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Anti-breast cancer triterpenoid saponins from the thorns of *Gleditsia sinensis*

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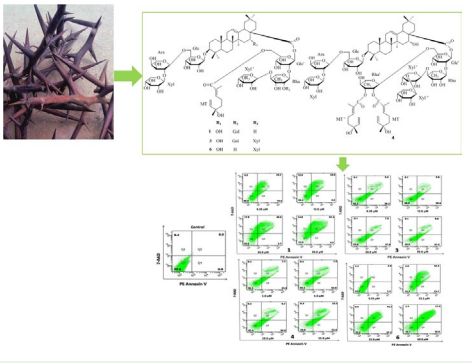
ABSTRACT

One new triterpenoid saponin (**1**), as well as six known ones (**2–7**), were isolated from the ethanol extract of the thorns of *Gleditsia sinensis*. Their structures were elucidated by extensive spectroscopic analysis in conjunction with chemical evidence. Cytotoxic activity of compounds **1–6** was evaluated against human breast cancer MCF 7 cells *in vitro* by the MTT method. Our results revealed moderate activities for compounds **1–6** with IC_{50} values of 18.43, 30.47, 18.46, 10.02, 30.76, and 17.32 μM, respectively. Furthermore, compounds **1**, **3**, **4**, and **6** induced apoptosis in MCF 7 cell, with **1** and **6** causing late apoptosis of MCF 7 cells, while **3** and **4** acting oppositely.



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KEYWORDS
Gleditsia sinensis; triterpenoid saponin; cytotoxic activity; apoptosis

1. Introduction

Gleditsia sinensis Lam. is a deciduous arbor from the Fabaceae family, which is widely cultivated in China, such as the Shandong, Hebei and Jiangsu provinces. Its thorns, known as 'Zao Jiao Ci', are traditional Oriental medicine as an efficacious therapeutic agent for the

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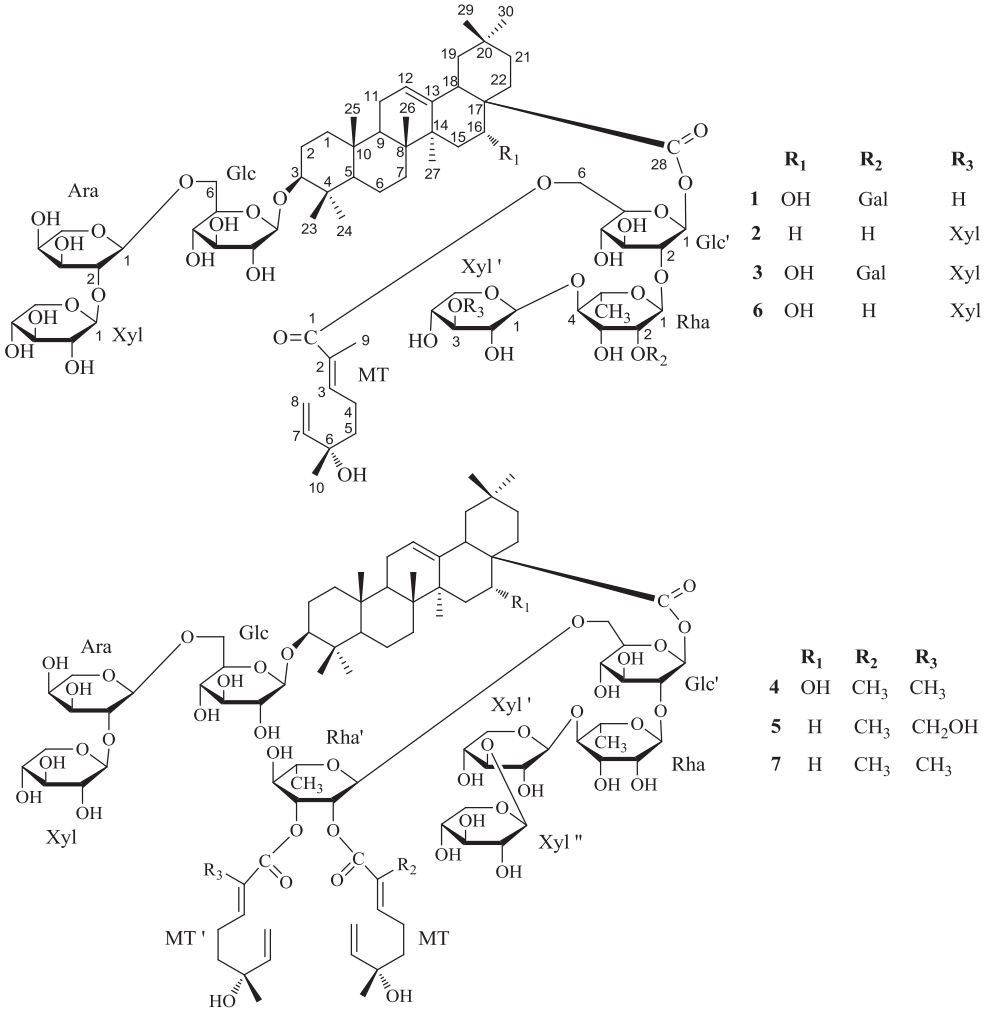
treatment of carbuncle, cancers, skin diseases and suppuration (Lee et al. 2013). In previous studies of *G. sinensis,* several classes of compounds were obtained, including triterpenoid saponins from the fruit (Zhong et al. 2004; Melek et al. 2014; Liu et al. 2016), flavonoids (Zhou et al. 2007a; Yu et al. 2017), ellagic acid glycosides and ferulic acid esters from the thorns (Zhou et al. 2007b; Yu et al. 2015). On the basis of the significant cytotoxicity of the ethanol extract of the thorns of *G. sinensis* against human breast cancer MCF 7 cells by our earlier phytochemical investigation, a search for novel anti-cancer metabolites were investigated and led to the isolation of one new triterpenoid saponin (**1**) and six known ones (**2–7**) for the first time from the thorns of *G. sinensis* (Figure 1), as well as the *in vitro* evaluation of their cytotoxic activities against MCF 7 tumor cells by the MTT method. Compounds **1–6** showed potential anticancer activity with IC_{50} values of 18.43, 30.47, 18.46, 10.02, 30.76, and 17.32 μM, respectively. Furthermore, the apoptotic activities of compounds **1**, **3**, **4**, and **6** were evaluated on MCF 7 cells, with **1** and **6** causing late apoptosis of MCF 7 cells, while **3** and **4** acting oppositely.



	R_1	R_2	R_3
1	OH	Gal	H
2	H	H	Xyl
3	OH	Gal	Xyl
6	OH	H	Xyl

	R_1	R_2	R_3
4	OH	CH_3	CH_3
5	H	CH_3	CH_2OH
7	H	CH_3	CH_3

Figure 1. Structures of compounds **1–7**.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder and gave positive Libermann–Burchard reaction, indicating its triterpenoid nature. Its molecular formula was determined to be $C_{79}H_{126}O_{37}$ by the molecular ion peak $[M + Na]^+$ at *m/z* 1689.7851 detected in the positive HRESIMS analysis (calcd. for $C_{79}H_{126}O_{37}Na$, 1689.7876), and indicative of 17 unsaturation degrees. The IR spectrum of **1** exhibited characteristic absorptions of hydroxy group (3411 cm^{-1}), ester carbonyl group (1709 cm^{-1}), and conjugated ester carbonyl group (1651 cm^{-1}). The ^{13}C NMR spectrum of **1** exhibited 79 carbon signals assignable to 10 methyls, 18 methylenes including one terminal double bond at δ_C 111.12 (MT-C-8), 40 methines including 34 oxygen-connected methines, three non oxygen-connected methines and three olefinic methines at δ_C 122.24 (C-12), 143.02 (MT-C-3) and 144.54 (MT-C-7), and 11 quaternary carbons including two ester carbonyl groups at δ_C 175.57 (C-28) and 168.08 (MT-C-1), and two olefinic carbons at 143.10 (C-13) and 127.02 (MT-C-2), with 30 signals being assigned as aglycone, 39 as the oligosaccharide moiety of three hexoses, one methylpentose and three pentoses, and the remaining 10 signals as a monoterpenic acid. The oligosaccharide moiety of **1** contained seven anomeric proton signals at δ_H 4.31 (1H, d, *J* = 6.0 Hz, Glc-H-1), 4.39 (1H, d, *J* = 7.8 Hz, Gal-H-1), 4.42 (1H, d, *J* = 7.2 Hz, Xyl-H-1), 4.52 (2H, overlapped, Ara-H-1, Xyl′-H-1), 5.36 (1H, d, *J* = 7.8 Hz, Glc′-H-1), and 5.69 (1H, d, *J* = 1.2 Hz, Rha-H-1), as well as their corresponding carbon signals at δ_C 105.13 (Glc-1), 101.89 (Ara-1), 105.22 (Xyl-1), 93.02 (Glc′-1), 99.05 (Rha-1), 105.08 (Xyl′-1), and 105.87 (Gal-1), respectively, as shown in the HSQC spectrum. The chemical shift of δ_C 88.54 (C-3) and 175.57 (C-28) indicated that **1** was a bisdesmosidic glycoside (Zhang et al. 1999a). Comparison of the NMR spectroscopic data of compound **1** with those of **3** (Zhang et al. 1999b) verified them to be almost consistent with each other, except for the sugar chain linked at C-28. Acid hydrolysis of **1** afforded D-xyloses, L-arabinose, D-glucoses, D-galactose, and L-rhamnose as sugar components by GC analysis. The trisaccharide chain linked at C-3 was unequivocally established by HMBC correlations from δ_H 4.42 (Xyl-H-1) to δ_C 79.91 (Ara-C-2), δ_H 4.52 (Ara-H-1) to δ_C 68.13 (Glc-C-6), δ_H 4.31 (Glc-H-1) to δ_C 88.54 (C-3); similarly, the tetrasaccharide chain attached to C-28 was also based on the HMBC correlations from δ_H 4.52 (Xyl′-H-1) to δ_C 82.03 (Rha-C-4), δ_H 4.39 (Gal-H-1) to δ_C 80.55 (Rha-C-2), δ_H 5.69 (Rha-H-1) to δ_C 75.52 (Glc′-C-2), δ_H 5.36 (Glc′-H-1) to δ_C 175.57 (C-28). Additionally, the monoterpenic acid in **1** was indicated the same as that in **3**, as well as the attachment position, which was substantiated by HMBC correlations of δ_H 3.47 (Glc′-H-6α) with 168.08 (MT-C-1). Therefore, the structure of **1** was elucidated as 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl echinocystic acid 28-*O*-β-D-xylopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)]-α-L-rhamnopyranosyl-(1→2)-[(6*S*,2*E*)-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl ester, named glespinoside A.

The known compounds, gleditsioside A (**2**) (Zhang et al. 1999b), gleditsioside D (**3**) (Zhang et al. 1999b), gleditsioside F (**4**) (Zhang et al. 1999a), gleditsioside G (**5**) (Zhang et al. 1999a), caspicaoside G (**6**) (Melek et al. 2014), and caspicaoside I (**7**) (Melek et al. 2014), were identified by comparison with NMR data in the literature. This study presents the isolation of these triterpenoid saponins from the thorns of *G. sinensis* for the first time, while some known saponins have previously been repoted from the fruit of *G. sinensis*. This could lead to a conclusion that the thorns of *G. sinensis* contain triterpenoid saponins, thus tending to interlink with the fruit.

To evaluate the cytotoxic activity of the isolated compounds (**1–6**), a MTT method was applied to test their *in vitro* growth inhibition against human breast cancer MCF 7 cells with cisplatin as positive control (IC_{50} value of 5.24 μM). Compounds **1–6** showed moderate activity with IC_{50} values of 18.43, 30.47, 18.46, 10.02, 30.76, and 17.32 μM, respectively, which provided some support for the application of the thorns of *G. sinensis* in some Chinese medicine prescriptions to treat breast cancer. Flow cytometry analysis was used to determine cell apoptosis induction of compounds **1**, **3**, **4**, and **6**, with IC_{50} values less than 20 μM. Measured results indicated that **1** and **6** mainly caused late apoptosis of MCF 7 cells, while **3** and **4** acting oppositely (Figure 2).

3. Experimental

3.1. General experimental procedures

The instruments used for IR, NMR, ESI-MS, HRESI-MS, and preparative HPLC experiments were the same as those in our previous study (Yu et al. 2017). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., China), D-101 resin (16–60 mesh, Bochum Tianjin Resin

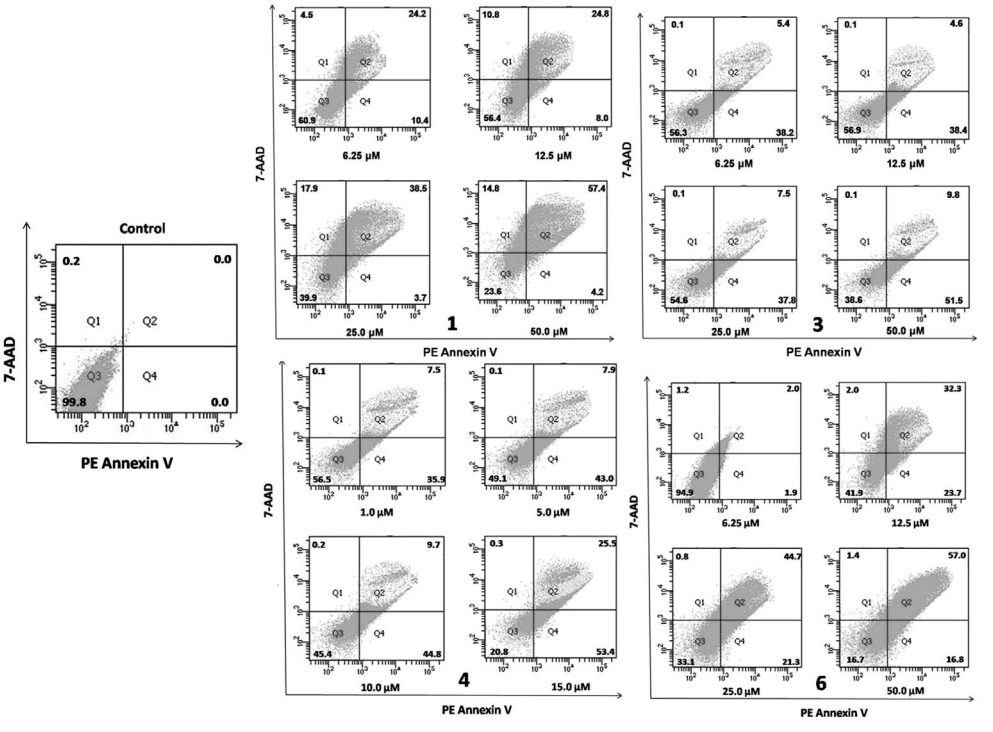


Figure 2. Compounds **1**, **3**, **4** and **6** caused apoptosis of MCF-7 cells). **1**, **3** and **6** at 6.25 μM caused early apoptosis of 10.4, 38.2, and 1.9%, respectively, and late apoptosis of 24.2, 5.4, and 2.0%, respectively; at 12.5 μM caused early apoptosis of 8.0, 38.4 and 23.7%, respectively, and late apoptosis of 24.8, 4.6, and 32.3%, respectively; at 25 μM caused early apoptosis of 3.7, 37.8 and 21.3%, respectively, and late apoptosis of 38.5, 7.5, and 44.7%, respectively; at 50 μM caused early apoptosis of 4.5, 51.5 and 16.8%, respectively, and late apoptosis of 57.4, 79.7, and 57.0%, respectively; while, at concentrations of 1.0, 5.0, 10 and 15 μM, **4** caused early apoptosis of 35.9, 43.0, 44.8, and 53.4%, respectively, as well as late apoptosis of 7.5, 7.9, 9.7, and 25.5%, respectively.

Technology Co. Ltd., China), and ODS (45 μm, YMC Co. Ltd., Japan) were used for Flash column chromatography (CC). Acetonitrile used in preparative HPLC was in HPLC grade, and other solvents were of analytical grade.

3.2. Plant material

The origin of the thorns of *G. sinensis* was the same as that reported in our previous study (Yu et al. 2017).

White amorphous powder. IR (KBr) ν_{max}: 3411, 2932, 1709, 1651, 1281, 1043 cm^{-1}; ESI-MS (positive-ion mode): *m/z* 1689.5 $[M + Na]^+$; HRESI-MS (positive-ion mode): *m/z* 1689.7851 $[M + Na]^+$ (calcd for $C_{79}H_{126}O_{37}Na$, 1689.7870). ^1H NMR (CD_3OD, 600 MHz) δ_H (ppm) aglycon: H-1*α* (1.58), H-1*β* (1.04), H-2*α* (1.82), H-2*β* (1.61), H-3 (3.26), H-5 (0.75), H-6*α* (1.53), H-6*β* (1.33), H-7*α* (1.69), H-7*β* (1.39), H-9 (1.59), H_{2-11} (1.82), H-12 (5.27, br s), H-15*α* (1.60), H-15*β* (1.40), H-16 (4.44, t, *J* = 4.2 Hz), H-18 (2.85), H-19*α* (2.27), H-19*β* (1.01), H-21*α* (1.88), H-21*β* (1.12), H-22*α* (1.81), H-22*β* (1.74), Me-23 (1.03, s), Me-24 (0.82, s), Me-25 (0.90, s), Me-26 (0.70, s), Me-27 (1.35, s), Me-29 (0.84, s), Me-30 (0.92, s); sugar moiety: Glc-H-1 (4.31, d, *J* = 6.0 Hz), Glc-H-2 (3.30), Glc-H-3 (3.31), Glc-H-4 (3.54), Glc-H-5 (3.42), Glc-H-6*α* (3.70), Glc-H-6*β* (3.99), Ara-H-1 (4.52, overlapped), Ara-H-2 (3.67), Ara-H-3 (3.68), Ara-H-4 (3.19), Ara-H-5*α* (4.16), Ara-H-5*β* (4.29), Xyl-H-1 (4.42, d, *J* = 7.2 Hz), Xyl-H-2 (3.27), Xyl-H-3 (3.48), Xyl-H-4 (3.45), Xyl-H-5*α* (3.90), Xyl-H-5*β* (3.16), Glc′-H-1 (5.36, d, *J* = 7.8 Hz), Glc′-H-2 (3.50), Glc′-H-3 (3.51), Glc′-H-4, 5 (3.27, overlapped), Glc′-H-6*α* (3.47), Glc′-H-6*β* (3.84), Rha-H-1 (5.69, d, *J* = 1.2 Hz), Rha-H-2 (3.99), Rha-H-3 (3.89), Rha-H-4 (3.51), Rha-H-5 (3.67), Rha-H_{3-6} (1.27, d, *J* = 7.8 Hz), Gal-H-1 (4.39, d, *J* = 7.8 Hz), Gal-H-2 (4.43), Gal-H-3 (3.47), Gal-H-4 (3.80), Gal-H-5 (3.45), Gal-H-6*α* (3.69), Gal-H-6*β* (3.83), Xyl′-H-1 (4.52, overlapped), Xyl′-H-2 (3.73), Xyl′-H-3 (3.29), Xyl′-H-4 (3.77), Xyl′-H-5*α* (3.91), Xyl′-H-5*β* (3.17), MT-H-3 (6.77, t, *J* = 7.8 Hz), MT-H_{2-4} (2.23), MT-H_{2-5} (1.60), MT-H-7 (5.88, dd, *J* = 10.8, 17.4 Hz), MT-H-8a (5.05, dd, *J* = 1.8, 10.8 Hz), MT-H-8b (5.21, dd, *J* = 1.8, 17.4 Hz), MT-H_{3-9} (1.79, s), MT-H_{3-10} (1.26, s). ^{13}C NMR (CD_3OD, 150 MHz) δ_C (ppm) aglycon: C-1 (38.50), C-2 (25.85), C-3 (88.54), C-4 (38.84), C-5 (55.66), C-6 (17.99), C-7 (32.84), C-8 (39.39), C-9 (46.47), C-10 (36.47), C-11 (23.16), C-12 (122.24), C-13 (143.10), C-14 (41.20), C-15 (34.97), C-16 (73.31), C-17 (48.58), C-18 (40.32), C-19 (46.68), C-20 (29.92), C-21 (34.97), C-22 (30.68), C-23 (27.07), C-24 (15.67), C-25 (14.86), C-26 (16.49), C-27 (25.85), C-28 (175.57), C-29 (31.97), C-30 (23.82); sugar moiety: Glc-C-1 (105.13), Glc-C-2 (74.29), Glc-C-3 (76.73), Glc-C-4 (71.44), Glc-C-5 (74.56), Glc-C-6 (68.13), Ara-C-1 (101.89), Ara-C-2 (79.91), Ara-C-3 (71.72), Ara-C-4 (67.08), Ara-C-5 (63.10), Xyl-C-1 (105.22), Xyl-C-2 (74.21), Xyl-C-3 (76.10), Xyl-C-4 (69.64), Xyl-C-5 (65.81), Glc′-C-1 (93.02), Glc′-C-2 (75.52), Glc′-C-3 (77.75), Glc′-C-4 (70.16), Glc′-C-5 (73.37), Glc′-C-6 (64.01), Rha-C-1 (99.05), Rha-C-2 (80.55), Rha-C-3 (70.38), Rha-C-4 (82.03), Rha-C-5 (67.43), Rha-C-6 (17.09), Gal-C-1 (105.87), Gal-C-2 (72.19), Gal-C-3 (73.42), Gal-C-4 (68.93), Gal-C-5 (75.22), Gal-C-6 (61.32), Xyl′-C-1 (105.08), Xyl′-C-2 (72.19), Xyl′-C-3 (76.62), Xyl′-C-4 (69.61), Xyl′-C-5 (65.81), MT-C-1 (168.08), MT-C-2 (127.02), MT-C-3 (143.02), MT-C-4 (23.12), MT-C-5 (40.78), MT-C-6 (70.69), MT-C-7 (144.54), MT-C-8 (111.12), MT-C-9 (11.09), MT-C-10 (26.46).

4. Conclusion

The phytochemical investigation of the active ethanol extract from the thorns of *G. sinensis* resulted in the isolation of seven triterpenoid saponins (**1–7**), one of which is new (**1**). Six

compounds (**1–6**) showed potential cytotoxic activity against MCF 7 cells, while only **1**, **3**, **4**, and **6** showed moderate cytotoxicities with IC_{50} values less than 20 μM. Obviously, an additional hydroxyl group with α-configuration at C-16 is favorable for the cytotoxic activity. Additionally, compounds **1**, **3**, **4**, and **6** induced apoptotic cell death in MCF 7 cells, with **1** and **6** causing late apoptosis, while **3** and **4** acting oppositely. Collectively, the results warrant further preclinical investigations of these saponins with α-OH at C-16 as potential novel anticancer agents.

Supplementary material

The extraction and isolation experiments, acid hydrolysis experiments, cytotoxicity assay and the original spectra of HR-ESI-MS, NMR data of compound **1** are available as Supporting Information, as well as the ^{1}H and ^{13}C NMR data of **2–7**.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

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References

Lee SJ, Ryu DH, Jang LC, Cho SC, Kim WJ, Moon SK. 2013. Suppressive effects of an ethanol extract of *Gleditsia sinensis* thorns on human SNU-5 gastric cancer cells. Oncol Rep. 29:1609–1616.

Liu C, Sun H, Wang WT, Zhang JB, Cheng AW, Guo X, Sun JY. 2016. A new triterpenoid saponin from *Gleditsia sinensis* and its antiproliferative activity. Nat Prod Res. 30:2065–2070.

Melek FR, Kassem IAA, Miyase T, Fayad W. 2014. Caspicaosides E-K, triterpenoid saponins and cytotoxic acylated saponins from fruits of *Gleditsia caspica* Desf. Phytochemistry. 100:110–119.

Yu JQ, Xian YX, Geng YL, Wang DJ, Zhou HL, Wang X. 2015. Anti-liver cancer constituents from the thorns of *Gleditsia sinensis*. Phytochem Lett. 13:343–347.

Yu JQ, Xian YX, Li G, Wang DJ, Zhou HL, Wang X. 2017. One new flavanocoumarin from the thorns of *Gleditsia sinensis*. Nat Prod Res. 31:275–280.

Zhang ZZ, Koike K, Jia ZH, Nikaido T, Guo D, Zheng JH. 1999a. Triterpenoidal saponins acylated with two monoterpenic acids from *Gleditsia sinensis*. Chem Pharm Bull. 47:388–393.

Zhang ZZ, Koike K, Jia ZH, Nikaido T, Guo D, Zheng JH. 1999b. Four new triterpenoidal saponins acylated with one monoterpenic acid from *Gleditsia sinensis*. J Nat Prod. 62:740–745.

Zhong L, Li P, Han J, Qu GQ, Guo D. 2004. Structure-activity relationships of saponins from *Gleditsia sinensis* in cytotoxicity and induction of apoptosis. Planta Med. 70:797–802.

Zhou LG, Li D, Wang JG, Liu YS, Wu JY. 2007a. Antibacterial phenolic compounds from the spines of *Gleditsia sinensis* Lam. Nat Prod Res. 21:283–291.

Zhou LG, Li D, Jiang W, Qin ZZ, Zhao S, Qiu MH, Wu JY. 2007b. Two ellagic acid glycosides from *Gleditsia sinensis* Lam. with antifungal activity on *Magnaporthe grisea*. Nat Prod Res. 21:303–309.