

Phytochemical analyses of *Ziziphus jujuba* Mill. var. *spinosa* seed by ultrahigh performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry

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Ziziphus jujuba Mill. var. *spinosa* (*Z. jujuba*) seeds have attracted much attention within the field of medicine due to their significant effects against disturbances of the central nervous system. Secondary metabolites composition is key to the influence of the pharmaceutical and commercial qualities of this plant. In this work, the phytochemical profile of *Z. jujuba* seeds was analysed by ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS). The UPLC-MS/MS information identified the main secondary metabolites in *Z. jujuba* seeds, including flavonoid C-glycosides, triterpene acids and unsaturated fatty acids. The leading chemical identified by UPLC-MS/MS was betulinic acid, and oleic acid was the leading volatile from the GC-MS results. All the samples tested showed similar phytochemical profiles, but levels of the chemical compounds varied. Principal component analysis revealed the principal secondary metabolites that could define the differences in quality. It was confirmed that the combination of UPLC-MS/MS and GC-MS was an effective technique to demonstrate the pharmaceutical quality of *Z. jujuba* seeds.

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1 Introduction

Ziziphus is a genus which has approximately 40 species of the *Rhamnaceae* family, and is distributed mainly in warm and subtropical regions throughout the world. As an important economic species of this genus, *Ziziphus jujuba* Mill. var. *spinosa* has generated much commercial value due to its significant pharmacological functions. Insomnia and anxiety are the main pharmaceutical activities attributed to the species in the wider application of this medicinal species.¹ In addition, it also has good bioactivities against weakness, hyperhidrosis, liver complaints, urinary trouble, fever, hypertension and hyperlipidemia.^{2,3} Compared to other hypotonic plants, such as kava,⁴ *Z. jujuba* (seeds) has a slow onset of effect, and has both a long-term effect on sleep architecture and low toxicity. As the commercial quality of the biological sources is readily affected by a number of factors, like the growing environment, processing conditions and genetic background the pharmaceutical capabilities of *Z. jujuba* seeds from various commercial sources vary.

Therefore, the pharmaceutical quality needs to be determined before use.

Natural bioactive compounds, including phenolics and carbohydrates, are responsible for the pharmaceutical activities of this medicinal herb.^{5,6} As an important ingredient of Chinese traditional medicines, *Z. jujuba* seeds have abundant bioactive secondary metabolites, including triterpene acids, unsaturated fatty acids and flavonoid C-glycosides,⁷⁻⁹ which are critical chemicals involved both in disease treatment and health benefits.

Metabolomics is being increasingly utilized to gain insight into the chemical composition of biological materials.¹⁰ Determination of metabolomic profiles by ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) has been recently adopted as an effective technique to understand the chemical nature of a medicinal plant. Though nuclear magnetic resonance spectroscopy (NMR) is also widely applied in metabolomics, the MS-based technique shows better precision and resolution than NMR. Therefore, in this work, the phytochemical profile of *Z. jujuba* seed was analysed by both UPLC-MS/MS and GC-MS to judge the quality of commercial *Z. jujuba* seed.

2 Materials and methods

2.1 Plant material

Six *Z. jujuba* seeds were purchased from Neatus Traditional Chinese Medicine Co. Ltd (Chengdu, China), Guanghong

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Pharmaceutical Co. Ltd (Guangzhou, China), and Yifang Chinese Medicine Co. Ltd (Anguo, China), respectively. Seed samples 2#, 4# and 6# were raw *Z. jujuba* seeds from Wenshang, Shandong province, Zhanhuang, Hebei province and Anguo, Hebei province, respectively. They were treated to obtain samples 1#, 3# and 5# by the following procedures. One kilogram of raw *Z. jujuba* seeds was heated at 200 °C with agitation. The seeds were toasted brown and when a pleasant aroma could be smelled, they were collected as the heat-treated sample. Each sample was pulverized into powder and sieved through 60-mesh sieve.

2.2 UPLC-MS/MS analysis

Two grams of *Z. jujuba* seed powder were extracted with 20 ml methanol during the course of one week in the dark at room temperature. The extract was centrifuged at 8000g for 10 min. Each sample was extracted in triplicate. The supernatant was collected and subjected to UPLC-MS/MS analysis. Acquity™ UPLC (Waters, Milford, MA, USA) equipped with an Acquity™ UPLC BEH C18 column (1.7 μm, 2.1 × 150 mm) was used for chemical isolation. A gradient elution programme was conducted as follows: 0–2 min, 5% solvent B; 2–17 min, from 5% to 30% solvent B; 17–20 min, from 30% to 55% solvent B; 20–35 min, from 55% to 100% solvent B; 35–37 min, from 100% to 5% solvent B; 37–39 min, 5% solvent B. Solvent A was 0.1% formic acid in water, and solvent B was acetonitrile. The flow rate was 0.25 ml min⁻¹. The column temperature was kept at 35 °C. Detection was performed on a triple quadrupole mass spectrometer detector using an electrospray ionization (ESI) interface. Ionization of the analytes was achieved using ESI in negative mode. The interface conditions were as follows: capillary voltage, 1.0 kV; cone voltage, 10 eV; collision voltage, 10 eV; source temperature, 150 °C; desolvation temperature, 500 °C; cone gas, nitrogen at a flow rate of 50 l h⁻¹; desolvation gas, nitrogen at a flow rate of 800 l h⁻¹; collision gas, argon at a flow rate of 0.2 ml min⁻¹. Mass scan was set in the range of *m/z* 50–2000. The daughter ion mode was monitored at a collision voltage in the range of 10–40 eV.

2.3 GC-MS analysis

One gram of *Z. jujuba* seed powder was extracted with 10 ml of hexane–acetone (7 : 3, v/v) during the course of one week in the dark at room temperature. The extracts were centrifuged at 8000g for 15 min. The supernatants were collected and subjected to trimethylsilyl derivatization due to the occurrence of organic acids. The trimethylsilyl derivatization was conducted by the same method as Yang *et al.*¹¹ A gas chromatography-mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyse the chemical composition. The derivatives were loaded into a RTX-5 capillary column. The temperature program was set as follows: the initial temperature of column was 50 °C, holding for 1 min, increasing to 250 °C at 3 °C min⁻¹, holding for 17 min, increasing to 280 °C at 10 °C min⁻¹; injection temperature: 250 °C. The ion source of the mass spectrometer was set at 250 °C. The scanning *m/z* range was 20–550 amu. 1 μl of sample was injected and the split ratio was 10 : 1. The carrier

gas was helium with flow rate of 1.0 ml min⁻¹. The peaks were identified by NIST database and retention index.

2.4 Data analysis

For each sample, the peak heights of chemicals in the mass spectra were recorded and averaged over three replicates. An unsupervised multivariate statistical method, principal component analysis (PCA), was used on UV-scaling data. PCA was performed to visualize the clustering of different samples without any knowledge of their group membership.

3 Results

3.1 The composition of semi-polar phytochemicals determined by UPLC-MS/MS

UPLC-MS/MS analyses were conducted on the methanolic extract of *Z. jujuba* seeds to identify semi-polar chemicals. Fig. 1 shows the full scan ESI-MS spectra in negative mode, which record the levels of secondary metabolites. Identified chemicals are listed in Table 1. Those chemicals with relatively high peak are interpreted here.

The leading peak was betulinic acid (retention time 30.55 min, Fig. 2). The MS spectra had an [M – H]⁻ at *m/z* 455.0. The fragment ions were interpreted as follows: *m/z* 437.0 (loss of H₂O), 410.9 (loss of carboxyl).¹² A base peak at *m/z* 188.9 was characteristic of the fragmentation of triterpenoid molecules with a lupane skeleton bearing a hydroxy group in position 3. It had arisen from the fragmentation of the C ring by cleavage of C9–C11 and C8–C14 bonds followed by the loss of H₂O.¹³ Moreover, two peaks at *m/z* 247.4 and 202.6 were observed, which indicated the formation of the [C₁₆H₂₄O₂] fragment ion and further loss of carboxyl.¹⁴

The peak at 29.79 min showed an [M – H]⁻ at *m/z* 454.9 and an [2M – H]⁻ at *m/z* 911.1, which indicated that the molecular weight (456) was equal to betulinic acid. The fragment ion at *m/z* 411.4 was formed by the loss of the carboxyl moiety. *m/z* 436.5 indicated the loss of H₂O due to the elimination of the hydroxyl group at position 3. Another two fragment ions at *m/z* 207.2 and 189.1 were detected. This fraction was identified as zizyphursolic acid based on above results and literature.¹⁵

Linoleic acid (molecular weight 280) was detected from the fraction at 32.06 min, which had an [M – H]⁻ at *m/z* 278.9. The fragment ion at *m/z* 260.6 was formed due to the loss of OH in the carboxyl group. *m/z* 58.7 represented the occurrence of acetic acid. A peak at *m/z* 70.6 was detected.

Spinoin, a flavonoid C-glycoside, is a characteristically bioactive compound in *Z. jujuba* seeds, which was detected at 11.78 min. The mass spectra showed an [M – H]⁻ at *m/z* 606.7 and a [2M – H]⁻ at *m/z* 1214.9 for the precursor ion. The fragment ion at *m/z* 427.2 represented the loss of glucose and H₂O. The difference (120) between *m/z* 606.7 and 486.8 was characteristic of the breakage of glucose at C₁–O and C₂–C₃. Other fragment ions were detected at *m/z* 366.9 and 118.8. They were characteristic of spinoin. This mass spectral information was confirmed in the literature.¹⁶



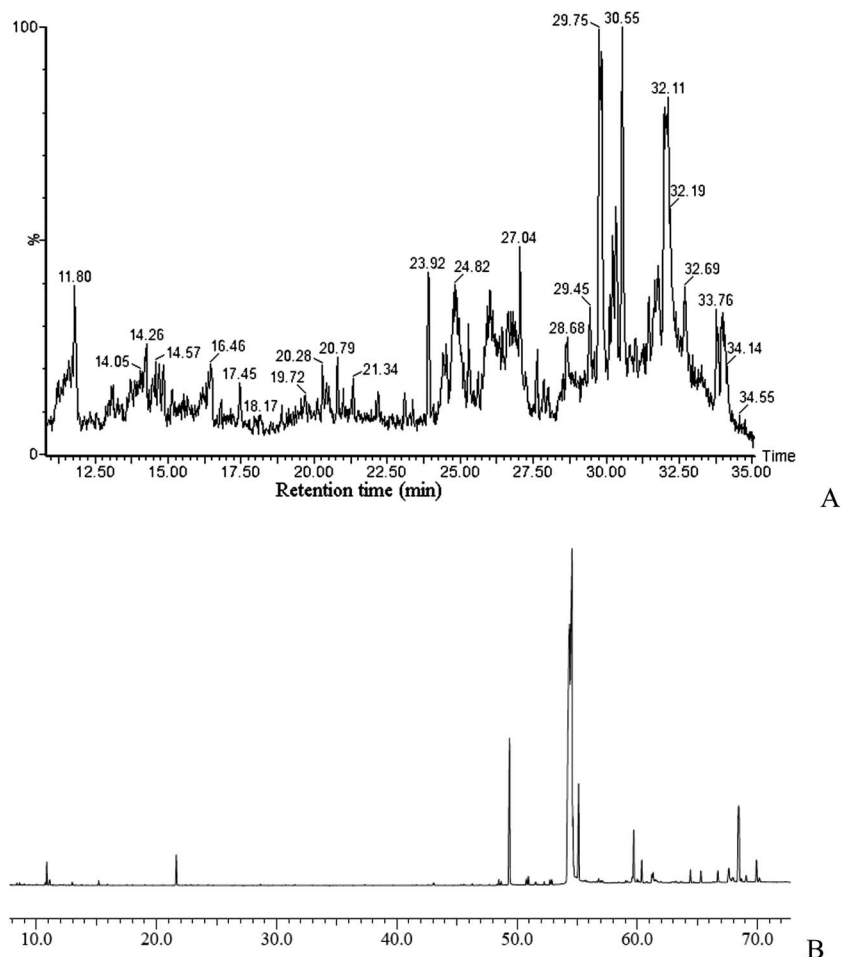


Fig. 1 UPLC-MS/MS and GC-MS profiles of *Z. jujuba* seeds. (A) Total ion chromatogram analysed by UPLC-MS/MS; (B) total ion chromatogram analysed by GC-MS.

Epiceanothic acid and ceanothic acid were detected at 27.07 and 23.92 min, respectively. They had the same distribution of precursor and fragment ions. An $[M - H]^-$ at m/z 485.0 and a $[2M - H]^-$ at m/z 971.2 were observed for the precursor ion. m/z 471.4 was formed by the loss of methyl. Fragment ions at m/z 441.0 was generated by the loss of one carboxyl moiety. m/z 423.4 indicated the loss of carboxyl and H_2O . In combination with the information in the literature,¹⁷ they were identified to be epiceanothic acid and ceanothic acid.

A peak at 20.79 min was analysed. An $[M - H]^-$ at m/z 1042.9 was found to be the precursor ion, which was consistent with the molecular weight of jujuboside B (1044). The fragment ion at m/z 910.9 indicated the loss of xylose, and m/z 310.6 represented the occurrence of xylose-(1,2)-glucose. An $[M - H]^-$ at m/z 1205.1 in the mass spectra at 20.28 min was consistent with the molecular weight of jujuboside A (1206). m/z 1187.4 was formed due to the loss of H_2O . m/z 751.0 indicated the penta-saccharide fragment ion.

3.2 Volatile chemicals composition determined by GC-MS

The volatile chemicals were extracted by hexane/acetone, trimethylsilylated and determined by GC-MS, which gave 22 volatile compounds (Table 2), including alcohols, carboxylic

acids, esters, and terpenoids. The leading volatile chemical was oleic acid in the GC-MS profile (Fig. 1B). Linoleic acid and stearic acid were the next most prolific volatile chemical. Other volatile compounds with percentage higher than 5% were palmitic acid and 2-monoecosanoylglycerol, respectively.

3.3 Principal component analyses

Principal component analysis was conducted on the semi-polar phytochemicals of *Z. jujuba* seed samples. The first two principal components explained 59.2% of the total variance, in which the first principal component explained 33.6% and the second explained 25.6%. The loading plot (Fig. 3A) revealed the principal compounds responsible for the sample difference. As shown in Table 3, the chemical with an absolute value higher than 0.8 was the representative compound. The first principal component was represented by kaempferol 3-rutinoside, ceanothic acid, 6,8-diglucopyranosyl-4',5,7-trihydroxyflavanone, 3',5'-di-C-beta-D-glucosylphloretin, linoleic acid derivative and glycerol ester 1. The second principal component was mainly characterized by 6''-feruloylspinosin, jujuboside A, jujuboside B, a-nor-2,20(29)-lupadiene-27,28-dioic acid and linoleic acid. The score plot showed the statistical similarity between samples. Sample 1# *Z. jujuba* seeds was characterized by large amounts of linoleic acid



Table 1 Semi-polar phytochemicals occurring in the methanolic extract of *Z. jujuba* seeds measured by UPLC-MS/MS^a

No.	Retention time (min)	Molecular weight	[M – H] [–] (m/z)	[2M – H] [–] (m/z)	Fragment ions (m/z)	Chemicals
1	11.22	594	592.8	1186.7	412.6, 352.7	Vicenin II
2	11.78	608	606.7	1214.9	486.8427.2, 366.9, 118.8	Spinosin
3	13.11	594	592.9		447.2, 340.8, 284.5	Kaempferol 3-rutinoside
4	14.22	784	782.8	1566.8	606.5, 193.2, 178.8	6''-Feruloylspinosin
5	14.84	942	941.8		470.8, 416.7, 325.4	Jujubasaponin V
6	16.49	1119	1117.8		836.9213.6	—
7	20.28	1206	1205.1		1187.4, 751.0	Jujuboside A
8	20.44	330	328.8		310.8, 178.6, 167.4	Pseudolaroside B
9	20.79	1044	1043.1		910.9, 310.6	Jujuboside B
10	23.92	486	484.9	971.3	471.0, 441.0, 423.4	Ceanothic acid
11	24.64	596	594.9		577.1, 559.4, 475.4	6,8-Diglucopyranosyl-4',5,7-trihydroxyflavanone
12	25.08	477	475.7	953.2		—
13	25.30	472	470.9		452.8, 393.1	Alphitolic acid
14	25.74	572	570.8		411.3390.5314.6254.9240.9152.7	—
15	26.46	598	596.9		506.9, 477.4	3',5'-Di-C-beta-D-glucosylphloretin
16	27.07	486	485	971.2	471.4, 441.0, 422.8	Epiceanothic acid
17	27.64	296	294.9		276.8, 194.8	—
18	29.43	298	296.8		278.9, 168.9	Linoleic acid derivative
19	29.79	456	455		436.5, 411.4, 207.2, 189.1	Zizyphursolic acid
20	30.25	834	833.1		553.1	Glycerol ester 1
21	30.55	456	455		437.0, 410.9, 247.4, 202.6188.9	Betulinic acid
22	31.67	454	452.9		408.7	A-nor-2,20(29)-lupadiene-27, 28-dioic acid
23	31.78	834	833.2		553.1	Glycerol ester 2
24	32.06	280	278.9		260.8, 70.6, 58.7	Linoleic acid
25	32.7	862	861.3	911.1	579.4	Glycerol ester 3

^a —: unknown.

and low amount of 6''-feruloylspinosin. Sample 2# and 4# seeds were located in the proximity of 1# seeds in the score plot (Fig. 3A), which indicated that they shared high similarity. Sample 3# root showed high levels of pseudolaroside B and chemical 12, an unknown compound, and low levels of glycerol ester 1. Sample 5# seeds was characterized by large amount of ceanothic acid. Sample 6# was isolated from the other five seeds in the score plot, which suggested that it was quite different to them based on the chemicals levels observed.

Fig. 3B shows the results of PCA on volatile chemicals levels. The first two principal components explained 74.0% of the total variance, 52.1% by the first component and 21.9% by the second component. Major chemicals defining the first principal component were tetradecanoic acid, palmitic acid, linoleic acid methyl ester, oleic acid methyl ester, 9-octadecenoic acid, 11-eicosenoic acid and eicosanoic acid. The second principal component was mainly characterized by linoleic acid ethyl ester, ethyl oleate, 2-(9,12-octadecadienyloxy)-ethanol, 1-monopalmitoylglycerol and thymol-glucoside (Table 4). Samples 1# and 6# were isolated from the other four seeds as shown in the score plot (Fig. 3B). Sample 1# *Z. jujuba* seeds was characterized by high levels of linoleic acid ethyl ester and thymol-glucoside. Sample 6# seed was characterized by high levels of 11-eicosenoic acid and eicosanoic acid.

4 Discussion

4.1 Phytochemical profile of *Z. jujuba* seeds

Metabolomics is a systemic investigation of small molecular chemicals profiles, which provides a good way to evaluate the quality of plant resources, like medicinal herbs. At present, UPLC-MS/MS and GC-MS are two efficient and precise techniques to resolve the metabolomic profile of plant resources.¹⁸ The former can identify semi-polar chemicals in a precise and time-saving manner. It has been widely applied to characterize plant secondary metabolites. GC-MS can reveal the chemical nature of volatile chemicals, which define the flavor of a plant.¹⁹ GC-MS also provides complementary data to UPLC-MS/MS analysis to obtain a complete metabolomic profile. From the phytochemical profile obtained in this work, it could be concluded that six *Z. jujuba* seeds had similar phytochemical profiles, but that the level of each peak varied.

Through phytochemical profile investigation, approximately 50 chemicals were identified from *Z. jujuba* seeds. As most of chemicals are difficult to obtain commercially, the structures identified in this paper identify the possible chemicals on the basis of fragment ions and the literature. It is generally accepted that *Z. jujuba* seeds are a good candidate to treat insomnia and anxiety. Thus, the distribution of pharmacological active



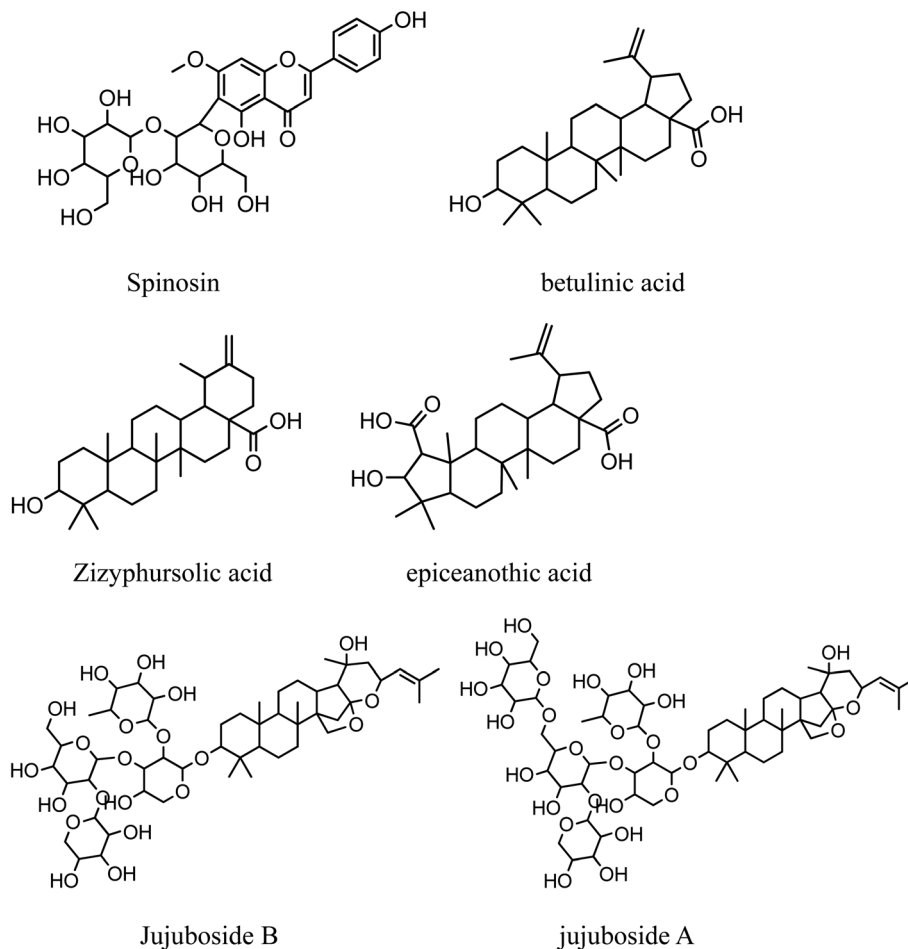


Fig. 2 Structures of characteristic chemicals in *Z. jujuba* seeds.

chemicals contributing to these effects should be investigated. Jujubosides, including jujubosides A and B, were detected as two independent peaks in the UPLC-MS/MS profile. Both contribute to the beneficial hypnotic effect of *Z. jujuba* seeds, and the mechanism of action for jujubosides is related to the serotonergic system.²⁰ Spinosin is a characteristic flavonoid C-glycoside, which occurred at a high levels in *Z. jujuba* seeds (Fig. 1). It is another important bioactive compound responsible for the hypnotic effect of this plant. Similar to jujubosides, spinosin regulates the serotonergic system to inhibit insomnia.²¹ Further evidence suggested that spinosin potentiates pentobarbital-induced sleep *via* the serotonin-1A (5-hydroxytryptamine, 5-[HT.sub.1A]) receptor.²² Though cyclopeptide alkaloids have been reported as another pharmacologically active chemical to contribute to the hypnotic activity,²³ they were not detected from the UPLC-MS/MS and GC-MS profiles in this work. *P. tenuifolia* root is another commonly used ingredient in traditional medicine to treat insomnia.²⁴ The responsible active chemicals are oligosaccharide esters and saponins, which are different to the chemicals in *Z. jujuba* seeds. Therefore, it is important to define pharmacological quality by quantifying the levels of specific chemicals. UPLC-MS/MS is a good choice of method to carry out the analysis.

In the UPLC-MS/MS profile, betulinic acid and zizyphursolic acid were the leading chemicals in *Z. jujuba* seeds.¹⁴ Both share similar structural characteristics as pentacyclic triterpene acid, which implies similar bioactivities. Comparing with zizyphursolic acid, betulinic acid is more common in the plant kingdom and has been proven to display diverse biological effects, including anticancer, antiinflammatory and antiviral activities.²⁵ It is also a potential biological response modifier capable of strengthening the immune system of the host.²⁶ The occurrence of these bioactive compounds in large amounts results in the good pharmacological and health-beneficial properties of *Z. jujuba* seeds.

GC-MS analysis showed that the major volatile compounds in *Z. jujuba* seeds were fatty acids. Among these, oleic acid was the leading volatile chemical, which is consistent with the determinations of Zhao *et al.*²⁷ It is also a pharmacologically active chemical. The essential oil of *Z. jujuba* seeds has been reported to have hair growth promoting activity,²⁸ and antiinflammatory activity.²⁹

Based on the level and importance for pharmacological function, spinosin, zizyphursolic acid, betulinic acid, ceanothic acid, epiceanothic acid and linoleic acid identified in the UPLC-MS/MS profile were selected to evaluate the quality of *Z. jujuba*



Table 2 Volatile chemicals occurring in hexane–acetone extract of *Z. jujuba* seed determined by GC-MS

No.	Retention time (min)	Chemicals	Molecular weight
1	10.87	Octyl 3-alcohol	130
2	43.1	Tetradecanoic acid	228
3	48.5	Palmitelaidic acid	254
4	49.4	Palmitic acid	256
5	50.8	Linoleic acid methyl ester	294
6	50.9	Oleic acid methyl ester	296
7	51.6	9-Octadecenoic acid	282
8	52.3	Heptadecanoic acid	270
9	52.7	Linoleic acid ethyl ester	308
10	53	Ethyl oleate	310
11	54.4	Linoleic acid	280
12	54.6	Oleic acid	282
13	55.1	Stearic acid	284
14	59.7	11-Eicosenoic acid	310
15	60.4	Eicosanoic acid	312
16	61.2	2-(9,12-Octadecadienyloxy)-ethanol	310
17	64.4	1-Monopalmitoylglycerol	330
18	65.3	Tetracosanoic acid	368
19	66.7	Thymol-glucoside	312
20	67.6	2-Monooctadecenoylglycerol	342
21	68.5	2-Monoicosanoylglycerol	386
22	69.9	Squalene	410

seeds. *Z. jujuba* seeds with relatively high levels of bioactive compounds are considered good quality. Through ANOVA analysis of representative chemicals levels (data not shown), the results indicated that the six seed samples studied had no significant difference in the levels of zizyphursolic acid. The levels of betulinic acid (2# > 4# = 6#), linoleic acid (1# > 6#), ceanothic acid (3# = 5# > 6# > 2#), epiceanothic acid (5# > 3# > 1#) were detected. It is not possible to define the total quality of *Z. jujuba* seeds through only the level of one bioactive compound. Therefore, PCA should be conducted to differentiate the quality between the samples.

4.2 PCA of *Z. jujuba* seeds phytochemicals

PCA is an unsupervised clustering technique for identifying patterns in data, expressing the data in such a way as to emphasize their similarities and differences. Through reducing the number of dimensions, it can define a limited number of principal components which describe independent variation in the results.^{10,30} In the present work, PCA was carried out on semi-polar and volatile chemicals of *Z. jujuba* seeds to find the principal phytochemicals defining the pharmacological quality. The loading plot displayed the influence of individual spectral peaks in each principal component and described the difference of the samples in the score plot.³¹ The points in the loading plot far from the zero point represented characteristic markers with the most confidence for each group. Fig. 3 shows the plots of PCA on semi-polar and volatile chemicals of *Z. jujuba* seeds. As most of bioactive compounds are present in the methanolic extract of *Z. jujuba* seeds, the UPLC-MS/MS results presented more information on

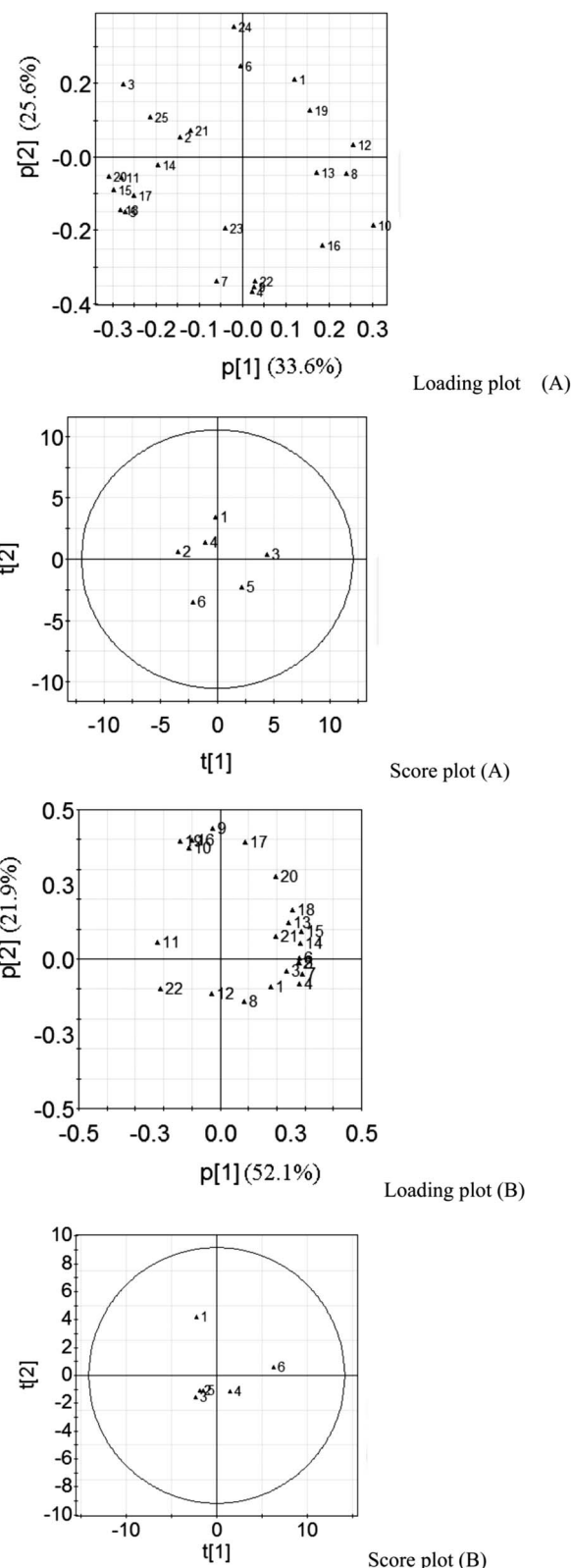


Fig. 3 Plots of PCA on semi-polar and volatile chemicals of *Z. jujuba* seeds. Loading plot (A) and score plot (A) are the results of semi-polar chemicals; loading plot (B) and score plot (B) are the results of volatile chemicals.



Table 3 Component matrix of PCA for the semi-polar phytochemicals of *Z. jujuba* seeds

Chemical number ^a	Components	
	1	2
1	-0.342	-0.534
2	0.418	-0.141
3	0.803	-0.502
4	-0.065	0.927
5	0.787	0.370
6	0.004	-0.631
7	0.177	0.856
8	-0.702	0.115
9	-0.084	0.891
10	-0.880	0.472
11	0.812	0.141
12	-0.746	-0.084
13	-0.499	0.114
14	0.591	0.055
15	0.863	0.228
16	-0.538	0.613
17	0.722	0.269
18	0.814	0.362
19	-0.447	-0.321
20	0.895	0.133
21	0.347	-0.179
22	-0.089	0.855
23	0.120	0.487
24	0.058	-0.898
25	0.622	-0.272

^a Represents the chemicals having the same number listed in Table 1.

Table 4 Component matrix of PCA for the volatile chemicals of *Z. jujuba* seeds

Chemical number ^a	Component	
	1	2
1	0.608	-0.199
2	0.942	-0.026
3	0.801	-0.090
4	0.949	-0.177
5	0.945	-0.028
6	0.950	0.008
7	0.988	-0.109
8	0.286	-0.310
9	-0.090	0.963
10	-0.374	0.817
11	-0.745	0.121
12	-0.105	-0.250
13	0.815	0.267
14	0.963	0.113
15	0.969	0.199
16	-0.338	0.878
17	0.299	0.860
18	0.869	0.365
19	-0.475	0.867
20	0.661	0.610
21	0.667	0.168
22	-0.712	-0.218

^a Represents the chemicals having the same number listed in Table 2.

quality. It was better to judge the quality on the basis of UPLC-MS/MS analyses, and the GC-MS results could also be used as a supplementary reference. Fig. 3A shows the characteristic compounds in each sample. The first two principal components explained most of the total variance. It was surprising that betulinic acid had the leading peak in the UPLC-MS/MS profile. However, it was not the representative chemical in the first two principal components due to the insignificant variance. Only those chemicals having significant variance are recorded in the PCA model. The loading plot showed that ceanothoic acid, 6''-feruloylspinosin, jujuboside B (second quadrant), glycerol ester 1, linoleic acid derivative, pseudolaroside B (third quadrant), kaempferol 3-rutinoside, linoleic acid (fourth quadrant) are the principal components which defined the quality differences between *Z. jujuba* seeds. The sample located in a quadrant of score plot is characterized by high amounts of bioactive compounds located in the same quadrant and low amounts of compounds in the opposite quadrant of the loading plot. In this work, samples 1#, 2# and 4# shared closely related similar qualities, which had high amounts of linoleic acid and kaempferol 3-rutinoside, and low amounts of ceanothoic acid. However, samples 3#, 5# and 6# showed significant differences between one another.

5 Concluding remarks

From the above results, it could be concluded that determination of the phytochemical profile is an efficient way to define the pharmaceutical quality of *Z. jujuba* seeds. By using analyses of UPLC-MS/MS and GC-MS, the semi-polar and volatile chemicals were identified and their levels were statistically compared. Betulinic acid was the leading semi-polar chemical in *Z. jujuba* seeds, and oleic acid was the leading volatile chemical. PCA could differentiate well between the quality of *Z. jujuba* seed samples.

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References

- 1 J. J. Liu, B. Chen and S. Z. Yao, *Talanta*, 2007, **71**, 668–675.
- 2 A. M. Pawlowska, F. Camangi, A. Bader and A. Braca, *Food Chem.*, 2009, **112**, 858–862.
- 3 C. H. Shou, Z. Y. Feng, J. Wang and X. X. Zheng, *Planta Med.*, 2002, **68**, 799–803.
- 4 D. Wheatley, *J. Psychopharmacol.*, 2005, **19**, 414–421.
- 5 B. Yang, F. Chen, Y. L. Hua, S. S. Huang, S. Lin, L. R. Wen and Y. M. Jiang, *Food Chem.*, 2012, **131**, 508–512.
- 6 Q. Q. Zhu, Y. M. Jiang, S. Lin, L. R. Wen, D. Wu, M. M. Zhao, F. Chen, Y. X. Jia and B. Yang, *Biomacromolecules*, 2013, **14**, 1999–2003.



- 7 Y. F. Sun, C. K. Song, H. Viemstein, F. Unger and Z. S. Liang, *Food Chem.*, 2013, **138**, 1998–2007.
- 8 Y. Wu, F. He, Q. Pan, Y. Shi, Z. D. Min and J. Y. Liang, *Chem. Nat. Compd.*, 2011, **47**, 369–372.
- 9 S. M. Lee, B. S. Min, C. G. Lee, K. S. Kim and Y. H. Kho, *Planta Med.*, 2003, **69**, 1051–1054.
- 10 S. Bernillon, B. Biais, C. Deborde, M. Maucourt, C. Cabasson, Y. Gibon, T. H. Hansen, S. Husted, R. C. H. de Vos, R. Mumm, H. Jonker, J. L. Ward, S. J. Miller, J. M. Baker, J. Burger, Y. Tadmor, M. H. Beale, J. K. Schjoerring, A. A. Schaffer, D. Rolin, R. D. Hall and A. Moing, *Metabolomics*, 2013, **9**, 57–77.
- 11 B. Yang, Y. M. Jiang, M. M. Zhao, F. Chen, R. Wang, Y. L. Chen and D. D. Zhang, *Food Chem.*, 2009, **115**, 609–614.
- 12 B. Rhourri-Frih, P. Chaimbault, B. Claude, C. Lamy, P. Andre and M. Lafosse, *J. Mass Spectrom.*, 2009, **44**, 71–80.
- 13 F. Modugno, E. Ribechini and M. P. Colombini, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 1787–1800.
- 14 A. M. Ayatollahi, M. Ghanadian, S. Afsharypour, O. M. Abdella, M. Mirzai and G. Askari, *Iran. J. Pharm. Res.*, 2011, **10**, 287–294.
- 15 H. M. Mukhtar, S. H. Ansari, M. Ali, T. Naved and Z. A. Bhat, *Pharm. Biol.*, 2005, **43**, 392–395.
- 16 W. Wang, J. Luo and L. Kong, *China J. Chin. Mater. Med.*, 2009, **34**, 2768–2773.
- 17 S. Guo, J. A. Duan, Y. P. Tang, Y. F. Qian, J. L. Zhao, D. W. Qian, S. L. Su and E. X. Shang, *J. Pharm. Biomed. Anal.*, 2011, **56**, 264–270.
- 18 A. Mari, D. Lyon, L. Fragner, P. Montoro, S. Piacente, S. Wienkoop, V. Egelhofer and W. Weckwerth, *Metabolomics*, 2013, **9**, 599–607.
- 19 C. Besada, G. Sanchez, A. Salvador and A. Granell, *Metabolomics*, 2013, **9**, 157–172.
- 20 J.-X. Cao, Q.-Y. Zhang, S.-Y. Cui, X.-Y. Cui, J. Zhang, Y.-H. Zhang, Y.-J. Bai and Y.-Y. Zhao, *J. Ethnopharmacol.*, 2010, **130**, 163–166.
- 21 L.-E. Wang, Y.-J. Bai, X.-R. Shi, X.-Y. Cui, S.-Y. Cui, F. Zhang, Q.-Y. Zhang, Y.-Y. Zhao and Y.-H. Zhang, *Pharmacol., Biochem. Behav.*, 2008, **90**, 399–403.
- 22 L. E. Wang, X. Y. Cui, S. Y. Cui, J. X. Cao, J. Zhang, Y. H. Zhang, Q. Y. Zhang, Y. J. Bai and Y. Y. Zhao, *Phytomedicine*, 2010, **17**, 404–409.
- 23 Y. Ma, H. Han, S.-Y. Nam, Y.-B. Kim, J.-T. Hong, Y.-P. Yun and K.-W. Oh, *J. Ethnopharmacol.*, 2008, **117**, 318–324.
- 24 L. C. Klein Junior, S. F. de Andrade and V. Cechinel Filho, *Chem. Biodiversity*, 2012, **9**, 181–209.
- 25 Y. Jine, M. Lis, M. Szczypka and B. Obminska-Mrukowicz, *Pol. J. Vet. Sci.*, 2012, **15**, 305–313.
- 26 Z. Dang, W. Lai, K. Qian, P. Ho, K.-H. Lee, C.-H. Chen and L. Huang, *J. Med. Chem.*, 2009, **52**, 7887–7891.
- 27 J. Zhao, S. P. Li, F. Q. Yang, P. Li and Y. T. Wang, *J. Chromatogr., A*, 2006, **1108**, 188–194.
- 28 J. I. Yoon, S. M. Al-Reza and S. C. Kang, *Food Chem. Toxicol.*, 2010, **48**, 1350–1354.
- 29 S. M. Al-Reza, J. I. Yoon, H. J. Kim, J. S. Kim and S. C. Kang, *Food Chem. Toxicol.*, 2010, **48**, 639–643.
- 30 M. K. Park, I. H. Cho, S. Lee, H. K. Choi, D. Y. Kwon and Y. S. Kim, *Food Chem.*, 2010, **122**, 1313–1319.
- 31 J. Yuk, K. L. McIntyre, C. Fischer, J. Hicks, K. L. Colson, E. Lui, D. Brown and J. T. Arnason, *Anal. Bioanal. Chem.*, 2013, **405**, 4499–4509.

