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ARTICLE

Characterization of Nucleosides and Nucleobases in Fruits of *Ziziphus jujuba* by UPLC-DAD-MS

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The fruit of *Ziziphus jujuba*, named dazao in Chinese, has been utilized as food as well as crude drugs in China for thousands of years. To explore the profiles of the nucleosides and nucleobases in this fruit, an ultraperformance liquid chromatograph coupled with a photodiode array detector and electrospray ionization−mass spectrometer method (UPLC-DAD-MS) has been established and validated in this paper. The validated method was successfully applied for the simultaneous characterization and quantitation of 9 nucleosides and nucleobases in 49 dazao samples, which comprised 43 cultivars from 26 cultivation regions. Furthermore, principal component analysis (PCA) was performed to classify the samples on the basis of the contents of the nine analyzed compounds. The results showed that almost all of these dazao samples were rich in nucleosides and nucleobases, although their contents were obviously various, and the proposed method could serve as a prerequisite for quality control of jujube products.

KEYWORDS: *Ziziphus jujuba*; nucleosides; nucleobases; UPLC-DAD-MS; quantitative analysis

INTRODUCTION

Ziziphus jujuba Mill., a thorny Rhamnaceous plant, is indigenous to China with a history of over 4000 years and is widely distributed in northern China, especially in Henan, Shandong, Hebei, Shanxi, Shaanxi, Ningxia, Xinjiang, and Gansu provinces. To date, more than 700 cultivars of *Z. jujuba* have been found in China. Its dried fruits, called dazao in China, has been commonly utilized as a food, food additive, and flavoring for thousands of years due to their high nutritional value (*1*). It has also been used as a traditional Chinese medicine (TCM) for the treatment of anorexia, lassitude, and loose stools in deficiency syndromes of the spleen and of hysteria in women (*2*).

Phytochemical studies have revealed that dazao contains various constituents, including triterpenic acids (*3*−*5*), flavonoids (*6*), cerebrosides (*7*), amino acids, phenolic acids (*8*), microelements (*1*), and polysaccharides (*9*, *10*). In addition, it was also found that dazao contained nucleosides, such as adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) (*11*, *12*), and the content of cAMP was very high in comparison with all other fruits (*13*). However, until now, no other nucleosides and nucleobases have been found except for these two compounds. It is well-known that nucleosides and their bases are involved in the regulation and modulation of various physiological processes in body (*14*, *15*) and exhibit multiple bioactivities, such as antiplatelet aggregation (*16*), antiarrhythmic (*17*), antioxidant (*18*), antiseizure (*19*), and antitumor effects (*20*). With these benefits for human health, recently, these compounds have held attraction in the scientific field, and many of them have been selected as quality control markers for several TCMs and foods (*21*−*24*). Therefore, exploration of the profiles of the nucleosides and nucleobases in dazao would be very helpful for improving their potential values as food and also be convenient for their quality control. For these purposes, 49 samples of dazao comprising 43 cultivars collected from 26 cultivation regions were simultaneously analyzed in this experiment. Ultraperformance liquid chromatography coupled with photodiode array detection (UPLC-DAD), a powerful technique with shortened run time and high sensitivity compared to routine HPLC (*25*), was utilized to separate and determine the nucleosides and their bases in the dazao samples. Furthermore, UPLC coupled with an electrospray ionization tandem mass spectrometer (ESI-MS/MS) method was used to further confirm the target compounds by comparing their MS data with those of reference standards. Finally, principal component analysis (PCA) was performed to evaluate and classify the samples according to the contents of the detected nucleosides and nucleobases.

MATERIALS AND METHODS

Chemicals and Reagents. Methanol (MeOH) was of HPLC grade from Merck (Darmstadt, Germany), and deionized water (H_2O) was purified by a superpurification system (Eped Technology Development Co., Ltd., Nanjing, China). Other reagent solutions, such as ammonium acetate and ammonia−water, were of analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Chemical standards of uracil (**1**), hypoxanthine (**2**), guanine (**3**), uridine (**5**), adenine (**6**), and cAMP (**9**) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Reference compounds of cytidine (**4**), cGMP (**7**), and guanosine (**8**) were obtained from Sigma Chemical Co. (St. Louis, MO). The purity of each compound was >98%, determined by HPLC analysis. The chemical structures of these reference compounds are shown in **Figure 1**.

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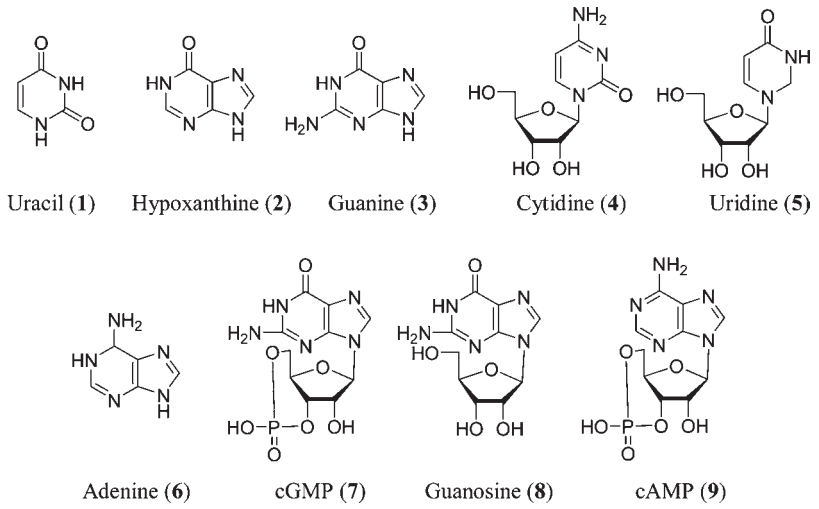

Figure 1. Chemical structures of the identified nucleosides and nucleobases.

Table 1. Cultivars and Cultivation Regions of 49 *Z. jujuba* Samples

sample	*Z. jujuba* cv.	cultivation region	sample	*Z. jujuba* cv.	cultivation region
1	Ruoqiangdazao	Ruoqiang, Xinjiang	26	Fupingdazao	Fuping, Hebei
2	Huizao	Hetian, Xinjiang	27	Chipingyuanzao	Chiping, Shandong
3	Huizao	Lingwu, Ningxia	28	Yuanlingzao	Chiping, Shandong
4	Huizao	Xinzheng, Henan	29	Yuanlingzao	Ningyang, Shandong
5	Lizao	Hetian, Xinjiang	30	Changhongzao	Ningyang, Shandong
6	Lizao	Yuncheng, Shanxi	31	Mangguozao	Heze, Shandong
7	Lizao	Taigu, Shanxi	32	Hetaowen	Heze, Shandong
8	Hupingzao	Taigu, Shanxi	33	Yantaihongzao	Yantai, Shandong
9	Junzao	Jiaocheng, Shanxi	34	Shouguangdazao	Shouguang, Shandong
10	Junzao	Hetian, Xinjiang	35	Sihongdazao	Sihong, Jiangsu
11	Hetianyuzao	Hetian, Xinjiang	36	Dongzao	Zhanhua, Shandong
12	Hamidazao	Hami, Xinjiang	37	Dalilongzao	Cangxian, Hebei
13	Gansuyuanzao	Pingliang, Gansu	38	Chahuzao	Cangxian, Hebei
14	Jinzao	Binxian, Shaanxi	39	Wuhezao	Cangxian, Hebei
15	Ningxiayuanzao	Zhongwei, Ningxia	40	Yiwuzao	Cangxian, Hebei
16	Lingwuchangzao	Lingwu, Ningxia	41	Mianzao	Cangxian, Hebei
17	Yazao	Liulin, Shanxi	42	Jidanzao	Cangxian, Hebei
18	Muzao	Liulin, Shanxi	43	Jinsixiaozao	Cangxian, Hebei
19	Banzao	Jishan, Shanxi	44	Guangyangdazao	Cangxian, Hebei
20	Lingbaoyuanzao	Lingbao, Henan	45	Xiaozao	Cangxian, Hebei
21	Jixinzao	Xinzheng, Henan	46	Malianxiaozao	Cangxian, Hebei
22	Lingzao	Xinzheng, Henan	47	Chuanlingzao	Cangxian, Hebei
23	Bianhesuanzao	Neihuang, Henan	48	Damuzao	Cangxian, Hebei
24	Zanhuangdazao	Zanhuang, Hebei	49	Xuanchengyuanzao	Cangxian, Hebei
25	Xingtangdazao	Xingtang, Hebei			

Chromatographic Conditions and Instrumentation. Analysis was performed on a Waters Acquity UPLC system (Waters, Milford, MA), consisting of a quaternary pump solvent management system, an online degasser, an autosampler, and an Acquity photodiode array detector. An Acquity UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm) column was applied for all analyses. The raw data were acquired and processed with MassLynx 4.1 software. The mobile phase was composed of A (MeOH) and B (5 mM ammonium acetate solution, adjusted to pH 8.0 with ammonia−water) with a gradient elution: 0−3 min, 0% A; 3−7 min, 0−6% A; 7−10 min, 6−15% A, 10−11 min, 15−50% A. The flow rate of the mobile phase was 0.3 mL min^{-1}, and the column temperature was maintained at 30 °C. Detection wavelength was set at 254 nm for compounds **1**, **2**, and **5**−**9**, at 273 nm for **3**, and at 269 nm for **4**. MS analysis was performed on a Micromass Q/TOF mass spectrometer connected to the Acquity UPLC 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 positive ion mode with a capillary voltage of 3 kV, a sampling cone voltage of 30 V, a cone gas flow of 50 L h^{-1}, a desolvation gas flow of 600 L h^{-1}, a desolvation temperature of 350 °C, a source temperature of 120 °C, a collision energy of 6 V, and full-scan spectra from 100 to 1000 Da.

Plant Materials. Forty-nine batches of dazao (samples 1−49), consisting of 43 cultivars from 26 cultivation regions, were collected in September 2008 and are summarized in **Table 1**. 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. After the cores had been removed, the dried fruits were pulverized to homogeneous powders (40 mesh). The dried powder (1.0 g,) was weighed accurately into a 50 mL conical flask with a stopper, and 20 mL of water was added. After accurate weighing, ultrasonication (40 kHz) was performed at room temperature for 30 min, and then the same solvent was added to compensate for the weight lost during the extraction. After centrifugation (13000 rpm, 10 min), the supernatant was stored at 4 °C and filtered through a 0.22 μm membrane filter before injection into the UPLC system for analysis.

Preparation of Standard Solutions. A mixed standard stock solution containing the reference compounds **1**−**9** was prepared in methanol/water (9:1, v/v). Working standard solutions for calibration curves were prepared by diluting the mixed standard stock solution with 10% methanol at different concentrations, and the concentration ranges for these nine

analytes were as follows: **1**, 0.10−10.10 μg mL^{-1}; **2**, 0.10−10.00 μg mL^{-1}; **3**, 0.15−15.10 μg mL^{-1}; **4**, 0.18−18.00 μg mL^{-1}; **5**, 0.30−29.80 μg mL^{-1}; **6**, 0.27−26.60 μg mL^{-1}; **7**, 0.12−11.50 μg mL^{-1}; **8**, 0.20−20.40 μg mL^{-1}; and **9**, 0.44−44.50 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.

UPLC Method Validation. For calibration, the linearity was obtained by plotting the peak areas versus the corresponding concentrations of each analyte. The lowest concentration of working solution for calibration use was diluted with water to a series of appropriate concentrations. They were analyzed until the signal-to-noise ratio (S/N) for each compound was about 3 for the limit of detection (LOD) and 10 for the limits of quantification (LOQ). The precision of the method was evaluated by analyzing the standard solutions containing the nine standard compounds. The experiment was repeated six times on the same day and additionally on three consecutive days to determine intra- and interday precision, respectively. Then, the relative standard deviation (RSD) of peak area for each of the marker compounds was calculated, respectively. To confirm the repeatability, six different sample solutions prepared from the same sample (sample 29) were analyzed and variations expressed by RSD. To evaluate the stability of the solution, one of the sample solutions mentioned above was stored at 25 °C and analyzed at 0, 2, 4, 8, 12, and 24 h, respectively. A recovery test was used to evaluate the accuracy of this method. The test was performed by adding known quantities of the nine standards into a certain amount (0.5 g) of *Z. jujuba* fruits (sample 29) separately. The spiked samples were then extracted, processed, and quantified in accordance with the methods mentioned above. Three replicates were performed for the test. The detected amounts (actual) were calculated by subtracting the total amount of each compound before spiking from the total amount after spiking. The ratio of detected amount (actual) to spiked amount (theoretical) was used to calculate the recovery percentage.

Identification and Quantification. Identification of the nucleosides and nucleobases was carried out by comparing the UPLC retention time and UV spectra of target peaks with those of the standards. To further confirm the structures of the constituents, standards and samples were analyzed by UPLC-ESI-MS/MS in positive ion mode. Quantification was performed on the basis of linear calibration plots of the peak areas versus the concentration.

Method for PCA of Samples. The PCA was done by SPSS 16.0 software (SPSS, Chicago, IL). In this study, the contents of the 9 markers analyzed from the 49 samples composed a data matrix with 49 rows and 9 columns, which was used for PCA after normalization. The first three principal components (PCs) were extracted, and the scatter plot were obtained by plotting the scores of PC 1 versus PC 2 and PC 3.

RESULTS AND DISCUSSION

Optimization of Extraction Procedure. In China, dazao is mainly used after drying, especially for medicinal purposes. To date, the drying method in cultivation regions is mostly to roast the fruits at 40−50 °C. For these reasons, we chose to dry the fruits at 45 °C after collection in this experiment, and at this temperature, the fruits could be dried for 6 days. In this study, extraction variables such as extraction method (refluxing and sonication), extraction solvent (water, 20% aqueous methanol, and 50% aqueous methanol), solvent volume (10, 20, and 30 mL), and extraction time (15, 30, 60, and 90 min) were investigated on sample 29 (1.0 g, 40 mesh) to obtain optimal extraction conditions. When one of the parameters was determined, the others were set at the default (extraction method, sonication; solvent, water; solvent volume, 20 mL; extraction time, 60 min).The results are shown in **Figure 2**, which indicates that ultrasonic bath extraction was more effective than refluxing, and the best solvent was found to be water, which could obtain the highest extraction efficiency for the nine constituents analyzed among these investigated solvents. Furthermore, it was found that the amounts of the compounds analyzed in dazao increased with extraction time extension and reached the maximum at 30 min; thereafter, a further increase of extraction time did not result in a significant increase in amount.

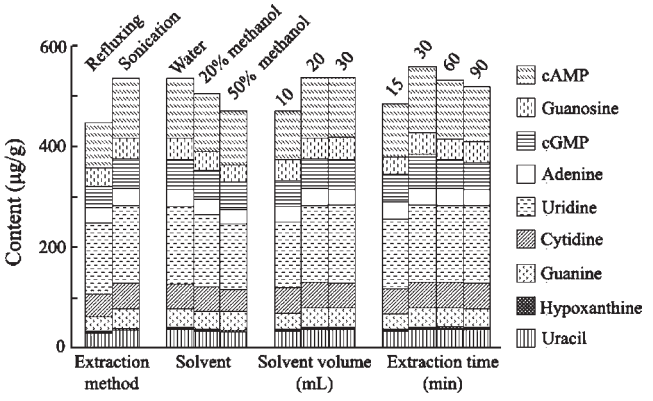

Figure 2. Effects of extraction method, solvent type, solvent volume, and extraction time on the extraction efficiency of investigated nucleosides and nucleobases from the dazao sample. When one of the parameters was determined, the others were set at the default (extraction method, sonication; solvent, water; solvent volume, 20 mL; extraction time, 60 min).

On the contrary, it could lead to dramatic decreases in yields of cAMP and cGMP. For example, compared with the amounts detected after an extraction of 30 min, the detected amount decreased about 9% for cAMP and 11% for cGMP when the extraction time was extended to 60 min. In addition, the volume of solvent was chosen as 20 mL, which was sufficient for sample extraction. Therefore, the final extraction conditions were as follows: each sample was extracted by sonication with 20 mL of water for 30 min, which was adequate and appropriate for the analysis.

Optimization of the UPLC Chromatographic Conditions. In our preliminary test, two columns, an Acquity BEH C_{18} (100 mm × 2.1 mm, 1.7 μm) and an Acquity HSS T3 (100 mm × 2.1 mm, 1.8 μm), were compared. The result showed that the latter has a stronger retention ability as well as better resolution for these hydrophilic components of dazao, with a high ratio of aqueous mobile phase; thus, the Acquity HSS T3 (100 mm × 2.1 mm, 1.8 μm) column was chosen for this analysis. As for the mobile phase, it was reported that 5 mM ammonium acetate aqueous solution can improve the separation of nucleobases and nucleosides for HPLC analysis (*21*). However, it was found that for the Acquity HSS T3 column, tailings were observed for most of the target peaks when only ammonium acetate was used as a mobile phase modifier. Hence, the aqueous ammonium acetate solutions (5 mM) with different pH values (6.0, 7.0, 7.5, and 8.0), which were adjusted with acetic acid/ammonia−water, were investigated. The result showed that the solution at pH 8.0 was better than those at pH 6.0, 7.0, and 7.5 for improving the peak shape. Also, it was found that methanol was better than acetonitrile for the separation of these target compounds. As a result, a mixed solution including methanol and aqueous ammonium acetate solution (5 mM, adjusted to pH 8.0 with ammonia−water) was chosen as the preferred mobile phase, and gradient elution was applied. It was also suggested that the separation was better when the flow rate was 0.3 mL min^{-1} and the column temperature was kept at 30 °C. According to the absorption maxima of the nine reference compounds on the UV spectra obtained by DAD detection, the wavelength was set at 254 nm for compounds **1**, **2**, and **5**−**9**, at 273 nm for **3**, and at 269 nm for **4**. Representative chromatograms for the standard analytes and the samples are shown in **Figure 3**.

UPLC Method Validation. The proposed UPLC method was validated by determining the linearity, LOD, LOQ, precision, repeatability, stability, and accuracy. The results demonstrated

that all calibration curves exhibited excellent linear regressions with the determination coefficients (r^2) ranging from 0.9995 to 0.9999, and the calibration ranges adequately covered variations in the amounts of the compounds investigated in the samples. The overall LODs and LOQs were <0.036 and 0.110 μg mL^{-1}, respectively (**Table 2**). Among the tested samples, the sample of cv. Yuanlingzao from Ningyang, Shandong (sample 29), exhibited the properties of good resolution and moderate contents of investigated compounds, which demonstrated that this sample was suitable for the repeatability, stability, and recovery tests. Therefore, we chose it for the experiments mentioned above. The intra- and interday variations, repeatability, and stability RSD values of the nine compounds were all <2.75% and are shown in **Table 3**. The overall recoveries lay between 95.4 and 102.2% for the nine reference compounds, with RSDs of <2.52%, which indicated that the established method was accurate enough for the determination of the nine nucleosides and nucleobases in dazao.

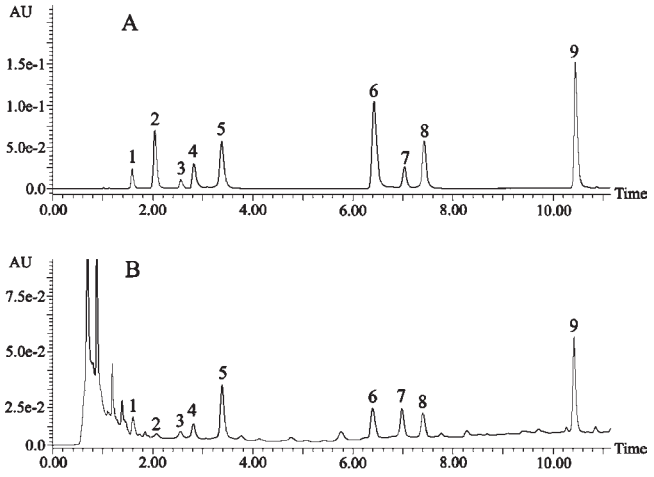

Figure 3. UPLC-DAD chromatograms of solution of standards (**A**) and samples (**B**). Peaks: 1, uracil (4.04 μg mL^{-1}); 2, hypoxanthine (4.00 μg mL^{-1}); 3, guanine (6.04 μg mL^{-1}); 4, cytidine (7.20 μg mL^{-1}); 5, uridine (11.92 μg mL^{-1}); 6, adenine (10.64 μg mL^{-1}); 7, cGMP (4.60 μg mL^{-1}); 8, guanosine (8.16 μg mL^{-1}); 9, cAMP (17.80 μg mL^{-1}).

Identification of Nucleosides and Nucleobases. By comparing their UPLC retention times and UV spectra as well as the MS data in positive ion mode with those of reference compounds, nine constituents in dazao were unequivocally identified as uracil, hypoxanthine, guanine, cytidine, uridine, adenine, cGMP, guanosine, and cAMP. The results are shown in **Table 4** and **Figure 3**. In the MS spectra, the most prominent mass-to-charge ratios corresponded to the molecular ions of $[M + H]^+$ and $[M + Na]^+$ for compounds **1**−**3**, **6**, **7**, and **9** and to the fragment ions of $[M + H - \text{ribose}]^+$ for **4** and **8**. Furthermore, fragments losing $-NH_3$ were also observed for compounds **3**, **6**, and **8**. In addition, for the compounds containing 3′,5′-cyclic monophosphate moieties,

Table 2. Calibration Curves and LOD and LOQ Data of Compounds Investigated by UPLC-DAD

	analyte	calibration curves[a]	r^2	linear range (μg mL^{-1})	LOD[b] (μg mL^{-1})	LOQ[b] (μg mL^{-1})
1	uracil	$y = 342.91x + 11.04$	0.9998	0.10−10.10	0.020	0.063
2	hypoxanthine	$y = 1337.40x - 8.51$	0.9999	0.10−10.00	0.012	0.040
3	guanine	$y = 132.63x + 17.73$	0.9996	0.15−15.10	0.030	0.094
4	cytidine	$y = 450.22x + 5.56$	0.9999	0.18−18.00	0.036	0.110
5	uridine	$y = 495.21x - 89.08$	0.9995	0.30−29.80	0.019	0.060
6	adenine	$y = 1163.70x - 27.90$	0.9999	0.27−26.60	0.017	0.053
7	cGMP	$y = 451.53x + 6.52$	0.9999	0.12−11.50	0.023	0.072
8	guanosine	$y = 614.19x - 6.89$	0.9999	0.20−20.40	0.026	0.080
9	cAMP	$y = 652.76x + 24.65$	0.9999	0.44−44.50	0.014	0.045

[a] y is the value of peak area, and x is the value of the reference compound's concentration (μg mL^{-1}). [b] LOD and LOQ were determined at S/N of about 3 and 10, respectively.

Table 3. Precision, Repeatability, Stability, and Recovery of the Nine Analytes

	precision (RSD, %)				recovery (%, n = 3)	
analyte	intraday (n = 6)	interday (n = 6)	repeatability (RSD, %, n = 6)	stability (RSD, %, n = 6)	mean	RSD,%
uracil	0.27	1.05	2.13	1.74	97.9	2.52
hypoxanthine	0.34	0.98	2.06	1.27	101.2	2.35
guanine	0.62	2.30	2.28	2.24	95.4	2.34
cytidine	0.28	1.59	1.84	1.68	99.4	1.72
uridine	0.42	2.75	2.24	1.80	102.2	1.55
adenine	0.25	1.09	1.56	0.85	97.6	1.98
cGMP	0.51	1.96	1.36	2.37	98.6	2.48
guanosine	0.35	1.71	0.57	1.01	96.4	2.12
cAMP	0.15	0.67	2.52	2.38	101.5	0.86

Table 4. Chromatographic and Spectrometric Data of Nucleosides and Nucleobases Found in *Z. jujuba* Fruits

peak	compound	t_R (min)	UV data (nm)	mass data (m/z)
1	uracil	1.59	258	113 $[M + H]^+$, 135 $[M + Na]^+$, 151 $[M + K]^+$
2	hypoxanthine	2.05	253	137 $[M + H]^+$, 159 $[M + Na]^+$, 175 $[M + K]^+$
3	guanine	2.56	245, 273	152 $[M + H]^+$, 174 $[M + Na]^+$, 135 $[M + H - NH_3]^+$
4	cytidine	2.82	269	244 $[M + H]^+$, 112 $[M + H - \text{ribose}]^+$, 266 $[M + Na]^+$, 487 $[2M + H]^+$
5	uridine	3.38	260	267 $[M + Na]^+$, 283 $[M + K]^+$, 113 $[M + H - \text{ribose}]^+$ 135 $[M + Na - \text{ribose}]^+$
6	adenine	6.42	259	136 $[M + H]^+$, 119 $[M + H - NH_3]^+$
7	cGMP	7.03	253	346 $[M + H]^+$, 152 $[M + H - 194]^+$, 384 $[M + K]^+$
8	guanosine	7.42	253	284 $[M + H]^+$, 152 $[M + H - \text{ribose}]^+$, 306 $[M + Na]^+$, 322 $[M + K]^+$, 135 $[M + H - \text{ribose} - NH_3]^+$
9	cAMP	10.45	258	330 $[M + H]^+$, 136 $[M + H - 194]^+$, 352 $[M + Na]^+$

Table 5. Contents of Nucleosides and Nucleobases in *Z. jujuba* Fruits

	contents of analytes (μg g^{-1}, n = 3)									
sample[a]	1	2	3	4	5	6	7	8	9	total
1	39.78 ± 0.73	nd[b]	23.61 ± 0.54	53.60 ± 0.36	135.13 ± 1.89	63.92 ± 1.37	107.40 ± 1.17	49.01 ± 0.99	381.88 ± 6.88	854.33 ± 2.58
2	56.10 ± 1.08	4.45 ± 0.08	19.44 ± 0.26	39.82 ± 0.61	83.16 ± 1.15	44.46 ± 1.06	56.66 ± 1.32	39.93 ± 0.71	120.43 ± 2.20	464.44 ± 3.90
3	22.77 ± 0.55	6.55 ± 0.18	47.23 ± 1.13	80.05 ± 0.68	142.26 ± 1.24	39.73 ± 0.62	63.36 ± 1.54	49.75 ± 0.72	168.32 ± 1.80	620.03 ± 5.36
4	21.83 ± 0.50	nd	16.14 ± 0.45	54.38 ± 1.09	171.43 ± 1.61	32.11 ± 0.63	31.46 ± 0.41	56.63 ± 0.87	38.55 ± 0.78	422.51 ± 0.34
5	62.03 ± 1.10	1.65 ± 0.05	20.11 ± 0.12	54.30 ± 0.19	99.29 ± 1.40	80.26 ± 2.01	91.80 ± 1.90	52.07 ± 0.61	272.60 ± 1.73	734.11 ± 1.37
6	34.14 ± 0.65	2.83 ± 0.07	47.56 ± 0.45	62.66 ± 0.65	159.27 ± 1.17	33.00 ± 0.68	87.93 ± 1.90	44.20 ± 1.22	168.05 ± 1.50	639.62 ± 4.47
7	25.86 ± 0.50	7.34 ± 0.06	51.08 ± 1.10	49.66 ± 0.81	128.61 ± 2.40	30.82 ± 0.34	116.26 ± 2.75	43.81 ± 0.81	292.36 ± 2.36	745.80 ± 3.41
8	43.82 ± 0.71	nd	72.78 ± 1.43	76.69 ± 1.34	137.45 ± 1.54	54.15 ± 1.41	124.45 ± 2.34	52.29 ± 1.37	465.09 ± 4.83	1026.72 ± 2.24
9	34.18 ± 0.84	3.05 ± 0.05	44.96 ± 0.42	96.39 ± 1.00	176.52 ± 3.54	64.86 ± 1.12	116.90 ± 2.59	59.30 ± 1.24	400.03 ± 4.23	996.19 ± 5.59
10	59.57 ± 1.20	5.43 ± 0.06	6.77 ± 0.13	44.35 ± 0.95	77.62 ± 1.18	85.49 ± 1.89	51.21 ± 0.76	48.48 ± 1.13	90.60 ± 1.72	469.52 ± 2.52
11	41.65 ± 0.69	nd	29.57 ± 0.40	79.95 ± 0.97	137.72 ± 2.82	65.23 ± 1.80	103.28 ± 2.28	57.08 ± 1.22	400.59 ± 4.24	915.07 ± 7.97
12	35.92 ± 0.85	4.60 ± 0.10	14.42 ± 0.32	75.12 ± 1.12	133.83 ± 1.19	45.03 ± 0.71	111.32 ± 1.35	70.17 ± 1.38	327.10 ± 3.06	817.51 ± 0.71
13	30.24 ± 0.73	7.12 ± 0.13	23.89 ± 0.42	89.96 ± 1.98	137.63 ± 1.62	37.70 ± 0.63	77.59 ± 0.57	53.35 ± 0.90	210.40 ± 1.73	667.87 ± 5.46
14	72.33 ± 1.29	21.26 ± 0.26	41.68 ± 0.53	115.80 ± 1.82	620.45 ± 4.61	106.84 ± 2.61	47.36 ± 0.98	147.05 ± 2.50	66.48 ± 1.45	1239.23 ± 11.04
15	31.77 ± 0.67	4.62 ± 0.05	19.89 ± 0.31	53.17 ± 0.51	117.90 ± 3.23	30.96 ± 0.67	80.64 ± 1.29	45.59 ± 0.61	164.81 ± 2.27	549.35 ± 2.49
16	52.68 ± 1.23	7.62 ± 0.17	28.16 ± 0.42	128.27 ± 2.40	189.38 ± 2.27	49.67 ± 0.67	117.79 ± 2.46	46.06 ± 0.92	346.83 ± 2.43	966.47 ± 6.75
17	28.94 ± 0.61	nd	15.28 ± 0.37	36.33 ± 0.35	73.33 ± 0.89	34.40 ± 0.58	44.67 ± 0.36	33.18 ± 0.42	97.81 ± 0.76	363.94 ± 0.37
18	33.75 ± 0.84	3.97 ± 0.09	11.66 ± 0.13	56.10 ± 0.87	88.46 ± 1.64	55.18 ± 1.49	32.75 ± 0.60	63.54 ± 1.47	46.53 ± 1.01	391.95 ± 1.02
19	46.34 ± 1.21	4.44 ± 0.10	10.76 ± 0.23	58.73 ± 0.72	83.81 ± 1.83	48.31 ± 0.33	50.94 ± 1.30	53.46 ± 0.62	81.48 ± 1.52	438.28 ± 3.08
20	37.70 ± 0.91	2.58 ± 0.05	26.97 ± 0.41	36.02 ± 0.12	159.58 ± 3.71	45.99 ± 0.68	43.03 ± 0.67	64.19 ± 1.19	68.70 ± 1.29	484.76 ± 5.72
21	26.79 ± 0.50	2.34 ± 0.06	34.50 ± 0.31	49.77 ± 0.67	171.58 ± 3.64	26.59 ± 0.63	50.01 ± 0.99	43.03 ± 1.09	91.67 ± 1.63	496.28 ± 2.05
22	24.54 ± 0.67	2.17 ± 0.06	41.01 ± 0.91	35.91 ± 0.91	119.28 ± 1.74	23.37 ± 0.58	93.65 ± 1.65	32.24 ± 0.55	197.39 ± 2.18	569.56 ± 4.80
23	43.46 ± 0.70	nd	36.80 ± 0.52	82.95 ± 0.93	101.08 ± 2.05	45.91 ± 0.79	146.74 ± 2.84	58.05 ± 0.93	407.52 ± 2.42	922.51 ± 1.85
24	37.67 ± 0.87	nd	23.43 ± 0.13	43.17 ± 0.83	133.51 ± 0.64	51.51 ± 0.82	45.78 ± 0.56	64.69 ± 1.44	84.23 ± 0.85	483.99 ± 2.03
25	18.26 ± 0.52	nd	23.17 ± 0.51	51.71 ± 0.85	126.69 ± 2.49	33.29 ± 0.80	52.92 ± 0.51	40.62 ± 0.56	80.44 ± 0.90	427.10 ± 3.32
26	67.43 ± 0.88	12.26 ± 0.32	31.62 ± 0.47	53.93 ± 1.06	151.94 ± 1.74	145.96 ± 2.39	43.65 ± 0.75	159.40 ± 2.06	70.28 ± 1.74	736.47 ± 2.95
27	24.52 ± 0.54	nd	62.18 ± 0.28	53.00 ± 0.35	121.78 ± 2.68	45.07 ± 0.74	127.57 ± 2.54	54.17 ± 1.07	357.50 ± 4.19	845.78 ± 9.13
28	37.08 ± 0.92	3.84 ± 0.11	40.53 ± 0.46	79.64 ± 0.66	18.25 ± 0.26	49.46 ± 0.89	40.17 ± 0.73	53.55 ± 1.30	66.21 ± 1.15	388.72 ± 1.19
29	37.93 ± 0.87	3.52 ± 0.04	38.40 ± 0.41	51.25 ± 0.80	153.71 ± 2.49	33.74 ± 0.49	65.72 ± 1.51	42.56 ± 0.79	132.09 ± 2.34	558.92 ± 6.31
30	27.75 ± 0.59	2.04 ± 0.03	41.13 ± 0.32	61.74 ± 0.71	132.51 ± 2.59	37.87 ± 1.07	49.59 ± 0.48	44.59 ± 0.47	81.52 ± 1.43	478.75 ± 1.01
31	20.42 ± 0.58	2.01 ± 0.05	27.43 ± 0.22	43.75 ± 1.22	178.17 ± 3.92	33.15 ± 0.41	121.25 ± 1.96	31.62 ± 0.62	268.95 ± 3.05	726.74 ± 3.68
32	90.46 ± 1.11	13.21 ± 0.24	56.61 ± 0.64	155.11 ± 2.09	320.90 ± 3.94	102.73 ± 1.38	8.41 ± 0.10	185.72 ± 4.39	8.10 ± 0.20	941.25 ± 5.20
33	30.22 ± 0.80	4.25 ± 0.05	41.40 ± 0.63	81.89 ± 0.62	161.31 ± 1.81	45.28 ± 0.90	155.62 ± 3.40	55.01 ± 0.62	350.61 ± 4.16	925.58 ± 8.61
34	42.24 ± 0.79	2.09 ± 0.04	44.85 ± 0.53	79.12 ± 1.54	152.88 ± 2.83	37.41 ± 0.52	46.11 ± 0.79	50.75 ± 0.69	90.86 ± 1.78	546.31 ± 6.35
35	20.54 ± 0.59	nd	63.24 ± 1.25	88.54 ± 0.61	199.48 ± 2.26	63.24 ± 1.21	50.98 ± 0.86	61.50 ± 1.28	84.13 ± 1.13	631.66 ± 3.09
36	44.86 ± 0.82	nd	nd	201.12 ± 2.78	119.47 ± 2.46	71.89 ± 1.45	122.62 ± 2.50	94.76 ± 1.22	408.61 ± 4.86	1063.33 ± 8.19
37	27.65 ± 0.67	2.66 ± 0.06	51.19 ± 0.38	99.87 ± 2.02	170.71 ± 3.84	77.05 ± 1.06	96.41 ± 1.71	57.07 ± 1.20	159.72 ± 2.61	742.32 ± 5.75
38	41.82 ± 0.54	nd	44.90 ± 0.81	67.05 ± 1.18	101.52 ± 1.47	30.69 ± 0.66	126.23 ± 2.32	40.15 ± 0.75	352.04 ± 6.03	804.39 ± 8.11
39	22.11 ± 0.43	11.02 ± 0.11	46.32 ± 1.12	37.49 ± 0.77	94.64 ± 2.21	49.14 ± 0.80	19.66 ± 0.43	34.12 ± 0.66	19.44 ± 0.32	333.93 ± 0.65
40	57.07 ± 0.60	4.28 ± 0.06	50.15 ± 0.60	88.63 ± 1.93	152.68 ± 2.18	56.52 ± 1.05	125.38 ± 1.84	68.12 ± 1.09	290.47 ± 3.47	893.30 ± 2.97
41	18.60 ± 0.47	nd	44.48 ± 0.27	40.92 ± 0.59	123.45 ± 1.44	25.71 ± 0.34	6.05 ± 0.17	28.58 ± 0.57	nd	287.79 ± 0.85
42	25.26 ± 0.53	4.60 ± 0.12	120.32 ± 0.66	66.17 ± 1.16	234.60 ± 4.30	38.80 ± 0.80	103.29 ± 2.17	16.62 ± 0.45	206.50 ± 3.41	816.17 ± 4.20
43	26.74 ± 0.40	7.30 ± 0.15	21.05 ± 0.52	60.68 ± 0.34	113.12 ± 1.11	39.98 ± 0.64	65.48 ± 0.50	34.26 ± 0.72	128.76 ± 1.64	497.38 ± 2.86
44	35.15 ± 0.51	7.26 ± 0.16	7.34 ± 0.08	51.50 ± 1.40	185.41 ± 4.41	32.04 ± 0.65	28.45 ± 0.52	44.15 ± 0.79	30.48 ± 0.59	421.78 ± 8.62
45	24.85 ± 0.49	7.66 ± 0.11	33.85 ± 0.71	73.61 ± 0.80	117.97 ± 0.43	44.44 ± 0.67	64.06 ± 0.72	42.14 ± 0.81	136.56 ± 2.60	545.13 ± 4.86
46	18.99 ± 0.31	3.67 ± 0.04	29.68 ± 0.33	54.86 ± 0.88	134.40 ± 3.56	35.66 ± 0.57	52.04 ± 1.20	51.54 ± 0.69	69.48 ± 0.95	450.32 ± 1.00
47	37.42 ± 0.79	nd	51.02 ± 0.67	62.99 ± 1.39	126.60 ± 2.41	32.83 ± 0.85	127.48 ± 3.55	43.63 ± 0.69	354.56 ± 3.68	836.53 ± 2.11
48	19.85 ± 0.42	3.42 ± 0.09	46.93 ± 0.63	33.38 ± 0.61	182.77 ± 0.69	41.05 ± 1.10	65.07 ± 1.07	30.49 ± 0.75	159.08 ± 2.40	582.04 ± 1.45
49	38.03 ± 0.47	9.12 ± 0.02	77.73 ± 0.69	107.08 ± 1.08	163.37 ± 4.03	48.74 ± 0.62	108.14 ± 2.07	60.43 ± 1.01	302.94 ± 5.62	915.59 ± 9.84

[a] The 49 samples are the same as in **Table 1**. [b] Not detected.

such as cGMP (**7**) and cAMP (**9**), the fragment ions of $[M + H - 194]^+$ were also presented in the MS spectra. All of the fragment ions mentioned above were consistent with those of reference standards **1**−**9**, which further confirmed the identification of the constituents in dazao.

Quantification of Nucleosides and Nucleobases. The established UPLC-DAD method was then subsequently applied to a simultaneous determination of the 9 markers in 49 samples of dazao, which comprised 43 cultivars from 26 cultivation regions. The results (**Table 5**) showed that almost all of these dazao samples were rich in the nucleosides and nucleobases, although their contents were obviously various. The total content of these investigated compounds in sample 14 (cv. Jinzao from Bingxian, Shaanxi) reached as high as 1239 μg g^{-1}, whereas it was only 288 μg g^{-1} in sample 41 (cv. Mianzao from Cangxian, Hebei). As for the individual compounds determined in the experiments, remarkable differences were also observed. For example, the compound cAMP was found to be a predominant constituent in many samples, and its highest content was 465 μg g^{-1} in sample 8 (cv. Hupingzao from Taigu, Shanxi), whereas it could not be detected in sample 41. Likewise, uridine, another compound found as a major constituent in many samples, varied from 18.25 to 620.45 μg g^{-1} Furthermore, the content of hypoxanthine was observed to be the least in all nine markers; its content was not more than 21.26 μg g^{-1}. In addition, samples 2−4, which were the same cultivar (Huizao) cultivated in different regions, demonstrated a

Table 6. Component Loading Matrix for PCA

	analytes								
component (PC)	1	2	3	4	5	6	7	8	9
1	0.802	0.753	0.022	0.573	0.695	0.840	−0.217	0.925	−0.170
2	0.146	−0.216	0.344	0.518	0.035	0.074	0.932	0.021	0.950
3	−0.326	0.297	0.818	−0.060	0.511	−0.236	−0.017	−0.139	−0.127

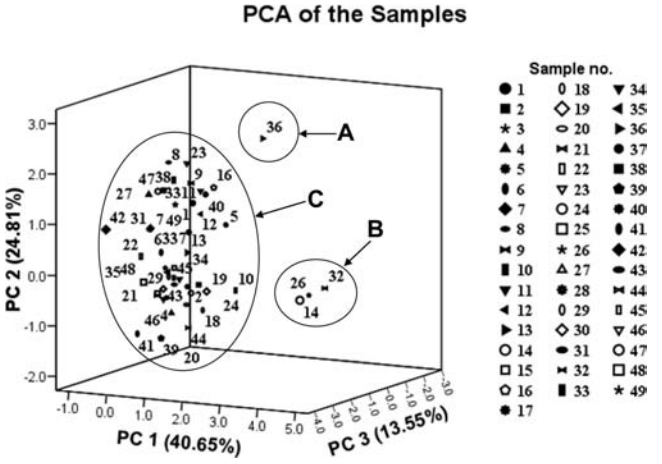

Figure 4. Scatter plot obtained by PCA of the 49 samples of *Z. jujuba*. The 49 samples are the same as in **Table 1**.

variance in the contents of these tested compounds, and the same variation could also be found in other samples. Analogously, for those samples belonging to different cultivars cultivated in the same region, such as samples 37−49, differences in the contents of these markers were also observed. These results revealed that in the different cultivars from various cultivation regions, the contents of these nucleosides and nucleobases were different and the variation may attribute to many factors, including genetic variation, plant origin, and climate or geography (soil or minerals).

PCA of the Samples. To evaluate the variation of dazao, PCA was performed on the basis of the contents of nine tested compounds from UPLC profiles. The first three principal components (PC 1, PC 2, and PC 3) with >79% of the whole variance, were extracted for analysis. Among them, PC 1 accounted for 40.65% of total variance, whereas PC 2 and PC 3 explained 24.81 and 13.55% of total variance, respectively. The remaining principal components, which had a minor effect on the model, were discarded. The components loading matrix is shown in **Table 6**. According to their loadings, PC 1 had good correlation with compounds **1**, **2**, **4**−**6**, and **8**, and PC 2 exhibited relationship with compounds **7** and **9**, whereas PC 3 was related mainly to compound **3**. The results mentioned above suggested that all nine compounds may contribute to the classification of the samples. The scatter plot is shown in **Figure 4**, where each sample is represented as a marker. It was noticeable that the samples were clearly clustered into three domains, with sample 36 in domain A, samples 14, 26, and 32 in domain B, and the others in domain C. These results indicated that samples with similar chemical profiles were commonly divided into one domain. Actually, this result was also in accordance with our previous report that sample 36, cv. Dongzao, with its chemical profiles of triterpenic acids, was obvious different from the other cultivars of dazao (*26*), which further supported the viewpoint that the genetic distance of the cultivar dongzao was remote from the other cultivars (*27*, *28*). As for samples 14 (cv. Jinzao), 26 (cv. Fupingdazao), and 32 (cv. Hetaowen), all of them exhibited the characteristics that the contents of those compounds with 3′,5′-cyclic monophosphate moieties were relatively lower, whereas the total contents of the other nucleosides and nucleobases analyzed in these experiments were higher.

In summary, 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 dazao is a food product rich in nucleosides and nucleobases, it could be a promising natural source for future industrial research of nucleosides and nucleobases with potential benefits for human health.

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LITERATURE CITED

(1) Li, J.-W.; Fan, L.-P.; Ding, S.-D.; Ding, X.-L. Nutritional composition of five cultivars of chinese jujube. *Food Chem.* **2007**, *103*, 454−460.
(2) Shen, X. C.; Tang, Y. P.; Yang, R. H.; Yu, L.; Fang, T. H.; Duan, J. A. The protective effect of *Zizyphus jujube* fruit on carbon tetrachloride-induced hepatic injury in mice by anti-oxidative activities. *J. Ethnopharmacol.* **2009**, *122*, 555−560.
(3) Guo., S.; Tang, Y.; Duan, J.-A.; Su, S.; Ding, A. Two new terpenoids from fruits of *Ziziphus jujuba*. *Chin. Chem. Lett.* **2009**, *20*, 197−200.
(4) Guo, S.; Duan, J.-A.; Tang, Y.-P.; Yang, N.-Y.; Qian, D.-W.; Su, S.-L.; Shang, E.-X. Characterization of triterpenic acids in fruits of *Ziziphus* species by HPLC-ELSD-MS. *J. Agric. Food Chem.* **2010**, *58*, 6285−6289.
(5) Lee, S. M.; Min, B. S.; Lee, C. G.; Kim, K. S.; Kho, Y. H. Cytotoxic triterpenoids from the fruits of *Zizyphus jujuba*. *Planta Med.* **2003**, *69*, 1051−1054.
(6) Pawlowska, A. M.; 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.
(7) Guo, S.; Tang, Y.-P.; Duan, J.-A.; Su, S.-L.; Qian, D.-W. Chemical constituents from the fruits of *Ziziphus jujuba*. *Chin. J. Nat. Med.* **2009**, *7*, 115−118.
(8) Hudina, M.; Liu, M.; Veberic, R.; Stampar, F.; Colaric, M. Phenolic compounds in the fruit of different varieties of Chinese jujube (*Ziziphus jujuba* Mill.). *J. Hortic. Sci. Biotechnol.* **2008**, *83*, 305−308.
(9) Huang, Y. L.; Yen, G. C.; Sheu, F.; Chau, C. F. Effects of water-soluble carbohydrate concentrate from Chinese jujube on different intestinal and fecal indices. *J. Agric. Food Chem.* **2008**, *56*, 1734−1739.
(10) Zhao, Z. H.; Liu, M. J.; Tu, P. F. Characterization of water soluble polysaccharides from organs of Chinese Jujube (*Ziziphus jujuba* Mill. cv. *Dongzao*). *Eur. Food Res. Technol.* **2008**, *226*, 985−989.
(11) Zhang, Y.; Lu, P.; Wang, H.; Zhang, J.-X.; Li, H.; Liu, J.-Z. Simultaneous HPLC determination of cAMP and cGMP in commercial jujube juice concentrate. *Food Sci* **2009**, *30*, 321−322.
(12) Zhao, A.-L.; Li, D.-K.; Wang, Y.-K.; Sui, C.-L.; Cao, Y.-Q.; Liang, Q. Study on the contents of cAMP and cGMP in different cultivars, growing periods and organs in Chinese jujube. *Acta Hortic. Sin.* **2009**, *36*, 1134−1139.
(13) Liu, M.; Wang, Y. cAMP contents of *Zizyphus jujuba* Mill., *Zizyphus spinosus* Hu. and other twelve horticural plants. *J. Agric. Univ. Hebei* **1991**, *14* (4), 20−23.
(14) Jacobson, K. A.; Jarvis, M. F.; Williams, M. Purine and pyrimidine (P2) receptors as drug targets. *J. Med. Chem.* **2002**, *45*, 4057−4093.
(15) Ralevic, V.; Burnstock, G. Receptors for purines and pyrimidines. *Pharmacol. Rev.* **1998**, *50*, 413−492.

(16) Anfossi, G.; Russo, I.; Massucco, P.; Mattiello, L.; Cavalot, F.; Balbo, A.; Trovati, M. Adenosine increases human platelet levels of 3′,5′-cGMP through nitric oxide: possible role in its antiaggregating effect. *Thromb. Res.* **2002**, *105*, 71−78.
(17) Conti, J. B.; Belardinelli, L.; Utterback, D. B.; Curtis, A. B. Endogenous adenosine is an antiarrhythmic agent. *Circulation* **1995**, *91*, 1761−1767.
(18) Virag, L.; Szabo, C. Purines inhibit poly(ADP-ribose) polymerase activation and modulate oxidant-induced cell death. *FASEB J.* **2001**, *15*, 99−107.
(19) Schmidt, A. P.; Lara, D. R.; de Faria Maraschin, J.; da Silveira Perla, A.; Onofre Souza, D. Guanosine and GMP prevent seizures induced by quinolinic acid in mice. *Brain Res.* **2000**, *864*, 40−43.
(20) Kinahan, J. J.; Kowal, E. P.; Grindey, G. B. Biochemical and antitumor effects of the combination of thymidine and 1-β-D-arabinofuranosylcytosine against leukemia L1210. *Cancer Res.* **1981**, *41*, 445−451.
(21) Gao, J. L.; Leung, K. S. Y.; Wang, Y. T.; Lai, C. M.; Li, S. P.; Hu, L. F.; Lu, G. H.; Jiang, Z. H.; Yu, Z. L. Qualitative and quantitative analyses of nucleosides and nucleobases in *Ganoderma* spp. by HPLC-DAD-MS. *J. Pharm. Biomed. Anal.* **2007**, *44*, 807−811.
(22) Qian, Z. M.; Wan, J. B.; Zhang, Q. W.; Li, S. P. Simultaneous determination of nucleobases, nucleosides and saponins in *Panax notoginseng* using multiple columns high performance liquid chromatography. *J. Pharm. Biomed. Anal.* **2008**, *48*, 1361−1367.
(23) Ranogajec, A.; Beluhan, S.; Smit, Z. Analysis of nucleosides and monophosphate nucleotides from mushrooms with reversedphase HPLC. *J. Sep. Sci.* **2010**, *33*, 1024−1033.
(24) Yang, F. Q.; Li, S. P. Effects of sample preparation methods on the quantification of nucleosides in natural and cultured *Cordyceps*. *J. Pharm. Biomed. Anal.* **2008**, *48*, 231−235.
(25) Churchwell, M. I.; Twaddle, N. C.; Meeker, L. R.; Doerge, D. R. Improving LC-MS sensitivity through increases in chromatographic performance: comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS. *J. Chromatogr., B* **2005**, *825*, 134−143.
(26) Guo, S.; Duan, J. A.; Tang, Y. P.; Su, S. L.; Shang, E. X.; Ni, S. M.; Qian, D. W. High-performance liquid chromatography-two wavelength detection of triterpenoid acids from the fruits of *Ziziphus jujuba* containing various cultivars in different regions and classification using chemometric analysis. *J. Pharm. Biomed. Anal.* **2009**, *49*, 1296−1302.
(27) Yu, C.-G.; Chen, J.-Z.; Yin, Y.-L. Study on peroxidase isoenzymes and esterase isoenzymes of leaves in five varieties of *Zizyphus jujuba* Mill. *J. Jiangsu For. Sci. Technol.* **2005**, *32* (5), 15−18.
(28) Gao, M.-X.; Zhang, J.-H. Activity analysis of 4 isozymes of 10 jujube cultivars. *J. Northwest Sci-Tech Univ. Agric. For.* **2004**, *42* (s1), 109−110.

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