Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Polyphenolic compounds from Malus hupehensis and their free radical scavenging effects

Qingwen Hu, Ying-Ying Chen, Qi-Yang Jiao, Afsar Khan, Jimiao Shan, Gui-Dong Cao, Feng Li, Chao Zhang & Hong-Xiang Lou

To cite this article: Qingwen Hu, Ying-Ying Chen, Qi-Yang Jiao, Afsar Khan, Jimiao Shan, Gui-Dong Cao, Feng Li, Chao Zhang & Hong-Xiang Lou (2017): Polyphenolic compounds from Malus hupehensis and their free radical scavenging effects, Natural Product Research, DOI: 10.1080/14786419.2017.1367784

To link to this article: http://dx.doi.org/10.1080/14786419.2017.1367784

View supplementary material

Published online: 13 Sep 2017.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=gnpl20

Download by: [Imperial College London Library] **Date:** 14 September 2017, At: 05:01

NATURAL PRODUCT RESEARCH, 2017
https://doi.org/10.1080/14786419.2017.1367784

Taylor & Francis
Taylor & Francis Group

Check for updates

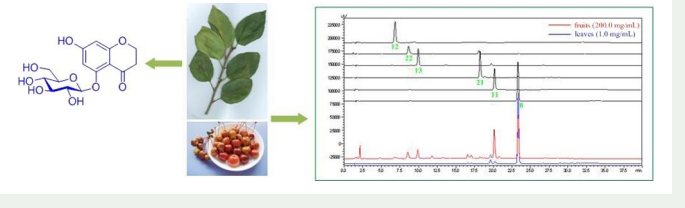
Polyphenolic compounds from *Malus hupehensis* and their free radical scavenging effects

Qingwen Hu[a], Ying-Ying Chen[a,b], Qi-Yang Jiao[b], Afsar Khan[c], Jimiao Shan[b], Gui-Dong Cao[b], Feng Li[b], Chao Zhang[b] and Hong-Xiang Lou[a]

[a]Key Laboratory of Chemical Biology of Ministry of Education, Department of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan, People's Republic of China; [b]Natural Products Laboratory, Research and Development Department, Reyoung Pharmaceutical Co., Ltd., Jinan, People's Republic of China; [c]Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad, Pakistan

ABSTRACT

One new 4-chromanone glycoside, 5-*O*-*β*-D-glucopyranoside-4-chromanone (**1**), together with 21 known polyphenols, was isolated from the leaves of *Malus hupehensis*. Their structures were elucidated on the basis of extensive spectroscopic methods including NMR (1D and 2D), mass (ESIMS and HRESIMS), IR, and by comparison with the data reported in the literature. Some of the isolated compounds were screened for antioxidant activity. Compounds **18** and **14** exhibited significant antioxidant activities with SC_{50} values 2.73 and 2.91 μg/mL, respectively, while **17**, **19**, **11**, **7**, **20**, **22**, **12** and **13** exhibited moderate activities with SC_{50} values ranging from 5.24–11.86 μg/mL. The HPLC fingerprint profiles of the leaves and fruits extracts were also analysed, which showed that the constituents were almost the same in both the extracts except for the content of phlorizin which was present in higher amount in the leaves.



ARTICLE HISTORY
Received 7 June 2017
Accepted 31 July 2017

KEYWORDS
Malus hupehensis; 4-chromanone; polyphenols; antioxidant activity; free radical scavenging

Downloaded by [Imperial College London Library] at 05:01 14 September 2017

1. Introduction

Previous phytochemical investigation on the genus *Malus* has revealed the secondary metabolites, mainly the polyphenols, including chromones, chalcones, flavones (Williams 1979; Roemmelt et al. 2003; Seeram et al. 2003; Sultana and Anwar 2008; Mari et al. 2010; Wang et al. 2013). Researches show that this genus possesses potential pharmacological activities

CONTACT Hong-Xiang Lou louhongxiang@sdu.edu.cn
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1367784.

© 2017 Informa UK Limited, trading as Taylor & Francis Group

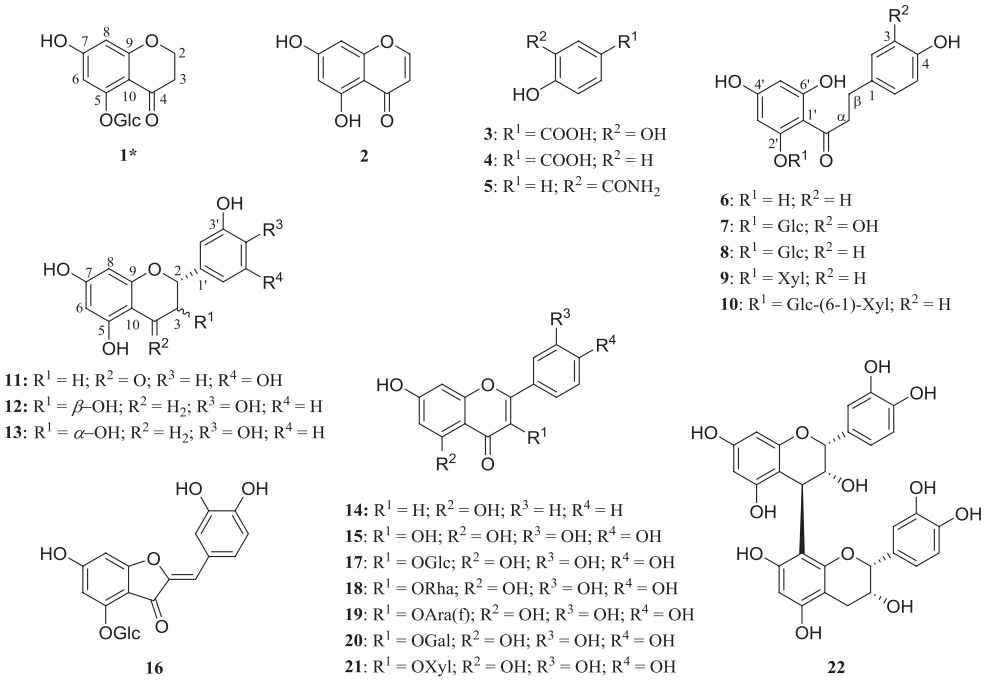


Figure 1. Structures of compounds **1**–**22** from *M. hupehensis*.

Table 1. DPPH radical scavenging activities of the compounds isolated from *M. hupehensis*.

Compound	SC_{50} (μg/mL)	Compound	SC_{50} (μg/mL)
1	>128	**12**	10.37
2	>128	**13**	11.86
3	NT[a]	**14**	2.91
4	NT[a]	**15**	21.04
5	>128	**16**	16.29
6	47.70	**17**	5.24
7	6.28	**18**	2.73
8	>128	**19**	5.88
9	>128	**20**	6.34
10	>128	**21**	NT[a]
11	5.96	**22**	9.07
Edaravone	4.72		

[a]NT: Not tested.

such as antioxidant, antihyperglycaemic, hepatoprotective and antiproliferative activities (Barbosa et al. 2010; Yang et al. 2010; Xue et al. 2011; Wang et al. 2013). Therefore, this genus has attracted much attention due to diverse structures of secondary metabolites and significant biological activities. *Malus hupehensis* (Rosaceae) is a well-known medicinal herb mainly distributed throughout southern China (Delectis Florae Reipublicae Popularis Sinicae Agenda Academiae Sinicae Edita 1974). The leaves of *M. hupehensis* have been used as an important class of tea with a local name "San-Pi-Guan" in Hubei province of China; three pieces of leaves make one pot of tea. Local people drink "San-Pi-Guan" for the treatment of hyperglycaemia (Williams 1979; Shen et al. 2013; Liu et al. 2015). Besides, phlorizin, a main component of the leaves, is effective to treat diabetes (Rossetti et al. 1987, 1987; Ehrenkranz

Downloaded by [Imperial College London Library] at 05:01 14 September 2017

Downloaded by [Imperial College London Library] at 05:01 14 September 2017

et al. 2005). Its fruits are sold in the market as health-caring liquor or immersed into the spirit to make the folk herbal liquor. Due to these uses, we were interested to find out whether the constituents are different in leaves and fruits. Therefore, we analysed the HPLC fingerprint profiles of the leaves and fruits extracts. The results showed that the constituents were almost the same in both the extracts except for the content of phlorizin which was present in higher amount in the leaves (see Supplementary material). Intrigued by the diverse chemical components and potential biological activities, we performed the photochemical investigation of the leaves of *M. hupehensis*. As a result, one new 4-chromanone glycoside, together with 21 known compounds, was isolated. Antioxidant activity assay showed that compounds **18** and **14** exhibited significant antioxidative activities with SC_{50} values 2.73 and 2.91 µg/mL, respectively, with edaravone as a positive control, while **17**, **19**, **11**, **7**, **20**, **22**, **12** and **13** showed moderate activities with SC_{50} values ranging from 5.24 to 11.86 µg/mL. The HPLC fingerprint analysis of the fruits of *M. hupehensis* showed that they contained mostly the same active components as those extracted from the leaves (see Supplementary material).

2. Results and discussion

The molecular formula of compound **1** was assigned as $C_{15}H_{18}O_9$ from the (+)-HRESIMS peak at *m/z* 365.0849 $[M + Na]^+$ (Calcd 365.0843), with 7 degrees of unsaturation, which was also consistent with its ^{13}C and DEPT NMR data. The ^{13}C NMR spectrum resolved 15 carbon resonances, of which 6 belonged to a sugar moiety (δ_C 103.8, 77.6, 75.7, 73.5, 69.7, 60.7) and the remaining 9 ascribed to a 4-chromanone moiety. The resonances assigned to the 4-chromanone unit included two methylenes (δ_C 65.9, 38.3), two aromatic methines (δ_C 101.2, 98.5) and five quaternary carbons (four aromatic δ_C170.2, 164.2, 160.9, 103.6 and one carbonyl δ_C 189.0). The structure of the 4-chromanone was established by HSQC and HMBC spectra (see Table S1 and Figure S1). In addition, the correlations of δ_H 4.57 (1H, d, J = 7.2 Hz, H-1′) with δ_C 160.9 (C-5) suggested the sugar moiety was linked to the C-5 of aglycone. Acid hydrolysis of **1** afforded D-glucose on the basis of GC analysis. The *β*-configuration of D-glucose was determined by the *J* coupling constant value (7.2 Hz) of H-1′. Thus, the structure of **1** was elucidated as 5-*O*-*β*-D-glucopyranoside-4-chromanone.

Besides, 21 known compounds were also isolated and identified as 5,7-dihydroxychromone (**2**) (Behery et al. 2013), protocatechuate (**3**) (Zhang et al. 1998), 4-hydroxybenzoic acid (**4**) (Saito et al. 1988), salicylamide (**5**) (Woods and Sherry 2003), phloretin (**6**) (Krishnamurty and Sathyanarayana 1989), 3′-hydroxyphloretin (**7**) (Xiao et al. 2017), phlorizin (**8**) (Xiao et al. 2017), phloretin-2′-*O*-*β*-D-xyloside (**9**) (Xiao et al. 2017), phloretin-2′-*O*-(*β*-D-xylopyranosyl-(1-6)-*β*-D-glucopyranoside) (**10**) (Lu and Foo 1997), 5,7,3′5,′-tetrahydroxy-dihydroflavone (**11**) (Nessa et al. 2004), (–)-catechin (**12**) (Kashiwada et al. 1986), (–)-epicatechin (**13**) (Lu and Foo 1997), chrysin (**14**) (Park et al. 2007), quercetin (**15**) (Sirat et al. 2010), cernuoside (**16**) (Güçlütürk et al. 2012), quercitrin (**17**) (Sirat et al. 2010), quercetin-3-*O*-*α*-L-rhamnoside (**18**) (Fukunaga et al. 1988), quercetin-3-*O*-*α*-L- arabinofuranoside (**19**) (Ek et al. 2006), hyperin (**20**) (Ek et al. 2006), quercetin-3-*O*-*β*-D-xyloside (**21**) (Ek et al. 2006), and procyanidin B2 (**22**) (Khan et al. 1997) by comparison of their spectroscopic data with those reported in the literature (Figure 1).

Some of the isolated compounds were evaluated for antioxidant activity Table 1. Compounds **18** and **14** exhibited significant DPPH free radical scavenging activities while

the compounds **17**, **19**, **11**, **7**, **20**, **22**, **12** and **13** showed moderate activities. The HPLC fingerprint analysis of the fruits showed that they contained mostly the same active components as those extracted from the leaves of *M. hupehensis* (see Supplemental material).

3. Experimental

3.1. General

UV spectra were recorded on a Shimadzu UV-2600 spectrophotometer. IR spectra were recorded on a Bruker FT-IR Tensor-27 infrared spectrophotometer with KBr pellets. One-dimensional (1D) and two-dimensional (2D) NMR spectra were recorded on a Bruker Avance DRX-600 spectrometer using the residual non-deuterated solvent signal as an internal standard. ESIMS and HRESIMS were recorded on an Agilent 6520 Q-TOF. HPLC was performed on a Shimadzu LC-6AD with a Shimadzu C18 (20 × 250 mm) column. Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co., Ltd., People's Republic of China) and MCI gel CHP20/P120 (75–150 μm, Mitsubishi Chemical Corporation, Japan) were used for column chromatography.

3.2. Plant material

The leaves of *M. hupehensis* were collected from Shansong Biological Products Co., Ltd. (Linyi, China), in September 2014. The leaves were identified by Prof. Lan Xiang, and a voucher specimen (MH201409) has been deposited in the Herbarium of the School of Pharmaceutical Sciences, Shandong University.

3.3. Extraction and isolation

The aired-dried leaves of *M. hupehensis* (10 Kg) were extracted with EtOH/H_2O (80/20 v/v, twice, 2 h each time) under reflux conditions to result a crude extract, which was further subjected to a HPD-100 macroporous adsorption resin chromatography eluted successively with aqueous EtOH gradient system (0, 30 and 95%). The 30% EtOH fraction (the total polyphenols) was separated on a polyamide column (EtOH/H_2O; 0, 15, 25, 50, 100%) to result five subfractions, fraction A1-A5. Fraction A1 was separated on a Sephadex LH-20 column and then on semi-preparative HPLC (15% ACN : H_2O) to yield compounds **10** (36 mg), **12** (9 mg), **13** (34 mg), and **22** (24 mg).The main component phlorizin (**8**; 300 g) was isolated from fraction A2. Fraction A3 was subjected to silica gel columns then to Sephadex LH-20 columns to yield compounds **7** (20 mg), **16** (80 mg), **17** (16 mg), **18** (60 mg) and **20** (20 mg). Fraction A4 was subjected to a Sephadex LH-20 column and then semi-preparative HPLC to yield compounds **19** (10 mg) and **21** (20 mg). Fraction A5 was subjected to a Sephadex LH-20 column to yield compounds **6** (1 g) and **15** (40 mg). The 95% EtOH fraction was suspended in H_2O, respectively, partitioned with petroleum ether, EtOAc, CH_2Cl_2 and *n*-BuOH to result four organic fractions. The CH_2Cl_2 soluble fraction was subjected to silica gel columns to yield compound **14** (20 mg).The EtOAc soluble fraction was separated by silica gel columns and RP-C18 columns to yield compounds **2** (15 mg) and **11** (25 mg). The *n*-BuOH soluble fraction was separated by RP-C18 columns and semi-preparative HPLC to isolate compounds **1** (15 mg), **3** (10 mg), **4** (6 mg), **5** (8 mg) and **9** (11 mg).

Downloaded by [Imperial College London Library] at 05:01 14 September 2017

5-*O*-*β*-D-glucopyranoside-4-chromanone (1):yellow amorphous powder, [α]25 D -55.10 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε):283 (3.22), 321 (2.95) nm; IR (KBr): ν_{max} 3368, 2917, 1584, 1403, 1311, 1248, 1194, 1028, 953, 843, 708, 651 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table S1; positive HRESIMS $[M + Na]^+$: *m/z* 365.0849 (calcd. for $C_{15}H_{18}O_9Na$, 365.0843).

3.4. Acid hydrolysis of compound 1

Compound **1** (2.0 mg) was dissolved in MeOH (3 mL), then treated with 2 M HCl (1,4-dioxane:H_2O, 1:1, 2 mL) and heated to 90 °C for 3 h following the literature method (Qin et al. 2016). After the solvent was removed under vacuum, the reaction mixture was extracted with $CHCl_3$ (5 mL × 3). The aqueous layer was neutralised using 0.5% ammonia solution, then, evaporated to dryness. The residue was dissolved in anhydrous pyridine (1 mL) followed by the addition of L-cysteine methyl ester hydrochloride (2 mg) and heated to 60 °C for 1 h. Then trimethylsilyimidazole solution (1.5 mL) was added and the reaction mixture was heated to 60 °C for 30 min. The dried product was partitioned with *n*-hexane and H_2O. The *n*-hexane layer was analysed by GC. The retention time of D-glucose (t_R, 16.95 min) was confirmed by comparison with an authentic sample.

3.5. DPPH radical scavenging activity

The DPPH assay was performed according to the method described by Huang (Huang et al. 2003).About 100 µL of the sample (concentration ranging from 0.25 to 256 µg/mL) were added to 100 µL of DPPH solution (150 µM in ethanol). The reaction was carried out at 25 °C in the dark for 30 min with shaking for 15 s, and then the absorbance of the reaction mixture was recorded with spectrophotometer at 517 nm. Pure ethanol was used as a control sample. The radical scavenging activities of the samples, expressed as partial inhibition of DPPH, were calculated according to the formula: inhibition (%) = $[(A_0–A_1)/A_0] \times 100$, where A_0 and A_1 are the absorbance values of the blank sample and of the tested samples, respectively. Edaravone was used as a positive control. The SC_{50} (the concentration required to scavenge 50% radicals) values of the tested compounds toward DPPH were calculated using the SPSS 21.0 software.

4. Conclusion

One new 4-chromanone glycoside named as 5-*O*-*β*-D-glucopyranoside-4-chromanone (**1**), together with 21 known polyphenols, was isolated from the leaves of *M. hupehensis*. Antioxidant activity assay showed that compounds **18** and **14** exhibited significant activities with SC_{50} values 2.73 and 2.91 µg/mL, respectively. The HPLC fingerprint profiles of the leaves and fruits extracts exhibited that the constituents were almost the same except for a higher content of phlorizin present in the leaves.

Disclosure statement

No potential conflict of interest was reported by the authors.

Downloaded by [Imperial College London Library] at 05:01 14 September 2017

Downloaded by [Imperial College London Library] at 05:01 14 September 2017

Funding

This work was financially supported by the National Natural Science Foundation of China [grant number 81630093].

References

Barbosa ACL, Pinto MdS, Sarkar D, Ankolekar C, Greene D, Shetty K. 2010. Varietal influences on antihyperglycemia properties of freshly harvested apples using *in vitro* assay models. J Med Food. 13:1313–1323.

Behery FAA, Naeem ZEM, Maatooq GT, Amer MMA, Ahmed AF. 2013. A novel antioxidant phenanthrenoid dimer from *Juncus acutus* L. Nat Prod Res. 27:155–163.

Delectis Florae Reipublicae Popularis Sinicae Agenda Academiae Sinicae Edita. 1974. Flora Reipublicae Popularis Sinicae. Beijing: Science Press. Vol 36; p. 378–380.

Ehrenkranz JRL, Lewis NG, Kahn CR, Roth J. 2005. Phlorizin: a review. Diabetes/Metab Res Rev. 21:31–38.

Ek S, Kartimo H, Mattila S, Tolonen A. 2006. Characterization of phenolic compounds from Lingonberry (*Vaccinium vitis-idaea*). J Agric Food Chem. 54:9834–9842.

Fukunaga T, Nishiya K, Kajikawa I, Watanabe Y, Suzuki N, Takeya K, Itokawa H. 1988. Chemical studies on the constituents of Hyphear tanakae HOSOKAWA from different host trees. Chem Pharm Bull. 36:1180–1184.

Güçlütürk I, Detsi A, Weiss EK, Ioannou E, Roussis V, Kefalas P. 2012. Evaluation of anti-oxidant activity and identification of major polyphenolics of the invasive Weed Oxalis pes-caprae. Phytochem Anal. 23:642–646.

Huang YL, Yeh PY, Shen CC, Chen CC. 2003. Antioxidant flavonoids from the rhizomes of *Helminthostachys zeylanica*. Phytochemistry. 64:1277–1283.

Kashiwada Y, Nonaka G, Nishioka I. 1986. Tannins and related compounds. XLV. Rhubarb. (5). Isolation and characterization of flavan-3-ol and procyanidin glucosides. Chem Pharm Bull. 34:3208–3222.

Khan ML, Haslam E, Williamson MP. 1997. Structure and conformation of the procyanidin B-2 dimer. Magn Reson Chem. 35:854–858.

Krishnamurty HG, Sathyanarayana S. 1989. Catalytic transfer hydrogenation, a facile conversion of hydroxyflavanones into hydroxydihydrochalcones. Synth Commun. 19:119–123.

Liu Q, Zeng H, Jiang S, Zhang L, Yang F, Chen X, Yang H. 2015. Separation of polyphenols from leaves of *Malus hupehensis* (Pamp.) Rehder by off-line two-dimensional high speed counter-current chromatography combined with recycling elution mode. Food Chem. 186:139–145.

Lu Y, Foo LY. 1997. Identification and quantification of major polyphenols in apple pomace. Food Chem. 59:187–194.

Mari A, Tedesco I, Nappo A, Russo GL, Malorni A, Carbone V. 2010. Phenolic compound characterisation and antiproliferative activity of "Annurca" apple, a southern Italian cultivar. Food Chem. 123:157–164.

Nessa F, Ismail Z, Mohamed N, Haris MRH. 2004. Free radical-scavenging activity of organic extracts and of pure flavonoids of *Blumea balsamifera* DC leaves. Food Chem. 88:243–252.

Park Y, Moon BH, Lee E, Lee Y, Yoon Y, Ahn JH, Lim Y. 2007. ^{1}H and ^{13}C-NMR data of hydroxyflavone derivatives. Magn Reson Chem. 45:674–679.

Qin XJ, Yu MY, Ni W, Yan H, Chen CX, Cheng YC, He L, Liu HY. 2016. Steroidal saponins from stems and leaves of *Paris polyphylla* var. *yunnanensis*. Phytochemistry. 121:20–29.

Roemmelt S, Zimmermann N, Rademacher W, Treutter D. 2003. Formation of novel flavonoids in apple (*Malusxdomestica*) treated with the 2-oxoglutarate-dependent dioxygenase inhibitor prohexadione-Ca. Phytochemistry. 64:709–716.

Rossetti L, Shulman GI, Zawalich W, DeFronzo RA. 1987. Effect of chronic hyperglycemia on *in vivo* insulin secretion in partially pancreatectomized rats. J Clin Invest. 80:1037–1044.

Rossetti L, Smith D, Shulman GI, Papachristou D, DeFronzo RA. 1987. Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. J Clin Invest. 79:1510–1515.

Saito H, Yokoi M, Aida M, Kodama M, Oda T, Sato Y. 1988. ^{13}C NMR spectra of para-substituted methoxybenzenes and phenols in the solid state: examination of chemical shift non-equivalence in ortho and meta carbons related to non-equivalent electron distribution, and application to assignment of peaks in meso-hexestrol and its derivatives. Magn Reson Chem. 26:155–161.

Seeram NP, Cichewicz RH, Chandra A, Nair MG. 2003. Cyclooxygenase inhibitory and antioxidant compounds from crabapple fruits. J Agric Food Chem. 51:1948–1951.

Shen T, Xiang L, Ren DM, Wang SQ, Yang MR, Lou HX. 2013. A protocol for the quality control of leaves in *Malus hupehensis* via seasonal variation, microscopic and chromatographic analysis. Chin Herb Med. 5:145–150.

Sirat HM, Rezali MF, Ujang Z. 2010. Isolation and identification of radical scavenging and tyrosinase inhibition of polyphenols from *Tibouchina semidecandra* L. J Agric Food Chem. 58:10404–10409.

Sultana B, Anwar F. 2008. Flavonols (kaempferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chem. 108:879–884.

Wang SQ, Zhu XF, Wang XN, Shen T, Xiang F, Lou HX. 2013. Flavonoids from *Malus hupehensis* and their cardioprotective effects against doxorubicin-induced toxicity in H9c2 cells. Phytochemistry. 87:119–125.

Williams AH. 1979. Dibenzoylmethanes and flavones of *Malus*. Phytochemistry. 18:1897–1898.

Woods M, Sherry AD. 2003. Synthesis and luminescence studies of aryl substituted tetraamide complexes of europium(III): a new approach to pH responsive luminescent europium probes. Inorg Chem. 42:4401–4408.

Xiao Z, Zhang Y, Chen X, Wang Y, Chen W, Xu Q, Li P, Ma F. 2017. Extraction, identification, and antioxidant and anticancer tests of seven dihydrochalcones from Malus 'Red Splendor' fruit. Food Chem. 231:324–331.

Xue B, Cao D, Zhou J, Zou K, Wang J. 2011. Effects of total flavonoids of the *Malus hupehensis* on proliferation and differentiation of osteoblasts and activity of osteoclasts. Chin J Tradit Med Traumatol Orthop. 19:12–14.

Yang J, Li Y, Wang F, Wu C. 2010. Hepatoprotective effects of apple polyphenols on CCl_4-induced acute liver damage in mice. J Agric Food Chem. 58:6525–6531.

Zhang HL, Nagatsu A, Okuyama H, Mizukami H, Sakakibara J. 1998. Sesquiterpene glycosides from cotton oil cake. Phytochemistry. 48:665–668.

Downloaded by [Imperial College London Library] at 05:01 14 September 2017