

## Chemical Constituents of the Seeds of *Pharbitis purpurea* and Laxative Effect of Methyl Caffeate on Rats

Fenqin Zhao , Yahui Yan , Jiawei Li , Yizhu Dong , Jin Xie ,  
Ruyue Chen  and Hui Yang \*

Institute of Pharmacy, School of Pharmacy, Henan University, Kaifeng, Henan 475004, P.R. China

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**Abstract:** The chemical constituents of the seeds of *Pharbitis purpurea* belonging to the Convolvulaceae family were studied. The anti-tumor activities of all isolated compounds on A549 and HepG2 tumor cell lines and effect of methyl caffeate (**8**) on intestinal excretion in rats were also studied. Fourteen compounds were isolated from the seeds of *P. purpurea* by column chromatography using silica gel, ODS, Sephadex LH-20 and HPLC. Their structures were determined by 1D and 2D NMR and HRMS. They were identified as (2*S*,3*S*)-5-[(1*E*)-2-carboxyethenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-3-benzofurancarboxylic acid dimethyl ester (**1**), 8-epiblechnic acid (**2**), dimethyl (1*R*,2*S*)-6,7-dihydroxy-1-(3,4-dihydroxy)-phenyl-1,2-dihydronaphthalene-2,3-dicarboxylate (**3**), dimethyl 6,9,10-trihydroxybenzo[*kl*]xanthene-1,2-dicarboxylate (**4**), syringaresinol-4'-*O*- $\beta$ -*D*-glucopyranoside (**5**), (+)-syringaresinol (**6**), (+)-pinoresinol (**7**), methyl caffeate (**8**), ethyl caffeate (**9**), osmanthuside J (**10**), methyl 6 $\beta$ ,7 $\beta$ ,16 $\beta$ ,17-tetrahydroxy ent-kauran-18-oate (**11**),  $\beta$ -sitosterol (**12**), 1-*O*-linoleoylglycol (**13**) and 1-*O*-linoleoyl-glycerol (**14**). Compounds **1** and **3** are new natural products. **4** is reported from the family Convolvulaceae for the first time and **8**, **10** and **11** from *P. purpurea* for the first time. Methyl caffeate (**8**), 10 and 30 mg/kg, significantly promote intestinal peristalsis in rats. Methyl caffeate (**8**) has no acute toxicity to rats at 5000 mg/kg.

**Keywords:** *Pharbitis purpurea*; chemical constituents; promoting intestinal peristalsis, methyl caffeate. © 2020 ACG Publications. All rights reserved.

### 1. Introduction

Qianniuzi is a traditional Chinese medicine, which is the dry mature seed of *Pharbitis nil* (L.) Choisy or *Pharbitis purpurea* (L.) Voigt. The 2015 Chinese Pharmacopoeia describes its efficacy as purging water, relieving constipation, removing phlegm, killing parasites and attacking accumulation. Qianniuzi is clinically used for oedema, constipation and abdominal distension, etc. In addition, it is often combined with Binlang (seed of *Areca catechu*), Daihuang (root of *Rheum palmatum*), Shanzha (fruit of *Crataegus pinnatifida*) or others to form a traditional Chinese medicine compound preparation for promoting digestion and easing constipation. According to pharmacological reports, *P. nil* can control plant diseases caused by *Ralstonia solanacearum* and *Xanthomonas* spp [1]. *P. nil* also inhibit MCF-F and human gastric cancer cells, and these effects are correlated with down- and up-regulation of growth-regulating apoptotic and tumor suppressor genes [2-4]. In addition, *P. nil* has alpha-glucosidase inhibitory activity, IC<sub>50</sub> = 0.35 mg/mL [5]. The chemical constituents of *P. nil* include lignans,

\* Corresponding author: E-Mail: [yanghui\\_wg@henu.edu.cn](mailto:yanghui_wg@henu.edu.cn); Phone:086-371-23880680 Fax:086-371-23880680

diterpenes, phenols, triterpenes, fatty acid methyl esters [6-13]. Many anti-tumor compounds are reported from the species. Pharbilignan C, pharbilignoside, pharbinilic acid, pharnilatins A and B inhibited A549, SK-OV-3, SK-MEL-2, HCT-15 and MDA-MB 231 cell lines by inducing apoptosis. Moreover, they inhibited NO production in the lipopolysaccharide-activated BV-2 microglia cell line [6-13]. There are also antifungal peptides (Pn-AMP1 and Pn-AMP2) in *P. nil* [14]. The seeds of *P. purpurea* and *P. nil* are used instead of each other in clinical practice. But there are still more comprehensive researches on the seeds of *P. purpurea* needed [15-16]. Therefore, the constituents of seeds of *P. purpurea* were studied in this experiment resulting in fourteen compounds identified. And it was clarified that the main component, methyl caffeate, played an important role in the laxative treatment with the seeds of *P. purpurea*.

## 2. Materials and Methods

### 2.1. General Experimental Procedures

<sup>1</sup>D and <sup>2</sup>D NMR spectra were obtained on Bruker AV-400 instruments (Bruker BioSpin Group, Faellanden, Switzerland) using TMS as internal standard for chemical shifts. Chemical shifts ( $\delta$ ) were expressed in ppm with reference to the TMS resonance. The HRESIMS spectra were recorded on an Agilent 1100 series LC/MSD ion trap mass spectrometer (Agilent Corporation, USA). The absorbance values were obtained on ELX-800UV absorbance microplate reader (Bio-Tek Corporation, USA). Optical rotations were measured with a SGW-1 polarimeter (Shanghai Jingke, China). The preparative HPLC was performed on a SHIMADZU series instrument (Shimadzu Inc., Kyoto, Japan) equipped with a Shim-pack PREP-ODS(H)KIT column (250 × 20 mm<sup>2</sup>, 5  $\mu$ m). Column chromatography (CC) was carried out on Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden), ODS (50  $\mu$ m, YMC Co., Ltd., Tokyo, Japan), and silica gel (300–400 mesh, Haiyang Chemical Co., Ltd., Qingdao, China). The preparative TLC was carried out on silica gel 60 F254 (for TLC, Haiyang Chemical Co., Ltd., Qingdao, China). Methanol (MeOH) for HPLC was purchased from Yuwang Industrial Co., Ltd. (Yucheng, China). Other organic solvents [petroleum ether, ethanol (EtOH), ethyl acetate (EtOAc) and dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>)] were analytical-grade from Fuchen Chemical Reagent Co., Ltd. (Tianjin, China). Doxorubicin hydrochloride (DOX) was purchased from Dalian Meilun Biology Technology Co., Ltd. Fetal bovine serum (FBS) was purchased from Zhejiang Tianhang Biological Technology Co., Ltd. and 3-[4,5dimethylthia-zol2yl]-2,5diphenyl tetrazolium bromide (MTT) from Beijing Solarbio Technology Co., Ltd. Mosapride citrate tablets (MC) was purchased from Taiyuan Yabao Pharmaceutical Co., Ltd.

### 2.2. Plant Material

The seeds of *P. purpurea* were collected at Jitun township, Huixian city in Henan Province, China, in September 2016 and identified by associate Prof. Zhao Yanli, Agricultural Scientific Institute in Kaifeng. A specimen (No. hd20160909) was deposited in the Herbarium of pharmacy college, Henan University, Kaifeng, China.

### 2.3. Cells and Animals

A549 (human lung carcinoma) and HepG2 (human hepatoma) tumor cell lines were obtained from the Type Culture Collection of the Chinese Academy of Sciences. (Shanghai Institute for Biological Sciences, Shanghai, China).

Male Sprague-Dawley rats (weight, 190-220 g) were purchased from Shandong Pengyue Experimental Animal Co., Ltd (lot number: SCXK 20140007). All the animals were housed in stainless steel cages in a room with controlled ambient temperature (23 ± 3°C), humidity (50 ± 20%), and lighting (12-hour light:dark cycle) conditions. The animals were free fed with water and a standard laboratory animal diet.

Our pharmacological experiments on rats were approved by the experimental animal Committee of Henan University.

#### 2.4. Extraction and Isolation

The powdered seeds of *P. purpurea* (10 kg) were extracted with 55% CH<sub>3</sub>OH (50 L) at room temperature for a week. The combined extracts were evaporated under reduced pressure to provide an CH<sub>3</sub>OH extract (101 g). The extract was fractionated by silica gel CC (7.0 × 120.0 cm) and successively eluted with CHCl<sub>3</sub>-CH<sub>3</sub>OH-H<sub>2</sub>O (95:5:0, 90:10:1, 85:15:1.5, 80:20:2, 70:30:3, 60:40:4, 50:50:5, 40:60:6, and 0:100:0, v/v/v) to obtain 10 fractions. Fr. 2 (5.2 g) was separated by silica gel CC (5.0 × 100.0 cm, eluted with petroleum ether-EtOAc 4:1, v/v) to afford **12** (193 mg) and Fr. 2.1-2.5. Compounds **13** (45 mg, *t<sub>R</sub>* 33.3 min) and **14** (50 mg, *t<sub>R</sub>* 42.5 min) were obtained from Fr. 2.4 by preparative HPLC (eluted with CH<sub>3</sub>OH-H<sub>2</sub>O, 83:17, v/v, flow rate 11 mL/min). Fr. 3 (13.2 g) was separated by silica gel CC (5.0 × 100.0 cm, eluted with CHCl<sub>3</sub>-CH<sub>3</sub>OH-H<sub>2</sub>O 25:0.95:0.05, v/v/v) to afford **8** (8.6 g). Fr. 4 (2.2 g) was treated successively by ODS CC (2.5 × 50.0 cm, successively eluted with CH<sub>3</sub>OH-H<sub>2</sub>O 0:100 to 100:0), silica gel CC (5.0 × 100.0 cm, successively eluted with CHCl<sub>3</sub>-CH<sub>3</sub>OH-H<sub>2</sub>O 30:0.95:0.05, 15:0.95:0.05, 7:0.95:0.05, v/v/v) and preparative HPLC (eluted with CH<sub>3</sub>OH-H<sub>2</sub>O, 42:58, v/v, flow rate 8 mL/min) to give **6** (2 mg, *t<sub>R</sub>* 26.9 min), **7** (2.1 mg, *t<sub>R</sub>* 31.8 min) and **9** (9.6 mg, *t<sub>R</sub>* 46.0 min). Fr. 4.4 was subjected to silica gel CC (5.0 × 100.0 cm, successively eluted with CHCl<sub>3</sub>-CH<sub>3</sub>OH-H<sub>2</sub>O 30:0.95:0.05, 15:0.95:0.05, 7:0.95:0.05, v/v/v) and then preparative HPLC (eluted with CH<sub>3</sub>OH-H<sub>2</sub>O, 45:55, v/v, flow rate 8 mL/min) to afford **2** (8.3 mg, *t<sub>R</sub>* 37.2 min). Fr. 5 (1.1 g) was also treated by preparative HPLC (eluted with CH<sub>3</sub>OH-H<sub>2</sub>O, 45:55, v/v, flow rate 10 mL/min) to afford **1** (123 mg, *t<sub>R</sub>* 45.6 min). Compounds **11** (3 mg) and **4** (30 mg) were obtained from Fr. 6 (1.2 g) by preparative HPLC (eluted with CH<sub>3</sub>OH-H<sub>2</sub>O, 50:50, v/v, flow rate 10 mL/min) and Sephadex LH-20 CC (successively eluted with CH<sub>3</sub>OH-H<sub>2</sub>O, 48:52 to 100:0, v/v). Fr. 7 (0.5 g) was separated by preparative HPLC (eluted with CH<sub>3</sub>OH-H<sub>2</sub>O, 41:59, v/v, flow rate 8 mL/min) and preparative TLC (EtOAc-EtOH-H<sub>2</sub>O, 5.6:0.95:0.05, v/v/v) to afford **3** (9.6 mg, *R<sub>f</sub>* 0.85) and **5** (12 mg, *R<sub>f</sub>* 0.15). Fr. 8 (0.3 g) was separated by preparative HPLC (eluted with CH<sub>3</sub>OH-H<sub>2</sub>O, 39:61, v/v, flow rate 8 mL/min) to yield **10** (5 mg, *t<sub>R</sub>* 18.5 min).

#### 2.5. (2*S*,3*S*)-5-[(1*E*)-2-carboxyethenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-3-benzofuran-carboxylic acid dimethyl ester (**1**)

Light yellow powders. mp 157.9-159.0°C. [ $\alpha$ ]<sub>D</sub><sup>20.3</sup> +78.6 (*c* 0.5, CH<sub>3</sub>OH). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD), <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>COCD<sub>3</sub>) data are given in Table 1; HRESIMS: *m/z* 387.1081 [M+H]<sup>+</sup> (calcd. for C<sub>20</sub>H<sub>19</sub>O<sub>8</sub>, 387.1080), 385.0928 [M-H]<sup>-</sup> (calcd. for C<sub>20</sub>H<sub>17</sub>O<sub>8</sub>, 385.0923). (see supporting information for the spectra)

#### 2.6. Cytotoxic Assay

Cytotoxicity of the 55% MeOH extract of the seeds of *P. purpurea* and compounds **1-14** against A549 and HepG2 tumor cell lines was evaluated with the MTT method. The tumor cells were seeded in 96-well (5 × 10<sup>4</sup> cells/mL) and incubated for 24 h, and then the medium were replaced with the tested compounds in different concentrations (100 μL/well). After incubating for 48 h, the medium group was exchanged with 100 μL of MTT (0.5 mg/mL in PBS medium) and incubated for 4 h. The supernatant was then removed and 100 μL of DMSO was added. The plate was placed in a shaker for dissolution. After 10 min, the optical density value was recorded with a micro plated reader at a wavelength of 570 nm.

Compounds **1-14** and DOX (positive control) were dissolved to 10 mM with DMSO. CH<sub>3</sub>OH extract was dissolved in DMSO at 10 mg/mL. Then they were diluted with the culture medium to different concentrations.

### 2.7. Effect of Methyl Caffate (**8**) on Intestinal Excretion in Rats

After a five-days adaptation period, each rat's own feces were mixed with water. The supernatant was injected intraperitoneally (5 mL/200g) into itself. Eighteen hours later, the infectious constipated model was well established when the rats showed vertical hair, curled body and the state of no eating or defecating.

Compound **8** and MC were suspended in 0.5% Sodium carboxymethyl cellulose (CMC-Na, containing 3% ink) to different **Q2** concentrations. The model rats were weighed and randomly divided into five groups ( $n = 16$ ). Compound **8** (10 or 30 mg/kg), MC (0.52 mg/kg) or physiological saline was orally administered to rats. We chose dosing volume (0.5 mL/100 g) that did not influence the food intake of the free-fed rats in this experiment. The rats were killed 45 minutes after administration. The whole intestine were took out to measure the length and weight. The propulsion rate of charcoal powder and the intestinal weight index were calculated as follows.

$$\text{Propulsion rate of charcoal powder} = V_c / V_0.$$

Where,

$V_c$  refers to the length of charcoal powder propulsion.

$V_0$  refers to the length of the whole intestine.

$$\text{Intestinal weight index} = W_i / W_0.$$

Where,

$W_i$  refers to the weight of the whole intestine.

$W_0$  refers to the weight of rat.

All values are presented as the mean  $\pm$  standard deviation (SD). The differences between the mean values of multiple groups were determined using a one-way analysis of variance. For all tests, statistical significance was set at \* $P$  values  $< 0.05$  or \*\* $P$  values  $< 0.01$ .

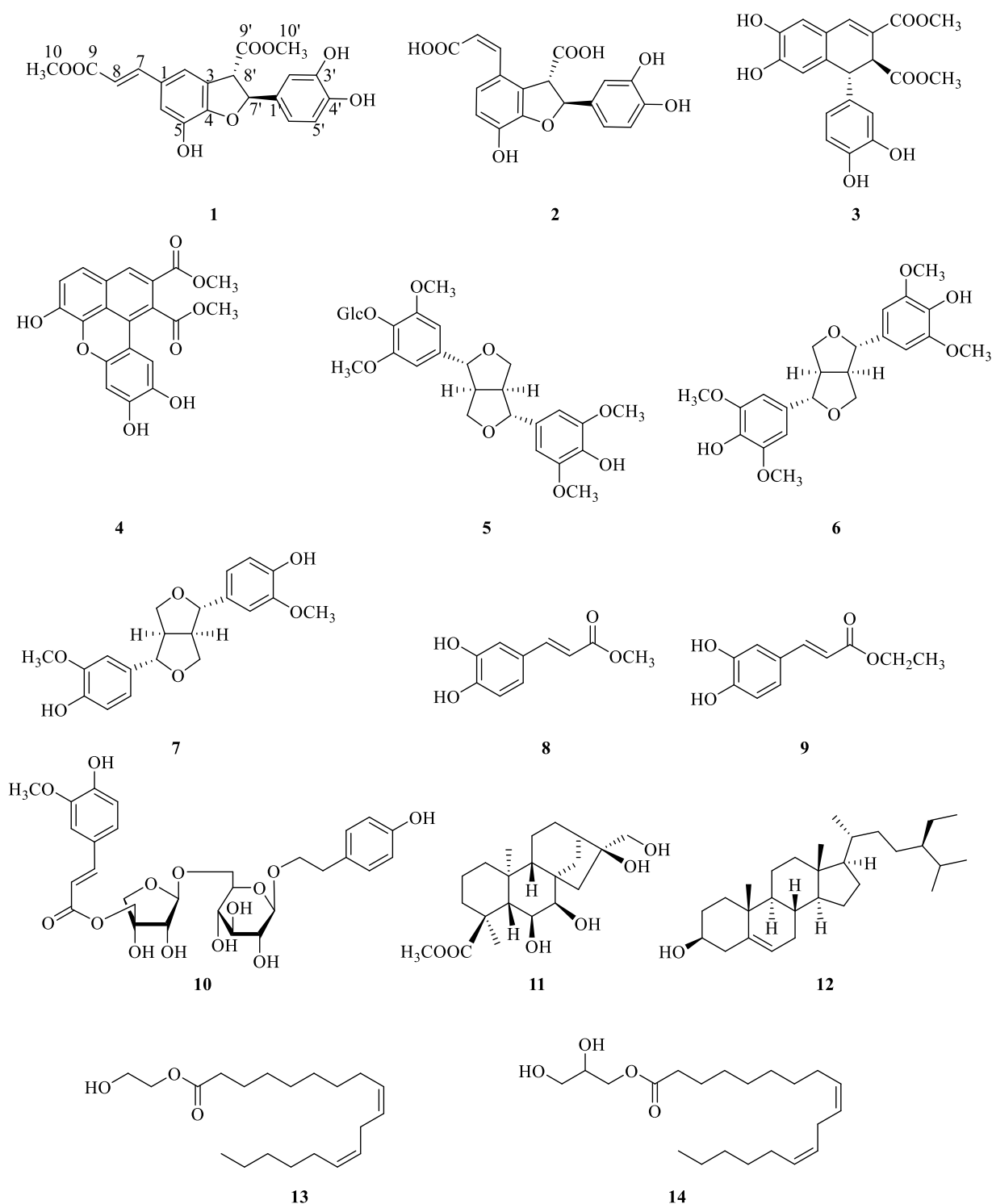
## 3. Results and Discussion

### 3.1. Structure Elucidation

Compound **1** was obtained as light yellow powder. mp. 157.9-159.0 °C.  $[\alpha]_D^{20.3} +78.6$  ( $c$  0.5, CH<sub>3</sub>OH). The molecular formula was determined as C<sub>20</sub>H<sub>18</sub>O<sub>8</sub> by its HRESIMS ( $[M+H]^+$ ,  $m/z$  387.1081; calcd for 387.1080) and HRESIMS ( $[M-H]^-$ ,  $m/z$  385.0928; calcd for 385.0923). On this basis, the <sup>1</sup>H NMR, <sup>13</sup>C NMR (in CD<sub>3</sub>OD, Table 1), DEPT and HSQC spectra of **1** showed the following groups: two methoxyl ( $\delta_H$  3.73, 3.77, each 3H, s;  $\delta_C$  52.1, 53.3), one oxymethine ( $\delta_H$  5.92, d,  $J = 7.2$  Hz;  $\delta_C$  88.6), a trans double bond ( $\delta_H$  6.27, 7.54, each 1H, d,  $J = 16.0$  Hz;  $\delta_C$  115.8, 146.5) and two carboxyl ester ( $\delta_C$  169.6 and 172.9). Comparing the NMR data of **1** with those of (2*S*,3*S*)-5-[(1*E*)-2-carboxyethenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-3-benzofurancarboxylic acid [17] showed that **1** was a derivative of caffeic acid dimers (Figure 1). The evident difference between **1** and (2*S*,3*S*)-5-[(1*E*)-2-carboxyethenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-benzofurancarboxylic acid was that **1** was two oxymethyl more than the known compound.

The HMBC (Figure 2) correlations of H-2 ( $\delta$  7.06) with C-4 ( $\delta$  150.7), C-6 ( $\delta$  117.3) and C-7 ( $\delta$  146.5), of H-6 ( $\delta$  6.99) with C-2 ( $\delta$  118.1), C-4, C-5 ( $\delta$  143.1) and C-7, of H-7 ( $\delta$  7.54) with C-2, C-6 and C-8 ( $\delta$  115.8), and of H-8 ( $\delta$  6.27) with C-1 ( $\delta$  129.8), together with the <sup>1</sup>H-<sup>1</sup>H COSY (Figure 2) correlations between H-7 ( $\delta$  7.54, d,  $J = 16.0$  Hz) and H-8 ( $\delta$  6.27, d,  $J = 16.0$  Hz), suggested there was a benzene ring linked to a double bond. The double bond was linked to a methyl carboxylate as well, which was proven by the HMBC (Figure 2) correlations of H-7, C-8 with C-9 ( $\delta$  169.6), and of H<sub>3</sub>-10 ( $\delta$  3.73) with C-9 ( $\delta$  169.6). The other phenylpropionic acid derivative methyl ester moiety in **1** were determined through correlations from H-2' ( $\delta$  6.78) to C-1' ( $\delta$  133.1), C-4' ( $\delta$  146.9) and C-7' ( $\delta$  88.6);

from H-5' ( $\delta$  6.73) to C-1', C-3' ( $\delta$  146.7) and C-4'; from H-6' ( $\delta$  6.69) to C-1', C-2' ( $\delta$  114.0), C-4' and C-7', from H-7' ( $\delta$  5.92) to C-1', C-2', C-6' ( $\delta$  118.7) and C-8' ( $\delta$  57.0), from H-8' ( $\delta$  4.26) to C-1', C-7' and C-9' ( $\delta$  172.9), and from H<sub>3</sub>-10' ( $\delta$  3.77) to C-9' in HMBC, and the <sup>1</sup>H-<sup>1</sup>H COSY correlations of H-7' ( $\delta$  5.92, d,  $J$  = 7.2 Hz)/H-8' ( $\delta$  4.26, d,  $J$  = 7.2 Hz) and H-5' ( $\delta$  6.73, d,  $J$  = 8.0 Hz)/H-6' ( $\delta$  6.69, dd,  $J$  = 8.0, 2.0 Hz)/H-2' ( $\delta$  6.78, d,  $J$  = 2.0 Hz). The HMBC spectrum of **1** also showed correlations with from H-2 to C-8', from H-7' to C-4, and from H-8' to C-3 ( $\delta$  127.6) and C-4. Based on the molecular formula, it was indicated that the two parts of phenylpropionic acid derivative methyl ester moiety were connected together, forming an oxygen-containing five-membered ring (C<sub>4</sub>→C<sub>3</sub>→C<sub>8'</sub>→C<sub>7'</sub>→O→C<sub>4</sub>). the geometry of the double bond was established to be *E* based on the coupling constant  $J_{7/8} = 16.0$  Hz.



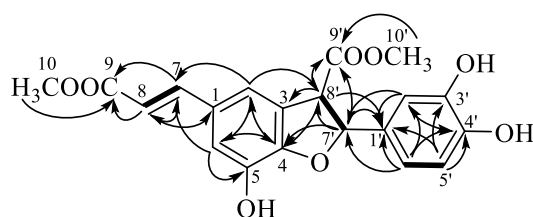
**Figure 1.** The chemical structures of compounds **1-14** from the seeds of *P. purpurea*.

The inferred structure above is the same as an organic synthetic product, (2*S*,3*S*)-5-[(1*E*)-2-carboxyethenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-3-benzofurancarboxylic acid dimethyl ester [18]. We measured the  $^{13}\text{C}$  NMR of **1** in acetone and found that it was consistent with the reported data of (2*S*,3*S*)-5-[(1*E*)-2-carboxyethenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-3-benzofurancarboxylic acid dimethyl ester (in acetone). Analysis of NOESY spectra of **1** also supported the structure. Hence, compound **1** was assigned the structure (2*S*,3*S*)-5-[(1*E*)-2-carboxyethenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-3-benzofurancarboxylic acid dimethyl ester and this is the first time that it has been obtained from a natural source.

The other isolated compounds (**2-14**) (Figure 1) were identified by comparing their spectroscopic data with those reported in the literature. They are 8-epiblechnic acid (**2**) [19], dimethyl (1*R*,2*S*)-6,7-dihydroxy-1-(3,4-dihydroxy)-phenyl-1,2-dihydronaphthalene-2,3-dicarboxylate (**3**) [20,21], dimethyl 6,9,10-trihydroxybenzo[*kl*]xanthene-1,2-dicarboxylate (**4**) [22], syringaresinol-4'-*O*- $\beta$ -*D*-glucopyranoside (**5**) [23], (+)-syringaresinol (**6**) [24,25], (+)-pinoresinol (**7**) [26-28], methyl caffeate (**8**) [29], ethyl caffeate (**9**) [30], osmanthuside J (**10**) [31], methyl 6 $\beta$ ,7 $\beta$ ,16 $\beta$ ,17-tetrahydroxy ent-kauran-18-oate (**11**) [32],  $\beta$ -sitosterol (**12**) [33], 1-*O*-linoleoyl-glycol (**13**) [34] and 1-*O*-linoleoyl-glycerol (**14**) [35].

Compound **3** is a new natural product, which is a dimer of methyl caffeate. It was reported to be obtained by the ferric chloride-catalysed condensation of methyl caffeate in the process of studying the structure of radosiin, which is a tetramer of caffeic acid with a lignan skeleton isolated from *Rabdosia japonica* [21].

Compound **4** is reported from the family Convolvulaceae and compounds **8**, **10** and **11** from *P. purpurea* for the first time.



**Figure 2.**  $^1\text{H}$ - $^1\text{H}$  COSY (—) correlations and HMBC (H→C) correlations of compound **1**

**Table 1.**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (100 MHz) NMR data for compound **1**

No.	$\delta_{\text{C}}$ (in $\text{CD}_3\text{OD}$ )	$\delta_{\text{C}}$ (in $\text{CD}_3\text{COCD}_3$ )	$\delta_{\text{H}}$ (in $\text{CD}_3\text{OD}$ )
1	129.8	129.5	
2	118.1	117.9	7.06 (br. s)
3	127.6	127.6	
4	150.7	150.2	
5	143.1	142.8	
6	117.3	117.5	6.99 (br. s)
7	146.5	145.7	7.54 (d, 16.0)
8	115.8	116.2	6.27 (d, 16.0)
9	169.6	168.0	
10	52.1	51.8	3.73 (s)
1'	133.1	132.8	
2'	114.0	114.2	6.78 (d, 2.0)
3'	146.7	146.4	
4'	146.9	146.7	
5'	116.4	116.4	6.73 (d, 8.0)
6'	118.7	118.9	6.69 (dd, 8.0, 2.0)
7'	88.6	88.1	5.92 (d, 7.2)
8'	57.0	56.4	4.26 (d, 7.2)
9'	172.9	171.9	
10'	53.3	53.2	3.77 (s)

### 3.2. Cytotoxicity Activity

The 55% MeOH extract of the seeds of *P. purpurea* exhibited moderate antitumor activities against A549 and HepG2 tumor cell lines (IC<sub>50</sub> 23.00 and 41.40 µg/mL respectively). All the tested compounds (1-14) were inactive in MTT tests.

### 3.3. Effect of Methyl Caffeate (8) on Intestinal Excretion in Rats

As shown in Table 2, the propulsion rates of charcoal powder in methyl caffeate (8) (10 or 30 mg/kg)-treated groups was 66.04 or 70.15 respectively, which was significantly higher than that in the blank group administered with physiological saline. There was significant difference between two doses of methyl caffeate (8) ( $P < 0.01$ ). The results showed that methyl caffeate (8) can promote intestinal peristalsis, which support the constipation-relieving effect of the seeds of *P. purpurea*. In addition, the content of methyl caffeate (8) in the seeds is relatively high. So methyl caffeate (8) may be considered as the main effective components of the seeds of *P. purpurea*, when used for treating constipation and abdominal distension.

As shown in Table 2, there was no significant difference among the methyl caffeate (10, 30 mg/kg)-treated groups and the blank group. So no more water is discharged when methyl caffeate (8) promoting defecation.

**Table 2.** Effect of methyl caffeate (8) on the propulsion rate of charcoal powder in rats ( $n = 16$ )

group	propulsion rate of charcoal powder
physiological saline	52.82±6.90
MC 0.52 mg/kg	73.23±7.79**
methyl caffeate 10 mg/kg	66.04±10.32**
methyl caffeate 30 mg/kg	70.15±3.33**

\*\* $P < 0.01$ , compared with the blank group

The seeds of *P. purpurea* has been recorded as poisonous in Traditional Chinese Medicine literature. In this study, its main chemical component, methyl caffeate, was orally administered to thirty-six rats and no poisoning symptoms was observed even at the maximum dose, 5000 mg/kg. Thus, methyl caffeate can be considered as an important functional component of the seeds of *P. purpurea* and it is non-poisonous. In vivo experiments of caffeic acid methyl ester in rats proved its anti-inflammatory, anti-diabetic and other effects previously [36-38]. However, those effects are not related to the clinical application of the seeds of *P. purpurea*. This is the first successful report on the pharmacological effects of methyl caffeate in combination with the actual clinical application of the seeds of *P. purpurea*.

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## Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

### ORCID

Fenqin Zhao: [0000-0002-2697-2567](https://orcid.org/0000-0002-2697-2567)

Yahui Yan: [0000-0001-6934-9874](https://orcid.org/0000-0001-6934-9874)

Jiawei Li: [0000-0002-0262-4138](https://orcid.org/0000-0002-0262-4138)

Yizhu Dong: [0000-0002-9676-7374](https://orcid.org/0000-0002-9676-7374)

Jin Xie: [0000-0002-3016-9996](https://orcid.org/0000-0002-3016-9996)

Ruyue Chen: [0000-0003-4411-749X](https://orcid.org/0000-0003-4411-749X)

Hui Yang: [0000-0003-2624-7899](https://orcid.org/0000-0003-2624-7899)

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