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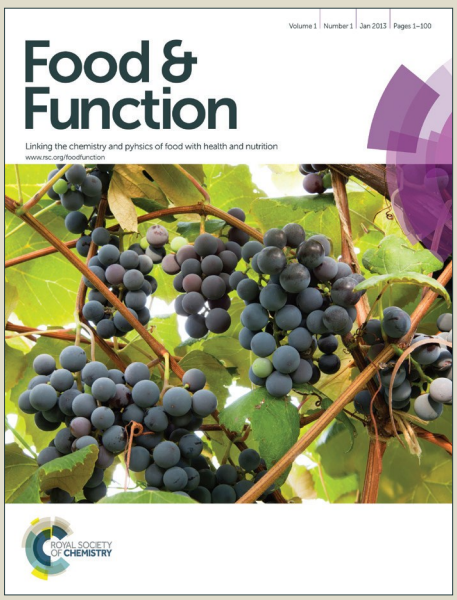

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Chemical Characterization of the Main Bioactive Constituents from Fruits of *Ziziphus jujuba*

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Abstract

The fruit of *Ziziphus jujuba* Mill., also called hongzao in Chinese, has long history of cultivation in China. From the fruit of *Z. jujuba,* twenty-seven known compounds were isolated and identified as the main constituents of the fruits. They were 3-*O*-(*trans*-*p*-coumaroyl)-alphitolic acid (**1**), 3-*O*-(*cis*-*p*-coumaroyl)-alphitolic acid (**2**), 3β-*O*-(*trans*-*p*-coumaroyl) maslinic acid (**3**), pomonic acid (**4**), 2-oxo-pomolic acid (**5**), benthamic acid (**6**), terminic acid (**7**), oleanic acid (**8**), betulinic acid (**9**), quercetin 3-*O*-rutinoside (**10**), quercetin 3-*O*-robinobioside (**11**), apigenin (**12**), traumatic acid (**13**), (*Z*)-4-oxotetradec-5-enoic acid (**14**), 7(*E*)-9-keto-hexadec-7-enoic acid (**15**), 9(*E*)-11-oxo-octadecenoic acid, (9CI) (**16**), and magnoflorine (**27**), etc. The HPLC fingerprint of *Z. jujuba* fruits was established at the same time. The compounds **4**, **5**, **7**, **11**, **14**, **15** and **16** were isolated from *Z. jujuba* for the first time. Compound **14** was isolated from the nature for the first time. Furthermore, cytotoxicity against four human tumor cell lines (MCF-7, A549, HepG2 and HT-29) of the isolated compounds (**1**-**17** and **27**) was evaluated. Among these compounds, compounds **1**, **2**, **3**, **6**, **7**, **9** and **12** had strong growth inhibitory effects on cancer cell lines. These results indicated that jujube extracts exhibited cytotoxicity on these cancer cell lines.

KEYWORDS: *Ziziphus jujuba* Mill., triterpenoids, cytotoxicity

1. Introduction

The fruit of *Ziziphus jujuba* Mill., also called hongzao in Chinese, has long history of cultivation in China. *Ziziphus jujuba* Mill., belonging to the Rhamnaceae family, was widely distributed in northern China. Hongzao is commonly used as food and in Chinese folk medicine for the treatment of fatigue, anorexia, and other health problems for thousands of years.[1]

Phytochemical studies have revealed that *Z. jujuba* contains various chemical constituents, including triterpenic acids,[2-4] flavonoids,[5] saponins,[6] alkaloids,[7] amino acids,[8] phenolic acids,[9,10] polysaccharides[11] and other constituents. The fruits of *Z. jujuba* were reported to have a variety of biological activities such as anti-tumor,[12-14] antioxidant,[8,15,16] anti-inflammatory,[17,18] hepatoprotective property,[19] gastrointestinal protective property,[20] and sedative effects.[21]

It has been reported that *Z. jujuba* fruit possesses hundreds of nutrients. Polysaccharides, cAMP and triterpenoid acids are the most characteristic and functional constituents.[22] The structure of triterpenoids is diversified, and it has a wide range of biological activities.[23,24] Much effort has been devoted to verify the effectiveness of *Z. jujuba* against cancer and the result shows that *Z. jujuba* exerts anticancer activities on several tumor cell lines.[2-4]

As part of our continuing research for bioactive components from nutritional fruits, we studied the jujube plant cultivated in Jiaxian, Shaan'xi province of China and isolated 27 compounds including nine triterpenoids (**1-9**) and 18 other compounds (**10-27**) as major constituents in the fruits of *Z. jujuba*. The HPLC fingerprint of *Z. jujuba* fruits was established at the same time. Structures of compounds 1-9, 11 and

14-17 are shown in Figure 1. Cytotoxicities against several human tumor cell lines (MCF-7, A549, HepG2, HT-29) of 18 isolated compounds are reported.

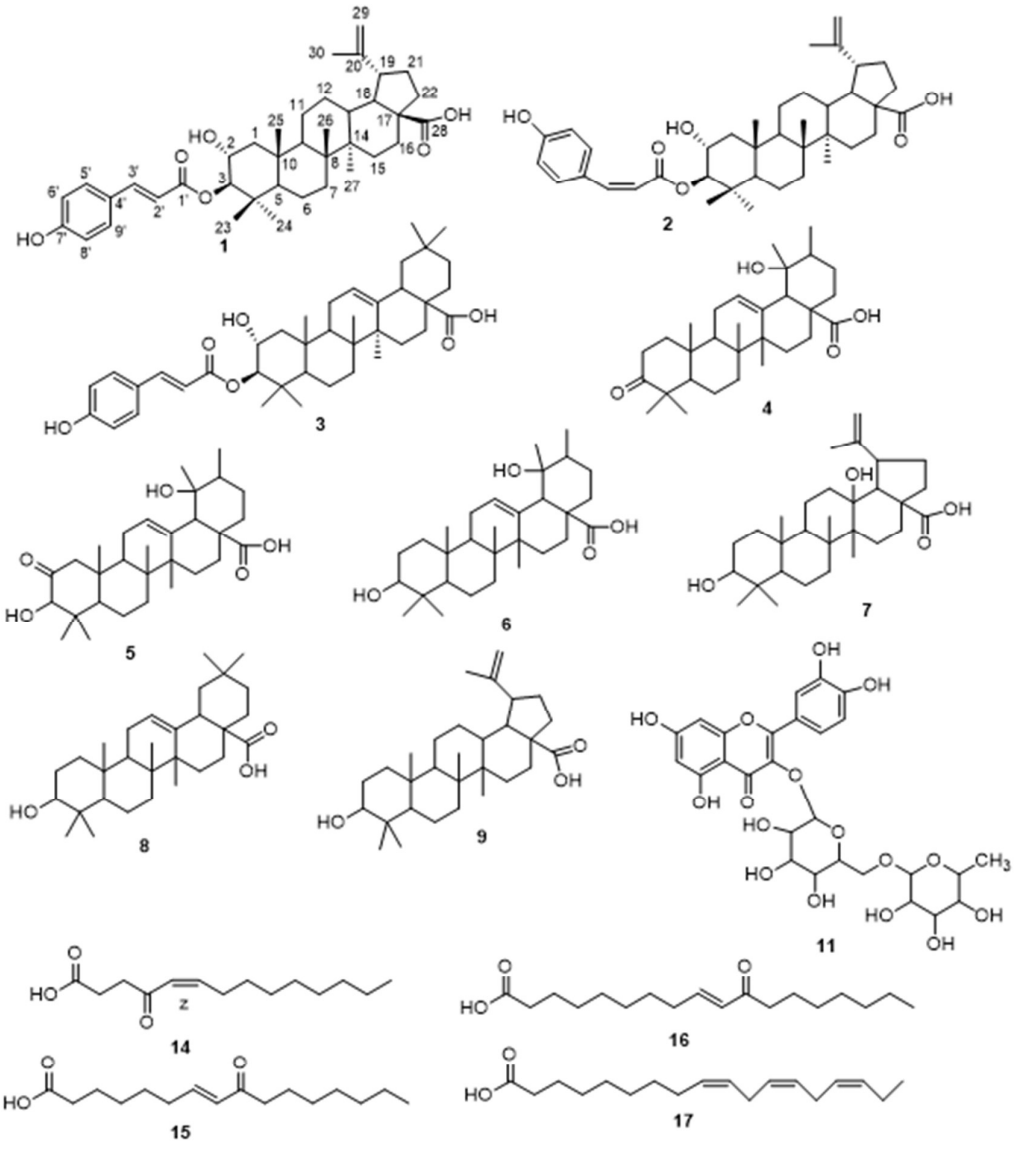

Fig. 1 Structure of compounds **1-9, 11** and **14-17** from *Ziziphus jujuba*.

2. Materials and methods

2.1 General experimental procedures.

Optical rotations were determined with a Perkin-Elmer Model 241 MC polarimeter. UV spectra were recorded on a Shimadzu UV-2401A. ^{1}H and ^{13}C nuclear magnetic

resonance (NMR) spectra were measured on a Bruker Avance 400 spectrometer (Bruker BioSpin GmbH, Beijing, China) with tetramethylsilane (TMS) as the internal standard, and chemical shifts were recorded in δ values. 2D NMR spectra include homonuclear ^{1}H correlation spectroscopy (COSY), through-space ^{1}H correlation spectroscopy (ROESY), one-bond heteronuclear ^{1}H-^{13}C correlation spectroscopy (HSQC), heteronuclear multiple-bond correlation spectroscopy (HMBC). Low- and High-resolution ESIMS were recorded on a Q-TOF Global mass spectrometer. Analytical HPLC was performed on an Agilent 1260 separation module connected to a G1315D DAD detector using a Phenomenex Luna C_{18} HPLC column (150 × 4.6 mm, 5 μm, 100 Å). Preparative HPLC was performed on a Shimadzu LC-20AD preparative chromatography system connected to an SPD-M20A DAD detector using Phenomenex Luna Silica and C_{18} HPLC columns (250 × 21.2 mm, 5 μm, 100 Å). LC-MS analysis was carried out on a Agilent 1200 series LC, equipped with G1367B Hip-ALS, G1315D DAD detector and 6110 Quadrupole MS. HPLC analysis was performed on an Agilent 1260 LC Series using Luna C-18 column (5 micron, 4.6 mm I.D. × 250, Phenomenex, Inc., Torrance, CA, USA) with a flow rate of 1.0 mL/min, and the column temperature was maintained at 30 ^{o}C. The mobile phase was composed of A (0.2% (v/v) phosphoric acid-water solution) and B (acetonitrile) with a gradient elution: 0 min, 100% A; 0-25 min, 100-85% A; 25-35 min, 85-80% A; 35-38 min, 80-75% A; 38-45 min, 75-70% A; 45-55 min, 70-45% A; 55-60 min, 45-30% A; 60-62 min, 30-95% A; 62-65 min, 95% A.

2.2 Chemicals and reagents.

CD_3OD, $CDCl_3$, and CF_3COOH (HPLC grade) were obtained from Merck (Darmstadt, Germany). CH_3OH (HPLC grade), CH_3CH_2OH (HPLC grade), and CH_3CN (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other organic solvents used in the current study, such as CH_3OH, ethyl acetate (EtOAc), acetone, and chloroform ($CHCl_3$) were of analytical grade. They are commercially available from Hengxing Chemical Reagent Co., Ltd. (Tianjin, China). CCK-8 and Annexin V/PI apoptosis detection kit were purchased from Qihai Biological Technology Ltd. (Shanghai, China).

2.3 Cell culture.

Human breast cancer cell line MCF-7, human alveolar basal epithelial cell line A549, human liver carcinoma cell line HepG2 and human colorectal adenocarcinoma cell line HT-29 were purchased from Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). These cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 at 37 ºC.

2.4 Materials.

The fruits of hongzao (*Z. jujuba*) were collected in October 2013 from Jiaxian, Shaan'xi Province, China. Their botanical origins were identified by the corresponding author, and voucher specimens were deposited in the herbarium of Northwest University, under number ZJ-01. Thin-layer chromatography (TLC) analysis was performed on precoated E. Merck Silica Gel 60 GF254 plates. Flash column chromatography (CC) was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), reversed phase C18 (octadecylsilyl, ODS)

123 silica gel (Silicycle, 50 μm, Canada), or Sephadex LH-20 (Sigma-Aldrich, St. Louis,
124 MO, USA). CCK-8 and PI detection kit were purchased from Qihai Biological
125 Technology Ltd. (Shanghai, China).

126 **2.5 Extraction and isolation.**

127 After the cores had been removed, the dried hongzao (20 kg) were pulverized to
128 homogeneous powders. The powders were extracted with EtOAc under cold soak for
129 two times (each for 48 h) and the ratio of material to solvent was 1:3. The resulting
130 solution was then filtered, and the filtrate was combined and concentrated under
131 vacuum at 40 ^{o}C to give an extracts (157 g). The crude residue was then extracted
132 with 60% EtOH under reflux for 2 hours and extracted twice with EtOAc, finally
133 combined the two EtOAc solutions and concentrated under vacuum to give the EtOAc
134 extract (167 g). The combined water solution was concentrated under vacuum to give
135 the water extract (200 g).

136 The EtOAc extract was subjected to a normal-phase silica gel column
137 chromatography (600 g of silica gel, 200-300 mesh), eluted with a stepwise gradient
138 of petroleum ether - acetone (from 50:1 to 0:1), to yield four major fractions (A-D).
139 The fractions were monitored using Agilent 1200 LC-MS and thin-layer
140 chromatography (TLC) with the developing solvent petroleum ether-EtOAc (from
141 20:1 to 0:1).

142 Fraction B (15 g) was purified by a flash ODS C_{18} silica gel column, eluted with a
143 gradient of CH_3CN-H_2O to afford three subfractions, B-1, B-2, and B-3. Fraction B-1
144 was passed through a Sephadex LH-20 column to obtain **8** (90 mg), and **9** (5 mg).

Fraction B-2 was separated by preparative HPLC to yield **14** (27 mg, t_R = 52.05 min), **15** (160 mg, t_R = 54.12 min), **16** (33 mg, t_R = 58.53 min), and **17** (20 mg, t_R = 59.67 min). Fraction B-3 was passed through a Sephadex LH-20 column to give **18** (190 mg, t_R = 31.82 min), and **19** (150 mg, t_R = 44.39 min).

Fraction C (9.8 g) was separated into four subfractions (C-1, C-2, C-3 and C-4) by a silica gel column chromatography using a stepwise gradient of petroleum ether-acetone (from 5:1 to 1:1) as an eluent. Fraction C-2 was separated by a flash ODS C_{18} silica gel column, eluted with CH_3CN-H_2O to afford two subfractions, C-2-1, and C-2-2. Fraction C-2-1 was subsequently purified by a Sephadex LH-20 column to give compound **13** (24 mg). C-2-2 was passed through a Sephadex LH-20 column to give **21** (23mg, t_R = 30.43 min) and **24** (195 mg, t_R = 11.75 min). Fraction C-3 was purified by a Sephadex LH-20 column to obtain fractions C-3-1 and C-3-2. Fraction C-3-1 was subsequently separated by a preparative HPLC to give compound **4** (4 mg), **5** (21 mg), and **7** (9 mg, t_R = 50.95 min). Fraction C-3-2 was further purified by a preparative HPLC to obtain **1** (20 mg, t_R = 61.02 min), **2** (25.3 mg, t_R = 47.10 min), **3** (9.2 mg, t_R = 51.89 min) and **6** (11.20 mg).

Fraction D (17 g) was also separated by a flash ODS C_{18} silica gel column, eluted with a gradient of CH_3OH-H_2O, to obtain three subfractions, D-1, D-2 and D-3. Fraction D-1 was purified by a Sephadex LH-20 column to obtain fractions D-1-1 and D-1-2, and fraction D-1-1 was further purified by a preparative HPLC to yield **10** (5.4 mg, t_R = 41.36 min) and **11** (7.0 mg, t_R = 40.66 min). Fraction D-2 was subsequently separated by a preparative HPLC to yield **25** (5 mg, t_R = 39.18 min) and **26** (6.3 mg, t_R

= 35.94 min). Fraction D-3 was further purified by a preparative HPLC to yield **20** (10 mg, t_R = 18.17 min) and **22** (4 mg, t_R = 13.22 min).

The water extract (200 g) was subjected to a macroporous resin D101 column chromatography (1 Kg of D101), eluted with a stepwise gradient of EtOH-H_2O, to yield three major fractions (I-III). Fraction II was further purified by a Sephadex LH-20 column to yield **12** (29.5 mg, t_R = 15.89 min) and **27** (120 mg, t_R = 24.92 min). The HPLC chromatograms of compounds and the extracts of *Ziziphus jujuba* is shown in Fig. 2.

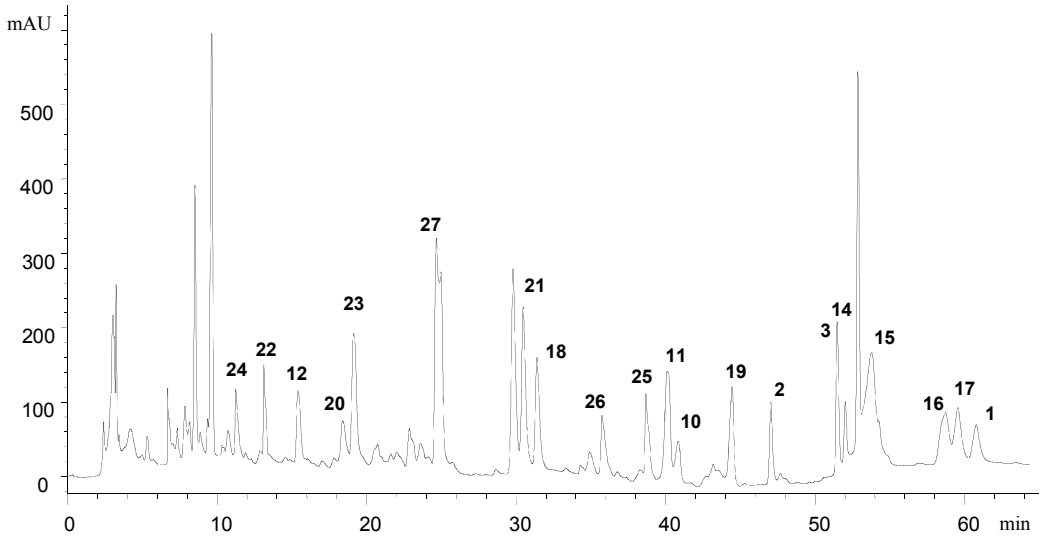

Fig. 2 HPLC chromatograms of compounds and the extracts of *Ziziphus jujuba*. Peaks: **1**, 3-*O*-(*trans*-*p*-Coumaroyl)-alphitolic acid (61.02 min); **2**, 3-*O*-(*cis*-*p*-Coumaroyl)-alphitolic acid (47.10 min); **3**, 3β-*O*-(*trans*-*p*-Coumaroyl) maslinic acid (51.89 min); **10**, Quercetin 3-*O*-rutinoside (41.36 min); **11**, Quercetin 3-*O*-robinobioside (40.66 min); **12**, Apigenin (15.89 min); **14**, (Z)-4-oxotetradec-5-enoic acid (52.05 min); **15**, 7(*E*)-9-keto-Hexadec-7-enoic acid (54.12 min); **16**, 9(*E*)-11-oxo-octadecenoic acid, (9CI) (58.53 min); **17**, (9*Z*,12*Z*,15*Z*)-Octadeca-9,12,15-trienoic acid (59.67 min); **18**, Benzoic acid (31.82 min); **19**, 2-Hydroxybenzoic acid (44.39 min); **20**, 4-Hydroxybenzoic acid (18.17 min); **21**, 4-Hydroxybenzaldehyde (30.43 min); **22**, 3,4-Dihydroxybenzoic acid (13.22 min); **23**, 4-Hydroxy-3-methoxybenzaldehyde (18.97 min); **24**, 1,2,4-Trihydroxybenzene (11.75 min); **25**, 4-Hydroxycinnamic acid (39.18 min); **26**, *p*-Hydroxycinnamic acid (35.94 min); **27**, Magnoflorine (24.92 min).

2.6 Cell inhibitory assay.

5×10^3 cells were seeded into 96-well plate one day before incubation. The final concentration of 100 μg/mL extracts was added into the cells on second day. After incubation for 24 h, 10 μL of CCK-8 was added and incubated for additional 3 h, and then OD value was measured by spectrophotometer under 450 nm. Cell inhibitory rate was calculated as follows: cell inhibitory rate= ($OD_{control}$ - $OD_{experiment}$) / ($OD_{control}$ - OD_{blank}) ×100%.

2.7 Cell death assay.

3×10^5 cells were seeded onto 6-well plate, 100 μg/mL extracts were added into the wells after the cell adherent. 24 h later, cells were harvested and washed, and then incubated with PI buffer for 15 min under 4 °C in a dark place, samples were subjected to flow cytometry analyses for cell death.

3. Results and discussion

3.1 Extraction and isolation.

The 10 kg air-dried fruit of *Z. jujuba* was extracted. The separation was conducted by using a combination of column chromatography of silica gel, Sephadex LH-20 and a flash ODS C_{18} silica gel column together with preparative HPLC to give twenty-seven compounds, namely 3-*O*-(*trans*-*p*-coumaroyl)-alphitolic acid (**1**),[25] 3-*O*-(*cis*-*p*-coumaroyl)-alphitolic acid (**2**),[25] 3β-*O*-(*trans*-*p*-coumaroyl)-maslinic acid (**3**),[26] pomonic acid (**4**),[27] 2-oxo-pomolic acid (**5**),[28] benthamic acid (**6**),[29] terminic acid (**7**),[30] oleanic acid (**8**),[31] betulinic acid (**9**),[32] quercetin 3-*O*-rutinoside (**10**),[33] quercetin 3-*O*-robinobioside (**11**),[33] apigenin (**12**),[34] traumatic acid (**13**),[35]

(*Z*)-4-oxotetradec-5-enoic acid (**14**),[36] 7(*E*)-9-keto-Hexadec-7-enoic acid (**15**),[37] 9(*E*)-11-oxo-octadecenoic acid, (9CI) (**16**),[38] (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid (**17**),[39] benzoic acid (**18**), 2-hydroxybenzoic acid (**19**), 4-hydroxybenzoic acid (**20**), 4-hydroxybenzaldehyde (**21**), 3,4-dihydroxybenzoic acid (**22**), 4-hydroxy-3-methoxybenzaldehyde (**23**), 1,2, 4-trihydroxybenzene (**24**), 4-hydroxycinnamic acid (**25**), *p*-hydroxycinnamic acid (**26**), and magnoflorine (**27**).[40] All structures were established by spectroscopic methods, including ^{1}H, ^{13}C NMR, DEPT, 2D correlation spectroscopy, ESI-MS, and chemical properties. The known compounds were identified by comparing NMR data with those reported in the literature. Compounds **10-27** were identified by direct comparison with commercial standards.

3.2 Structural elucidations.

Compound **1** was obtained as a white powder, and its molecular formula was $C_{39}H_{55}O_6$ according to the ESI-MS (positive) data: *m/z* 619 $[M + H]^+$. The UV spectrum of **1** exhibited maximum absorptions at 230 and 315 nm. The ^{13}C NMR spectrum combined with a DEPT revealed the presence of 39 carbon signals, including a carboxyl carbon (δ 180.0), an ester group (δ 169.8), 10 aromatic or olefinic (δ 110.4 - 169.8), and 2 oxygenated methane (δ 68.0, 85.7). All proton resonances were assigned with the aid of ^{1}H-^{1}H COSY and TOCSY spectra. The ^{13}C NMR signals were assigned by HMQC, HMBC, and DEPT spectra. The ^{1}H-NMR spectrum exhibited six tertiary methyl signals at δ_H 1.73, 1.18, 1.00, 0.99, 0.95, 0.90. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of alphitolic acid[41] established the presence of the same backbone, except that position 3 was linked with

a *p*-hydroxycinnamic acid. The *p*-hydroxycinnamic acid group was determined by the signals of C-1′ (δ 169.8), C-2′ (δ 116.0), C-3′ (δ 146.4), C-4′ (δ 127.5), C-5′ (δ 131.0), C-6′ (δ 117.0), C-7′ (δ 161.3), C-8′ (δ 117.0), C-9′ (δ 131.2) and H-2′ at δ_H 6.42 (1H, d, J = 16.0 Hz), H-3′ at δ_H 7.63(1H, d, J = 16.0 Hz), H-5′, 9′ at δ_H 7.49 (2H, d, J = 8.8 Hz), H-6′, 8′ at δ_H 6.83 (2H, d, J = 8.8 Hz). From the DEPT, we could clearly see that position 3 (δ 85.7) was a CH and the *p*-hydroxycinnamic acid group was located here due to the correlation between C-1′(δ 169.8) and H-3 (δ_H 4.63, 1H, s) in the HMBC. There was also HMBC correlation between C-1′ (δ 169.8) and H-2' (δ 6.42, 1H, d, J = 16.0 Hz), H-3′ (δ 7.63, 1H, d, J = 16.0 Hz) in the spectrum. In addition, H-3 (δ 4.63, 1H, s) had correlations with C-2 (δ 68.0), C-5 (δ 48.6), C-1 (δ 42.1), C-4 (δ 39.6), C-24 (δ 29.3), C-23 (δ 18.3) through the HMBC. The relative stereochemistry of **1** was confirmed on the basis of the NOESY correlations. There was no obvious correlation between H-2′ at δ_H 6.42 and H-3′ at δ_H 7.63 in the NOESY spectrum, and the coupling constant of J is 16.0 Hz in the methanol-d_6, suggesting a *trans* configuration of the double bond. As for compound **2,** very similar chemical shifts of all carbon and hydrogen signals to **1** were found, except that H-2′ at δ_H 5.88 (1H, d, J = 8.8 Hz) and H-3′ at δ_H 6.90 (1H, d, J = 8.8 Hz). There was strong cross-peak between H-2′ at δ_H 5.88 and H-3′ at δ_H 6.90 in the NOESY spectrum, and the coupling constant of J between 2′ and 3′ is 8.8 Hz in the methanol-d_6, suggesting that the double bond in 2 is *cis* configuration. As for compound **3**, the ^{1}H and ^{13}C NMR spectra reveal that it has the same *p*-hydroxycinnamic acid group as compound **1,** but the skeleton was maslinic acid[42] and the *p*-hydroxycinnamic acid group was

also located at position 3 due to the correlation between C-1′ (δ 169.2) and H-3 (δ_H 4.63, 1H, s) in HMBC. There was no obvious correlation between H-2′ at δ_H 6.39 and H-3′, at δ_H 7.63 in the NOESY spectrum, and the coupling constant of J was 16.0 Hz in the methanol-d_6, suggesting a *trans* configuration of the double bond. Thus, the structure of compound **1** was determined to be 3-*O*-(*trans*-*p*-coumaroyl)-alphitolic acid, **2** was identified to be 3-*O*-(*cis*-*p*-coumaroyl)-alphitolic acid, and **3** was identified to be 3β-*O*-(*trans*-*p*-coumaroyl)-maslinic acid. The complete ^{1}H and ^{13}C NMR of **1** and **2** assignments are given in Table 1.

Table 1 ^{1}H, ^{13}C , ^{1}H-^{1}H COSY and HMBC Data for **1** and **2** (DMSO-d_6)[a]

No.	**1**			**2**		
	δ_C	δ_H (J in Hz)	HMBC (H to C)	δ_C	δ_H (J in Hz)	HMBC (H to C)
1	42.1 t	1.68 m	2,10	42.1 t	1.65 m	2,5,10
2	68.0 d	3.98 dd(12.0,6.0)	3	67.9 d	3.92 dd(12.0,6.0)	3
3	85.7 d	4.63 s	1′,1,2,4,5,23, 24	85.3 d	4.65 s	1′,1,2,4,5,23, 24
4	39.4 s			39.6 s		
5	48.6 d	1.02 s	3,4,6,10	50.5 d	1.04 s	3,4,610
6	35.6 t	1.72 m	5,7,8	35.5 t	1.72 m	5,7,8
7	33.9 t	1.43 m	5,6,8,9	33.9 t	1.45 m	5,6,8,9
8	39.8 s			39.7 s		
9	48.9 d	1.06 s	8,10,11	52.0 d	1.05 m	8,10,11
10	40.8 s			40.6 s		
11	21.4 t	1.65 m	9,10,12	19.7 t	1.65 m	9,10,12
12	26.9 t	1.68 m	11,13	26.9 t	1.70 m	11,13
13	37.5 d	1.06 s	12,14,18	37.4 d	1.05 m	12,14,18
14	43.8 s			43.8 s		
15	30.5 t	1.49 m	14,16,17,27	30.9 t	1.48 m	14,16,17,27
16	32.3 t	1.97 m	15,17	32.4 t	1.95 m	15,17
17	57.6 s			57.6 s		
18	50.6 d	1.40 m	13,17,19	56.7 d	1.38 m	13,17,19
19	48.0 d	2.08 m	18,20,29	48.6 d	2.10 m	18,20,21
20	152.1 s			152.0 s		

21	31.8 t	1.52 m	19,22	31.8 t	1.50 m	19,22
22	38.3 t	1.70 m	17,21	38.2 t	1.65 m	17,21
23	18.3 q	1.00 s	3,4,24	18.1 q	0.99 s	3,4,24
24	29.3 q	0.99 s	4,5,23	29.3 q	0.96 s	4,5,23
25	18.0 q	1.18 s	1,9,10	18.0 q	1.16 s	1,9,10
26	16.8 q	0.90 s	8,9,14	16.8 q	0.83 s	8,9,14
27	15.3 q	0.95 s	8,13,14,15	15.3 q	0.88 s	8,13,14,15
28	180.0 s			180.1 s		
29	110.4 t	4.74 s, 4.65 s	19,30	110.4 t	4.74 s, 4.66 s	19,30
30	19.7 q	1.73 s	19,20,29	19.5 q	1.76 s	19,20,29
1’	169.8 s			168.7 s		
2’	116.0 d	6.42 d (16.0)	1′,4′	117.5 d	5.88 d (8.8)	1′,3′,4′,5′
3’	146.4 d	7.63 d (16.0)	1′,2′,4′,5′	144.8 d	6.90 d (8.8)	1′,2′,5′
4’	127.5 s			127.9 s		
5’	131.0 d	7.49 d (8.8)	3′,7′	133.8 d	7.69 d (8.8)	3′,6′,7′
6’	117.0 d	6.83 d (8.8)	4′,7′,8′	115.9 d	6.77 d (8.8)	4′,7′,8′
7’	161.3 s			160.0 s		
8’	117.0 d	6.83 d (8.8)	4′,6′,7′	115.9 d	6.77 d (8.8)	4′,6′,7′
9’	131.2 d	7.49 d (8.8)	3′,7′	133.8 d	7.69 d (8.8)	3′,7′,8′

[a]Carbon multiplicities were determined by DEPT experiments (s = C, d= CH, t = CH_2, q = CH_3);

Figures in parentheses denote *J* values (Hz).

3.3 Jujube extracts inhibit proliferation of different tissue derived cancer cells.

Anti-tumor activities of 18 compounds (**1-17** and **27**) were evaluated on four human cancer cell lines: MCF-7, A549, HepG2 and HT-29. As shown in Fig. 3, majority of compounds could inhibit different tissues derived cancer proliferation. Compounds **1**, **2**, **3**, **6**, **7**, **9** and **12** exhibited stronger inhibitory effect on all four cancer cell lines, the highest inhibitory rate was reached to above 99%. Lee et al.[3] also showed that compounds **1**, **2**, **3** and **9** had high cytotoxic activities against K562, B16 (F-10), SK-MEL-2, PC-3, LOX-IMVI and A549 tumor cell lines by the sulforhodamin B (SRB) method. Compound **6** previously isolated from rosemary extract has been reported to have antiproliferative effects on colon cancer cells.[43] Some other

compounds, like **5**, **8** and **16** possessed a selective inhibitory effect on cancer cells, compound **16** only exhibited stronger inhibitory effect on A549 cells, it could hardly inhibit the proliferation of MCF-7, HT-29 and HepG2 cells. Similarly, compound **1** strongly inhibited the proliferation of MCF-7 and A549 cells, but it barely inhibited HT-29 and HepG2 cells proliferation. Meanwhile, flow cytometry assay revealed that Compounds **1**, **2**, **3**, **6**, **7**, **9** and **12** significantly promoted cell death on all aforementioned cell lines, but compounds **8** and **16** only induced cell death on one or two cell lines (Fig. 4). Taken together, these data suggested that jujube extracts are potent candidates for cancer prevention.

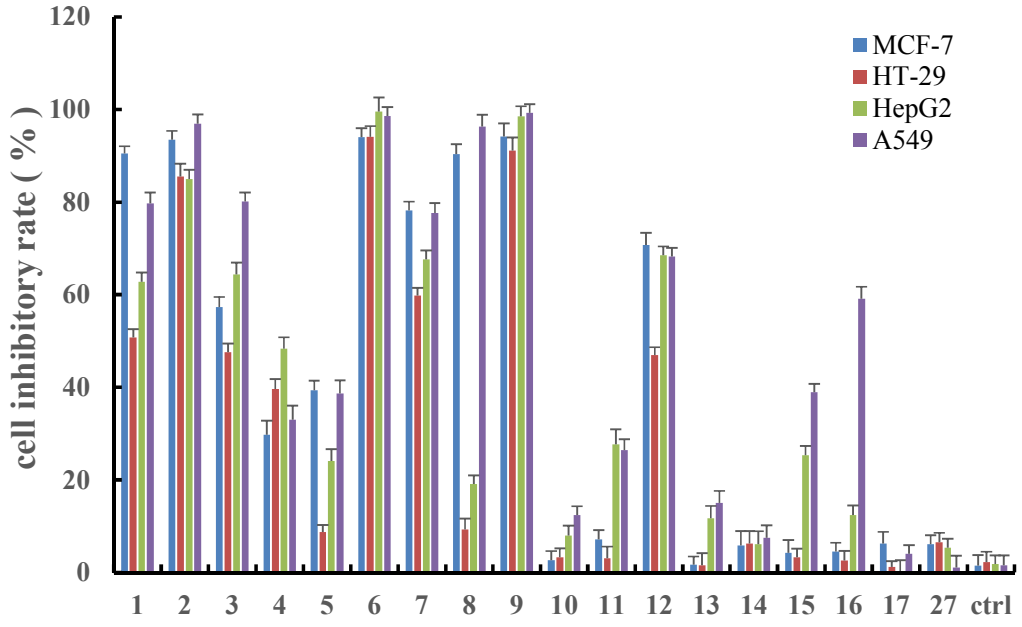

Fig. 3 Jujube extracts inhibited the proliferation of various cancer cell lines. MCF-7, A549, HepG2 and HT-29 cells were exposed to different Jujube isolates, and cell inhibitory rate was determined with CCK-8 assay.

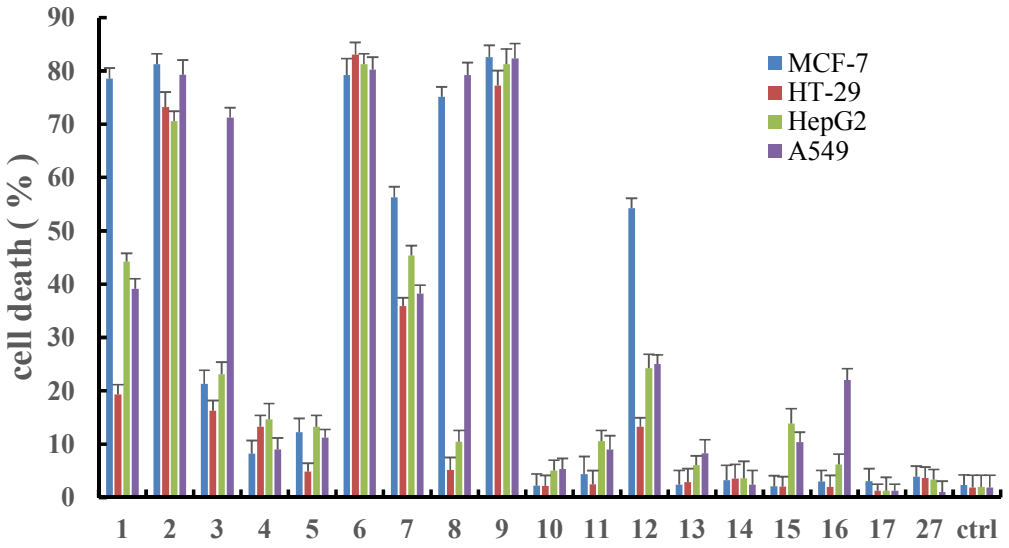

Fig. 4 Jujube extracts induced cell death on various human cancer cells. MCF-7, A549, HepG2 and HT-29 cells were harvested after exposing to different Jujube isolates, and cell death was determined by flow cytometry assay.

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Chemical Characterization of the Main Bioactive Constituents from Fruits of *Ziziphus jujuba*

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