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Original research paper

Chemical fingerprinting and quantitative analysis of two common *Gleditsia sinensis* fruits using HPLC-DAD

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Gleditsiae Fructus Abnormalis and Gleditsiae Sinensis Fructus are obtained from different developmental stages of fruits from *Gleditsia sinensis* Lam. (Leguminosae). The possible interchangeable usage of the two fruits, however, has long been very controversial. Here, high performance liquid chromatography coupled with diode array detection was developed to explore their chemical fingerprinting profiles. Besides, the amounts of aglycones of saponin compounds, echinocystic acid and oleanolic acid in both fruits were quantified. The results indicated that there was no significant difference in the content of aglycones from the two types of fruits. However, their chromatographic fingerprints showed distinct characteristics. Therefore, the interchangeable application of these fruits has to be taken with a specific precaution.

Keywords: Gleditsiae Fructus Abnormalis, Gleditsiae Sinensis Fructus, HPLC-DAD, fingerprinting

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Gleditsia sinensis Lam. (Leguminosae) is a saponin-rich plant. Two main types of fruits can be generated from this plant, namely Gleditsiae Sinensis Fructus (GSF), the bigger-sized, normal fruit, and Gleditsiae Fructus Abnormalis (GFA), the abnormal fruit of older or damaged fruits. As they are rich in saponins with antibacterial functions (1–6), both fruits have been widely used in soaps, shampoos, detergents or toothpastes in our daily life. Herbal products containing triterpene saponins of *G. sinensis* are available in Europe, America, Asia and Africa, and are mainly imported from China where the herb is dominantly cultivated.

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In the herbal market, the price of GFA is much higher than that of GSF, which is mainly due to the availability of the latter. Thus, herbal traders suggest replacing GFA by GSF for daily application (7). Whether the two fruits could be used in similar applications is still not known. There are many types of triterpenoidal saponins found in GFA or GSF: about 19 saponins have been isolated and identified, which were also reported to possess numerous bioactivities (8–13). The aglycones of these saponins were identified as echinocystic acid and oleanolic acid and were proposed to be active ingredients in numerous medicinal herbs (14–17). At present, the aglycons of saponin compounds are considered to be appropriate chemical compounds for the quality control of herbal medicines, since they are convenient in terms of method development and marker availability (18–20). Besides, HPLC fingerprint has been considered to be a comprehensive approach for assessing the quality of herbal medicines (21). Today, this technique is widely used for quality control and species differentiation of herbs (22, 23). We therefore employed the HPLC fingerprint and quantitative analysis approaches to reveal the variation between the two fruits.

EXPERIMENTAL

Chemicals and materials

Twelve herb samples were collected from different geographical regions of China. Ten batches of GFA and two batches of GSF were collected from the same plants. Plant materials were authenticated according to their morphological characteristics. Voucher specimens were deposited in the Centre for Chinese Medicine at Hong Kong University of Science & Technology, Hong Kong, China.

The chemical standards of echinocystic acid and oleanolic acid were purchased from the ChromaDex (ChromaDex, USA) and the National Institute for the Control of Pharmaceutical & Biological Products (NICPBP, China). Their purity was determined to be over 98 % by normalization of peak areas, as revealed by HPLC-DAD.

Gleditsioside A was isolated from the roots of GFA, and its structure was established by comparison of its spectral data (mass spectrum, ^{1}H NMR and ^{13}C NMR) with the literature data (8, 12). In general, the purity of a chemical marker should be over 98 %, which could be used for quantification. As for gleditsioside A, the main saponin in the fruits, used as a marker for HPLC fingerprint, was purified in-house. This chemical was rather unstable during storage, and the purity was around 95 %. Thus, this chemical could be used as a reference standard for qualitative HPLC fingerprint only.

HPLC-grade acetonitrile was purchased from Merck (Germany) and ultra-pure water was prepared using a Milli-Q purification system (Millipore S.A.S., France). Other reagents used were of analytical grade.

Preparation of sample solutions

Stock solutions of echinocystic acid, oleanolic acid and gleditsioside A were prepared in concentrations of 1000, 800 and 200 mg L^{-1}, respectively, by accurately weighing

each compound into a volumetric flask and dissolving it in methanol. Stock solutions were stored at –20 °C.

GFA was pulverized and dried to constant mass and then passed through a 0.25-mm sieve before use. For fingerprint analysis, 0.1 g of sample was accurately weighed and placed into a 15-mL Falcon tube and extracted with 10 mL 25 % methanol in an ultrasonic bath for 30 min. The solution was filtered through a 0.45-μm Millipore filter before injection of 10 μL into the HPLC.

For quantitative analysis, 0.5 g of herbal powder was accurately weighed and placed into a 250-mL round-bottomed flask, reflux was extracted with 25 mL of methanol for 30 min, the solution was transferred into a 50-mL centrifuge tube and centrifuged at 4,000 x *g* for 5 min. Ten mL of the supernatant was pipetted into a 250-mL round-bottomed flask and concentrated to dryness under vacuum using a rotary evaporator. The residue was dissolved in 10 mL of diluted hydrochloric acid/ethanol solution and reflux was extracted for 30 min. After cooling, the solution was transferred into a 15-mL centrifuge tube and centrifuged at 4,000 x *g* for 5 min. The supernatant was discarded and the residue was transferred to a 25-mL volumetric flask and made up to the mark with methanol. The solution was filtered through a 0.45-μm Millipore filter before HPLC analysis.

Apparatus and chromatographic conditions

The HPLC system consisted of an Agilent 1200 binary pump (Agilent, Germany), an auto sampler and a DAD detector at 210 nm for all analyses. Chromatographic separation was carried out on an Alltima C_{18} column (4.6 x 250 mm, 5 μm). For fingerprint analysis, the mobile phase consisted of acetonitrile (A) and water (B) using the following gradient program: 0–60 min, 25.0–40.0 % A. A pre-equilibration period of 10 min was used between individual runs. For quantitative analysis, the mobile phase consisted of acetonitrile and water using the following gradient program: 0–10 min, 65.0 % A, 10–20 min, 65.0–85.0 % A, 20–30 min, 85 % A. A pre-equilibration period of 10 min was used between individual runs. The column temperature was 25 °C with a flow rate of 1.0 mL min^{-1} at 210 nm and the injection volume of 10 μL.

For the MS analysis, an Agilent QQQ-MS/MS (6410B, Germany) equipped with an ESI (electrospray ionization) ion source was operated in positive ion mode. The drying gas, nitrogen temperature and flow were 325 °C and 10 L min^{-1}, respectively, nebulizer pressure was 241 kPa, capillary voltage 4.0 kV; delta electro multiplier voltage 400 V. Agilent Mass Hunter workstation software version B.01.00 was used for data acquisition and processing.

Method validation (18, 19, 24)

Linearity. – Linearity was established by injection of 10 μL of mixed standard solution at six different concentrations. Calibration curve was plotted subsequently based on linear regression analysis of the integrated peak *vs.* concentration (μg mL^{-1}). Each calibration curve included six data points.

Limit of detection and limit of quantification. – The limits of detection (*LOD*) and quantification (*LOQ*) were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively.

Precision. – The precision of the method was validated by determining of intra- and inter-day variability. The intra- and inter-day precision was determined by analyzing six replicates of the standard solution of the two analytes during a single day and six replicates of the sample examined on 3 successive days, respectively. The relative standard deviation (RSD) was taken as a measure of intermediate precision.

Recovery. – A recovery test was used to evaluate the accuracy of method. An exact amount of each standard was added to accurately weighed 0.5 g of GFA (sample C), which was then extracted and analysed as described. For comparison, a blank sample (not spiked with standard compounds) was prepared and analyzed.

HPLC fingerprinting. – In HPLC fingerprinting, a well resolved peak corresponding to an available chemical reference substance can be used as a marker peak for the calculation of RRTs (relative retention times) of other peaks in the same chromatogram. The RRT of a characteristic peak is calculated retention time of a chosen marker peak. For positive identification, the sample must give all characteristic peaks with the RRTs falling within the acceptable range.

RESULTS AND DISCUSSION

Extraction procedure and chromatographic conditions

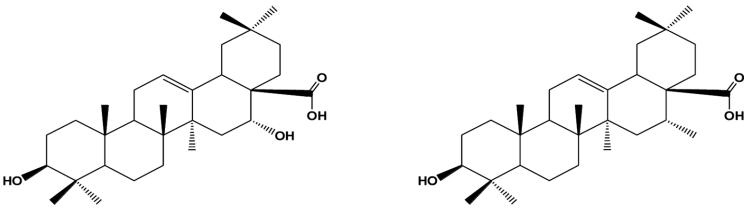
The contents of echinocystic acid and oleanolic acid (Fig. 1a) were determined in a hydrochloric acid hydrolysed herbal extract. Fig. 1b shows a typical HPLC profile of mixed standards and an extract of GFA or GSF at 210 nm. In order to obtain a quantitative extraction of analytes from GFA or GSF, the variables involved in the extraction procedure were optimized. Different extraction solvents (methanol, 70 % methanol, absolute ethanol and 70 % ethanol) and different concentrations of hydrochloric acid/50 % ethanol (0.5, 1.0 and 2.0 mol L^{-1} HCl) were tested. Extraction by methanol and hydrolysis by 2 mol L^{-1} HCl/50 % ethanol were found to be optimal (Fig. 2). Different ratios of acetonitrile/water and methanol/water were investigated for the optimal mobile phase, and the quantitative analysis was achieved by gradient elution using a binary mixture of acetonitrile and water.

Method validation

Linearity. – Linearity was observed in the concentration range 1.0–800 μg mL^{-1} for echinocystic acid and 1.9–1000 μg mL^{-1} for oleanolic acid. Calibration curves depict excellent correlations for echinocystic acid (R^2 = 0.9998) and oleanolic acid (R^2 = 0.9990).

LOD and *LOQ*. – The *LOD*s for two analytes were 0.05 and 0.06 μg mL^{-1}. Their *LOQ*s were 1.00 and 1.50 μg mL^{-1}, respectively.

a)



b)

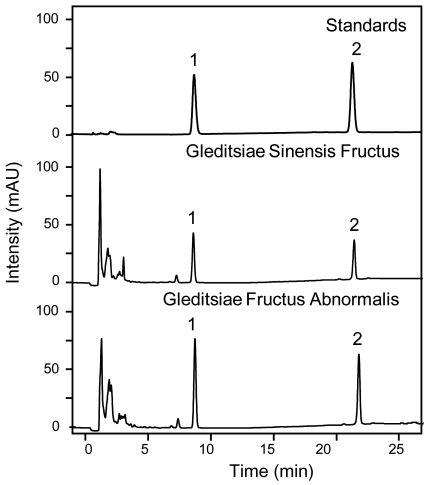


Fig. 1. a) Chemical structures of echinocystic acid and oleanolic acid analyzed in Gleditsiae Fructus Abnormalis and Gleditsiae Sinensis Fructus. b) Representative HPLC chromatograms of mixed standards and extract of Gleditsiae Fructus Abnormalis and Gleditsiae Sinensis Fructus. 1 – echinocystic acid, 2 – oleanolic acid.

Precision. – Precise values evaluated on model samples were as follows. The intra-day RSD values for two analytes were 0.7 and 0.4 % (n = 6), and inter-day RSD values were 3.8 and 3.4 %, respectively (n = 6) (Table I).

Recovery. – The average recoveries (n = 6) for echnocystic acid and oleanolic acid were 95.8 ± 2.3 % and 93.2 ± 2.2 %, respectively (Table I).

Results indicated that the developed HPLC-DAD method was precise, accurate and sensitive enough for simultaneous quantitative evaluation of echinocystic acid and oleanolic acid in GFA.

Determination of echinocystic acid and oleanolic acid

The validated HPLC-DAD method was applied to quantify two chemical markers, echinocystic acid and oleanolic acid, in 12 batches of samples. The results of quantitative analyses are summarized in Table II. We found that GSF (K and L) contained both echinocystic acid and oleanolic acid. The average contents of echinocystic acid for GFA and GSF were 19.76 ± 4.52 and 19.70 ± 1.67 g kg^{-1}, respectively, while those of oleanolic acid were 21.25 ± 1.95 g kg^{-1} and 15.38 ± 0.86 g kg^{-1}, respectively. As described, GSF (K and

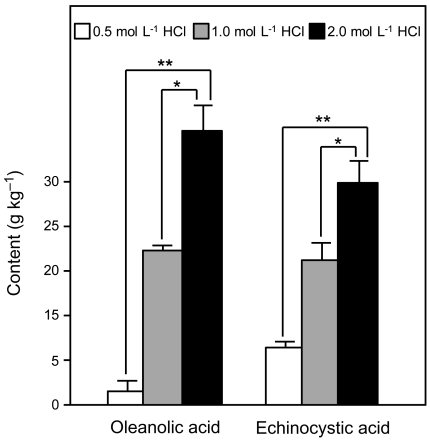


Fig. 2. Comparison of different hydrolytic concentrations of hydrochloric acid during extraction. Values are expressed in g kg^{-1} of dried single herb. Mean ± SD, $n = 3$. Significant difference: $^{*}p < 0.05$, $^{**}p < 0.01$.

L) and GFA (E and J) were collected from the same plants, yet the amount of aglycons hydrolyzed from saponins was different. Specifically, GFA (E) with echinocystic acid to oleanolic acid ratio was 1.1, while GSF (K) with two markers ratio was 1.3. Likewise, the ratios of two markers were 1.0 and 1.3, respectively. These results suggested a different ratio of saponins contained in two different types of fruits: GSF contained a higher ratio of echinocystic acid-type saponins to oleanolic acid-type ones than GFA. Similarly, GFA contained a higher ratio of oleanolic acid-type saponins to echinocystic acid-type ones than GSF. We speculated that these different ratios of both analytes in two fruits, this might be due to the different developmental stages of the fruit. A research for saponins by Xia *et al.* (24) reported that the contents in GFA were higher than that in GSF, and their ratios were 2.4 : 1.8 : 0.9 : 1.0 and 2.3 : 2.3 : 1.0 : 1.0 for GFA and GSF, respectively. Indeed, a research by Li and Hu (25) reported that the amount of oleanolic acid decreased during the development of fruits. However, further study should be conducted to investigate how these two analytes changed in the fruits.

Table I. Precision, repeatability and recovery of two analytes in model samples

Analyte	Precision				Recovery ($n = 6$)	
	Intra-day ($n = 6$)		Inter-day ($n = 6$)[a]		Mean (%)	RSD (%)
	Mean (μg mL^{-1})	RSD (%)	Mean (μg mL^{-1})	RSD (%)		
Echinocystic acid	30.10	0.2	29.32	1.1	95.85	2.3
Oleanolic acid	27.40	0.1	26.83	0.9	93.23	2.2

[a] The inter-day analysis refers to six replicates of the sample examined on three consecutive days.

Table II. Content of echinocystic acid and oleanolic acid in Gleditsiae Fructus Abnormalis and Gleditsiae Sinensis Fructus

Item	Sample	Production/ collection area	Content (g kg^{-1}) [a]	
			Echinocystic acid	Oleanolic acid
A	Gleditsiae Fructus Abnormalis	Shandong (HK Market)	17.81 ± 2.31	22.78 ± 2.37
B	Ditto	Shandong (HK Market)	20.99 ± 2.86	22.67 ± 0.61
C	Ditto	Shandong (HK Market)	27.95 ± 1.73	19.22 ± 2.68
D	Ditto	Shandong (HK Market)	12.91 ± 3.42	19.42 ± 1.91
E	Ditto	Guangzhou, Guangdong	24.18 ± 1.82	21.51 ± 0.48
F	Ditto	Yuncheng, Shanxi	21.05 ± 2.60	24.08 ± 2.83
G	Ditto	Anguo, Hebei	15.46 ± 0.46	18.22 ± 1.33
H	Ditto	Taian, Shandong	16.44 ± 3.21	20.24 ± 2.08
I	Ditto	Shangqiu, Henan	17.88 ± 0.11	21.12 ± 1.43
J	Ditto	Lintong, Shanxi	22.93 ± 3.13	23.24 ± 3.03
K	Gleditsiae Sinensis Fructus	Guangzhou, Guangdong	20.88 ± 2.23	15.99 ± 0.81
L	Ditto	Lintong, Shanxi	18.52 ± 0.92	14.77 ± 1.69

[a] Values are expressed in g kg^{-1} of dried fruit mass.
Mean ± SD, $n = 3$.

Although the literature demonstrates that echinocystic acid, one of the aglycones, was employed to quantitatively control the quality of GFA (26, 27), previous methods, especially for sample preparation, were time-consuming, *i.e.* around 20 hours. Besides, hazardous solvents such as chloroform were used in the extraction. In comparison with these methods, our current method is simpler and more practical to control the quality of the fruits. For instance, the preparation procedure could be done within 3 hours. Moreover, less hazardous solvent was employed in this study.

HPLC fingerprint analysis

In order to clarify the chemical properties of the two fruits, chromatograms of different samples were generated. The process of standardization included the selection of »characteristic peaks« in chromatograms and normalization of retention times of all characteristic peaks. Gleditsioside A, one of the major saponins found in both fruits, was used as the reference standard in the fingerprint analysis. Due to similar physicochemical properties of saponins, in this paper we identified the reference peak in samples by comparing the HPLC retention time and MS data in positive mode with the reference standard. In the MS spectra of samples, the molecular ion of $[M+Na]^+$ at *m/z* 1643.0 was deduced from gleditsioside A, which further confirmed the identity of gleditsioside A (Fig. 3a). As shown in Fig. 3b, the chromatograms of both fruits from different regions contained 5 characteristic peaks. Among these peaks, gleditsioside A (peak 5), which

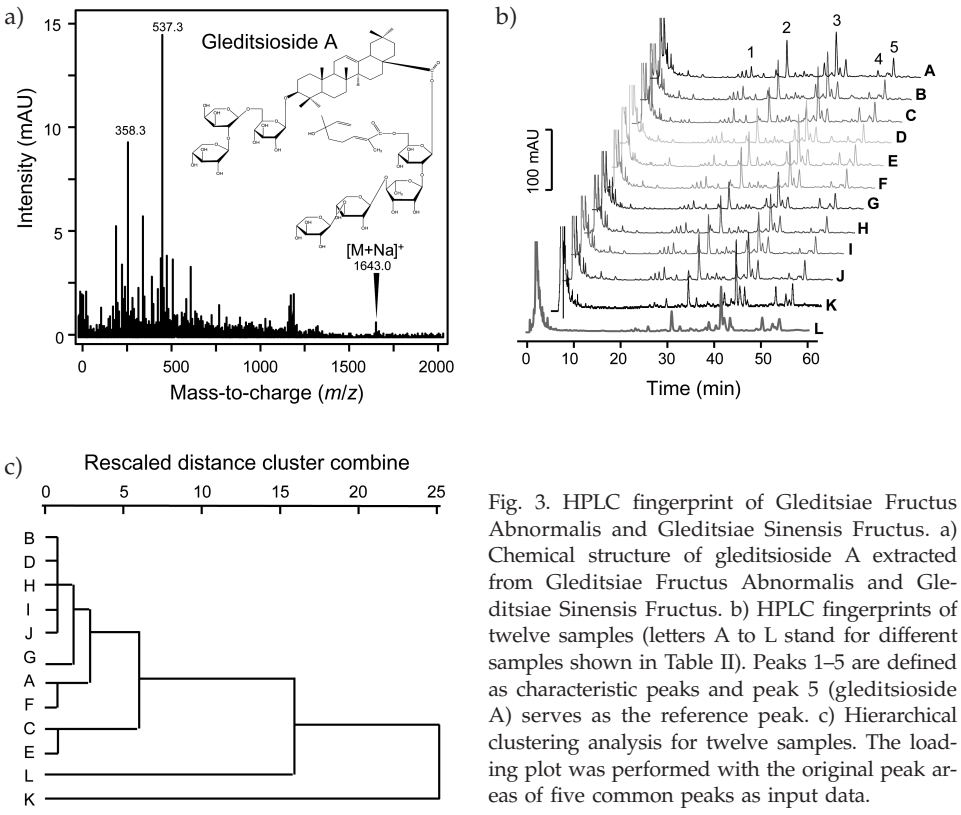


Fig. 3. HPLC fingerprint of Gleditsiae Fructus Abnormalis and Gleditsiae Sinensis Fructus. a) Chemical structure of gleditsioside A extracted from Gleditsiae Fructus Abnormalis and Gleditsiae Sinensis Fructus. b) HPLC fingerprints of twelve samples (letters A to L stand for different samples shown in Table II). Peaks 1–5 are defined as characteristic peaks and peak 5 (gleditsioside A) serves as the reference peak. c) Hierarchical clustering analysis for twelve samples. The loading plot was performed with the original peak areas of five common peaks as input data.

eluted at a retention time of 54.7 min, was chosen as the marker peak. The relative retention time (*RRT*) of the 5 characteristic peaks with reference to peak 5 (gleditsioside A) were: for peak 1 (0.42 ± 0.11), 2 (0.57 ± 0.02), 3 (0.77 ± 0.06), 4 (0.94 ± 0.02). The RSD values of *RRTs* of the 5 peaks in 12 sample batches were less than 3.0 %, which confirmed the high stability and reproducibility of the fingerprints.

In the hierarchical clustering analysis, method called average linkage between groups was applied, and Pearson correlation was selected as measurement. The result is shown in Fig. 3c. The tested populations of fruits were divided into two main clusters: samples A-J (GFA) as cluster one and samples K and L (GSF) as cluster two. This clustering agreed well with the results of two distinct types of fruits. The results here suggested that the difference between two fruits might be the result from the different ratios of saponins between two developmental stages. This is in agreement with the study reporting that saponin contents could be changed due to the developmental stages of herbs (25).

Both fruits were recognized as the same herbal medicine in *Compendium of Materia Medica* by Li Shizhen in the Ming dynasty (A.D. 1578) of China. However, GFA was de-

scribed as a drug of higher quality than GSF. Nowadays, they are recorded as different medicinal drugs in *Chinese pharmacopoei*a (18). In line with this, the study by Xiao (28) has suggested that GFA and GSF cannot be substitutes for each other because of the difference of their growth habitats as well as their chemical compositions.

As regards to the content of total saponins, no significant difference was found between GFA and GSF (26, 29). Total amounts of four saponins, including gleditsioside A, gleditsioside B, gleditsioside D and gleditsioside I were 52.7 and 52.4 mg g^{-1} for GFA and GSF, respectively (24). However, some findings revealed that there were differences between two fruits when comparing the amount of echinocystic acid and metals, *i.e.*, Cu, Zn, Fe, Mn, Mg, K and Ca, as well as their antibacterial effect (26, 30). Here, we found that the chemical fingerprints of both fruits are rather different, and therefore the interchangeable usage of GFA and GSF as a medicine should be undertaken with precaution, unless full chemical composition has been revealed.

CONCLUSIONS

The established HPLC-DAD method was found to be accurate and precise enough to be adopted for routine quality control of GFA and GSF. GSF was found to be very similar to GFA in the content of echinocystic acid and oleanolic acid. On the other hand, HPLC fingerprinting revealed a different ratio of echinocystic acid to oleanolic acid type saponins. Further chemical and pharmaceutical study should be conducted to reveal whether GFA might be replaced by GSF in clinical applications.

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REFERENCES

1. G. Francis, Z. Kerem, H. P. Makkar and K. Becker, The biological action of saponins in animal systems: A review, *Br. J. Nutr.* **88** (2002) 587–605; DOI: 10.1079/BJN2002725.
2. S. G. Sparg, M. E Light and J. van Staden, Biological activities and distribution of plant saponins, *J. Ethnopharmacol*. **94** (2004) 219–243; DOI: 10.1016/j.jep.2004.05.016.
3. J. D. Zhang, Y. B. Cao, Z. Xu, H. H. Sun, M. M. An, L. Yan, H. S Chen, P. H. Gao, Y. Wang, X. M. Jia and Y. Y. Jiang, *In vitro* and *in vivo* antifungal activities of the eight steroid saponins from *Tribulus terrestris* L. with potent activity against fluconazole-resistant fungal pathogens, *Biol. Pharm. Bull*. **28** (2005) 2211–2215; DOI: 10.1248/bpb.28.2211.
4. B. Yu, Y. Zhang and P. Tang, Carbohydrate chemistry in the total synthesis of saponins, *Eur. J. Org. Chem*. **31** (2007) 5145–5161; DOI: 10.1002/ejoc.200700452.
5. Y. F. Chen, C. H. Yang, M. S. Chang, Y. P. Ciou and Y. C. Huang, Foam properties and detergent abilities of the saponins from *Camellia oleifera*, *Int. J. Mol. Sci.* **11** (2010) 4417–4425; DOI: 10.3390/ijms11114417.

6. X. Crebassa, H. Caruel, F. Mercier and G. R. M. G. San Martin, *Use of Saponines from Gleditsia Against Mollusc Pests*, E.P. Pat. 2,533,646, 29 Dec 2012.
7. X. P. Yang, A comparative study of chemical components of Fructus Gleditsiae Sinensis and Fructus Gleditsiae Abnormalis, *J. Huaiyin Teachers Coll. (Nat. Sci. Ed.)*, 2004, 143–146.
8. Z. Zhang, K. Koike, Z. Jia, T. Nikaido, D. Guo and J. Zheng, Gleditsiosides N-Q, new triterpenoid saponins from *Gleditsia sinensis*, *J. Nat. Prod.* **62** (1999) 877–881; DOI: 10.1021/np990060m.
9. L. M. Chow, J. C. Tang, I. T. Teo, C. H. Chui, F. Y. Lau, T. W. Leung, G. Cheng, R. S. Wong, I. L. Wong, K. M. Tsang, W. Q. Tan, Y. Z. Zhao, K. B. Lai, W. H. Lam, D. A. Guo and A. S. Chan, Antiproliferative activity of the extract of *Gleditsia sinensis* fruit on human solid tumour cell lines, *Chemotherapy* **48** (2002) 303–308; DOI: 10.1159/000069713.
10. Y. Dai, Y. P. Chan, L. M. Chu and P. P. Bu, Antiallergic and anti-inflammatory properties of the ethanolic extract from *Gleditsia sinensis*, *Biol. Pharm. Bull.* **25** (2002) 1179–1182; DOI: 10.1248/bpb.25.1179.
11. L. J. Fu, Y. Dai, Z. T. Wang and M. Zhang, Inhibition of experimental allergic rhinitis by the *n*-butanol fraction from the anomalous fruits of *Gleditsia sinensis*, *Biol. Pharm. Bull.* **26** (2003) 974–977; DOI: 10.1248/bpb.26.974.
12. L. F. Hou, Y. Dai, Y. F. Xia and Z. N. Gong, Alleviation of picryl chloride-induced delayed type hypersensitivity reaction by saponin fraction of *Gleditsia sinensis*, *Biol. Pharm. Bull.* **29** (2006) 1056–1059; DOI: 10.1248/bpb.29.1056.
13. Z. Z. Gao, Y. F. Xia, X. J. Yao, Y. Dai and Q. Wang, A new triterpenoid saponin from *Gleditisia sinensis* and structure-activity relationships of inhibitory effects on lipopolysaccharide-induced nitric oxide production, *Nat. Prod. Res.* **22** (2008) 320–332; DOI: 10.1080/14786410701766422.
14. X. Tong, S. Lin, M. Fujii and D. X. Hou, Molecular mechanisms of echinocystic acid-induced apoptosis in HepG2 cells, *Biochem. Biophys. Res. Commun.* **321** (2004) 539–546; DOI: 10.1016/j.bbrc.2004.07.004.
15. B. Plohmann, G. Bader, K. Hiller and G. Franz, Immunomodulatory and antitumoral effects of triterpenoid saponins, *Pharmazie* **52** (1997) 953–957.
16. J. Liu, Pharmacology of oleanolic acid and ursolic acid, *J. Ethnopharmacol.* **49** (1995) 57–68; DOI: 10.1016/0378-8741(95)90032-2.
17. C. Gauthier, J. Legault, K. Girard-Lalancette, V. Mshvildadze and A. Pichette, Hemolytic activity, cytotoxicity and membrane cell permeabilization of semi-synthetic and natural lupane- and oleanane-type saponins, *Bioorg. Med. Chem.* **17** (2009) 2002–2008; DOI: 10.1016/j.bmc.2009.01.022.
18. *Pharmacopoeia of the People's Republic of China*, The State Pharmacopoeia Commission of P. R. of China, Beijing 2010.
19. *Hong Kong Chinese Materia Medica Standards*, Department of Health, Government of the Hong Kong Special Administrative Region, Hong Kong 2010, pp. 203–204.
20. Z. Q. Yin, R. F. Zhang, J. Zhang, M. Kong and Y. Dai, Quality evaluation of Gleditsiae Fructus Abnormalis, *J. China Pharm. Univ.* **42** (2011) 428–430.
21. Y. Z. Liang, P. S. Xie and K. Chan, Chromatographic fingerprinting and metabolomics for quality control of TCM, *Comb. Chem. High T. Scr.* **13** (2010) 943–953; DOI: 10.2174/138620710793360310.
22. W. Lu, F. Yang and S. Wang, Development of an HPLC fingerprint for quality control and species differentiation of *Uncaria rhynchophylla* (Miq.) ex Havil, *Acta Chromatogr.* **24** (2012) 643–651; DOI: 10.1556/AChrom.24.2012.4.10.
23. Q. F. Zhang, H. Y. Cheung and L. B. Zeng, Development of HPLC fingerprint for species differentiation and quality assessment of Rhizoma Smilacis Glabrae, *J. Nat. Med.* **67** (2013) 207–211; DOI: 10.1007/s11418-012-0648-9.

24. Y. F. Xia, Z. Z. Gao, Y. Dai and Q. Wang, Analysis of bioactive saponins in Fructus Gleditsiae Abnormalis and Fructus Gleditsiae Sinensis by LC-ELSD, *Chromatographia* **70** (2009) 1361–1366; DOI: 10.1365/s10337-009-1312-8.
25. J. T. Li and Z. H. Hu, Accumulation and dynamic trends of triterpenoid saponin in vegetative organs of *Achyranthus bidentata*, *J. Integr. Plant Biol.* **51** (2009) 122–129; DOI: 10.1111/j.1744-7909.2008.00764.x.
26. N. Li, W. F. Bao and G. Song, The quantitative determination of echinocystic acid in *Gleditsia sinensis* L. by HPLC, *J. Shenyang Pharm. Univ.* **18** (2010) 198–200.
27. J. H. Qu, Z. H. Li and P. Li, Determination of echinocystic acid in Fructus Gleditsiae Abnormalis from different regions by HPLC, *China Pharmacist* **13** (2010) 385–386.
28. Y. J. Xiao, Gleditsiae Sinensis Fructus should not be confused with Gleditsiae Fructus Abnormalis, *Capital Med.* **16** (2009) 49.
29. Y. Y. Jia and G. F. Zhang, Comparison of total saponins between Gleditsia Sinensis Fructus and Gleditsiae Fructus Abnormalis, *J. Chin. Med. Mat.* **4** (1987) 19–21.
30. Y. Y. Jia, S. Z. Wang and G. F. Zhang, Preliminary comparative analysis of the fruit of *Gleditsia sinensis* Lam. and *G. officinalis* Hemsl., *China J. Chin. Mat. Med.* **12** (1987) 35–37.