Neuroprotective effects of triterpenoid saponins from *Medicago sativa* L. against H$_2$O$_2$-induced oxidative stress in SH-SY5Y cells

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ABSTRACT

Medicago sativa L. is a forage legume plant widely distributed in all continents. Six new triterpenoid saponins, Medicagosides A-F (**1–6**) and five known ones (**7–11**) were isolated from *M. sativa*. Their structures were determined via HRESIMS, 1D and 2D NMR analysis. Biologically, all the isolates displayed neuroprotective activities against H$_2$O$_2$-induced damage in SH-SY5Y cells. Among them, compounds **1**, **3–5** and **10** exhibited striking neuroprotective activities at 100 μM, restoring cell viability range from 79.66% to 89.03%, relative to 79.46% (100 μM) of Trolox used as the positive control.

1. Introduction

The incidence of neurodegenerative diseases has been increasing due to the rising aging population. These diseases, primarily Alzheimer's and Parkinson's diseases, are characterized by progressive and irreversible degeneration neuronal cells [1]. With the advancements in research on neurodegenerative diseases, more factors related to the occurrence and development of neurodegenerative diseases are beginning to be revealed. The most striking risk factor of neurodegenerative diseases is aging, as it is a major cause of poor memory, communication difficulties, bad learning ability and changes in personality, which seriously affects human health [2,3]. Synthetic neuroprotective agents used to treat neurodegenerative diseases have some side effects including dry mouth, fatigue, somnolence, dysphoria and difficulty walking [4]. Thus, more attention has been paid to natural products as potential neuroprotective agents because they possess fewer side effects and leave no drug residues in the human body [4–6]. Previous studies indicate that neurodegenerative diseases are caused by mitochondrial DNA mutation, oxidative stress damage, excitatory toxin and immune-related inflammation, of which oxidative stress plays an important role [7]. Oxidative stress occurs when reactive oxygen species containing H$_2$O$_2$ are excessively generated [8]. Oxidative stress can lead to the neuronal cell injury because these cells possess low oxidative resistance, high metabolic capacity and non-replicative nature [9]. Therefore, H$_2$O$_2$-induced damage in the neuron-like cells, such as the human neuroblastoma SH-SY5Y cells [10], is used as a model to investigate the neuroprotective effects of various compounds in neurodegenerative diseases.

Medicago sativa L. (Leguminosae) was initially discovered in Iran and is now extensively grown in Europe, USA, Canada, Australia, China and India [11]. *M. sativa* is a perennial plant of 30–100 cm height. Its roots are thick and strong, and grow deep into the soil layers. Its leafy stems are erect, clumped and even supine. *M. sativa* is used as a forage crop and its leaves are used to prepare proteins as nutritional supplement, hence *M. sativa*. is an important plant in agricultural sectors and food industries [12]. *M. sativa*, is also used as a medicinal plant to boost memory, treat cough, kidney stones, gastric ulcers, arthritis and central nervous system diseases [13,14]. Previous phytochemical investigations on *M. sativa* have reported the presence of several chemical compounds such as saponins [15], flavonoids [12], alkaloids [16], polysaccharides [17], coumarins [18] and amino acids [19], of which saponins are among the major chemical components.

Saponins are a class of glycosides in which aglycones are triterpenes or steroid. These compounds have been extensively isolated from natural plants, of which triterpenoid-type compounds are the main constituents. Saponins possess various biological activities including antitumor, antifungal, anti-inflammatory, antiviral and immunomodulation activities [20,21]. Saponins isolated from *M. sativa* are pentacyclic triterpenoid glycosides, of which hederagenin-type and medicagenic acid-type saponins are present in high quantities [14,22]. Based on the previous findings that saponins confer neuroprotection against neurodegenerative disorders [23,24], we performed neuroprotective assays

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Table 1

^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) for aglycone moieties of compounds **1–6** in Pyridine-d_5.

NO.	1 δ_H (J in Hz)	δ_C	2 δ_H (J in Hz)	δ_C	3 δ_H (J in Hz)	δ_C	4 δ_H (J in Hz)	δ_C	5 δ_H (J in Hz)	δ_C	6 δ_H (J in Hz)	δ_C
1	1.59 m; 1.10 m	38.8	1.56 m; 1.04 m	38.7	1.44 m; 0.93 m	38.6	1.52 m; 0.96 m	38.7	2.31 m; 1.31 m	44.0	2.24 m; 1.19 m	43.4
2	2.21 m; 2.00 m	26.2	2.18 m; 2.01 m	26.0	2.26 m; 1.94 m	25.8	2.10 m; 1.91 m	25.8	4.87 m	70.2	4.65 m	69.0
3	4.30 m	81.7	4.15 m	82.7	4.10 m	83.4	4.13 m	82.2	4.75 d (3.2)	85.5	4.10 d (3.5)	81.9
4	–	43.5	–	43.5	–	43.3	–	43.4	–	52.6	–	54.3
5	1.74 m	47.5	1.60 m	48.1	1.50 m	48.5	1.56 m	47.8	1.99 m	52.5	1.44 m	47.8
6	1.79 m; 1.44 m	18.2	1.71 m; 1.36 m	18.3	1.69 m; 1.35 m	18.3	1.65 m; 1.33 m	18.2	1.83 m; 1.66 m	20.8	1.55 m; 1.02 m	20.2
7	1.47 m; 1.70 m	32.9	1.58 m; 1.34 m	32.8	1.58 m; 1.30 m	32.8	1.82 m; 1.75 m	32.5	1.60 m; 1.29 m	33.0	1.84 m; 1.77 m	32.5
8	–	39.9	–	39.9	–	39.9	–	39.9	–	40.1	–	40.2
9	1.78 m	48.1	1.76 m	48.1	1.70 m	48.1	1.71 m	48.1	1.77 m	48.7	1.67 m	48.4
10	–	36.9	–	36.9	–	36.8	–	36.9	–	36.8	–	36.2
11	1.93 m; 1.81 m	23.9	1.74 m; 1.01 m	23.8	2.03 m; 1.90 m	23.8	1.92 m; 1.94 m	23.8	2.12 m; 2.03 m	23.9	1.97 m	23.3
12	5.46 t	122.9	5.45 t	122.9	5.42 t	122.8	5.43 t	122.9	5.50 t	122.4	5.44 t	122.7
13	–	144.1	–	144.1	–	144.1	–	144.1	–	144.8	–	144.2
14	–	42.1	–	42.1	–	42.1	–	42.1	–	42.2	–	42.3
15	2.10 m; 1.26 m	28.3	2.35 m; 1.13 m	28.2	2.32 m; 1.08 m	28.2	2.33 m; 1.09 m	28.2	2.14 m; 1.11 m	28.2	2.33 m; 1.15 m	28.1
16	2.08 m; 2.00 m	23.1	2.06 m; 1.96 m	23.4	2.03 m; 1.92 m	23.3	2.03 m; 1.93 m	23.3	2.09 m; 1.93 m	23.6	2.08 m	23.9
17	–	47.3	–	47.0	–	46.9	–	46.9	–	46.6	–	46.9
18	3.29 dd (13.7, 3.9)	41.6	3.21 dd (13.9, 3.9)	41.7	3.18 dd (13.2, 3.4)	41.7	3.19 dd (13.7, 4.1)	41.7	3.31 dd (13.3, 2.94)	41.9	3.21 dd (13.4, 3.7)	41.7
19	1.76 m; 1.26 m	46.1	1.74 m; 1.26 m	46.1	1.73 m; 1.24 m	46.1	1.73 m; 1.24 m	46.1	1.79 m; 1.31 m	46.4	1.79 m; 1.27 m	46.1
20	–	30.9	–	30.7	–	30.7	–	30.7	–	30.9	–	30.8
21	1.37 m; 1.16 m	34.1	1.58 m; 1.34 m	33.9	1.32 m; 1.06 m	33.9	1.33 m; 1.07 m	33.9	1.44 m; 1.20 m	34.1	1.36 m; 1.09 m	33.9
22	2.02 m; 1.74 m	32.7	1.82 m; 1.76 m	32.5	1.80 m; 1.74 m	32.5	1.55 m; 1.31 m	32.7	2.05 m; 1.82 m	33.1	1.43 m; 1.22 mm	32.5
23	4.35 m; 3.77 d (10.5)	64.0	4.30 m; 3.78 d (10.5)	65.2	4.32 m; 3.76 d (10.6)	65.9	4.08 m; 3.73 d (10.6)	65.0	–	180.8	9.81 s	208.2
24	0.97 s	13.7	1.11 s	13.4	1.11 s	13.3	0.95 s	13.4	2.06 s	14.1	1.76 s	11.6
25	0.95 s	16.1	0.97 s	16.1	0.91 s	16.1	0.93 s	16.1	1.54 s	16.8	1.47 s	16.7
26	1.12 s	17.5	1.15 s	17.5	1.11 s	17.5	1.13 s	17.5	1.05 s	17.3	1.12 s	17.5
27	1.22 s	26.0	1.19 s	26.0	1.19 s	26.0	1.18 s	26.0	1.26 s	26.2	1.24 s	26.1
28	–	176.3	–	176.4	–	176.4	–	176.4	–	176.4	–	176.4
29	0.92 s	33.1	0.91 s	33.1	0.88 s	33.1	0.89 s	33.1	0.94 s	33.2	0.93 s	33.1
30	1.00 s	23.7	0.90 s	23.6	0.87 s	23.6	0.88 s	23.6	1.01 s	23.7	0.91 s	23.6

on the isolated triterpenoid saponins from *M. sativa*. Specifically, neuroprotective properties of the saponins from *M. sativa* were studied for the first time. Here, we report that compounds **1–11** isolated and characterized from *M. sativa* have neuroprotective effects against H_2O_2-induced injury in SH-SY5Y cells.

2. Materials and methods

2.1. General experimental procedures

^{1}H NMR, ^{13}C NMR, HMBC, HSQC, ^{1}H-^{1}H COSY, TOCSY and NOESY spectra were accomplished by Bruker AVIII 600 MHz spectrometers (Bruker Co., Billerica, MA, USA) in pyridine-d_5. HRESIMS analysis was conducted using an Agilent 6540 Q-TOF MS (Agilent Technologies, USA). LC-20AR HPLC fitted with an RID-20A detector (Shimadzu Corporation, Japan) was employed for analysis and preparation of Promosil C18 column and YMC-pack ODS-A column, respectively. HP-Amino column (Sepax Technologies, USA) was used for acid hydrolysis experiment. The isolation and purification of compounds were performed via D101 macroporous resin (Tianjin Ruijin Special Chemicals Co., China), silica gel (Qingdao Haiyang Chemical Co., China), octadecylsilanized (ODS) (ODS-A-HG, YMC Co. Ltd.). Methanol, acetonitrile, 30% H_2O_2 and all other solvents were purchased from Shenyang-Lab Science and Trade Co., Ltd. (Shenyang, China). Trolox was obtained from Biotopped Science and Technology Co., Ltd. (Beijing, China). The microplate reader made by Bio-Rad Laboratories (Hercules, California, USA) was used.

2.2. Plant material

M. sativa used in this study was obtained from Inner Mongolia Prataculture (Chifeng, China) and authenticated by Professor Jin-Cai Lu (Shenyang Pharmaceutical University). The sample (No. 2016-MSL) was kept in our research group laboratory (Shenyang University of Chemical Technology).

2.3. Extraction and isolation

M. sativa (7.0 kg) was extracted with EtOH-H$_2$O (70:30, v/v) by refluxing three times for two hours and concentrated to obtain the residue, which was successively partitioned three times with CH$_2$Cl$_2$. The aqueous phase (1.5 kg) was analyzed by a macroporous resin D101 column (EtOH/H$_2$O, 0, 30%, 50%, 70% and 95%, v/v) to obtain Fr. 1–5. The combined fractions 4–5 (600.0 g) based on TLC profiles were subjected to vacuum-liquid chromatography over silica gel with CH$_2$Cl$_2$/MeOH (50:1–0:1, v/v) as an eluent, which yielded eight fractions (A-H). Fr. C (43.4 g) was analyzed by a silica gel column (CH$_2$Cl$_2$/MeOH, 50:1–0:1, v/v) to form Fr. C1-C4. Subsequently, Fr. C3 (14.7 g) was repeatedly applied to the silica gel column and eluted using CH$_2$Cl$_2$/MeOH (30:1–0:1, v/v) to produce Fr. C3-1-C3-4. Fr. C3-3 (4.7 g) was separated through a column chromatography with ODS using MeOH/H$_2$O (40%, 60%, 80% and 100%, v/v) to form Fr. C3-3a-C3-3e. Fr. C3-3c was chromatographed over HPLC (70% MeOH/H$_2$O, v/v) to obtain compounds **7** (392.9 mg), **2** (4.9 mg), **8** (12.9 mg) and **4** (6.1 mg). Compounds **10** (93.8 mg), **3** (33.0 mg) and **6** (4.5 mg) from Fr. C3-3b were separated under identical conditions as for Fr. C3-3c. Separation of Fr. C3-3d via HPLC (72% MeOH/H$_2$O, v/v) produced compounds **5** (5.5 mg), **1** (4.3 mg) and **9** (25.0 mg).

In addition, Fr. D (34.2 g) was separated on a silica gel column (CH$_2$Cl$_2$/MeOH, 10:1–0:1, v/v) to obtain Fr. D1-D5. Fr. D2 (3.1 g) was then purified via an ODS column (MeOH/H$_2$O, 40%, 60%, 80% and 100%, v/v), yielding three fractions (Fr. D2-1-D2-3). Finally, compound **11** (9.0 mg) was obtained from Fr. D2-3 via HPLC with MeOH/H$_2$O (80%, v/v).

Table 2

^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) for sugar moieties of compounds **1–6** in Pyridine-d_5.

NO.	1 δ$_H$ (J in Hz)	δ$_C$	2 δ$_H$ (J in Hz)	δ$_C$	3 δ$_H$ (J in Hz)	δ$_C$	4 δ$_H$ (J in Hz)	δ$_C$	5 δ$_H$ (J in Hz)	δ$_C$	6 δ$_H$ (J in Hz)	δ$_C$
3-O-	Glc		Xyl		Glc		Ara		Glc		Glc	
1	5.02 d (7.3)	106.4	5.12 d (6.9)	104.3	5.53 d (7.4)	103.4	5.37 d (4.1)	103.6	5.21 d (7.7)	102.7	4.97 d (7.7)	101.1
2	4.07 m	76.0	4.17 m	82.3	4.10 m	83.5	4.59 m	80.0	3.87 m	84.5	3.93 m	83.3
3	4.01 m	88.3	4.27 m	77.8	4.30 m	78.7	4.66 m	72.7	4.24 m	77.4	4.16 m	77.8
4	4.20 m	71.0	4.12 m	70.4	4.12 m	70.7	4.56 m	67.8	4.11 m	70.1	4.08 m	70.5
5	4.32 m	78.3	4.32 m; 3.63 m	66.5	4.20	77.7	4.35 m; 3.88 m	63.6	3.94 m	78.5	3.95 m	78.4
6	4.58 m; 4.33 m	62.5			4.46 m; 4.42 m	62.1			4.49 m; 4.45 m	61.6	4.54 m; 4.26 m	62.5
	Xyl		Glc		Glc		Glc		Glc		Glc	
1	5.30 d (7.8)	105.8	5.59 d (7.7)	103.4	5.11 d (7.6)	103.3	5.33 d (7.9)	103.5	5.30 d (7.7)	104.5	5.37 d (7.7)	103.6
2	4.06 m	74.2	4.22 m	84.2	4.21 m	84.6	4.16 m	85.4	3.90 m	85.6	4.21 m	84.7
3	4.11 m	78.6	4.28 m	77.9	4.27 m	77.8	4.28 m	77.7	4.45 m	78.0	4.20 m	77.9
4	4.10 m	69.5	4.24 m	71.2	4.37 m	70.9	4.21 m	71.3	4.25 m	69.9	4.32 m	70.8
5	4.31 m; 3.58 m	66.4	3.86 m	77.9	3.84 m	78.2	3.79 m	78.0	3.73 m	77.6	3.91 m	78.0
6			4.51 m; 4.44 m	62.2	4.48 m; 4.40	62.3	4.44 m; 4.28 m	62.3	4.49 m; 4.26 m	62.5	4.53 m; 4.45 m	62.4
			Ara		Xyl		Glc		Glc		Xyl	
1			5.33 d (6.9)	106.3	5.35 d (7.0)	106.5	5.34 d (8.0)	106.7	5.09 d (7.6)	106.6	5.40 d (7.1)	106.6
2			4.61 m	73.6	4.08 m	76.0	4.11 m	76.9	4.04 m	76.9	4.13 m	76.1
3			4.20 m	74.2	4.11 m	77.7	4.22 m	77.8	4.15 m	77.3	4.32 m	78.3
4			4.28 m	69.1	4.12 m	70.6	4.14 m	71.0	4.10 m	71.0	4.15 m	70.7
5			4.46 m; 3.83 m	67.3	4.33 m; 3.71 m	67.5	4.04 m	79.1	3.93 m	79.5	4.33 m; 3.72 m	67.5
6							4.48 m; 4.44 m	62.1	4.58 m; 4.30 m	62.5		
28-O-	Ara		Glc		Glc		Glc				Glc	
1	6.51 d (2.3)	93.5	6.37 d (8.2)	95.7	6.32 d (8.0)	95.7	6.35 d (8.2)	95.7			6.36 d (8.1)	95.7
2	4.62 m	75.2	4.26 m	74.2	4.23 m	74.1	4.22 m	74.1			4.23 m	74.1
3	4.58 m	70.0	4.34 m	78.9	4.35 m	78.9	4.31 m	78.9			4.41 m	78.9
4	4.44 m	66.2	4.41 m	71.0	4.29 m	71.0	4.37 m	70.9			4.39 m	71.0
5	4.53 m; 3.98 m	63.1	4.06 m	79.4	4.04 m	79.3	4.04 m	79.3			4.08 m	79.4
6			4.49 m; 4.38 m	62.5	4.50 m; 4.35 m	62.7	4.64 m; 4.39 m	62.6			4.49 m; 4.46 m	62.2
	Rha											
1	5.85 brs	101.1										
2	4.64 m	72.7										
3	4.65 m	71.9										
4	4.41 m	84.4										
5	4.45 m	68.6										
6	1.84 d (5.8)	18.4										
	Xyl											
1	5.17 d (7.1)	107.2										
2	4.12 m	75.6										
3	4.09 m	78.7										
4	4.24 m	71.6										
5	4.26 m; 3.54 m	67.5										

2.3.1. Medicagoside A (1)

White amorphous powder; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data in C$_5$D$_5$N, as shown in Tables 1 and 2; HRESIMS, *m/z* 1211.5417 [M + Cl]$^-$ (calcd for C$_{57}$H$_{92}$O$_{25}$Cl, 1211.5611).

2.3.2. Medicagoside B (2)

White amorphous powder; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data in C$_5$D$_5$N, as shown in Tables 1 and 2; HRESIMS, *m/z* 1059.5387 [M − H]$^-$ (calcd for C$_{52}$H$_{83}$O$_{22}$, 1059.5371).

2.3.3. Medicagoside C (3)

White amorphous powder; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data in C$_5$D$_5$N, as shown in Tables 1 and 2; HRESIMS, *m/z* 1089.5474 [M − H]$^-$ (calcd for C$_{53}$H$_{85}$O$_{23}$, 1089.5476).

2.3.4. Medicagoside D (4)

White amorphous powder; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data in C$_5$D$_5$N, as shown in Tables 1 and 2; HRESIMS, *m/z* 1089.5476 [M − H]$^-$ (calcd for C$_{53}$H$_{85}$O$_{23}$, 1089.5476).

2.3.5. Medicagoside E (5)

White amorphous powder; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz)

data in C$_5$D$_5$N, as shown in Tables 1 and 2; HRESIMS, *m/z* 1011.4778 [M + Na]$^+$ (calcd for C$_{48}$H$_{76}$O$_{21}$Na, 1011.4771).

2.3.6. Medicagoside F (6)

White amorphous powder; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data in C$_5$D$_5$N, as shown in Tables 1 and 2; HRESIMS, *m/z* 1103.5303 [M − H]$^-$ (calcd for C$_{53}$H$_{83}$O$_{24}$, 1103.5269).

2.4. Acid hydrolysis of triterpenoid saponins

New compounds (**1–6**) were hydrolyzed with 2 M CF$_3$COOH for 6 h at 95 °C. The hydrolysates were concentrated with CH$_3$OH repeatedly to obtain sugar residues. The residues were analyzed by HPLC on an HP-Amino column, using acetonitrile-water (84:16) as an eluent [25]. The saccharide composition of compounds were confirmed through comparisons with standard sugars [t_R (L-rhamnose) = 18.6 min; t_R (D-xylose) = 22.2 min; t_R (L-arabinose) = 24.8 min; t_R (D-glucose) = 34.9 min; t_R (D-galactose) = 37.2 min].

2.5. CCK-8 cell viability assay

Neuroprotective properties of compounds **1–11** against H$_2$O$_2$-induced
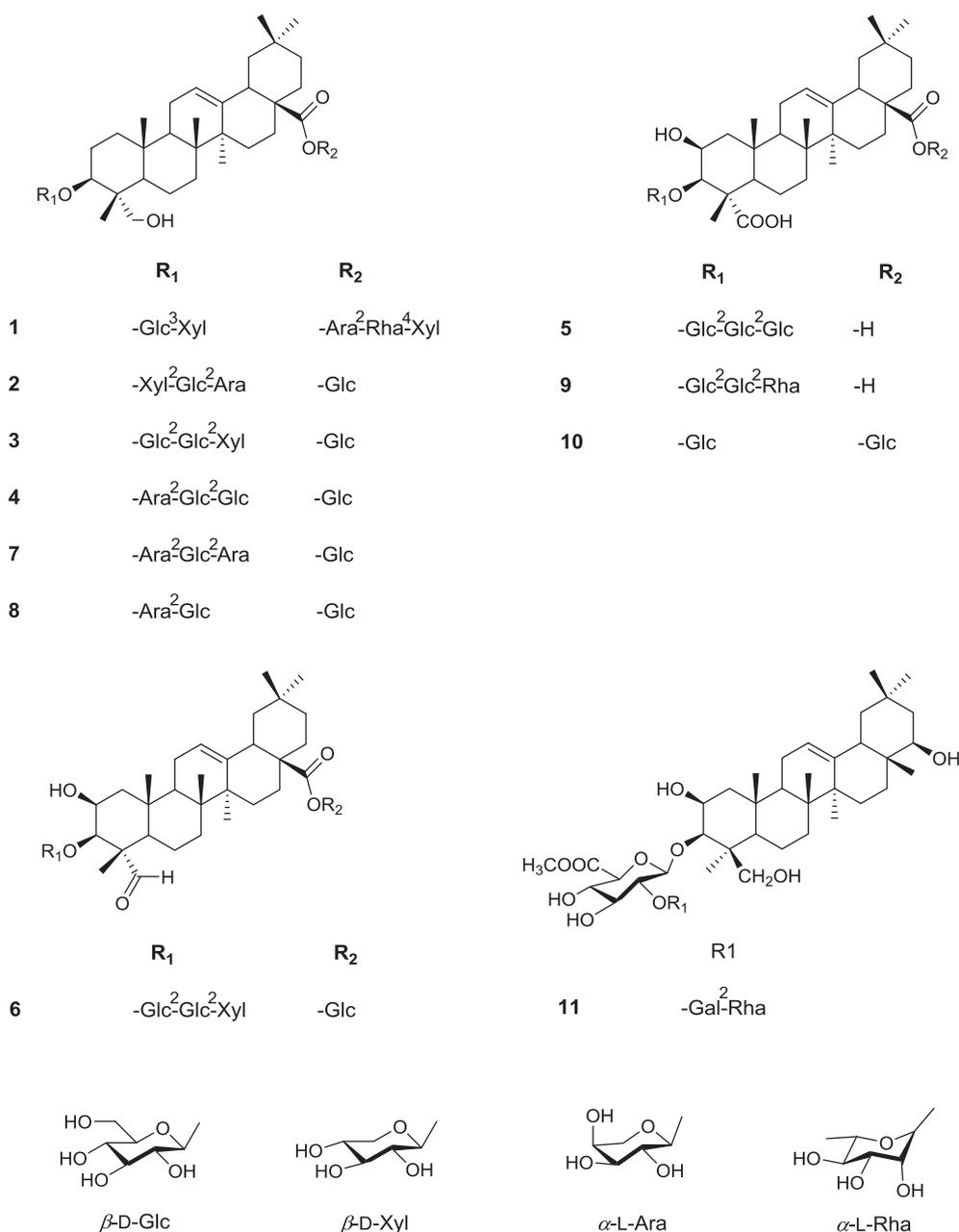
Fig. 1. Structures of triterpenoid saponins (**1–11**) from *M. sativa*.

damage in SH-SY5Y cells were measured by the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan) method as reported previously [5,26]. SH-SY5Y cells, initially introduced in 2016, and 1:1 mixture of MEM and F12 medium were provided by Stem Cell Bank, Chinese Academy of Sciences. SH-SY5Y cells were added into 96-well microplates (1×10^4/ well) and placed in the incubator for 24 h at 37 °C. Different concentrations of compounds (25, 50 and 100 μM) freshly prepared with medium were added to plates for 1 h before they were damaged with H_2O_2 (600 μM) for 4 h. Thereafter, CCK-8 (10 μL) was added into the 96-well microplates. The absorbance readings were measured at 450 nm by the microplate reader. Trolox, which was used as the reference group, was handled with the same methods as the drug group. Finally, cell viability was compared with that of the control group of 100%.

3. Results and discussion

The 70% ethanol extract of *Medicago sativa* L. was isolated and purified through column chromatography (D101 macroporous resin,

silica gel and ODS) and HPLC method which yielded six new compounds (**1–6**) and five known ones (**7–11**) (Fig. 1). Compounds (**7–11**) were identified as 3-*O*-[α-L-arabinopyranosyl(1 → 2)-*β*-D-glucopyranosyl(1 → 2)-α-L-arabinopyranosyl]-28-*O*-[*β*-D-glucopyranoside] he-deragenin (**7**) [27,28], 3-*O*-[*β*-D-glucopyranosyl(1 → 2)-α-L-arabinopyranosyl] hederagenin 28-*O*-*β*-D-glucopyranosyl ester (**8**) [29], 3-*O*-[α-L-rhamnopyranosyl(1 → 2)-*β*-D-glucopyranosyl(1 → 2)-*β*-D-glucopyranoside]medicagenic acid (**9**) [22], medicagenic acid 3,28-di(*O*-glucopyranoside) (**10**) [27] and soyasaponin I methyl ester (**11**) [30].

Compound **1** formed a white amorphous powder. Its molecular formula was confirmed as $C_{57}H_{92}O_{25}$ by the negative-ion HRESIMS at *m/z* 1211.5417 [M + Cl]$^-$ (calcd for $C_{57}H_{92}O_{25}Cl$, 1211.5611). The ^1H NMR spectrum (Table 1) showed six single methyl signals at δ_H 0.92 (H-29), 0.95 (H-25), 0.97 (H-24), 1.00 (H-30), 1.12 (H-26) and 1.22 (H-27), one primary hydroxymethyl group at δ_H 4.35 and 3.77 (H-23) and one olefinic proton signal at δ_H 5.46 (H-12). The ^{13}C NMR spectrum (Tables 1 and 2) together with the HSQC spectrum showed 30 carbon signals of the sapogenin portion and 27 of the sugar component. From

the ^{13}C NMR spectrum, six tertiary methyl carbons at δc 13.7 (C-24), 16.1 (C-25), 17.5 (C-26), 23.7 (C-30), 26.0 (C-27), 33.1 (C-29), and two olefinic carbons at δ_C 122.9 (C-12) and 144.1 (C-13) showed a $\triangle^{12}$ oleanane skeleton. The C-3 and C-28 positions were glycosylated according to the downshift of δc 81.7 and upshift of δc 176.3, respectively, indicating that compound **1** was a 3,28-bidesmosidic saponin. Five anomeric protons were found at δ_H 5.02, 5.17, 5.30, 5.85 and 6.51 corresponding to five anomeric carbons at δ_C 106.4, 107.2, 105.8, 101.1 and 93.5 in the HSQC spectrum, respectively. Furthermore, acid hydrolysis experiment and NMR analysis confirmed the presence of D-glucose, D-xylose, L-arabinose and L-rhamnose in compound **1**. Coupling constants ($^3J_{H-1, H2}$ = 7.1–7.8 Hz) revealed a β-configuration for the glucose and xylose [31]. The α-anomeric configuration of the arabinose was identified by the $^3J_{H-1, H-2}$ value of 2.3 Hz on the basis of a study by Ishii et al. [32] and the NOESY correlations from δ_H 6.51 (Ara-H-1) to δ_H 4.58 (Ara-H-3) and δ_H 4.44 (Ara-H-4) [33]. The correlations of δ_H 5.85 (Rha-H-1) with δ_H 4.41 (Rha-H-4) and δ_H 1.84 (Rha-H-6) in the NOESY spectrum (Fig. 3) represented the α-anomeric configuration of the rhamnose unit [34]. All proton signals were assigned by the COSY, TOCSY, HMBC and HSQC spectra. The HMBC correlations (Fig. 2) from δ_H 5.30 (Xyl-H-1) to δ_C 88.3 (Glc-C-3), and δ_H 5.02 (Glc-H-1) to δ_C 81.7 (C-3) indicated that the sequence of the disaccharide at the C-3 position was β-D-xylopyranosyl(1 → 3)-β-D-glucopyranosyl moiety. The sequence of the saccharide portion at C-28 was β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranosyl moiety as supported by the HMBC spectrum, showing correlations from δ_H 5.17 (Xyl-H-1′) to δ_C 84.4 (Rha-C-4), δ_H 5.85 (Rha-H-1) to δ_C 75.2 (Ara-C-2), and δ_H 6.51 (Ara-H-1) to δ_C 176.3 (C-28). From the NOESY spectrum, the α-configuration of H-3 of the aglycone was identified by the observation of the correlations from δ_H 4.30 (H-3) to δ_H 1.74 (H-5), and δ_H 1.74 (H-5) to δ_H 1.22 (H-27) [35]. Based on these data sets, compound **1** resembled the known compound **7** [27,28] except for the sugar types assigned to C-3 and C-28. Accordingly, compound **1** was identified as 3-O-[β-D-xylopyranosyl(1 → 3)-β-D-glucopyranosyl]-28-O-[β-D-xylopyranosyl (1 → 4)-α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranoside]hederagenin and named as Medicagoside A.

Compound **2**, a white amorphous powder, was determined as $C_{52}H_{84}O_{22}$ by the negative-ion HRESIMS (m/z 1059.5387 [M − H]$^-$, calcd for $C_{52}H_{83}O_{22}$, 1059.5371). Its ^{1}H NMR spectrum (Tables 1 and 2) exhibited signals for six angular methyl groups at δ_H 0.90 (H-30), 0.91 (H-29), 0.97 (H-25), 1.11 (H-24), 1.15 (H-26) and 1.19 (H-27), one primary oxygenated methylene at δ_H 4.30 and 3.78 (H-23) and one olefinic proton at δ_H 5.45 (H-12). The NMR data were similar to those of **1**, showing $\triangle^{12}$ and 3,28-bidesmosidic triterpenoid saponin of **2**. Moreover, according to the HSQC spectrum, four anomeric protons at δ_H 5.12, 5.33, 5.59 and 6.37 were assigned to four anomeric carbons at δ_C 104.3, 106.3, 103.4 and 95.7, respectively, showing the existence of four sugar molecules in compound **2**. The sugar constituents of **2** were defined as D-glucose, D-xylose and L-arabinose after acid hydrolysis experiment. The β-anomeric configuration for xylose and glucose moieties were identified by the $^3J_{H1, H2}$ values of 6.9–8.2 Hz [31]. The arabinose unit was deduced by the α-anomeric configuration depending on the $^3J_{H1, H2}$ value and NOESY data (Fig. 3) [32,33]. The saccharide sequences were determined from HMBC, HSQC, COSY and TOCSY experiments. Based on the HMBC cross-peaks (Fig. 2) from δ_H 5.33 (Ara-H-1) to δ_C 84.2 (Glc-C-2), δ_H 5.59 (Glc-H-1) to δ_C 82.3 (Xyl-C-2), and δ_H 5.12 (Xyl-H-1) to δ_C 82.7 (C-3), the α-L-arabinopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-xylopyranosyl portion was assigned to C-3. Additionally, the β-D-glucopyranosyl unit was labeled at C-28 of compound **2** through the HMBC cross-peak from δ_H 6.37 (Glc-H-1′) to δ_C 176.4 (C-28). The α-configuration of H-3 was identified via NOESY cross-peaks of δ_H 4.15 (H-3) with δ_H 1.60 (H-5), and δ_H 1.60 (H-5) to δ_H 1.19 (H-27) [35]. The NMR data resembled those of the isolated compound **7** [27,28] except for the α-L-arabinopyranosyl portion attached to C-3. Therefore, compound **2** was determined as 3-O-[α-L-arabinopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-

xylopyranosyl]hederagenin 28-O-β-D-glucopyranosyl ester and given the trivial name Medicagoside B.

Compound **3** was a white amorphous powder. Its molecular formula was determined as $C_{53}H_{86}O_{23}$ by the negative-ion HRESIMS (m/z 1089.5474 [M − H]$^-$, calcd for $C_{53}H_{85}O_{23}$, 1089.5476). Compared with the NMR data (Tables 1 and 2) of compound **2**, compound **3** was identified as a bidesmosidic hederagenin saponin. The only difference between compounds **2** and **3** was the types of saccharide units assigned to C-3 of compound **3**. From acid hydrolysis experiment, D-glucose and D-xylose were found in compound **3**. Moreover, the saccharide portions were defined with β orientation by coupling constants [31]. The saccharide portion at C-3 of the aglycone was verified as β-D-xylopyranosyl (1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl moiety based on HMBC cross-peak (Fig. 2) of δ_H 5.35 (Xyl-H-1) with δ_C 84.6 (Glc-C-2′), δ_H 5.11 (Glc-H-1′) with δ_C 83.5 (Glc-C-2), and δ_H 5.53 (Glc-H-1) with δ_C 83.4 (C-3). The existence of β-D-glucopyranosyl ester at C-28 of compound **3** was confirmed on the basis of HMBC correlations from δ_H 6.32 (Glc-H-1″) to δ_C 176.4 (C-28). Thus, compound **3** was identified as 3-O-[β-D-xylopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl]hederagenin 28-O-β-D-glucopyranosyl ester and named as Medicagoside C.

Compound **4** was isolated as a white amorphous powder and had the same molecular formula ($C_{53}H_{86}O_{23}$) as compound **3** in accordance with the negative-ion HRESIMS (m/z 1089.5476 [M − H]$^-$, calcd for $C_{53}H_{85}O_{23}$, 1089.5476). Compound **4** was almost identical to compound **3** except for the presence of L-arabinose unit instead of the D-xylose unit as revealed by the NMR (Tables 1 and 2) analyses and acid hydrolysis experiment. Coupling constants ($^3J_{H1, H2}$ = 4.1 Hz) and NOESY (Fig. 3) analysis showed the α-configuration for arabinose unit [32,33]. β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-α-L-arabinopyranosyl portion and β-D-glucopyranosyl portion were bound to C-3 and C-28 of compound **4**, respectively, which were confirmed by the HMBC cross-peaks (Fig. 2) from δ_H 5.34 (Glc-H-1′) to δ_C 85.4 (Glc-C-2), δ_H 5.33 (Glc-H-1) to δ_C 80.0 (Ara-C-2), δ_H 5.37 (Ara-H-1) to δ_C 82.2 (C-3), and δ_H 6.35 (Glc-H-1″) to δ_C 176.4 (C-28). Thus, compound **4** was found to be 3-O-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-α-L-arabinopyranosyl]hederagenin 28-O-β-D-glucopyranosyl ester and given the trivial name Medicagoside D.

Compound **5** was obtained as a white amorphous powder with the molecular formula $C_{48}H_{76}O_{21}$ by the positive-ion HRESIMS at m/z 1011.4778 [M + Na]$^+$ (calcd for $C_{48}H_{76}O_{21}Na$, 1011.4771). In the ^{1}H NMR spectrum (Tables 1 and 2) and HSQC spectra, six tertiary methyl singlets [δ_H 0.94 (H-29), 1.01 (H-30), 1.05 (H-26), 1.26 (H-27), 1.54 (H-25) and 2.06 (H-24)], one olefinic proton [δ_H 5.50 (H-12)], as well as three anomeric protons (δ_H 5.09, 5.21 and 5.30) were found. The ^{13}C NMR spectrum showed representative olefinic carbon signals (δ_C 122.4 and 144.8) attributable to olean-12-ene moiety, two carboxyl groups (δ_C 180.1 and 180.8) and three anomeric carbons (δ_C 102.7, 104.5 and 106.6). It was evident from the NMR data that only C-3 position of aglycone was glycosylated and C-28 position contained one free carboxyl group. The presence of D-glucose in compound **5** was verified via acid hydrolysis experiment, NMR analysis along with the reported literature [36]. Their β-anomeric configurations were confirmed by the $^3J_{H1, H2}$ coupling constants [37]. All sugar signals of compound **5** were revealed by a combination of HMBC, HSQC, COSY and TOCSY spectra. The sequence of three sugar molecules localized at C-3 of compound **5** was inferred via the HMBC correlations (Fig. 2) between δ_H 5.09 (Glc-H-1″) and δ_C 85.6 (Glc-C-2′), δ_H 5.30 (Glc-H-1′) and δ_C 84.5 (Glc-C-2), δ_H 5.21 (Glc-H-1) and δ_C 85.5 (C-3), showing that the linkage was β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl portion. From the NOESY spectrum (Fig. 3), the stereochemistry of H-2 and H-3 of the aglycone was determined by long-range correlations of δ_H 4.87 (H-2) with δ_H 1.99 (H-5) and δ_H 1.77 (H-9), δ_H 4.75 (H-3) with δ_H 1.99 (H-5) and δ_H 1.77 (H-9), and δ_H 1.99 (H-5) with δ_H 1.26 (H-27) [38], suggesting the presence of the 2β,3β-dihydroxyoleanane skeleton. From the NMR analysis, the aglycone moiety of **5** was confirmed as
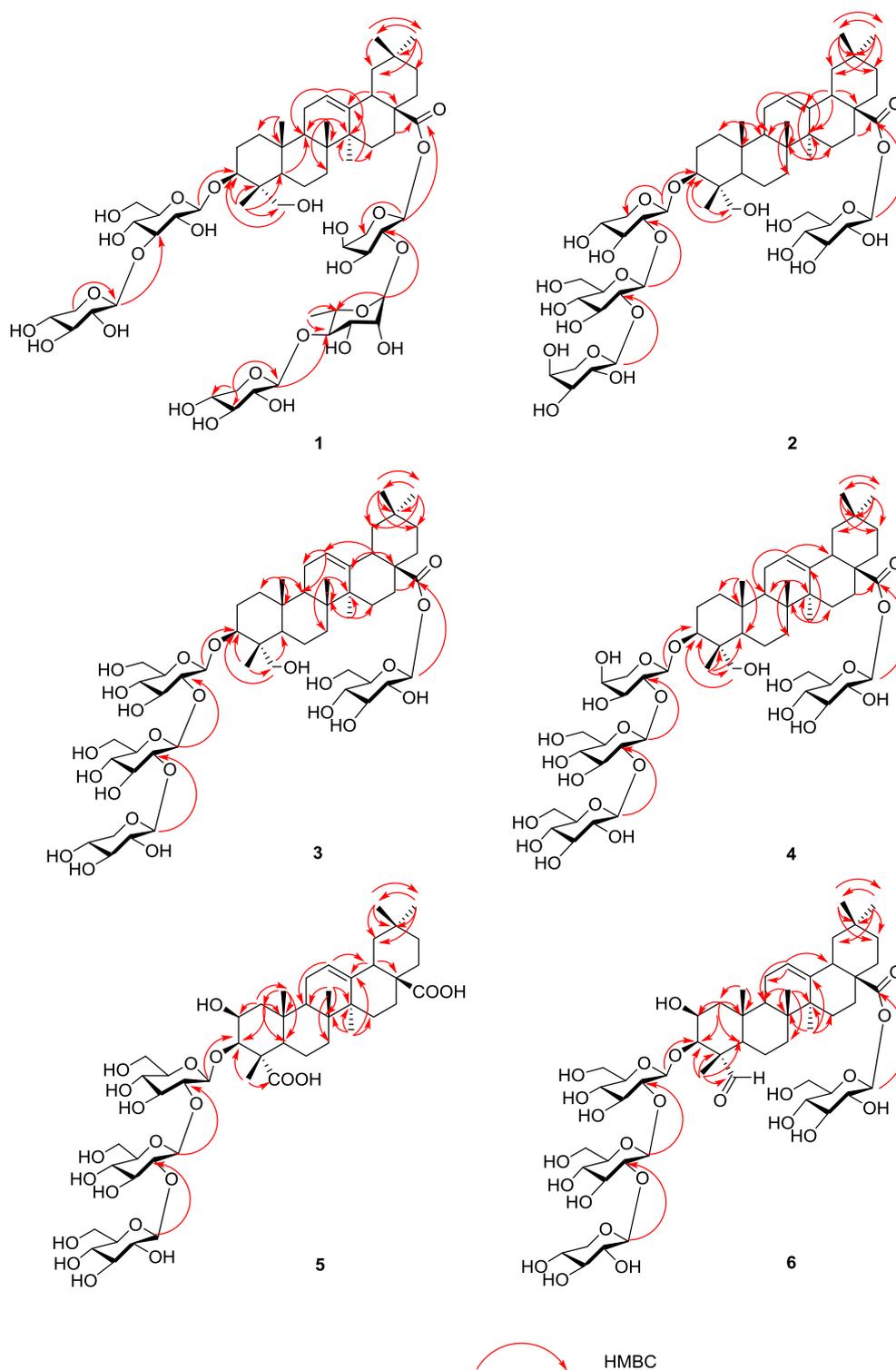
Fig. 2. Key HMBC correlations of compounds **1–6**.

$2\beta,3\beta$-dihydroxy medicagenic acid similar to that of the isolated compound **9** [22]. Consequently, compound **5** was determined as 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyrano-side]medicagenic acid and given the trivial name Medicagoside E.

Compound **6** was isolated as a white amorphous powder. Its molecular formula, $C_{53}H_{84}O_{24}$, was identified via the HRESIMS data (*m/z* 1103.5303 [M − H]⁻, calcd for 1103.5269). The ¹H NMR (Table 1) and HSQC data revealed the presence of six methyl singlets [δ_H 0.91 (H-30), 0.93 (H-29), 1.12 (H-26), 1.24 (H-27), 1.47 (H-25) and 1.76 (H-24)],

one aldehydic proton [δ_H 9.81 (H-23)] with a corresponding carbon (δ_C 208.2), and one olefinic proton [δ_H 5.44 (H-12)] with two typical olefinic carbon signals (δ_C 122.7 and 144.2), which were consistent with one $\triangle^{12}$ pentacyclic triterpene derivative. The ¹³C NMR results (Tables 1 and 2) showed 30 carbon signals of the aglycone and 23 of the saccharide unit. One hydroxyl group was bound to C-2 of the aglycon as revealed by the 1D NMR data and the HMBC correlation (Fig. 2) from δ_H 2.24 (H-1) to δ_C 69.0 (C-2). The α-configuration for H-2 and H-3 were identified by analysis of the NOESY correlations (Fig. 3) from δ_H
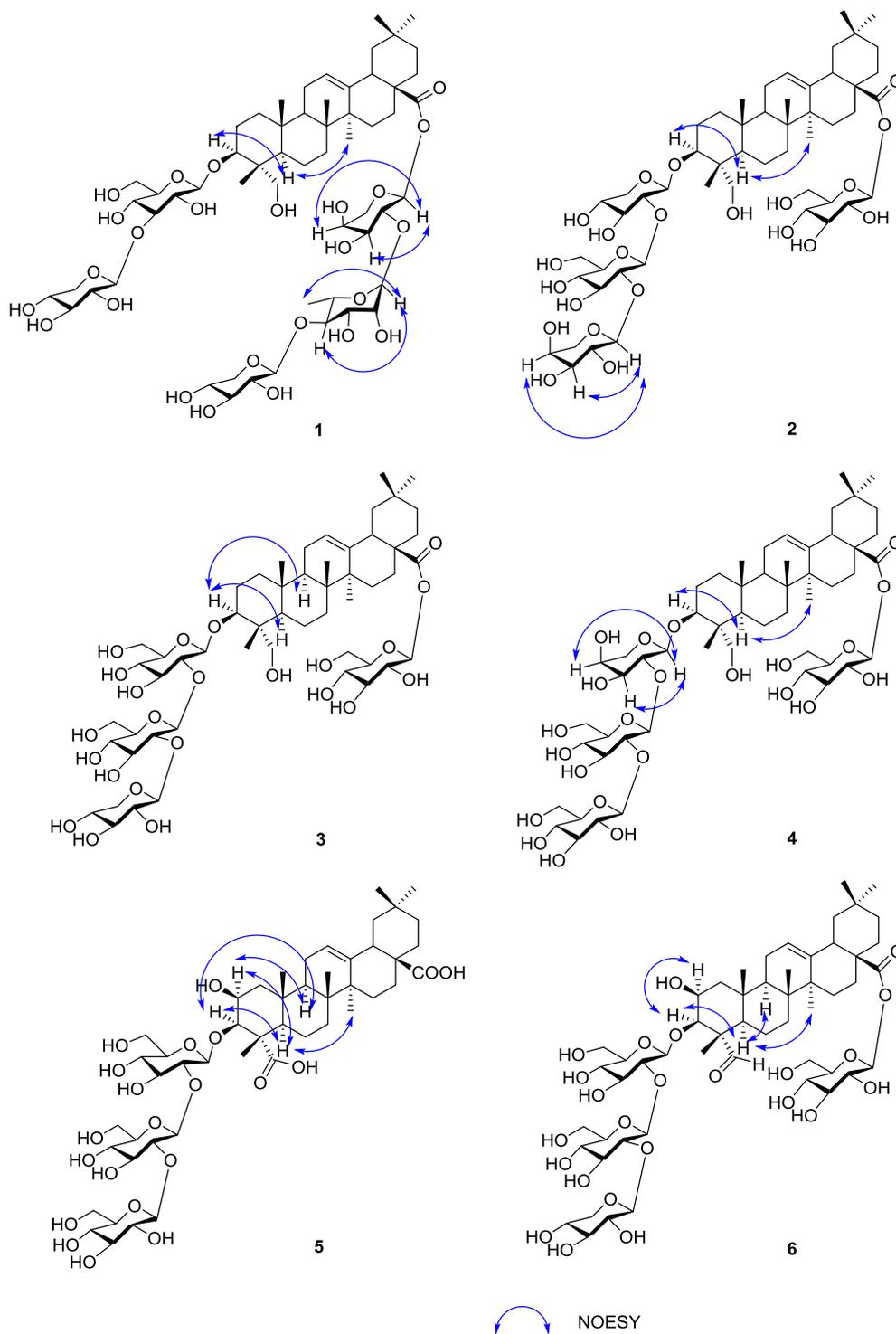
Fig. 3. Key NOESY correlations of compounds **1–6**.

4.65 (H-2) to δ_H 4.10 (H-3), δ_H 4.10 (H-3) to δ_H 1.44 (H-5), δ_H 1.44 (H-5) to δ_H 1.67 (H-9), and δ_H 1.44 (H-5) to δ_H 1.24 (H-27) [38]. One aldehyde group was assigned to C-4 of the aglycon by the HMBC cross-peak of δ_H 1.76 (H-24) with δ_C 208.2 (C-23). Therefore, the aglycone portion of compound **6** was designated as 2β,3β-dihydroxyolean-12-en-23-ale-28-oic acid [39]. Four sugar units were further determined using acid hydrolysis experiment, which confirmed the existence of the D-glucose and D-xylose. The β-anomeric configurations for these sugar units were also identified by the $^3J_{H1, H2}$ values of 7.1–8.1 Hz [31]. The HMBC correlation peaks were observed from δ_H 5.40 (Xyl-H-1) to δ_C

84.7 (Glc-C-2′), δ_H 5.37 (Glc-H-1′) to δ_C 83.3 (Glc-C-2), and δ_H 4.97 (Glc-H-1) to δ_C 81.9 (C-3), suggesting that β-D-xylopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl portion was bound to C-3. Likewise, the β-D-glucopyranosyl portion was bound to C-28. Therefore, compound **6** was identified as 3-*O*-[β-D-xylopyranosyl(1 → 2)-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside-2β, 3β-dihydroxyolean-12-en-23-ale-28-oic acid and named as Medicago-side F.

The neuroprotective effects of compounds **1–11** isolated from *M. sativa* were examined *in vitro* by the typical model of H$_2$O$_2$-induced
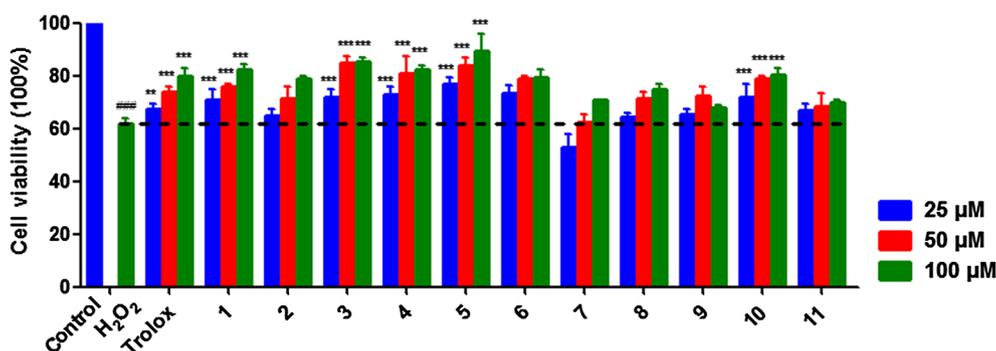
Fig. 4. Neuroprotective effects of compounds **1–11** against H_2O_2-induced injury in SH-SY5Y cells using CCK-8 assay. Detailed data were showed in the Supplementary Information Table S1. $^{###}P < 0.001$ vs. the control group, $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. the H_2O_2-treated cells group.

damage in SH-SY5Y cells. Cell viability was remarkably reduced to around 61% in H_2O_2-treated cells compared to the cells of the control group in which cell viability was 100%. As shown in Fig. 4, cell viability of H_2O_2-treated cells was improved following treatment with the isolated compounds compared with the H_2O_2-treated group. Compounds **2** and **6** restored the cell viability to 78.23% (100 μM) and 78.71% (100 μM), respectively, which was nearer to that of Trolox (79.46%, 100 μM). Notably, compounds **1**, **3–5** and **10** exhibited striking protective effects as the concentration was increased. Accordingly, cell viability at 100 μM reached 81.94%, 84.84%, 81.94%, 89.03%, and 79.66%, respectively, compared to Trolox. Among the compounds, the neuroprotective activity of compound **5** was the highest, while compound **9** (62.13%, 100 μM) showed a weak protective effect. Interestingly, the structures of compounds **5** and **9** only differed by the types of saccharide molecules bound to C-3 of the aglycon, suggesting that these types of saccharide molecules had an important influence on the neuroprotective effects of the compounds against the H_2O_2-induced damage in SH-SY5Y cells. In recent years, some researchers investigated the neuroprotective effects of oleanane-type triterpenoid saponins [40–41]. Zhang et al. [5] showed that congmusaponin I acted as a protective agent in neurodegenerative disease by inhibiting H_2O_2-induced apoptosis and fighting against oxidative stress. Wang et al. [24] indicated that autophagy was conducive to the protective effect of Eclalbasaponin I. Although mechanisms of neuroprotection by triterpenoid saponins remain obscure, those findings about neuroprotective activities of saponins (**1–11**) from *M. sativa* may provide some ideas for the development of agents to treat neurodegenerative diseases.

4. Conclusions

Six novel triterpenoid saponins (**1–6**) and five known ones (**7–11**) were isolated from *M. sativa*. The neuroprotective activities against H_2O_2-induced injury in SH-SY5Y cells of compounds **1–11** were evaluated using CCK-8 assay. The result indicated that all the isolates displayed neuroprotective activities. Among them, the protective activities of compounds **2** and **6** were nearer to that of Trolox used as the positive control. Notably, compounds **1**, **3–5** and **10** exhibited striking protective activities in contrast with Trolox. Taken together, these data sets show that triterpenoid saponins can be used to treat neurodegenerative diseases. Further studies are warranted to explore the structure-activity relationships of these compounds.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.11.008.

References

[1] M. Goldsmith, L. Abramovitz, P. Dan, Acs Nano 8 (2014) 1958–1965, https://doi.org/10.1021/nn501292z.
[2] J.N. Mazon, A.H. de Mello, G.K. Ferreira, G.T. Rezin, Life Sci. 182 (2017) 22–28, https://doi.org/10.1016/j.lfs.2017.06.002.
[3] G. Shevchenko, M. Wetterhall, J. Bergquist, K. Höglund, L.I. Andersson, K. Kultima, J. Proteome Res. 11 (2012) 6159–6174, https://doi.org/10.1021/pr300808h.
[4] G.P. Kumar, K.R. Anilakumar, S. Naveen, Pharmacognosy J. 7 (2015) 1–17, https://doi.org/10.5530/pj.2015.7.1.
[5] Y. Zhang, W. Wang, H. He, X.Y. Song, G.D. Yao, S.J. Song, J. Funct. Foods 45 (2018) 313–320, https://doi.org/10.1016/j.jff.2018.04.026.
[6] Y. Li, J.L. Xu, C.H. Yuan, H. Ma, T.T. Liu, F.F. Liu, N.P. Seeram, Y. Mu, X.S. Huang, L.Y. Li, J. Funct. Foods 28 (2017) 1–10, https://doi.org/10.1016/j.jff.2016.10.021.
[7] M.T. Lin, M.F. Beal, Nature 443 (2006) 787–795, https://doi.org/10.1038/nature05292.
[8] A. González-Sarrías, M.Á. Núñez-Sánchez, F.A. Tomás-Barberán, J.C. Espín, J. Agric. Food Chem. 65 (2017) 752–758, https://doi.org/10.1021/acs.jafc.6b04538.
[9] L. Jisun, G. Samantha, J. Zhang, Biochem. J. 441 (2012) 523–540, https://doi.org/10.1042/bj20111451.
[10] J. Ruffels, M. Griffin, J.M. Dickenson, Eur. J. Pharmacol. 483 (2004) 163–173, https://doi.org/10.1016/j.ejphar.2003.10.032.
[11] E. Gaweł, M. Grzelak, M. Janyszek, J. Herb. Med. 10 (2017) 8–16, https://doi.org/10.1016/j.hermed.2017.07.002.
[12] A. Stochmal, S. Piacente, C. Pizza, F. De Riccardis, R. Leitz, W. Oleszek, J. Agric. Food Chem. 49 (2001) 753–758, https://doi.org/10.1021/jf000876p.
[13] A. Mielmann, Brit. Food J. 115 (2013) 590–600, https://doi.org/10.1108/00070701311317865.
[14] R. Katarzyna, P. Paweł, W. Olga, G. Ryszard, B. Bogusław, Phytochem. Lett. 20 (2016) 520–539, https://doi.org/10.1016/j.phytol.2016.12.006.
[15] S. Sen, H.P.S. Makkar, K. Becker, J. Agric. Food Chem. 46 (1998) 131–140, https://doi.org/10.1021/jf970389i.
[16] B.P. Naidu, L.G. Paleg, G.P. Jones, Phytochemistry 31 (1992) 1195–1197, https://doi.org/10.1016/0031-9422(92)80259-h.
[17] L. Chen, J. Liu, Y. Zhang, B. Dai, Y. An, L.L. Yu, J. Agric. Food Chem. 63 (2015) 3219–3228, https://doi.org/10.1021/acs.jafc.5b00494.
[18] J.H. Wang, L.G. Zhou, J.G. Han, Z. Yu, Nat. Prod. Res. Dev. 21 (2009) 346–353 https://doi.org/10.16333/j.1001-6880.2009.02.019.
[19] K.S. Bora, A. Sharma, Pharm. Biol. 49 (2011) 211–220, https://doi.org/10.3109/13880209.2010.504732.
[20] G. Francis, Z. Keem, H.P.S. Makkar, K. Becker, Brit. J. Nutr. 88 (2002) 587–605, https://doi.org/10.1079/bjn2002725.
[21] J. Milgate, D.C.K. Roberts, Nutr. Res. 8 (1995) 1223–1249, https://doi.org/10.1016/0271-5317(95)00081-s.
[22] Z. Bialy, M. Jurzysta, W. Oleszek, S. Piacente, C. Pizza, J. Agric. Food Chem. 47 (1999) 3185–3192, https://doi.org/10.1021/jf9901237.
[23] A. Sun, X. Xu, J. Lin, X. Cui, R. Xu, Phytother. Res. 29 (2014) 187–200, https://doi.org/10.1002/ptr.5246.
[24] W. Wang, G.D. Yao, X.Y. Shang, J.C. Gao, Y. Zhang, S.J. Song, Biomed. Pharmacother. 97 (2018) 152–161, https://doi.org/10.1016/j.biopha.2017.10.

106.

[25] Y.K. Cheng, L.I. Lei, Z.K. Meng, A.L. Hou, Y.J. Wu, L.R. Teng, Chem. Res. Chinese Universities 24 (2008) 167–170, https://doi.org/10.1016/s1005-9040(08)60034-3.

[26] Y. Ni, L. Li, W. Zhang, D. Lu, C. Zang, D. Zhang, Y. Yu, X. Yao, J. Agric. Food Chem. 65 (2017) 2936–2946, https://doi.org/10.1021/acs.jafc.6b03866.

[27] W. Oleszek, K.R. Price, I.J. Colquhoun, M. Jurzysta, M. Ploszynski, G.R. Fenwick, J. Agric. Food Chem. 38 (1990) 1810–1817, https://doi.org/10.1021/jf00099a006.

[28] G. Massiot, C. Lavaud, L.L. Menolivier, G.V. Binst, S.P.F. Miller, H.M. Fales, J. Chem. Soc. Perkin Trans. I (12) (1988) 3071–3079, https://doi.org/10.1039/p19880003071.

[29] S.M.A. Khalik, T. Miyase, H.A. El-Ashaal, F.R. Melek, Phytochemistry 54 (2000) 853–859, https://doi.org/10.1016/s0031-9422(00)00168-0.

[30] B. Cui, J. Inoue, T. Takeshita, J. Kinjo, T. Nohara, Chem. Pharm. Bull. 40 (1992) 3330–3333, https://doi.org/10.1248/cpb.40.3330.

[31] F.R. Melek, I.A. Kassem, T. Miyase, W. Fayad, Phytochemistry 100 (2014) 110–119, https://doi.org/10.1016/j.phytochem.2014.01.019.

[32] H. Ishii, I. Kitagawa, K. Matsushita, K. Shirakawa, K. Tori, T. Tozyo, M. Yoshikaw, Y. Yoshimur, Tetrahedron Lett. 22 (1981) 1529–1532, https://doi.org/10.1016/S0040-4039(01)90369-7.

[33] S. Li, J. Zhao, Y. Liu, Z. Chen, Q. Xu, I.A. Khan, S. Yang, J. Agric. Food Chem. 62 (2014) 488–496, https://doi.org/10.1021/jf4046667.

[34] X.N. Han, C.Y. Liu, Y.L. Liu, Q.M. Xu, X.R. Li, S.L. Yang, J. Agric. Food Chem. 61 (2013) 12692–12699, https://doi.org/10.1021/jf405384r.

[35] Y. Wang, Y.F. Liu, C.L. Zhang, D. Liang, H. Luo, Z.Y. Hao, R.Y. Chen, D.Q. Yu, Phytochem. Lett. 15 (2016) 204–209, https://doi.org/10.1016/j.phytol.2016.01.007.

[36] W. Oleszek, M. Jurzysta, M. Poloszynski, I.J. Colquhoun, K.R. Price, G.R. Fenwick, J. Agric. Food Chem. 40 (1992) 191–196, https://doi.org/10.1021/jf00014a005.

[37] M. Lehbili, A.M. Alabdul, A. Kabouche, L. Voutquennenazabadioko, A. Abedini, H. Morjani, T. Sarazin, S.C. Gangloff, Z. Kabouche, Phytochemistry 144 (2017) 33–42, https://doi.org/10.1016/j.phytochem.2017.08.015.

[38] M. Lehbili, A.A. Magid, A. Kabouche, L. Voutquenne-Nazabadioko, H. Morjani, D. Harakat, Z. Kabouche, Phytochemistry 150 (2018) 40–49, https://doi.org/10.1016/j.phytochem.2018.03.005.

[39] A. Tava, M. Mella, P. Avato, M.P. Argentieri, Z. Bialy, M. Jurzysta, J. Agric. Food Chem. 53 (2005) 9954–9965, https://doi.org/10.1021/jf052468x.

[40] X. Yu, L.N. Wang, L. Ma, R. You, R. Cui, D. Ji, Y. Wu, C.F. Zhang, Z.L. Yang, H. Ji, Pharmacol. Biochem. Be. 101 (2012) 479–486, https://doi.org/10.1016/j.pbb.2012.02.014.

[41] J.Z. Wan, L.L. Deng, C.C. Zhang, Q. Yuan, J. Liu, Y.Y. Dun, Z.Y. Zhou, H.X. Zhao, C.Q. Liu, D. Yuan, T. Wang, Can. J. Physiol. Pharmacol. 94 (2016) 919–928, https://doi.org/10.1139/cjpp-2015-0262.