Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

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

A new phenol glycoside from Physalis angulata

Cheng-Peng Sun, Xiu-Fang Nie, Ning Kang, Feng Zhao, Li-Xia Chen & Feng Qiu

To cite this article: Cheng-Peng Sun, Xiu-Fang Nie, Ning Kang, Feng Zhao, Li-Xia Chen & Feng Qiu (2016): A new phenol glycoside from Physalis angulata, Natural Product Research, DOI: 10.1080/14786419.2016.1269102

To link to this article: http://dx.doi.org/10.1080/14786419.2016.1269102

NATURAL PRODUCT RESEARCH, 2016
http://dx.doi.org/10.1080/14786419.2016.1269102

Taylor & Francis
Taylor & Francis Group

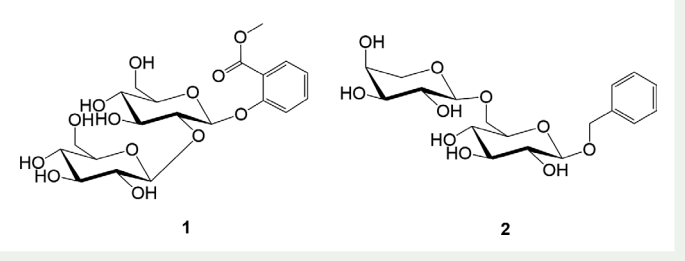
A new phenol glycoside from *Physalis angulata*

Cheng-Peng Sun[a,b], Xiu-Fang Nie[b], Ning Kang[a], Feng Zhao[c], Li-Xia Chen[b] and Feng Qiu[a,b]

[a]Tianjin State Key Laboratory of Modern Chinese Medicine and School of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Tianjin, China; [b]Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Department of Natural Products Chemistry, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China; [c]Key Laboratory of Molecular Pharmacology and Drug Evaluation, Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, School of Pharmacy, Yantai University, Yantai, China

ABSTRACT

A new phenol glycoside, physanguloside A (**1**), was isolated from *Physalis angulata* together with four known compounds. We report herein, for the first time, the presence of compounds **2**–**5** in the genus *Physalis*. The structures of all the compounds were established by NMR, IR, UV and HRESIMS spectroscopic analyses, and comparison with the literature data. All isolated compounds were assayed for inhibitory activity on nitric oxide production by LPS-induced in RAW 264.7 macrophages.



ARTICLE HISTORY
Received 14 July 2016
Accepted 20 November 2016

KEYWORDS
Physalis angulata; phenol glycoside; nitric oxide

1. Introduction

Physalis angulata L. (known in Chinese as '*ku-zhi*') is a member of the genus *Physalis* (Solanaceae) comprising approximately 120 species widely distributed in tropical and subtropical regions (Institute of Botany of the Chinese Academy of Sciences & Kunming Institute of Botany of the Chinese Academy of Sciences 1978). As a traditional folk medicine, *P. angulata* has been used to cure inflammatory-related diseases, such as dermatitis, asthma, hepatitis and malaria in other countries such as Peru, Mexico and Brazil (Di Stasi et al. 1989). Phytochemical investigations of the genus *Physalis* have led to the isolation of withanolides (Chen et al. 2007; Qiu et al. 2008a; Guan et al. 2014; Nicolás et al. 2015), labdane-type

CONTACT Li-Xia Chen syzyclx@163.com; Feng Qiu fengqiu20070118@163.com
Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/14786419.2016.1269102.

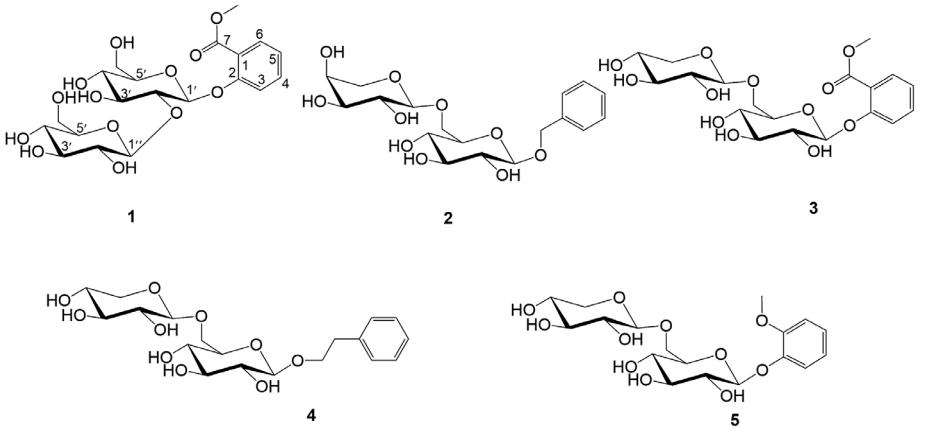


Figure 1. Chemical constituents of *P. angulata*.

diterpenes (Pérez-Castorena et al. 2006, 2010; Maldonado et al. 2015), flavonoids (Qiu et al. 2008a; Maldonado et al. 2012), megastigmane glycosides (Qiu et al. 2008b), alkaloids (Basey et al. 1992; Asano et al. 1995), ceramides (Su et al. 2002) and acylsucroses (Maldonado et al. 2006). Some of the isolated compounds have shown significant pharmacological activities, including anti-inflammatory (Choi & Hwang 2003; Bastos et al. 2008; He et al. 2014), antitumor (Hsieh et al. 2006; Hseu et al. 2011; He, Zang, Feng, Wang, 2013; He, Zang, Feng, Chen, 2013; Zhang et al. 2014), immunomodulatory (Kusumaningtyas et al. 2015) and antinociceptive (Bastos et al. 2006) activities.

As part of our ongoing search to obtain potential anti-inflammatory agents from *P. angulata* (Sun et al. 2016a, 2016b), 75% EtOH extract of *P. angulata* was subjected to various chromatographic techniques to afford a new phenol glycoside, physanguloside A (**1**) and four known compounds including a phenylcarbinol glycoside (**2**), two phenol glycosides (**3** and **5**) and a phenethanol glycoside (**4**) (Figure 1). Their structural elucidations were identified by NMR, IR, UV and HRESIMS analysis. All isolated compounds were evaluated for inhibitory effects on nitric oxide production in RAW 264.7 macrophages.

2. Results and discussion

Physanguloside A (**1**) was obtained as a pale yellow amorphous powder. Its molecular formula was deduced as $C_{20}H_{28}O_{13}$ by the positive HRESIMS *m/z* 499.1434 $[M + Na]^+$ and ^{13}C NMR data, indicating 7 degrees of unsaturation. The IR spectrum of **1** showed absorption bands of hydroxy (3424 cm^{-1}) and aromatic (1646 and 1468 cm^{-1}) functionalities. The ^1H NMR data of **1** (Table S1) showed signals of a methoxy group at δ_H 3.88 (3H, s) and four aromatic protons at δ_H 7.69 (1H, dd, J = 7.9, 1.8 Hz, H-6), 7.52 (1H, td, J = 7.9, 1.8 Hz, H-4), 7.25 (1H, br d, J = 7.9 Hz, H-3) and 7.07 (1H, br t, J = 7.9 Hz, H-5), suggesting the presence of 12-disubstituted benzene. In addition, two anomeric signals at δ_H 5.34 (1H, d, J = 7.4 Hz, H-1′) and 4.82 (1H, d, J = 7.7 Hz, H-1″) indicated the presence of two glycoses. The ^{13}C NMR spectrum of **1** revealed 20 carbon resonances, including a carboxyl carbon (δ_C 168.1), 6 aromatic carbons (δ_C 157.7, 135.0, 132.5, 122.6, 121.5 and 116.4), 2 anomeric carbons (δ_C 104.5 and 99.8), 10 oxygenated carbons (δ_C 83.1, 77.9, 77.8, 77.7, 77.5, 76.2, 71.0, 70.9,

62.5 and 61.6) and a methoxy carbon (δ_C 52.8). The above NMR data indicated that **1** was a phenol glycoside.

HPLC with an optical rotation detector (HPLC-OR) is widely used to determine enantiomers (Yamamoto et al. 2008), and can also be performed for the configuration of glycoses (Sun et al. 2016b; Wang et al. 2016). Two glycoses were identified by HPLC-OR analysis for hydrolysed products of **1** to be D-glucose. The anomeric configurations of two glucoses were deduced as a *β*-orientation based on the coupling constants [δ_H 5.34 (1H, d, J = 7.4 Hz, H-1′) and 4.82 (1H, d, J = 7.7 Hz, H-1′′)] (Sun et al. 2016b). In the HMBC spectrum of **1**, long-range correlations of H-1′ with C-2, H-1′′ with C-2′, H-6 with C-7, and OCH_3 with C-7 (Figure S1) indicated the locations of two glucoses, the methoxy group and the ester carbonyl group. The NOESY cross-peaks of H-3 with H-1′/H-4, H-4 with H-5, and H-5 with H-6 (Figure S1) further confirmed the above conclusion. Taking all above data into consideration, the structure of physanguloside A (1) was defined as methyl 2-(2′-*O*-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyloxybenzoate.

In addition, the investigation of the 75% EtOH extract of *P. angulata* led to the isolation of four known compounds, including benzylalchohol *O*-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**2**) (Kawahara et al. 2005), gaultherin (**3**) (Wang et al. 2011), phenethanol-*β*-vicianoside (**4**) (Wang et al. 2012) and guaiacyl-*β*-D-primeveroside (**5**) (Mayr et al. 2014). To the best of our knowledge, this is the first report of compounds **2**–**5** in the genus *Physalis*.

Nitric oxide (NO) is a well-known cellular signalling molecule and is considered as an important regulator in many physiological mechanisms (Turtay et al. 2015; Bahnson et al. 2016; Ren et al. 2016). Pharmacological studies have indicated that inflammation is related to overproduction of NO (Mulligan et al. 1991). *P. angulata* is a traditional folk medicine used to treat inflammatory-related conditions, such as dermatitis, asthma, hepatitis and malaria. In our previous studies, some compounds isolated from *P. angulata*, such as physangulatins I, J and K, physagulides I and K and physagulin I, displayed a potent inhibition of NO production (Sun et al. 2016a, 2016b). Therefore, all isolated compounds (**1**–**5**) were evaluated for inhibitory effects on NO production induced by LPS in RAW 264.7 macrophages (Table S2). However, none of the isolates displayed significant inhibitory effect against NO production.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter (Perkin-Elmer, Waltham, MA, USA). UV spectra were measured with a Shimadzu UV 2201 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR spectra were carried out on a Bruker IFS 55 spectrometer (Bruker Optics, Ettlingen, Germany). NMR experiments were performed on Bruker AV-400 and AV-600 spectrometers (Bruker Biospin, Fallanden, Switzerland). Chemical shift values are expressed in δ (ppm) using the peak signals of the solvent MeOH-d_4 (δ_H 3.31 and δ_C 49.2) as the reference. HRESIMS were obtained on an Agilent 6210 TOF mass spectrometer (Palo Alto, USA). AB-8 resins were purchased from the Chemical Plant of NanKai University (Tianjin, China). Silica gel GF254 used to prepare TLC plates and silica gel (200–300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Factory (Qingdao, China). Sephadex LH-20 was a product of Pharmacia (Amersharm, Sweden).

Octadecyl silica gel was purchased from Merck Chemical Company Ltd (Darmstadt, German). RP-HPLC equipped with an LC-6AD liquid chromatograph, SPD-20A UV detector (Shimadzu, Kyoto, Japan) and ODS column (250 × 20 mm, 5 μm, 120 Å, YMC Co. Ltd) was used for the separation. All reagents were purchased from Tianjin Damao Chemical Company (Tianjin, China). Spots were detected on TLC plates under UV light or heated after spraying with anisaldehyde-H_2SO_4 reagent.

3.2. Plant material

The stems and leaves of *P. angulata* were collected in Nanning, Guangxi Province, China, in July 2013. It was identified by Jia-Fu Wei, Guangxi Institute for Food and Drug Control, and a voucher specimen (PA-20130826) has been deposited in the herbarium of the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

3.3. Extraction and isolation

The dried stems and leaves of *P. angulata* (9.5 kg) were extracted with 75% EtOH (110 L × 2 h × 2). The extract (1.3 kg) was concentrated *in vacuo*, suspended with H_2O (5 L), and partitioned with petroleum ether (5 L × 3), EtOAc (5 L × 3) and *n*-BuOH (5 L × 3), successively. The *n*-BuOH extract (102.0 g) was subjected to AB-8 resin CC (10 × 80 cm) eluted with EtOH/H_2O (0:100, 30:70, 60:40 and 95:5) to obtain four fractions (B1–B4). Fraction B2 (10.0 g) was separated by ODS CC (3 × 50 cm), MeOH–H_2O as eluent (1:9 to 1:0), yielding three subfractions (B21–B23). Subfraction B23 (2.0 g) was purified by a Sephadex LH-20 column (3 × 80 cm) using MeOH as eluent to afford three subfractions (B231-B233). Finally, compounds **1** (15 mg, t_R = 20 min), **2** (38 mg, t_R = 15 min), **3** (21 mg, t_R = 16 min), **4** (5 mg, t_R = 34 min) and **5** (3 mg, t_R = 24 min) were obtained from subfraction B232 (870 mg) by preparative a HPLC analysis (CH_3CN-H_2O, 20:80).

Physanguloside A (**1**): pale yellow amorphous powder; $[\alpha]_D^{25}$ − 29.3 (*c* 0.075, MeOH); UV (MeOH) λ_{max} (log ε) 222 (3.9), 289 (3.9) nm; IR (KBr) ν_{max} 3396, 2921, 2850, 1717, 1646, 1468, 1384, 1122 cm^{-1}; ^1H (400 MHz, MeOH-d_4) and ^{13}C NMR (100 MHz, MeOH-d_4) data, see Table S1; HRESIMS *m/z* 499.1434 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_{13}Na$, 499.1428).

3.4. Acid hydrolysis of compound 1

Compound **1** (4 mg) was dissolved in 2 M HCl (3.0 mL) and stirred at 90 °C for 3 h (Sun et al. 2016b; Wang et al. 2016). The reaction mixture was extracted with $CHCl_3$, and the aqueous layer was evaporated to give a monosaccharide. The residue was analysed by HPLC using a Jasco LC-NetII/ADC system, equipped with a Jasco OR-4090 detector, a gradient pump, and a Shodex Asahipak NH2P-50 4E column (250 × 4.6 mm, 5 μm). A 20 μL sample was injected, and the mobile phase was a solvent of CH_3CN-H_2O (75:25). The HPLC spectrum of D-glucose (No. 492–61-5, ChromaDex Inc., USA) showed the positive peak at 12.5 min, and that of L-glucose (No. 921–60-8, Tokyo Chemical Industry Co., LTD, Japan) showed the negative peak at 12.5 min.

3.5. NO Production Bioassay

All compounds were assayed for the inhibition of NO production according to Griess method (Li et al. 2010; Sun et al. 2016a). 1×10^6 Cells/well of RAW 264.7 cells were placed into 96-well plates, and incubated at 37 °C for 24 h by the stimulation of LPS (1 μg/mL) with or without test compounds. After the addition of Griess reagent [0.1% *N*-(1-naphthyl)ethylenediamine (50 μL); 1% sulphanilamide in 5% H_3PO_4 (50 μL)], absorbance (540 nm) was recorded using a microplate reader. The standard curve was used to calculate the NO concentrations and inhibitory rates.

4. Conclusion

In summary, a new phenol glycoside (**1**) and four known compounds (**2**–**5**) were isolated from the stems and leaves of *P. angulata*. This is the first report about the presence of phenol glycoside derivatives (**2**–**5**) in the genus *Physalis*. Furthermore, compounds **1**–**5** were evaluated for their effects on NO production in RAW 264.7 macrophages.

Supplementary material

Supplementary material relating to this article is available online, alongside with Figures S1–S10.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by grants from the National Natural Science Foundation of China (NSFC) [grant number 81430095]; Fund of the Educational Department of Liaoning Province [grant number L2011177]; Liaoning Baiqianwan Talents Program [grant number 2013921043]; 2015 Career Development Program for Young and Middle-aged Teachers of Shenyang Pharmaceutical University [grant number ZQN2015015].

References

Asano N, Kato A, Oseki K, Kizu H, Matsui K. 1995. Calystegins of *Physalts alkekengi* var. *francheti* (Solanaceae). Structure determination and their glycosidase inhibitory activities. Eur J Biochem. 229:369–376.

Bahnson ES, Havelka GE, Koo NC, Jiang Q, Kibbe MR. 2016. Periadventitial adipose tissue modulates the effect of PROLI/NO on neointimal hyperplasia. J Surg Res. 205:440–445.

Basey K, McGaw BA, Woolley JG. 1992. Phygrine, an alkaloid from *Physalis* species. Phytochemistry. 31:4173–4176.

Bastos GNT, Santos ARS, Ferreira VMM, Costa AMR, Bispo CI, Silveira AJA, Do Nascimento JLM. 2006. Antinociceptive effect of the aqueous extract obtained from roots of *Physalis angulata* L. on mice. J Ethnopharmacol. 103:241–245.

Bastos GNT, Silveira AJA, Salgado CG, Picanço-Diniz DLW, do Nascimento JLM. 2008. *Physalis angulata* extract exerts anti-inflammatory effects in rats by inhibiting different pathways. J Ethnopharmcol. 118:246–251.

Chen R, Liang JY, Liu R. 2007. Two novel neophysalins from *Physalis alkekengi* L. var. *franchetii*. Helvetica Chimica Acta. 90:963–966.

Choi EM, Hwang JK. 2003. Investigations of anti-inflammatory and antinociceptive activities of *Piper cubeba*, *Physalis angulata* and *Rosa hybrid*. J Ethnopharmcol. 89:171–175.
Di Stasi LC, Santos EMG, Moreira dos Santos C, Hiruma CA. 1989. In Plantas Medicinais da Amazonia. Editora Unesp, Sao Paulo; p. 45–46.
Guan YZ, Shan SM, Zhang W, Luo JG, Kong LY. 2014. Withanolides from *Physalis minima* and their inhibitory effects on nitric oxide production. Steroids. 82:38–43.
He H, Zang LH, Feng YS, Wang J, Liu WW, Chen LX, Kang N, Tashiro S, Onodera S, Qiu F, Ikejima T. 2013. Physalin A induces apoptotic cell death and protective autophagy in HT1080 human fibrosarcoma cells. J Nat Prod. 76:880–888.
He H, Zang LH, Feng YS, Chen LX, Kang N, Tashiro S, Onodera S, Qiu F, Ikejima T. 2013. Physalin A induces apoptosis via p53-Noxa-mediated ROS generation, and autophagy plays a protective role against apoptosis through p38-NF-κB survival pathway in A375-S2 cells. J Ethnopharmacol. 148:544–555.
He H, Feng YS, Zang LH, Liu WW, Ding LQ, Chen LX, Kang N, Hayashi T, Tashiro S, Onodera S, Qiu F. 2014. Nitric oxide induces apoptosis and autophagy; autophagy down-regulates NO synthesis in physalin A-treated A375-S2 human melanoma cells. Food Chem Toxicol. 71:128–135.
Hseu YC, Wu CR, Chang HW, Kumar KJS, Lin MK, Chen CS, Cho HJ, Huang CY, Lee HZ, Hsieh WT, et al. 2011. Inhibitory effects of *Physalis angulata* on tumor metastasis and angiogenesis. J Ethnopharmacol. 135:762–771.
Hsieh WT, Huang KY, Lin HY, Chung JG. 2006. *Physalis angulata* induced G_2/M phase arrest in human breast cancer cells. Food Chem Toxicol. 44:974–983.
Institute of Botany of the Chinese Academy of Sciences, and Kunming Institute of Botany of the Chinese Academy of Sciences. Flora of China (Zhongguo Zhiwu Zhi). 1978. Beijing: Science Press; p. 50–59.
Kawahara E, Fujii M, Kato K, Ida Y, Akita H. 2005. Chemoenzymatic synthesis of naturally occurring Benzyl 6-*O*-glycosyl-*β*-D-glucopyranosides. Chem Pharm Bull. 53:1058–1061.
Kusumaningtyas RW, Laily N, Limandha P. 2015. Potential of ciplukan (*Physalis Angulata* L.) as source of functional ingredient. Procedia Chem. 14:367–372.
Li J, Zhao F, Li MZ, Chen LX, Qiu F. 2010. Diarylheptanoids from the rhizomes of *Curcuma kwangsiensis*. J Nat Prod. 73:1667–1671.
Maldonado E, Torres FR, Martínez M, Pérez-Castorena AL. 2006. Sucrose esters from the fruits of *Physalis nicandroides* var. *attenuata*. J Nat Prod. 69:1511–1513.
Maldonado E, Gutiérrez R, Pérez-Castorena AL, Martínez M. 2012. Orizabolide, a new withanolide from *Physalis orizabae*. J Mex Chem Soc. 56:128–130.
Maldonado E, Pérez-Castorena AL, Romero Y, Martínez M. 2015. Absolute configuration of labdane diterpenoids from *Physalis nicandroides*. J Nat Prod. 78:202–207.
Mayr CM, Parker M, Baldock GA, Black CA, Pardon KH, Williamson PO, Herderich MJ, Francis IL. 2014. Determination of the importance of in-mouth release of volatile phenol glycoconjugates to the flavor of smoke-tainted wines. J Agric Food Chem. 62:2327–2336.
Mulligan MS, Hevel JM, Marletta MA, Ward PA. 1991. Tissue injury caused by deposition of immune complexes is L-arginine dependent. Proc Natl Acad Sci USA. 88:6338–6342.
Nicolás FG, Reyes G, Audisio MC, Uriburu ML, González SL, Barboza GE, Nicotra VE. 2015. Withanolides with antibacterial activity from *Nicandra john-tyleriana*. J Nat Prod. 78:250–257.
Pérez-Castorena AL, Oropeza RF, Vázquez AR, Martínez M, Maldonado E. 2006. Labdanes and withanolides from *Physalis coztomatl*. J Nat Prod. 69:1029–1033.
Pérez-Castorena AL, Martînez M, Maldonado E. 2010. Labdanes and Sucrose Esters from Physalis sordida. J Nat Prod. 73:1271–1276.
Qiu L, Zhao F, Jiang ZH, Chen LX, Zhao Q, Liu HX, Yao XS, Qiu F. 2008a. Steroids and flavonoids from *Physalis alkekengi* var. *franchetii* and their inhibitory effects on nitric oxide production. J Nat Prod. 71:642–646.
Qiu L, Zhao F, Liu HW, Chen LX, Jiang ZH, Liu HX, Wang NL, Yao XS, Qiu F. 2008b. Two new megastigmane glycosides, physanosides A and B, from *Physalis alkekengi* L. var. *franchetii*, and their effect on NO release in macrophages. Chem Biodivers. 5:758–763.
Ren H, Bull JL, Meyerhoff ME. 2016. Transport of nitric oxide (NO) in various biomedical grade polyurethanes: measurements and modeling impact on NO release properties of medical devices. ACS Biomater Sci Eng. 2:1483–1492.

Su BN, Misico R, Jung Park E, Santarsiero BD, Mesecar AD, Fong HHS, Pezzuto JM, Douglas Kinghorn A. 2002. Isolation and characterization of bioactive principles of the leaves and stems of *Physalis philadelphica*. Tetrahedron. 58:3453–3466.

Sun CP, Qiu CY, Yuan T, Nie XF, Sun HX, Zhang Q, Li HX, Ding LQ, Zhao F, Chen LX, Qiu F. 2016a. Antiproliferative and anti-inflammatory withanolides from *Physalis angulata*. J Nat Prod. 79:1586–1597.

Sun CP, Yuan T, Wang L, Kang N, Zhao F, Chen LX, Qiu F. 2016b. Anti-inflammatory labdane-type diterpenoids from *Physalis angulata*. RSC Adv. 6: 76838–76847.

Turtay MG, Karabas M, Parlakpinar H, Colak C, Sagir M. 2015. The analgesic effect of apelin-13 and its mechanism of action within the nitric oxide and serotonin pathways. Hippokratia. 19:319–323.

Wang C, Zhang TT, Du GH, Zhang DM. 2011. Synthesis and anti-nociceptive and anti-inflammatory effects of gaultherin and its analogs. J Asian Nat Prod Res. 13:817–825.

Wang LB, Wang JW, Wang G, Sun SC, Xu B, Wu LJ. 2012. Chemical constituents in the lipid-lowering fraction of flos *Helichrysum arenarium*. Chin J Med Chem. 22:220–226.

Wang D, Su D, Li XZ, Liu D, Xi RG, Gao HY, Wang XB. 2016. Barrigenol triterpenes from the husks of *Xanthoceras sorbifolia* Bunge and their antitumor activities. RSC Adv. 6:27434–27446.

Yamamoto A, Kawai M, Miwa T, Tsukamoto T, Kodama S, Hayakawa K. 2008. Determination of adulteration in apple juice by HPLC with novel optical rotation detector. J Agric Food Chem. 56:7302–7304.

Zhang HP, Cao CM, Gallagher RJ, Timmermann BN. 2014. Antiproliferative withanolides from several solanaceous species. Nat Prod Res. 28:1941–1951.