Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Chemopreventive Agents from *Physalis minima* Function as Michael Reaction Acceptors

Ruizhi Men[1,2], Ning Li[1,2], Chihong Ding[3], Yingzhan Tang[1,2], Yachao Xing[1,2], Wanjing Ding[3], Zhongjun Ma[3]

[1]School of Traditional Chinese Materia Medica, [2]Key Laboratory of Structure-Based Drug Design and Discovery (Shenyang Pharmaceutical University), Ministry of Education, Wenhua Road 103, Shenyang 110016, [3]Institute of Marine Biology, Ocean College, Zhejiang University, Zijingang Campus, No. 866 Yuhangtang Road, Hangzhou 310058, PR China

Submitted: 24-05-2015 Revised: 14-07-2015 Published: 11-05-2016

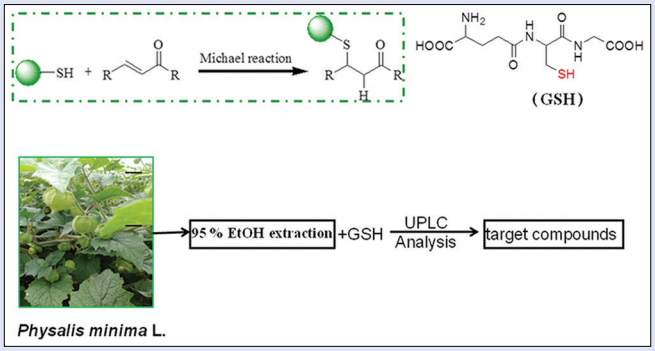
ABSTRACT

Background: The fruits of some varieties of genus *Physalis* have been used as delicious fruits and functional food in the Northeast of China. **Materials and Methods:** To reveal the functional material basis, we performed bioactivity-guided phytochemical research and chemopreventive effect assay of the constituents from *Physalis minima*. **Results:** It was demonstrated that the ethyl acetate extract of *P. minima* L. (EEPM) had potential quinone reductase (QR) inducing activity with induction ratio (IR, QR induction activity) value of 1.47 ± 0.24, and glutathione binding property as potential Michael reaction acceptors (with an α, β-unsaturated ketone moiety). Furthermore, bioactivity-guided phytochemical research led eight compounds (1–8), which were elucidated as 3-isopropyl-5-acetoxycyclohexene-2-one-1 (1), isophysalin B (2), physalin G (3), physalin D (4), physalin I (5), physordinose B (6), stigmasterol-3-O-β-D-glucopyranoside (7) and 5α-6β-dihydroxyphysalin R (8) on the basis of nuclear magnetic resonance spectroscopy analyses and HRESIMS. Then, isophysalin B (2) and physordinose B (6) showed significant QR inducing activity with IR value of 2.80 ± 0.19 and 2.38 ± 0.46, respectively.

Key words: Glutathione, Michael reaction acceptors, *Physalis minima*, *Physalis*, quinone reductase inducing activity

SUMMARY

- An ultra-performance liquid chromatographic method with glutathione as the substrate was used to detect the Michael reaction acceptors in extracts of *Physalis minima* (EPM)
- We investigated the chemical constituents of EPM guided by biological activity method
- Isophysalin B (1) and physordinose B (6) showed strong quinone reductase inducing activity with induction ratio values of 2.80 ± 0.19 and 2.38 ± 0.46
- This study generated useful information for consumers and many encourage researchers to utilize edible fruits from *Physalis* as a source of phytochemicals.



Abbreviations used: EPM: Extracts of *Physalis minima*, EEPM: Ethyl acetate extract of Physalis minima L., GSH: Glutathione, MRAs: Michael reaction acceptors, QR: Quinone reductase.

Correspondence:

Dr. Ning Li,
Key Laboratory of Structure-Based
Drug Design and Discovery, Ministry of Education,
Shenyang Pharmaceutical University,
Wenhua Road 103,
Shenyang 110016, China.
E-mail: liningsypharm@163.com

DOI: 10.4103/0973-1296.182153

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

The fruits of some varieties of genus *Physalis* have been used as delicious fruits and functional food in the Northeast of China. For example, the fruit of *Physalis alkekengi* has been used as a nutritious fruit as well as a treatment of aplastic anemia.[1] And extractor infusions from *Physalis* have also been used in various countries in folk medicine as a treatment for different illnesses, such as spleen disorder,[2] or as a tonic, diuretic, purgative,[3] anticancer or anti-mycobacterial agent.[4]

Quinone reductase (QR), a phase II enzyme, plays an important role in anticancer, detoxification pathways and antioxidant defense. It is currently included as a key member of a panel of *in vitro* bioassays directed toward the discovery of new plant-derived cancer chemopreventive agents.[5] Many natural products have been found to have activities of inducing QR.[6-8] The QR induction is regulated by the Keap1/Nrf2/ARE pathway in which Michael reaction acceptors (MRAs) (with α, β-unsaturated ketone moiety) can alkylate highly reactive cysteine residues in Keap1, and upregulate the expressions of phase II detoxifying enzymes, such as heme oxygenase 1 (HO^{-1}), QR, and glutathione (GSH)-S-transferase.[9]

In our previous studies on active constituents with QR inducing the activity of plants of the Solanaceae family,[10,11] with anolides isolated from *Physalis angulata* L. var. villosa Bonati could induce QR activity. As the plant from the same genus, *Physalis minima* L., which is widely distributed throughout tropical and subtropical regions of the world, had been investigated in this paper for the search of active constituents with QR inducing activity. An ultra-performance liquid chromatographic (UPLC) method with GSH as the substrate was used to detect the MRAs in the ethyl extract of *P. minima* L. (EEPM). The

Cite this article as: Men R, Li N, Ding C, Tang Y, Xing Y, Ding W, *et al*. Chemopreventive agents from *Physalis minima* function as michael reaction acceptors. Phcog Mag 2016;12:S231-6.

result revealed that the EEPM had electrophiles that could induce QR activity. Then, we investigated the chemical constituents of EEPM guided by biological activity method. Finally, eight compounds, 3-isopropyl-5-acetoxycyclohexene-2-one-1[12] (1), isophysalin B[13] (2), physalin G[14] (3), physalin D[15] (4), physalin I[16] (5) physordinose B[17] (6), stigmasterol-3-O-β-D-glucopyranoside[18] (7) and 5α-6β-dihydroxyphysalin R[19] (8), were isolated from the EEPM. QR induction activity was measured for these compounds to evaluate their cancer chemopreventive properties. In this paper, we described the isolation, structural elucidation, and the quinine reductase activity of these components.

MATERIALS AND METHODS

General methods

A UPLC mass system (UPLC, Waters, Milford, MS, USA) and a Bridge Ethylidene Hybrid Particle C18 column (2.1 mm × 50 mm, 1.7 μm) were used to perform the chromatographic analysis. The high-performance liquid chromatography (HPLC) analysis was performed on Shimadzu HPLC (DGU-20A5 Degasser, LC-20AT Liquid Chromatograph, SIL-20AC Autosampler, SPD-M20A Diode Array Detector, CTO-20AC Column Oven) using Inerstil ODS-SP (5 μm, 4.6 mm × 250 mm) column. Preparative HPLC was performed on Beijing Chuangxintongheng LC3000 Semi-preparation Gradient HPLC System using Sepax Amethyst C-18 (5 μm, 21.2 × 250 mm) column. Ultraviolet (UV) spectra were recorded on a UV-2550 (PC) spectro photometer, Shimadzu Corporation. One- and two-dimensional nuclear magnetic resonance (NMR) experiments were performed on a Ultra shield Plus 400 MHz spectrometer (Bruker Corporation, Germany). Chemical shifts were referred to tetramethylsilane. J values were given in Hz. HR-ESIMS spectra detection was performed on a Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer (Waters, Manchester, UK) using an electrospray ionization source in positive mode. Thin-layer chromatography was carried out on Qingdao Puke Sil G/UV254 plates of 0.25 thickness, and spots were visualized by spraying with 20% H_2SO_4/EtOH followed by heating.

Plant material

The whole plants of *P. minima* (3.3 kg dried weight) were collected in August 2011 in Guangzhou, Guangdong Province of China. The plant material was identified by Dr. Bo Yang (Zhejiang Chinese Medical University, China) and deposited at Zhejiang Chinese Medical University, under the acquisition number A20120923.

Ultra-performance liquid chromatographic method analysis of the ethyl acetate extract of *Physalis minima* L. reacted with glutathione

To analyze, we used UPLC with GSH as the substrate and we detected electrophiles in EEPM. The 20 mg of EEPM was suspended in methanol (1 mL). After centrifugal separation, 40 μL of supernatant was added to 360 μL of 5 mM GSH dissolved by 25 mM Tris-HCl buffer (pH 8.0). For another 40 μL, GSH was taken out as a control. Then they were both incubated at 37°C for 2 h. In the whole UPLC system, A (H_2O) and B (CH_3CN) were used as the mobile phase. For EEPM, a gradient program was applied according to the following method: 0–3 min, 10–30% B, 3–5 min, 20–30% B, 5–7 min 30–45% B, 7–9 min 45–75% B, and 9–13 min 75–85% B. The injection volume was 1 μL with the flow rate of 0.5 mL/min, and the column and sample temperatures were maintained at 35°C and 25°C, respectively.

Extraction and isolation

The dried and powdered whole plants of *P. minima* (3.3 kg) were extracted with 95% EtOH under reflux 3 times (3 L × 10 L). Evaporation of the solvent under reduced pressure provided the ethanolic extract (240 g). The extract was dissolved and suspended in H_2O (1.5 L), and then partitioned with petroleum ether (3 L × 1.5 L), EtOAc (3 L × 1.5 L), respectively, to yield petroleum ether extract (20.0 g) and ethyl acetate extract (65 g).

Approximately 60 g of the EEPM was subjected to column chromatography on silica gel (200–300 mesh, 130 cm × 8 cm, 600 g) gradiently eluted with petroleum ether/ethyl acetate (15:1, 10:1, 5:1, 3:1, 2:1, 1:1, 1:2, 0:1) to give 18 fractions (Fr.A–Fr.R). Fr.K (0.5 g) was subjected to preparative HPLC using MeOH/H_2O as the mobile phase (0–50 min, 37%; 50–120 min, 45%; flow rate 10 mL/min) to yield 6 sub-fractions (Kfr 1–Kfr 6), Kfr. 5 was reseparated by preparative HPLC using 30% MeOH/H_2O as the mobile phase (10 mL/min) to give compound 1 (7 mg, t_R = 59.0 min). Fr.O (3.0 g) was first subjected to silica gel chromatography eluted with dichloromethane/methanol (from 1:0–0:1) yielding Ofr. 1–Ofr10. Ofr. 2 (170 mg) was purified by preparative HPLC using 57% MeOH–H_2O (10 mL/min) to give compound 2 (25 mg). Ofr. 5 (210 mg) was separated by Sephadex LH-20 eluted with CH_2Cl_2/MeOH (1:1) and preparative HPLC using 40% MeOH–H_2O (10 mL/min) to afford compound 3 (50 mg). Ofr. 10 was purified over preparative HPLC with 40% MeOH–H_2O to give compound 4 (50 mg, t_R = 60.0 min). Fr.P (4.0 g) was first applied to silica gel chromatography eluted with CH_2Cl_2/MeOH (50:1, 30:1, 20:1) to give Pfr. 1–Pfr. 9. Pfr. 1 was purified over preparative HPLC with 50% MeOH–H_2O to give compound 5 (10 mg, t_R = 64.0 min). Fr.Q (4.0 g) was first subjected to silica gel chromatography eluted with CH_2Cl_2/MeOH (50:1, 30:1, 20:1, 15:1, 0:1) yielding Qfr. 1–Qfr. 12. Qfr. 10 was separated by preparative HPLC using MeOH/H_2O as the mobile phase (0–35 min, 30–100%; 35–55%, 100%; flow rate 10 mL/min) to get sub-fractions Qfr. 10-A–I. Qfr. 10-F was further purified by preparative HPLC using MeOH/H_2O as the mobile phase (0–35 min, 30–100%; 35–55%, 100%; flow rate 10 mL/min) to get compound 6 (30 mg, t_R = 43.0 min) and compound 7 (54 mg, t_R = 55.0 min). Qfr. 10-A was applied to preparative HPLC using 30% MeOH-H_2O (10 mL/min) to afford compound 8 (45 mg).

Bioassay for cytotoxic activity and quinine reductase activity

QR inducing activity was assessed using hepa 1c1c7 cells (obtained from ATCC). The cells were maintained in α-minimum essential medium supplemented with 0.1% penicillin-streptomycin, 10% fetal bovine serum (GIBCO, NY, USA) and incubated in 5% CO_2 at 37°C. Hepa lclc7 cells were seeded in 96-well plates at a density of 1.0×10^5 cells/mL in 100 μL of media. After 24 h, the test compounds were added, and the cells were cultured for another 48 h. The medium was decanted, and the cells were incubated with 50 μL of 0.8% digitonin and 2 mM EDTA solution (pH 7.8) for 10 min at 37°C. Then, 200 μL of a mixed solution containing bovine serum albumin(0.67mg/mL),3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.72 mM), 1.5% Tween 20, 0.5 M tris-HCl, 5 μM FAD, 150 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate dehydrogenase, 50 mM NADP, and 50 mM menadione were added into each well. After incubation for 3 min, the plates were scanned at 550 nm. Stock solution of the compound was dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO was kept at 0.5% (v/v). 4'-bromoflavone in DMSO and DMSO were used as the positive and negative control, respectively, and their final concentrations of DMSO were both kept at 0.5% (v/v). The cytotoxicity

of the compound was determined by a crystal violet assay as previously described. Induction ratio (IR) = $(OD_{NOQ1DrugGroup} - OD_{NOQ1BlankGroup})$ $(OD_{CrystalVioletSolventGroup} - OD_{CrystalVioletBlankGroup})/(OD_{NOQ1SolventGroup} - OD_{NOQ1BlankGroup})$ $(OD_{CrystalVioletDrugGroup} - OD_{CrystalVioletBlankGroup}) \times 100\%$. Experiments were carried out 3 times on separate occasions.

RESULTS AND DISCUSSION

Ultra-performance liquid chromatographic method analysis of ethyl acetate extract of *Physalis minima* L. conjugated with glutathione

In Figure 1, it was obvious that more than three peaks were weakened and three peaks disappeared by comparing UPLC chromatograms of EEPM before and after incubating with GSH, which suggested that the constituents disappeared or reduced might represent electrophiles to potentially induce QR activities as natural MARs. Among the eight compounds isolated from EEPM, compound 6 conjugated well with GSH. The UPLC chromatograms before and after incubated with GSH are shown in Figure 2.

Phytochemical investigation

The EEPM of the whole plant of *P. minima* was subjected to repeated column chromatography and HPLC to afford eight compounds (1–8) [Figure 3]. They were identified as 3-isopropyl-5-acetoxycyclohexene-2-one-1 (1), isophysalin B (2), physalin G (3), physalin D (4), physalin I (5), physordinose B (6), stigmasterol-3-O-β-D-glucopyranoside (7) and 5α-6β-dihydroxyphysalin R (8) by analysis of spectral data.

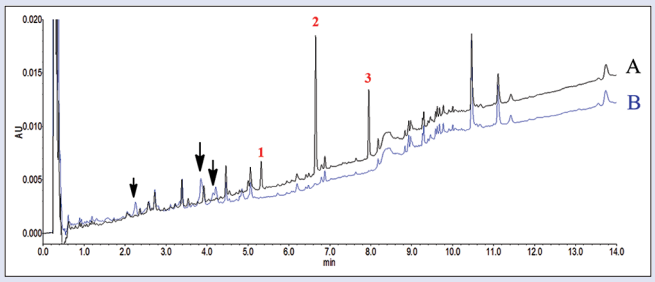


Figure 1: UPLC fingerprinting of the ethyl acetate extract of *Physalis minima* L. (EEPM) before and after the combination with GSH (A: before, B: after)

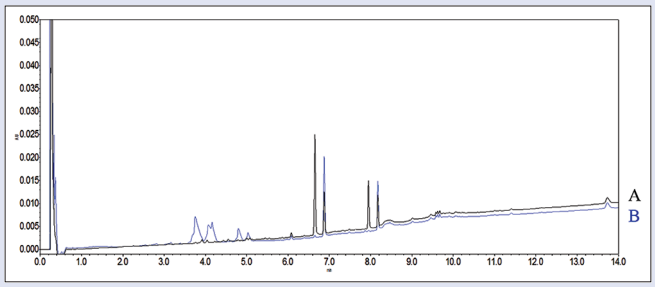


Figure 2: UPLC fingerprinting of compound 2 (isophysalin B) before and after the combination with GSH (A: before, B: after)

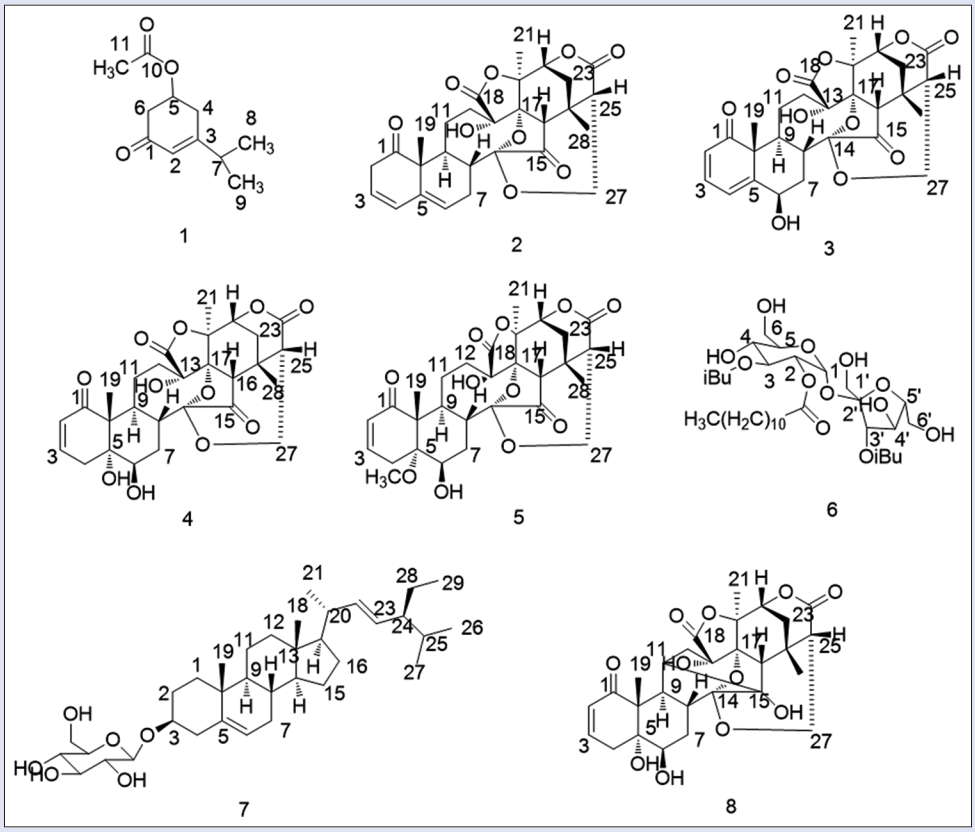


Figure 3: The structures of compounds 1-8

Table 1: The ^{1}H-NMR spectroscopic data of compounds 2, 3 and 4 (400 MHz, DMSO-d_6)

No.	2	3	4
	δ_H, mult(*J* in Hz)	δ_H, mult(*J* in Hz)	δ_H, mult(*J* in Hz)
1	-	-	-
2	2.78 m	5.92 d (9.6)	5.69 dd (10.2, 2.6)
3	5.58 m	7.02 dd (9.6, 5.5)	6.61 ddd (10.1, 5.0, 2.1)
4α	6.05 dd (10.5, 2.6)	6.15 d (5.5)	1.97dd (20.6, 5.1)
4β		-	3.11dd (7.2, 3.4)
5	-	-	4.23 s
6α	5.66 d (3.4)	4.47 m	3.47 dd (6.6, 3.0)
6β	-	5.10 d (2.3) (6-OH)	4.89 d (4.2) (6-OH)
7α	2.45 m	2.25 dd (14.0, 2.6)	1.79 m
7β	2.45 m	1.30 dd (14.0, 2.0)	1.79 m
8	2.29 m	2.69 m	2.19 dt (11.0, 11.0, 5)
9	2.99 m	2.08 m	3.11 dd (7.2, 3.4)
10	-	-	-
11α	1.28 m	2.52 m	0.93 m
11β	1.28 m	0.91 m	
12α	1.58 m	1.80 m	2.11 m
12β	1.58 m	1.39 dd (15.5, 9.8)	1.44 dd (16.4, 10.1)
13	-	6.40 s (OH)	5.77 s (OH)
14	-	-	-
15	-	-	-
16	2.27 s	2.82 s	2.78 s
17	-	-	-
18	-	-	-
19	1.26 s	1.20 s	1.09 s
20	-	-	-
21	1.96 s	1.70 s	1.79 s
22	4.50 d (4.6)	4.52 t (2.1)	4.56 t (2.3)
23α	2.24 m	2.08 m	1.90 dd (14.0, 2.3)
23β	2.24 m	1.88 dd (14.5, 2.5)	
24	-	-	-
25	2.43 m	2.87 d (3.6)	2.87 d (3.8)
26	-	-	-
27α	3.77 d (4.5)	4.25 dd (13.3, 4.3)	3.56 d (12.9)
27β	4.55 dd (14.5, 4.6)	3.59 d (13.3)	4.23 m
28	1.32 s	1.15 s	1.15 s

Table 2: The ^{1}H-NMR spectroscopic data of compounds 5 and 8 (400 MHz, DMSO-d_6 or MeOD)

No.	5	8
	δ_H, mult(*J* in Hz)	δ_H, mult(*J* in Hz)
1	-	-
2	5.68 dd (10.5, 2.8)	5.81 dd (10.0, 2.3)
3	6.63 ddd (10.6, 5.2, 2.0)	6.76 ddd (10.0, 5.2, 2.3)
4α	2.32 m	2.18 dd (19.3, 5.2)
4β	2.87 m	3.21 dt (19.7, 2.6)
5	-	-
6α	3.80 m	3.65 t (2.4)
6β	4.95 d (3.4) (6-OH)	
7α	1.60 td (11.5, 3.4)	2.09 m
7β	1.89 m	1.79 dt (13.3, 2.6)
8	2.20 td (11.2, 3.4)	2.61 dt (12.5, 2.6)
9	3.16 m	2.79 dd (13.0, 6.7)
10	-	-
11α	1.42 m	-
11β	1.42 m	2.32 t (6.1)
12α	1.61 m	1.89 m
12β	2.01 m	2.05 m
13	5.77 s (13-OH)	-
14	-	-
15	-	-
16	2.78 s	3.35 s
17	-	-
18	-	-
19	1.14 s	1.35 s
20	-	-
21	1.80 s	1.76 s
22	4.56 t (2.4)	4.46 t (2.3)
23α	1.89 m	1.89 m
23β	2.09 m	1.96 m
24	-	-
25	2.87 d (4.1)	2.73 m (β)
26	-	-
27α	4.24dd (13.4, 4.6)	4.24 br.d (12.8)
27β	3.57d (13.4)	4.92 br.d (12.4)
28	1.15 s	1.44 s

3-isopropyl-5-acetoxycyclohexene-2-one-1

White amorphous powder; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.69 (1H, brs, H-2), δ1.97 (1H, m, H-4α), δ1.53 (1H, dd, *J* = 14.8, 3.8 Hz, H-4β), δ4.33 (1H, m, H-5), δ2.46 (1H, m, H-6α), δ1.78 (1H, dd, *J* = 13.2, 3.9 Hz, H-6β), δ1.60 (1H, br.s, H-7), δ 1.47 (3H, s, H-8), δ 1.27 (3H, s, H-9), δ 1.78 (3H, s, H-11); ^{13}C NMR (100 MHz, $CDCl_3$): 182.7 (C-1), 113.0 (C-2), 86.9 (C-3), 45.8 (C-4), 67.0 (C-5), 47.5 (C-6), 36.1 (C-7), 26.6 (C-8), 27.2 (C-9), 172.1 (C-10), 30.8 (C-11).

Isophysalin B

White needle crystal; ^{1}H NMR (DMSO-d_6, 400 MHz) [Table 1]; ^{13}C NMR (DMSO-d_6, 100 MHz) [Table 3].

Physalin G

Light yellow powder; ^{1}H NMR (DMSO-d_6, 400 MHz) [Table 1]; ^{13}C NMR (DMSO-d_6, 100 MHz) [Table 3].

Physalin D

White amorphous powder; ^{1}H NMR (DMSO-d_6, 400 MHz) [Table 1]; ^{13}C NMR (DMSO-d_6, 100 MHz) [Table 3].

Physalin I

White needle crystal; HREIMS [M + Na] $^+$m/z: 581.1978 (calcd for $C_{29}H_{34}O_{11}$, 581.1999).^{1}H NMR (DMSO-d_6, 400 MHz) [Table 2]; ^{13}C NMR (DMSO-d_6, 100 MHz) [Table 3].

Physordinose B

Colorless oil; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.40 (1H, m, H-1), δ 4.99 (1H, t, *J* = 4.9 Hz, H-2), δ 5.17 (1H, m, H-3), δ 4.54 (1H, m, H-5), δ 2.34 (1H, m, H-2"α), δ 2.24 (1H, m, H-2"β), δ 5.30 (1H, d, *J* = 8.0 Hz, H-3'), δ 1.25 (16H, br.s, CH_2-5"-CH_2-11"), δ 0.88 (3H, t, *J* = 7 Hz, H-12"); ^{13}C NMR (DMSO-d_6, 100 MHz):.88.8 (C-1), 67.4 (C-2), 71.9 (C-3), 70.5 (C-4), 72.8 (C-5), 61.7 (C-6), 62.5 (C-1'), 103.2 (C-2'), 76.6 (C-3'), 71.8 (C-4'), 82.8 (C-5'), 59.9 (C-6'), 172.6 (Lauroyl O-2 C-1"), 33.4 (CH_2-2'), 24.6 (CH_2-3"), 28.6 (CH_2-4"), 29.1 (CH_2-5"-CH_2-11"), 29.0 (CH_2-5"-CH_2-11"), 28.8 (CH_2-5"-CH_2-11"), 28.6 (CH_2-5"-CH_2-11"), 29.1 (CH_2-5"-CH_2-11"), 31.4 (CH_2-5"-CH_2-11"), 22.2 (CH_2-5"-CH_2-11"), 14.0 (CH_3-12"), 175.1 (iBu-3 C-1""), 33.5 (CH-C-2""), 18.6 (CH_3-C-3""), 18.7 (CH_3-C-4""), 175.2 (iBu-3' C-1""), 34.1 (CH-C-2""), 15.8 (CH_3-C-3""), 18.9 (CH_3-C-4"").

Stigmasterol-3-O-β-D-glucopyranoside

White amorphous powder; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.36 (1H, m, H-2α), δ2.13 (1H, t, *J* = 11.8 Hz, H-2 β), δ3.42 (1H, m, H-3α), δ2.88 (1H, m, H-4α), δ3.02 (1H, m, H-4 β), δ5.32 (1H, br. s, H-6), δ0.64 (3H, s, H-18), δ0.95 (3H, s, H-19), δ1.22 (3H, br.s, H-21), δ4.85 (1H, d, *J* = 5.0 Hz, H-22), δ 4.81 (1H, d, *J* = 5.0 Hz, H-23), δ 4.21 (1H, d, *J* = 7.8 Hz, H-1'), 2.90–3.66 (5H, m, H-Glc). ^{13}C NMR (DMSO-d_6, 100 MHz): 36.9 (C-1), 31.3 (C-2), 70.2 (C-3), 41.9 (C-4), 140.6 (C-5), 121.3 (C-6), 31.3 (C-7), 31.5 (C-8), 48.7 (C-9), 36.3 (C-10), 20.1 (C-11),

Table 3: The ^{13}C-NMR spectroscopic data of compounds 2, 3, 4, 5 and 8 (100 MHz for ^{13}C, DMSO-d_6 or MeOD)

No.	δC				
	2	3	4	5	8
1	208.3	204.9	204.5	203.5	206.8
2	39.6	125.9	127.4	127.9	128.8
3	121.2	140.0	143.0	141.3	145.5
4	126.8	116.6	35.3	27.0	32.6
5	139.8	158.0	76.5	81.9	79.3
6	129.2	71.7	72.7	66.3	76.2
7	26.0	34.3	26.8	27.2	29.7
8	39.3	39.1	38.4	38.0	41.7
9	32.2	30.6	30.0	30.1	43.3
10	55.2	53.7	53.6	54.2	56.2
11	24.8	18.7	24.8	24.9	49.2
12	25.4	25.2	25.9	26.0	36.0
13	79.8	78.2	78.8	78.6	87.5
14	107.8	106.5	107.0	106.9	114.6
15	214.3	209.6	210.0	209.9	78.0
16	56.4	53.9	53.6	54.1	49.6
17	81.2	80.8	80.6	80.6	83.6
18	172.4	171.8	1719	172.0	176.0
19	26.6	17.0	13.4	13.8	14.9
20	80.5	80.5	80.6	80.8	84.0
21	21.7	21.7	21.8	21.8	21.2
22	77.0	76.4	76.5	76.4	78.3
23	33.1	31.4	31.4	31.4	36.6
24	31.3	30.6	30.6	30.6	33.0
25	51.0	49.4	49.5	49.5	51.9
26	166.8	167.3	167.5	167.5	172.2
27	60.9	60.8	60.6	60.7	61.5
28	19.4	24.5	24.6	24.6	29.5
5-OCH_3				49.6	

Table 4: Cytotoxic activities and quinine reductase activities of compounds 1-8 in hepa 1c1c7 cells

Compound	Concentration (μM)	IR
1	20	0.90±0.06
2	20	2.80±0.19
3	20	1.35±0.14
4	20	1.30±0.06
5	20	1.23±0.13
6	20	2.38±0.46
7	20	1.40±0.04
8	20	0.88±0.07
The combined residue	20 μg/mL	1.47±0.24
DMSO	0.125%	1
4'-bromoflavone[a]	10 μg/mL	2.15±0.09

a: 4'-Bromoflavone was tested as the positive control

38.4 (C-12), 41.9 (C-13), 56.3 (C-14), 23.9 (C-15), 29.3 (C-16), 55.6 (C-17), 11.8 (C-18), 19.2 (C-19), 40.1 (C-20), 20.7 (C-21), 138.1 (C-22), 128.5 (C-23), 49.6 (C-24), 31.9 (C-25), 20.4 (C-26), 18.6 (C-27), 23.9 (C-28), 12.1 (C-29), 100.8 (C-1'), 76.8 (C-2'), 76.8 (C-3'), 70.2 (C-4'), 73.5 (C-5'), 61.2 (C-6').

5α-6β-dihydroxyphysalin R

White amorphous powder; ^{1}H NMR (MeOD, 400 MHz) [Table 2]; ^{13}C NMR (MeOD, 100 MHz) [Table 3].

Quinone reductase induction activities of isolated compounds

As the EEPM of *P. minima* showed potential QR induction value of 1.47 ± 0.24 at the final concentration of 20 μg/mL, we measured the QR induction activity of constituents (1–8) isolated from the EEPM. In the *in vitro* bioactivity assay, compounds 2 (20 μM) and 6 (20 μM) showed strong QR inducing activity with IR value of 2.80 ± 0.19 and 2.38 ± 0.46, respectively, using 4-bromoflavone as a positive control substance (2.15 ± 0.09, 10 μg/mL) [Table 4].

GSH conjugating results showed that compound 2 conjugated well with GSH, which was in agreement with the result of QR inducing assay.

CONCLUSION

The results showed that the UPLC method with GSH as the substrate could be used to detect the MRAs in extracts of *P. minima*. Isophysalin B (1) and physordinose B (6) showed strong QR inducing activity with IR value of 2.80 ± 0.19 and 2.38 ± 0.46. This study generated useful information for consumers and many encourage researchers to utilize edible fruits from *Physalis* as a source of phytochemicals.

Acknowledgments

The work was supported partially by National Natural Science Foundation of China (81173531), Fund for long-term training of young teachers in Shenyang Pharmaceutical University (ZCJJ2013409).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Wang W. The health care function and medical value of *Physalis alkekengi*. Food Nutr China 2008;3:55-6.
2. Sastri BN. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. 1956; NISCAIR 4.
3. Oshima Y, Bagchi A, Hikino H, Sinha SC, Ray AB. Withaphysalin E, a withanolide of *Physalis minima* var. indica. Phytochemistry 1987;26:2115-7.
4. Sahai M, Kirson I. Withaphysalin D, a new withaphysalin from *Physalis minima* Linn. var. indica. J Nat Prod 1984;47:527-9.
5. Su B, Gu JQ, Kang YH, Park EJ, Pezzuto JM, Kinghorn AD. Induction of the phase II enzyme, quinone reductase by with anolides and norwithanolides from *Solanaceous* species. Mini Rev Org Chem 2004;1:115-23.
6. Zhang Y, Talalay P, Cho CG, Posner GH. A major inducer of anticarcinogenic protective enzymes from broccoli: Isolation and elucidation of structure. Proc Natl Acad Sci U S A 1992;89:2399-403.
7. Luo LP, Wang RH, Wang XJ, Ma ZJ, Li N. Compounds from *Angelica keiskei* with NQO1 induction, DPPH scavenging and α-glucosidase inhibitory activities. Food Chem 2012;13:992-8.
8. Colucci MA, Moody CJ, Couch GD. Natural and synthetic quinones and their reduction by the quinone reductase enzyme NQO1: From synthetic organic chemistry to compounds with anticancer potential. Org Biomol Chem 2008;6:637-56.
9. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, *et al.* Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci U S A 2002;99:11908-13.
10. Ding H, Hu Z, Yu L, Ma Z, Ma X, Chen Z, *et al.* Induction of quinone reductase (QR) by withanolides isolated from *Physalis angulata* L. var. villosa Bonati (*Solanaceae*). Steroids 2014;86:32-8.
11. Ji L, Yuan Y, Luo L, Chen Z, Ma X, Ma Z, *et al.* Physalins with anti-inflammatory activity are present in *Physalis alkekengi* var. franchetii and can function as Michael reaction acceptors. Steroids 2012;77:441-7.
12. Kong C, Xu X, Zhou B, Hu F, Zhang C, Zhang M. Two compounds from allelopathic rice accession and their inhibitory activity on weeds and fungal pathogens. Phytochemistry 2004;65:1123-8.
13. Choudhary MI, Yousuf S, Anjum S, Fun HK, Ali S. Biotransformation of physalin H and leishmanicidal activity of its transformed products. Chem Pharm Bull 2006;54:927-30.

14. Chen R, Liang J, Yang Y. Chemical constituents from *Physalis alkekengi* and structural revision of physalin G. Chin J Nat Med 2007;5:186-9.
15. Januário AH, Filho ER, Pietro RC, Kashima S, Sato DN, França SC. Antimycobacterial physalins from *Physalis angulata* L. (*Solanaceae*). Phytother Res 2002;16:445-8.
16. Row LR, Sarma NS, Matsuura T, Physalins EH. New physalins from *Physalis Angulata* and *P. lancifolia*. Phyochemrstry 1978;17:1641-5.
17. Pérez-Castorena AL, Martínez M, Maldonado E. Labdanes and sucrose esters from *Physalis sordida*. J Nat Prod 2010;73:1271-6.
18. Costa HN, Santos MC, Alcântara AF. Constituintes químicos e atividade anti-edematogênica de Peltodon radicans (LAMIACEAE). Quím Nova 2008;31:744-50.
19. Li X, Zhang C, Wu D, Tang L, Cao X, Xin Y. *In vitro* effects on intestinal bacterium of physalins from *Physalis alkekengi* var. Francheti. Fitoterapia 2012;83:1460-5.

Ning Li

ABOUT AUTHOR

Ning Li, Professor at the College of traditional Chinese medicine, Shenyang Pharmaceutical University. Her scientific interest includes isolate active ingredient and its quality control from traditional Chinese medicine and natural medicine