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 aromatic glycoside and its anti-proliferative activities from the leaves of Bergenia purpurascens

Shan-Shan Zhang, Zhi-Xin Liao, Ri-Zhen Huang, Cheng-Cheng Gong, Lan-Ju Ji & Hong-Fa Sun

To cite this article: Shan-Shan Zhang, Zhi-Xin Liao, Ri-Zhen Huang, Cheng-Cheng Gong, Lan-Ju Ji & Hong-Fa Sun (2017): A new aromatic glycoside and its anti-proliferative activities from the leaves of Bergenia purpurascens, Natural Product Research, DOI: 10.1080/14786419.2017.1338278

To link to this article: http://dx.doi.org/10.1080/14786419.2017.1338278

NATURAL PRODUCT RESEARCH, 2017
https://doi.org/10.1080/14786419.2017.1338278

Taylor & Francis
Taylor & Francis Group

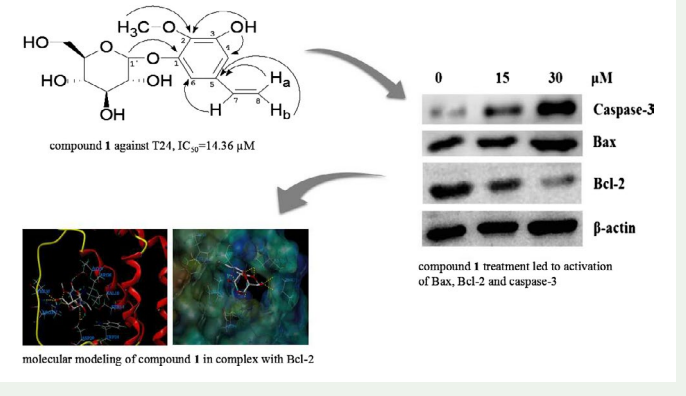
A new aromatic glycoside and its anti-proliferative activities from the leaves of *Bergenia purpurascens*

Shan-Shan Zhang[a], Zhi-Xin Liao[a], Ri-Zhen Huang[a], Cheng-Cheng Gong[a], Lan-Ju Ji[b] and Hong-Fa Sun[b]

[a]School of Chemistry and Chemical Engineering, Southeast University, Nanjing, PR China; [b]Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, PR China

ABSTRACT

Chemical investigation of the ethanolic extracts of the dried leaves of *Bergenia purpurascens* led to the isolation and identification of a new aromatic glycoside, 1-O-β-D-glucopyranosyl-2-methoxy-3-hydroxyl-phenylethene (**1**), along with other 19 known compounds (**2–20**). The structure of compound **1** was determined by a detailed analysis using various analytical techniques, including 1D and 2D NMR. *In vitro* anti-proliferative activities of compound **1** on five human cancer cell lines were evaluated. The results showed that compound **1** possessed the most potent effects with the IC_{50} values of 14.36 ± 1.04 μM against T24 cells. The further bioactivity analysis showed that compound **1** induced apoptosis of T24 cells, and altered anti- and pro-apoptotic proteins, leading to mitochondrial dysfunction and activation of caspase-3 for causing cell apoptosis. The present investigation illustrated compound **1** might be used as a potential antitumour chemotherapy candidate.



ARTICLE HISTORY

Received 19 November 2016
Accepted 12 May 2017

KEYWORDS

Bergenia purpurascens; aromatic glycoside; cancer cell lines; anti-proliferative activities; docking model

CONTACT Zhi-Xin Liao zxliao@seu.edu.cn

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1338278.

1. Introduction

The genus *Bergenia* (Saxifragaceae) includes about 10 species mainly distributed in Central and East Asia, are evergreen perennial herbs. The rhizomes have been used in herbal formulations to dissolve kidney and bladder stones, and to treat leucorrhoea, pulmonary afflictions for centuries (Asolkar et al. 1992). Alcohol extracts of the genus *Bergenia* have been shown to have analgesic, anti-inflammatory, antibacterial and anti-tussive (Sinha et al. 2001), diuretic properties (Gehlot et al. 1976), and antilithiatic (Seth et al. 1974; Garimella et al. 2001), antiviral (Rajbhandari et al. 2001, 2003). There are many reports of ethnobotanical use of different *Bergenia* species for treatment of urinary calculi and other urinary diseases; for treatment of heart disease, stomach disorders, and anti-diabetic properties (Srivastava et al. 1986). Furthermore, the genus *Bergenia* have been discovered to possess antimicrobial, antioxidant and urease inhibitory activities in recent studies (Agnihotri et al. 2015; Sadat et al. 2015).

Bergenin which was isolated from the genus *Bergenia* has now been successfully developed as a listing of drug (Compound Bergenin Tablets) for treating chronic bronchitis in China (Zhang 2008). Previous chemical compositions study of the genus *Bergenia* mainly focused on the extraction technology, content determination and preparation of bergenin.

Bergenia purpurascens, one of the main resource of bergenin, grows in moist stone crevices of alpine distributes in Tibet, has long been used as a Tibetan medicine for tonic, haemostatic and anti-tussive actions (Lv & Wang 2003). Systematic studies on the chemical compositions of this genus were rarely found in literatures, especially for its leaves, so this research aims to investigate the constitutions of the leaves of *B. purpurascens* comprehensively; to find more biological active components for further medicinal application. As a result, a new compound was obtained from its ethanolic extract which was rarely found in nature, and was tested for its antitumour and anti-proliferative activities, as well as docking model.

2. Results and discussion

Compound **1** was obtained as white powder, which was analysed for the molecular formula $C_{15}H_{20}O_8$ by HR-ESI-MS (*m/z* for [M-H]: 327.0736 Calcd 327.1035); α_D^{20}:+37.88 (*c* = 0.033, MeOH); IR (KBr) ν_{max} cm^{-1}: 3227, 1650, 1600, 1509, 1439, 894, 823, 776, 752; UV λ_{max} (CH_3OH) (log ε) 230 (1.78), 283 (2.21), 361 (0.23). The ^1H NMR spectrum of **1** showed terminal alkenyl protons at δ_H 6.97 (1H, dd, *J* = 6.8, 12.8 Hz, Ha-8), and 6.84(1H, dd, *J* = 6.8, 8.92 Hz, H_b-8), one alkenyl proton at δ_H 6.64 (1H, dd, *J* = 8.92, 12.8 Hz, H-7), corresponding to two olefinic carbons at C-8 (δ_c 118.0) and C-7 (δ_c 114.8) respectively, with the help of HSQC spectrum. The position of the double bond group was assigned at C-5 (δ_c 151.9) due to the proton signal of H-7 (δ_H 6.64) associated with C-4 (δ_c 115.9), C-6 (δ_c 118.1), and the proton signals of Ha-8, H_b-8 (δ_H 6.97 and 6.84) relating to C-5 (δ_c 151.9) from the HMBC. According to δ_H 3.69 (3H, s) and δ_c 55.8, deduced that there was a methoxy group, and it correlated with C-2 (δ_c 152.6) from HMBC spectrum. In the low field of ^1H NMR, one proton at δ_H 9.0 (1H, s), it was the character of hydroxyl group, and the hydroxyl proton signals was associated with C-2 (δ_c 152.6) and C-4 (δ_c 115.9) from the HMBC analysis, so the hydroxyl group was assigned at C-3 (δ_c 154.7). Key HMBC correlations were shown in Figure 1.

Compound **1** was hydrolysed with dilute hydrochloric acid, paper TLC showed the R_f value of sugar moiety was equal to the glucose standard sample. The terminal proton signal of glucose H-1′ δ_H 4.63 (1H, d, *J* = 7.6 Hz) could judge as β-configuration and it was connected

to C-1 (δ_c 150.8) from the HMBC. In the TOCSY spectrum, from the glucose terminal hydrogen (δ_H 4.63), this sugar spin-coupled system was got, which could be attributed other hydrogen protons, 4.63-3.12-3.15-3.23-3.25-3.45 (glucose). Hence, the structure of new compound **1** was confirmed as 1-O-β-D-glucopyranosyl-2-methoxy-3-hydroxyl-phenylethene.

The known compounds (**2–20**) were ursolic acid (**2**) (Ibrahim et al. 2014), 3β,5α-dihydroxy-15-cinnamoyloxy-14-oxolathyra-6Z,12E-diene (**3**) (Wang et al. 2008), oleanolic acid (**4**) (Guo et al. 2008), 2α-hydroxyursolic acid (**5**) (Seo et al. 1975), hydroquinone (**6**) (Song et al. 2007), gallic acid (**7**) (Deng & Qin 2008), Ferulic Acid (**8**) (Choi et al. 2005), 11-O-galloylbergenin (**9**) (Zhang et al. 2007), 11-O-(4′-hydroxybenzoyl) bergenin (**10**) (Kashima & Miyazawa 2012), ardimerin (**11**) (Ryu et al. 2002), taraxerol (**12**) (Feng et al. 2008), β-sitosterol (**13**) (Shen et al. 2012), betulinic acid (**14**) (Zhang, Li et al. 2014), ocimol (**15**) (Siddiqui et al. 2007), kaempferol (**16**) (Kuroyanagi et al. 1978), bergenin (**17**) (Chen & Yang 2008), daucosterol (**18**) (Kim et al. 1996), β-sitosterol palmitate (**19**) (Wei et al. 2013), arbutin (**20**) (Sun et al. 2006). All the above compounds were identified by the spectral data (UV, IR, ESI-MS, ^{1}H and ^{13}C NMR) with those reported in the literature.

2.1. Cytotoxic effects of compounds

Seven compounds against the five cell lines (MGC-803, T24, HepG2, NCI-H460 and HL-7702) were evaluated with MTT assay (Table 1). Compared with other compounds, **1** showed good

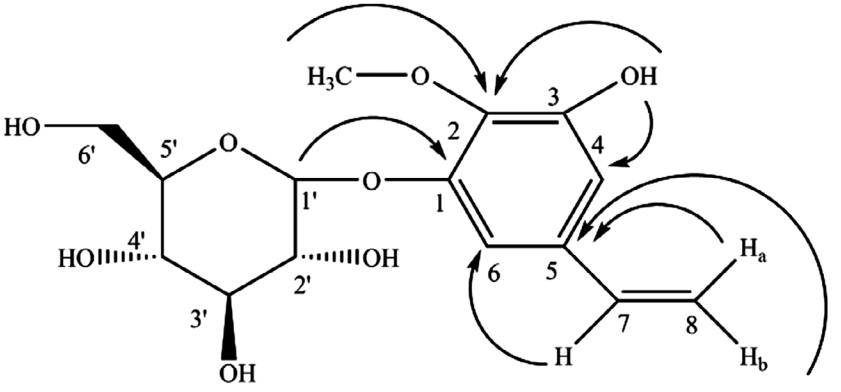


Figure 1. Key HMBC correlations of the new compound **1.**

Table 1. Cell growth inhibition activity of compounds.

Compound	IC_{50} (μM)				
	MGC-803	HepG2	NCI-H460	T24	HL-7702
1	18.10 ± 1.15	20.93 ± 1.07	17.69 ± 1.17	14.36 ± 1.04	>100
3	27.95 ± 1.42	42.78 ± 1.44	53.89 ± 1.36	48.15 ± 1.09	>100
9	18.81 ± 1.04	26.42 ± 0.87	25.68 ± 1.04	46.54 ± 1.23	>100
10	35.16 ± 0.78	>100	>100	31.29 ± 1.35	>100
11	>100	>100	>100	>100	>100
15	83.56 ± 0.98	>100	>100	56.72 ± 0.84	>100
17	26.95 ± 1.12	60.15 ± 1.28	36.47 ± 0.93	26.95 ± 1.35	>100
HCPT[a]	5.06 ± 1.21	5.03 ± 0.85	38.55 ± 1.16	4.37 ± 0.57	69.47 ± 1.84

[a]Used as positive control.

inhibitory activity, especially for cancer cell lines NCI-H460 and T24, reduced NCI-H460 cells percentages significantly, with IC_{50} values of 17.69 μM. Furthermore, compound **1** showed the strongest inhibitory effect on the growth of T24 cells (IC_{50} = 14.36 μM). However, compound **1** barely inhibited the growth of HL-7702, which is a normal cell line, at concentrations up to 100 μM.

2.2. Morphological characterisation of cell apoptosis of T24 cells by Hoechst 33258

In order to further validate the cell apoptosis upon treatment of compound **1**, T24 cells were stained with Hoechst 33258 staining after the treatment for 24 h at different concentrations (0, 15, 30 μM). Our experimental observation showed that, in the control group, most of the cells exhibited the weak blue fluorescence of normal cells (Figure S1). After the treatment of compound **1**, some cells emitted brilliant blue fluorescence, and nuclei of more T24 cells appeared hyper condensed (brightly stained). Remarkably, the numbers of apoptotic nuclei containing condensed chromatin increased significantly after the T24 cells were treated with compound **1** for 24 h, indicating that apoptosis of the T24 cells was induced by compound **1** in a concentration-dependent manner.

2.3. Effects of compound **1** *on the induction of apoptosis*

In order to confirm whether compound **1** induced reduction in cell viability due to the induction of apoptosis, the apoptotic rates of T24 cells treated with compound **1** at various concentrations of 0, 15, 30 μM (Figure S2). As shown in Figure S2, few (5.46%) apoptotic cells were present in the control panel, in contrast, the population rose to 17.99% at the concentration of 15 μM after treatment with compound **1** for 24 h. Further increase to 49.8% occurred after treatment with **1** at the concentration of 30 μM. These results clearly confirmed that compound **1** could cause a notable increase of cellular apoptosis in dose-dependent manner from 0 to 30 μM.

2.4. Compound **1** *induced expression of pro- and anti-apoptotic proteins*

The Bcl-2 family members are important regulators of the mitochondrial apoptotic pathway. Two most important members of Bcl-2 family, the anti-apoptotic protein Bcl-2 and the pro-apoptotic protein Bax, are key regulators of this progress (Zhang, Nie et al. 2014). The effects of compound **1** on the constitutive levels of Bax, Bcl-2 and caspase-3 in T24 cells are given in Figure S3, compared with the control group, **1** induced a significant increase of Bax level and an inhibition on the expression of Bcl-2, in a dose-dependent manner.

2.5. Docking model

As biological activity studies suggested that compound **1** could dramatically inhibit the expression of Bcl-2 (Figure S3), compound **1** was docked into the available crystal structure of Bcl-2 (PDB: 1bxl) in order to rationalise the observation. The binding modes of compound **1** in the binding site of Bcl-2 are depicted in Figure 2. The Surflex docking scores is 9.00 for **1**, where higher scores indicate greater binding affinity. Figure 2 shows that the interacting

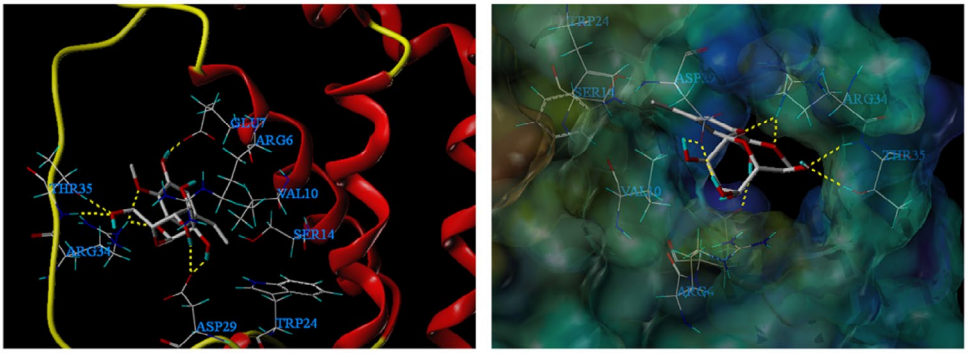


Figure 2. Molecular modelling of compound **1** in complex with Bcl-2.

mode of compound **1** in the binding site is surrounded by Arg6, Glu7, Val10, Ser14, Trp24, Asp29, Arg34 and Thr35. In particular, compound **1** forms some hydrogen bonds with the polar amino acids Glu7, Asp29, Arg34 and Thr35, respectively, suggesting a probable strong electrostatic interaction with the protein. Molecular docking analysis showed that compound **1** has inhibition effect on expression of Bcl-2 protein may be related with the binding to Bcl-2.

3. Experimental

3.1. General methods

^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance DRX-400 spectrometer. IR spectra were recorded on a NICOLET IR200 FT-IR spectrophotometer. HR-ESI-MS was carried out on an Agilent Technologies 6224 TOF LC-MS apparatus. TLC was conducted on silica gel HSGF254 plates (10–40 μm; Yan tai Jiang you Chemical, Inc.). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc.), MCI Gel (CHP 20P, 75–150um, Mitsubishi Kasei Corporation).

3.2. Plant material

The leaves of *B. purpurascens* were collected in September 2015 from Jiuzhi County, Guoluo City, Qinghai Province, China, and the plant was identified by Prof. Zhu-Nan Gong of College of Life Science, Nanjing Normal University, Nanjing, China. A voucher specimen (No. 15-09-01-01) was deposited at the Natural Products Chemistry Laboratory of Southeast University, Nanjing, China.

3.3. Extraction and isolation

The leaves of *B. purpurascens* (5.0 kg) were extracted with 95% ethanol (30.0 L × 5) at room temperature. After removal of the solvent, the extractum (327.0 g) was suspended in water (3000 mL), partitioned sequentially with petroleum ether (PE) (2000 mL × 5 times), EtOAc (2000 mL × 5 times) to yield crude PE (100.3 g), EtOAc (183.3 g) extracts respectively. The crude PE extract was decolorised on MCI GEL, then a sample (19.0 g)

was subjected to silica gel CC using a gradient system with increasing polarity of PE/EtOAc (from 50:1 to 1:6, *v*/*v*) to yield **12** (18 mg), **13** (347 mg), **14** (165 mg), **15** (12 mg), **16** (23 mg), **17** (1003 mg), **18** (368 mg), **19** (16 mg), **20** (27 mg). The EtOAc extract decolorized on MCI GEL (90.0 g), then used the same method to afford **1** (610 mg), **2** (338 mg), **3** (10 mg), **4** (18 mg), **5** (23 mg), **6** (189 mg), **7** (207 mg), **8**(20 mg), **9** (903 mg), **10** (486 mg), **11** (11 mg).

3.4. Docking model

All the docking studies were carried out using Sybyl-X 2.0 on a windows workstation. The crystal structure of the tubulin in complex with colchicine was retrieved from the RCSB Protein Data Bank (PDB: 1bxl.pdb) (Enyedy et al. 2001). The 3D structures of these selected compounds were first built using Sybyl-X 2.0 sketch followed by energy minimisation using the MMFF94 force field and Gasteiger-Marsili charges. We employed Powell's method for optimising the geometry with a distance dependent dielectric constant and a termination energy gradient of 0.005 kcal/mol. All the selected compounds were automatically docked into the colchicine binding pocket of Bcl-2 by an empirical scoring function and a patented search engine in the Surflex docking program. The polar hydrogen atoms were added and also the automated docking manner was applied in the present work. Other parameters were established by default to estimate the binding affinity characterised by the Surflex-Dock scores in the software. A higher score represents stronger binding affinity. The optimal binding pose of the docked compounds was selected based on the Surflex scores and visual inspection of the docked complexes.

3.5. 1-O-β-D-glucopyranosyl-2-methoxy-3-hydroxyl-phenylethene

White powder; m.p.145–147°C; α_D^{20}:+37.88°(*c* = 0.033, MeOH); IR (KBr) ν_{max} cm^{-1}: 3227, 1650, 1600, 1509, 1439, 894, 823, 776, 752; UV λ_{max} (CH_3OH) (log ε) 230 (1.78), 283 (2.21), 361 (0.23); HR-ESI-MS: *m/z* 327.0736 $[M-H]^-$ ($C_{15}H_{20}O_8$ Calcd 327.1035). ^1H NMR (400 MHz, DMSO) δ_H (ppm, J Hz): 9.0 (1H, s, OH-3), 6.97 (1H, dd, *J* = 6.8, 12.8 Hz, Ha-8), 6.84 (1H, dd, *J* = 6.8, 8.92 Hz, H_b-8), 6.66 (1H, d, *J* = 2.0 Hz, H-4), 6.86 (1H, d, *J* = 3.2 Hz, H-6), 6.64 (1H, dd, *J* = 8.92, 12.8 Hz, H-7), 3.69 (3H, s, $-OCH_3$), 4.63 (1H, d, *J* = 7.6 Hz, H-1′), 4.55 (1H, t, *J* = 5.6 Hz, OH-6′), 4.99 (1H, d, *J* = 5.2 Hz, OH-4′), 5.05 (1H, d, *J* = 4.4 Hz, OH-3′), 5.25 (1H, d, *J* = 4.8 Hz, OH-2′), 3.45 (1H, m, H-6′), 3.25 (1H, m, H-3′), 3.23 (1H, m, H-5′), 3.15 (1H, m, H-2′), 3.12 (1H, m, H-4′); ^{13}C NMR (100 MHz, DMSO) δ_c (ppm): 55.8 ($-OCH_3$), 61.2 (C-6′), 70.2 (C-4′), 73.7 (C-2′), 77.1 (C-5′), 77.4 (C-3′), 102.2 (C-1′), 114.8 (C-7), 115.9 (C-4), 118.0 (C-8), 118.1 (C-6), 150.8 (C-1), 151.9 (C-5), 152.6 (C-2), 154.7(C-3).

4. Conclusions

A new aromatic glycoside **(1)**, and 19 known compounds (**2–20**), were isolated from the leaves of *B. purpurascens*. The structure of the new compound was identified on the basis of spectroscopic analysis. The novel compound demonstrated potent cytotoxic activities against the T24 cancer cell lines. The further bioactivity analysis showed that compound **1** induced apoptosis of T24 cell lines.

Supplementary material

The biological experiments and original spectra of NMR, HR-ESI-MS data of the new compound (**1**) are available as Supporting Information.

Acknowledgment

We are grateful to Prof. Zhu-Nan Gong of College of Life Science, Nanjing Normal University, Nanjing, China, for the identification of the plant material.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The project was funded by State Key Laboratory for Chemistry and Molecular Engineering of Medicinal Resources (Guangxi Normal University) [grant number CMEMR 2016-B06].

References

Agnihotri V, Sati P, Jantwal A, Pandey A. 2015. Antimicrobial and antioxidant phytochemicals in leaf extracts of *Bergenia ligulata*: a Himalayan herb of medicinal value. Nat Pro Res. 29:1074–1077.

Asolkar LV, Kakkar KK, Chakre OJ. 1992. Glossary of Indian medicinal plants with active principles. New Delhi: PID, CSIR; p. 122.

Chen P, Yang JS. 2008. Chemical constituents from the *Caesalipinia millettii*. China J Chin Mater Med. 33:1852–1855.

Choi BR, Hong SS, Han XH, Hwang JS, Lee MH, Hur JD, Hwang BY, Ro JS. 2005. Antioxidant constituents from *Portulaca oleracea*. Nat Prod Sci. 11:229–232.

Deng AJ, Qin HL. 2008. Studies on chemical constituents of fruits of *Bridelia tomentosa*. China J Chin Mater Med. 33:158–160.

Enyedy IJ, Ling Y., Nacro K, Tomita Y, Wu X, Cao, Y, Guo R, Li B, Zhu X, Huang Y, Long YQ. 2001. Discovery of small-molecule inhibitors of Bcl-2 through structure-based computer screening. J Med Chem. 44:4313–4324.

Feng B, Gong XJ, Shi LY, Jian G, Pei YH, Wang YQ. 2008. Studis on phenolic compounds from *Stellera chamaejasme*. China J Chin Mater Med. 33:405–408.

Garimella TS, Jolly CI, Narayanan S. 2001. In vitro studies on antilithiatic activity of seeds of *Dolichos biflorus Linn* and rhizomes of *Bergenia ligulata Wall*. Phytother Res. 15:351–355.

Gehlot NK, Sharma VN, Vyas DS. 1976. Some pharmacological studies on ethanolic extract of roots of *Bergenia ligulata*. Indian J Pharmacol. 8:92–94.

Guo N, Chen XQ, Zhao QS. 2008. A new polyisoprenylated benzoylphloroglucinol derivative from *Hypericum henryi subsp. uraloides* (Guttiferae). *Acta Bot Yunnan*. 30: 515–518.

Ibrahim M, Ambreen S, et al. 2014. Phytochemical investigation on *Eucalyptus globulus Labill*. Asian J Chem. 26:1011–1014.

Kashima Y, Miyazawa M. 2012. Synthesis and biological evaluation of bergenin analogues as mushroom tyrosinase inhibitors. Arch Pharmacal Res. 35:1533–1541.

Kim DK, Kwak JH, Song KW, Kwon HC, Zee OP, Lee KR. 1996. Phytochemical constituents from *A. conitum pseudolaeve Var. erectum*. Saengyak Hakhoechi. 27:75–79.

Kuroyanagi M, Fukuoka M, Yoshihira K, Natori S, Yamasaki K. 1978. Confirmation of the structure of tiliroside, an acylated kaempferol glycoside, by ^{13}C-Nuclearmagnetic resonance. Chem Pharm Bull. 26:3594–3596.

Lv XM, Wang JX. 2003. Research progress in genus *Bergenia* plants. Chin Med Mat. 26:58–60.
Rajbhandari M, Wegner U, Jülich M, Schöpke T, Mentel R. 2001. Screening of Nepalese medicinal plants for antiviral activity. J Ethnopharmaco. 74:251–255.
Rajbhandari M, Wegner U, Schopke T, Lindequist U, Mentel R. 2003. Inhibitory effect of *Bergenia ligulata* on influenza virus A. Pharmazie. 58:268–271.
Ryu GS, Lee SY, Kim BS, Ryu SY, Hwang HJ, Choi BW, Lee BH, Jung DS. 2002. Ardimerin, a new dimeric lactone from the herb of *Ardisia japonica*. Nat Prod Sci. 8:108–110.
Sadat A, Uddin G, Alam M, Ahmad A, Siddiqui BS. 2015. Structure activity relationship of bergenin, p-hydroxybenzoyl bergenin, 11-O-galloylbergenin as potent antioxidant and urease inhibitor isolated from *Bergenia ligulata*. Nat Pro Res. 29:2291–2294.
Seo S, Tomita Y, Tori K. 1975. Biosynthesis of ursene-type triterpenes from sodium [1,2-^{13}C] acetate in tissue cultures of *Isodon japonicushara* and reassignments of ^{13}C NMR signals in urs-12-enes. J Chem Soc Chem Commun. 1:954–955.
Seth SDS, Prabhakar MC, Bapna BC, Arora RB. 1974. Studies on the antilithiatic property of *Berginia ligualata*. Res Indian Med. 9:1–3.
Shen L, Jiang SP, Zhu HJ. 2012. Chemical constituents in herb of *Incravillea younghusbandii Sprague*. Nat Prod Res Dev. 24:1210–1213.
Siddiqui BS, Aslam H, Ali ST, Begum S, Khatoon N. 2007. Two new triterpenoids and a steroidal glycoside from the aerial parts of *Ocimum basilicum*. Chem Pharm Bull. 55:516–519.
Sinha S, Murugesan T, Maiti K, Gayen JR, et al. 2001. Antibacterial activity of *Bergenia ciliata* rhizome. Fitoterapia. 72:550–552.
Song YL, Feng ZB, Cheng YX, Gao JM. 2007. Chemical components of *Chaenomeles speciosa* (Sweet) Nakai. Acta Bot Boreal Occident Sin. 27:0831–0833.
Srivastava TN, Rajasekhren S, Badola DP, Shah DC. 1986. An index of the available medicinal plants, used in Indian system of medicine from Jammu and Kashmir State. Ancient Sci Life. 6:49–63.
Sun XH, Shen GM, Tian X. 2006. Chemical components of *Scrofella Chinensis*. Acta Bot Boreal Occident Sin. 26:0412–0415.
Wang H, Zhang XF, Zhou Y, Peng SL, Zhou DW, Ding LS. 2008. Two new diterpenes from *Euphorbia kansuensis*. Fitoterapia. 79:262–266.
Wei XX, Li YT, Jin YQ. 2013. Studies on chemical constituents from *Husks of Tatary Buckwheat*. Nat Prod Res Dev. 25:44–46.
Zhang N, Li N, Sun YN, Li JL, Xing SS, Tuo ZD, Cui L. 2014. Diacylglycerol compounds from *Barks of Betula platyphyllawith* inhibitory activity against acyltransferase. Chin Herb Med. 6:164–167.
Zhang SS, Nie SP, Huang DF, Feng YL, Xie MY. 2014. A novel polysaccharide from *Ganoderma atrum* exerts antitumor activity by activating mitochondria-mediated apoptotic pathway and boosting the immune system. J Agric Food Chem. 62:1581–1589.
Zhang YH. 2008. Determination of bergeninum and chlorphenamine maleate in compound bergeninum tablets by HPLC. China Pharmaceuticals. 17:25–26.
Zhang W, Xu KP, Zeng GY, Tan JB, Li FS, Zhou YJ, Tan GS. 2007. Studies on chemical constituents of *Ardisiabicolor Walker*. Chin Pharm J. 42:656–658.