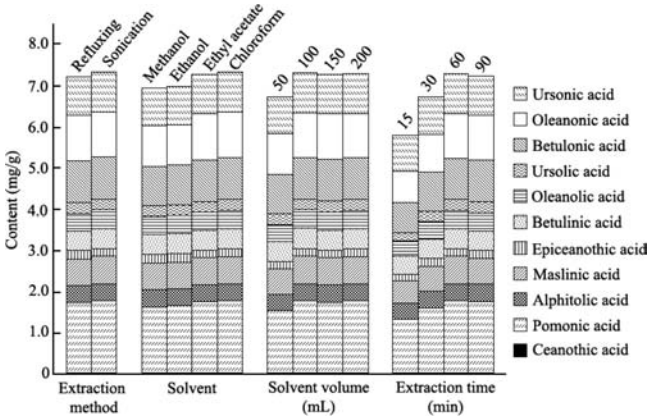

Figure 2. Effects of extraction method, solvent type, solvent volume, and extraction time on the extraction efficiency of investigated triterpenic acids from the sarcocarps of suanzao collected from Ningxia, China. When one of the parameters was determined, the others were set at the default (solvent, chloroform; solvent volume, 200 mL; extraction time, 60 min).

time, the chromatographic conditions were optimized. The resolutions of these compounds were tested and compared with different reversed-phase conditions using a variety of analytical columns such as Hypersil C_{18} (250 mm × 4.6 mm, 5 μm), Diamonsil C_{18} (250 mm × 4.6 mm, 5 μm), and Waters SunFire C_{18} (250 mm × 4.6 mm, 5 μm). In this experiment, a gradient solvent system composed of MeOH/H_2O was selected as a mobile phase, because it afforded a better separation and resolution of target peaks (especially for the oleanolic acid and ursolic acid) from other constituents than acetonitrile/H_2O. Considering all of the reference compounds as the derivates of triterpenic acids, the investigation was commenced with a buffer at acidic pH to improve the peak shape and eliminate the tailing of the target peak. Hence, acetic acid was used, because it was easy to clean out, which is better for use with the ELSD and MS detector. In addition, the good resolution of oleanolic acid and ursolic acid, which were difficult to separate, was achieved when the triethylamine was used as a mobile phase modifier. As a result, a Hypersil C_{18} (250 mm × 4.6 mm, 5 μm) column with a mixed solution including methanol, 0.3% acetic acid, and 0.15% triethylamine in H_2O (v/v) as the mobile phase was chosen as the preferred chromatographic conditions, and gradient elution was applied. It was also suggested that the separation was better when the flow rate was 0.5 mL min^{-1} and the column temperature was kept at 25 °C. Operating conditions for ELSD such as the nebulizing gas flow rate and the drift tube temperature were optimized to obtain the best signals and S/N ratio. The optimum nebulizing gas flow rate in this case was set at 2.7 L min^{-1}, and the optimal drift tube temperature was determined to be 80 °C. Representative chromatograms for the standard analytes and for the samples are shown in **Figure 3**.

HPLC Method Validation. The proposed chromatographic method was validated to determine the linearity, LOD, LOQ, intraday and interday precisions, and accuracy. All calibration curves showed good linearity ($r^2 > 0.9979$) within relatively wide concentration ranges, and the overall LODs and LOQs were less than 7.03 and 14.05 μg mL^{-1}, respectively (**Table 1**). The intra- and interday variations, repeatability, and stability RSD values of the 11 compounds were all < 3.5% and are shown in **Table 2**. The overall recoveries lay between 94.6 and 102.0% for the 11 reference compounds, with RSDs of < 3.5%, which indicated that the established method was accurate enough for the determination of the 11 triterpenic acids in suanzao and dazao.

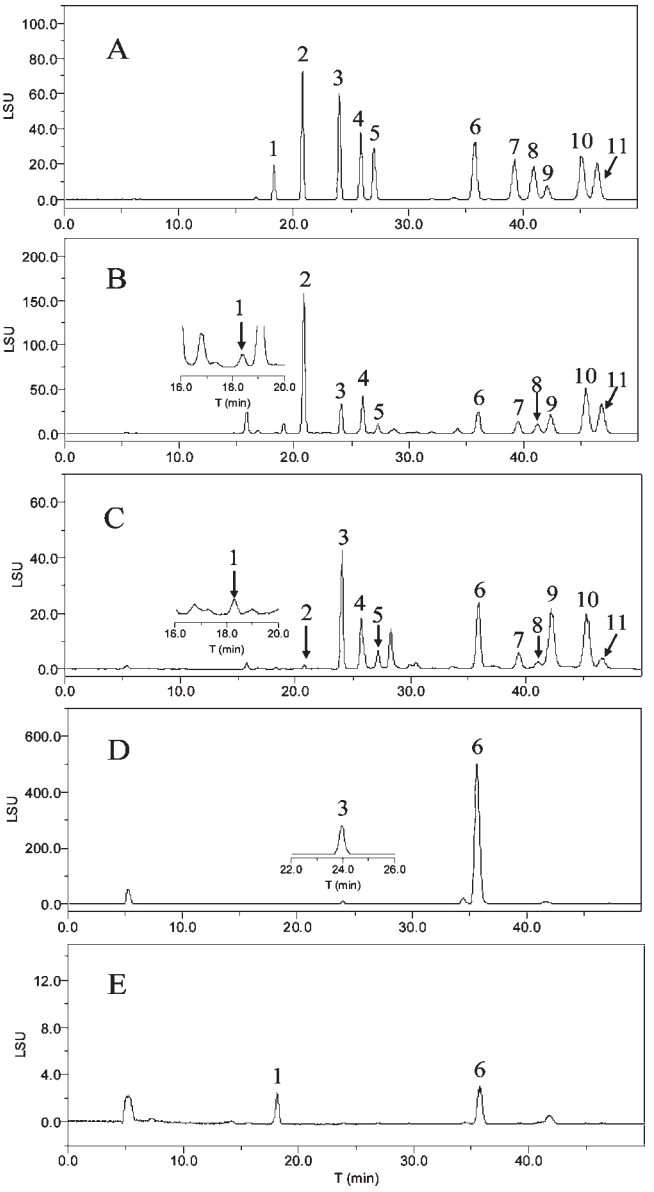

Figure 3. HPLC-ELSD chromatograms of solution of standards (**A**) and samples (**B**−**E**): sarcocarp of *Z. jujuba* var. *spinosa* (**B**); sarcocarp of *Ziziphus jujuba* (**C**); seed of *Z. jujuba* var. *spinosa* (**D**); hard core of *Z. jujuba* var. *spinosa* (**E**). Peaks: 1, ceanothic acid (**1**); 2, pomonic acid (**2**); 3, alphitolic acid (**3**); 4, maslinic acid (**4**); 5, epiceanothic acid (**5**); 6, betulinic acid (**6**); 7, oleanolic acid (**7**); 8, ursolic acid (**8**); 9, betulonic acid (**9**); 10, oleanonic acid (**10**); 11, ursonic acid (**11**).

Identification of Triterpenic Acids. HPLC-ELSD and HPLC-MS/MS analyses of the samples revealed that 11 triterpenic acids were found and recognized as ceanothic acid, pomonic acid, alphitolic acid, maslinic acid, epiceanothic acid, betulinic acid, oleanolic acid, ursolic acid, maslinic acid, betulonic acid, oleanonic acid, and ursonic acid in the sarcocarps of suanzao and dazao by comparison of their HPLC retention times, elution orders, and ESI-MS/MS spectrometric data with those of reference standards. However, only two triterpenic acids were found in the hard core and seed of suanzao, respectively. The results are shown in **Table 3** and **Figure 3**. In the MS spectra, the most prominent mass-to-charge ratios corresponded to deprotonated molecular ions for all 11 compounds. Furthermore, the fragments of $[M - H - HCOOH]^-$ at m/z 439 and $[M - H_2O - COOH]^-$ at m/z 423 were found by tandem mass spectrometry (MS/MS) for

Table 1. Calibration Curves and LOD and LOQ Data of Investigated Compounds by HPLC-ELSD

peak	analyte	calibration curve[a]	r^2	linear range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
1	ceanothic acid	$y = 1.6487x + 2.3115$	0.9985	4.10−328.00	2.05	4.10
2	pomonic acid	$y = 1.5917x + 1.8201$	0.9979	22.00−1760.00	4.40	8.80
3	alphitolic acid	$y = 1.6042x + 2.1418$	0.9985	13.10−1048.00	2.62	5.24
4	maslinic acid	$y = 1.5932x + 1.9974$	0.9982	12.8−1024.00	3.20	6.40
5	epiceanothic acid	$y = 1.6559x + 2.1790$	0.9992	8.00−640.00	4.00	8.00
6	betulinic acid	$y = 1.6505x + 1.9795$	0.9991	13.7−1096.00	3.43	6.85
7	oleanolic acid	$y = 1.6274x + 1.9610$	0.9994	12.70−1016.00	5.08	10.16
8	ursolic acid	$y = 1.6049x + 2.2608$	0.9997	9.15−732.00	3.66	7.32
9	betulonic acid	$y = 1.6454x + 1.5347$	0.9992	14.05−1124.00	7.03	14.05
10	oleanonic acid	$y = 1.6344x + 1.8761$	0.9994	17.00−1360.00	6.80	13.60
11	ursonic acid	$y = 1.6667x + 1.8330$	0.9994	16.00−1280.00	6.40	12.80

[a] y is the logarithmic value of peak area, and x is the logarithmic value of the reference compound's concentration (μg mL^{-1}).

Table 2. Precision, Repeatability, Stability, and Recovery of 11 Analytes

	precision (RSD, %)		repeatability	stability	recovery (%, $n = 3$)	
analyte	intraday ($n = 6$)	interday ($n = 6$)	(RSD, %, $n = 6$)	(RSD, %, $n = 5$)	mean	RSD (%)
ceanothic acid	1.1	2.9	2.9	2.8	98.6	0.9
pomonic acid	1.6	2.0	1.9	2.3	94.6	3.3
alphitolic acid	1.3	1.6	2.0	3.2	97.6	2.2
maslinic acid	1.6	2.5	2.2	3.4	101.3	3.0
epiceanothic acid	2.6	3.0	1.9	1.1	99.9	1.5
betulinic acid	0.8	0.7	2.7	2.3	99.8	1.3
oleanolic acid	1.6	2.0	2.3	2.1	98.8	2.7
ursolic acid	1.2	1.4	1.9	2.2	98.5	2.6
betulonic acid	1.5	1.6	2.4	3.0	95.2	2.8
oleanonic acid	1.5	1.9	2.2	2.9	96.1	2.5
ursonic acid	2.7	2.6	2.5	1.7	102.0	2.4

Table 3. Chromatographic and Spectrometric Data of Triterpenic Acids Found in *Z. jujuba var. spinosa* and *Z. jujuba* Fruits

peak	compound	t_R (min)	$[M - H]^-$ (m/z)	MS/MS fragments (m/z)
1	ceanothic acid	18.3	485	439, 423
2	pomonic acid	20.8	469	451, 409, 407, 389
3	alphitolic acid	24.0	471	393
4	maslinic acid	25.9	471	393, 391
5	epiceanothic acid	27.0	485	439, 423
6	betulinic acid	35.9	455	
7	oleanolic acid	39.3	455	
8	ursolic acid	41.0	455	
9	betulonic aicd	42.2	453	407, 391
10	oleanonic acid	45.1	453	407, 391
11	ursonic acid	46.5	453	407, 391

compounds **1** and **5**. The MS/MS spectrum of compound **2** showed fragment ions at m/z 451 $[M - H_2O - H]^-$, 425 $[M - COOH]^-$, 407 $[M - H_2O - COOH]^-$, and 389 $[M - 2H_2O - COOH]^-$. Likewise, for compounds **9**−**11**, the fragment ions were mainly located at m/z 407 $[M - H - HCOOH]^-$ and 391 $[M - H_2O - COOH]^-$, respectively. All of the fragment ions mentioned above were consistent with those of reference standards **1**−**11**, which further confirmed the identification of the constituents in suanzao and dazao.

Quantification of Triterpenic Acid. The established HPLC-ELSD method was then subsequently applied to a simultaneous determination of the 11 markers in the samples of suanzao and dazao. The results (**Table 4**) showed that the sarcocarps of suanzao demonstrated a higher quantitative content of triterpenic acids than the sarcocarps of dazao (7.31 versus 3.46 mg g^{-1} of dried samples), which were all collected from the same region (Ningxia, China). Furthermore, the sum of ursane-type triterpenic acid contents, such as pomonic acid (**2**), ursolic (**8**), and ursonic acid (**11**), in the sarcocarps of suanzao was > 7 times that in the sarcocarps of dazao. Especially for the compound pomonic acid (which was a predominant triterpenic acid in the sarcocarps of suanzao), its content reached 1.75 mg g^{-1} in the sarcocarps of suanzao, whereas it was only 0.11 mg g^{-1} in the sarcocarps of dazao. It is well-known that these pentacyclic triterpenes are all biosynthesized through the isoprenoid pathway. For oleanane- and ursane-type triterpenes, the cyclization mechanism is identical up to the C-19 oleanyl cation stage, at which migration of the methyl group from C-20 to C-19, followed by three successive 1,2-hydride shifts and deprotonation, gives ursane-type triterpenes, whereas a direct hydride shift from C-18 to C-19, followed by another hydride shift and proton loss, gives oleanane-type triterpenes (*23*). All of these reactions are catalyzed by the corresponding enzymes (*24*). Thus, more ursane-type triterpenic acids being determined in suanzao than in dazao may due to the fact that the relevant enzyme activities of promoting the rearrangement of the C-29 methyl group were higher in suanzao than in dazao. Additionally, it was reported that the functions of these triterpenic acids in plants are likely to protect plants against attack by microbes (*25*). Compared to *Z. jujuba*, which is cultivated in an orchard, *Z. jujuba* var. *spinosa* are wild trees and are more easily infested by microbes. Therefore, the higher content of triterpenic acids in suanzao could be the result of adaptation to the environment.

Besides, the contents of the triterpenic acids varied in the samples collected from different regions. For the sample of suanzao, the total content of the 11 triterpenic acids in its sarcocarps collected from Ningxia was more than twice that of the sample collected from Xinjiang. These results revealed that the differences in the contents of triterpenic acids depended not only on the species but also on the growing conditions, such as soil, geographical, and environmental conditions. Quantitative analysis of the different parts of suanzao revealed that there are many kinds of triterpenic acids in the sarcocarps, but only two in the hard cores. Although only two triterpenic acids were also found in the seeds of suanzao, the content of betulinic acid reached 3.62 mg g^{-1} of the seeds, which was nearly 9 and 50 times those in the sarcocarps and hard cores, respectively. These results further supported the opinion of traditional Chinese medicine that the seed of *Z. jujuba* var. *spinosa* was different in clinic usage with the sarcocarps of *Z. jujuba* var. *spinosa*.

The results mentioned above showed that the proposed method could serve as a prerequisite for quality control and standardization of jujube products. On the basis of the analytical results that the suanzao is a food product rich in

Table 4. Contents of 11 Investigated Compounds in Different Samples

	contents of analyte (mg/100 g, $n = 3$)											
sample[a]	1	2	3	4	5	6	7	8	9	10	11	total
1	4.3 ± 0.1	175.4 ± 2.3	41.3 ± 1.2	66.3 ± 0.9	19.6 ± 0.7	48.7 ± 1.0	41.7 ± 1.0	26.0 ± 0.6	101.8 ± 1.8	110.5 ± 2.7	95.0 ± 1.1	730.6 ± 5.3
2	nd[b]	76.2 ± 1.7	25.0 ± 1.0	43.1 ± 1.2	14.4 ± 0.6	22.8 ± 1.1	18.3 ± 0.5	14.7 ± 0.4	37.7 ± 0.9	37.5 ± 0.6	38.6 ± 1.0	328.3 ± 6.3
3	2.6 ± 0.2	10.7 ± 0.5	43.2 ± 0.7	41.5 ± 0.6	13.8 ± 0.5	43.3 ± 0.8	20.6 ± 0.6	6.5 ± 0.3	87.0 ± 1.1	54.4 ± 1.3	22.3 ± 0.9	345.9 ± 7.0
4	nd	nd	20.6 ± 0.6	nd	nd	362.2 ± 4.2	nd	nd	nd	nd	nd	382.8 ± 4.7
5	3.5 ± 0.2	nd	nd	nd	nd	7.8 ± 0.4	nd	nd	nd	nd	nd	11.3 ± 0.5

[a] Samples: 1, sarcocarps of *Z. jujuba* var. *spinosa* collected from Ningxia; 2, sarcocarps of *Z. jujuba* var. *spinosa* collected from Xinjiang; 3, sarcocarp of *Z. jujuba*; 4, seed of *Z. jujuba* var. *spinosa*; 5, hard core of *Z. jujuba* var. *spinosa*. [b] Not detected.

triterpenic acids, it could be a promising natural source for future industrial research of triterpenic acids with potential benefits for human health.

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LITERATURE CITED

(1) Romero, C.; García, A.; Medina, E.; Ruíz-Méndez, M.; Castro, A.; Brenes, M. Triterpenoid acids in table olives. *Food Chem*. **2010**, *118*, 670–674.
(2) Máñez, S.; Recio, M.; Giner, R.; Ríos, J. Effect of selected triterpenoids on chronic dermal inflammation. *Eur. J. Pharmacol*. **1997**, *334*, 103–105.
(3) Recio, M.; Giner, R; Máñez, S. Investigations on the steroidal anti-inflammatory activity of triterpenoids from *Diospyros leucomelas*. *Planta Med*. **1995**, *61*, 9–12.
(4) Li, X; Cai, L.; Wu, C. Antimicrobial compounds from *Ceanothus americanus* against oral pathogens. *Phytochemistry* **1997**, *46*, 97–101.
(5) Suksamrarn, S.; Panseeta, P.; Kunchanawatta, S.; Distaporn, T.; Ruktasing, S.; Suksamrarn, A. Ceanothane- and lupane-type triterpenes with antiplasmodial and antimycobacterial activity from *Ziziphus cambodiana*. *Chem. Pharm. Bull*. **2006**, *54*, 535–537.
(6) Liu, Y.; Hartley, D.; Liu, J. Protection against carbon techachloride hepatotoxicity by oleanolic acid is not mediated though metallothionein. *Toxicol. Lett*. **1998**, *95*, 77–85.
(7) Bowen-Forbes, C.; Mulabagal, V.; Liu, Y.; Nair, M. Ursolic acid analogues: non-phenolic functional food components in Jamaican raspberry fruits. *Food Chem*. **2009**, *116*, 633–637.
(8) Liu, C.; Yen, M.; Tsang, S.; Gan, K.; Hsu, H.; Lin, C. Antioxidant triterpenoids from the stems of *Momordica charantia*. *Food Chem*. **2010**, *118*, 751–756.
(9) Fulda, S. Betulinic acid: a natural product with anticancer activity. *Mol. Nutr. Food Res*. **2009**, *53*, 140–146.
(10) He, X.; Liu, R. Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple′s anticancer activity. *J. Agric. Food Chem*. **2007**, *55*, 4366–4370.
(11) Kassi, E.; Papoutsi, Z.; Pratsinis, H.; Aligiannis, N.; Manoussakis, M.; Moutsatsou, P. Ursolic acid, a naturally occurring triterpenic, demonstrates anticancer activity on human prostate cancer cells. *J. Cancer Res. Clin. Oncol*. **2007**, *113*, 493–500.
(12) Kundu, A.; Barik, B.; Mondal, D.; Dey, A.; Banerji, A. Zizyberanalic acid, a pentacyclic triterpenoid of *Zizyphus jujuba*. *Phytochemistry* **1989**, *28*, 3155–3158.
(13) Li, J.; Fan, L.; Ding, S.; Ding, X. Nutritional composition of five cultivars of Chinese jujube. *Food Chem*. **2007**, *103*, 454–460.
(14) Guo, S.; Tang, Y.; Duan, J.; Su, S.; Ding, A. Two new terpenoids from fruits of *Ziziphus jujuba*. *Chin. Chem. Lett*. **2009**, *20*, 197–200.
(15) Lee, S.; Min, B.; Lee, C.; Kim, K.; Kho, Y. Cytotoxic triterpenoids from the fruits of *Zizyphus jujuba*. *Planta Med*. **2003**, *69*, 1051–1054.
(16) Lee, S.; Park, J.; Lee, Y.; Lee, C.; Min, B.; Kim, J.; Lee, H. Anti-complementary activity of triterpenoides from fruits of *Zizyphus jujuba*. *Biol. Pharm. Bull*. **2004**, *27*, 1883–1886.
(17) Cheng, G.; Bai, Y.; Zhao, Y.; Tao, J.; Liu, Y.; Tu, G.; Ma, L.; Liao, N.; Xu, X. Flavonoids from *Ziziphus jujuba* Mill var. *spinosa*. *Tetrahedron* **2000**, *56*, 8915–8920.
(18) Li, L.; Liao, X.; Peng, S.; Ding, L. Chemical constituents from the seeds of *Ziziphus jujuba* var. *spinosa* (Bunge) Hu. *J. Integr. Plant Biol*. **2005**, *47*, 494–498.
(19) Pawlowska, A.; Camangi, F.; Bader, A.; Braca, A. Flavonoids of *Zizyphus jujuba* L. and *Zizyphus spina-christi* (L.) Willd (Rhamnaceae) fruits. *Food Chem*. **2009**, *112*, 858–862.
(20) Bao, K.; Li, P.; Li, H.; Qi, L. Simultaneous determination of flavonoids and saponins in semen ziziphi spinosae (Suanzaoren) by high performance liquid chromatography with evaporative light scattering detection. *Chin. J. Nat. Med*. **2009**, *7*, 47–53.
(21) Liu, J.; Chen, B.; Yao, S. Simultaneous analysis and identification of main bioactive constituents in extract of *Zizyphus jujuba* var. *sapinosa* (Zizyphi spinosi semen) by high-performance liquid chromatography−photodiode array detection−electrospray mass spectrometry. *Talanta* **2007**, *71*, 668–675.
(22) Zhao, J.; Li, S.; Yang, F.; Li, P.; Wang, Y. Simultaneous determination of saponins and fatty acids in *Ziziphus jujuba* (suanzaoren) by high performance liquid chromatography−evaporative light scattering detection and pressurized liquid extraction. *J. Chromatogr., A* **2006**, *1108*, 188–194.
(23) Abe, I.; Rohmer, M.; Prestwich, G. D. Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. *Chem. Rev*. **1993**, *93*, 2189–2206.
(24) Morita, M.; Shibuya, M.; Kushiro, T.; Masuda, K.; Ebizuka, Y. Molecular cloning and functional expression of triterpene synthases from pea (*Pisum sativum*). *Eur. J. Biochem*. **2000**, *267*, 3453–3460.
(25) Kubo, I.; Matsumoto, A.; Takase, I. A multichemical defense mechanism of bitter olive *Olea europaea* (Oleaceae). Is oleuropein a phytoalexin precursor? *J. Chem. Ecol*. **1985**, *11*, 251–263.

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