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Original article

Isolation and Chemotaxonomic Significance of Chemical Constituents from *Rubus parvifolius*

Quan-xi Mei[1]*, Xiao-lu Chen[1], Xue Xia[2], Zhi-jian Fang[3], Hong-bo Zhou[3], Yu-qiao Gao[1], Wei-bo Dai[1], Ren-wang Jiang[2]*

1. Zhongshan Hospital, Guangzhou University of Chinese Medicine, Zhongshan 528400, China

2. College of Pharmacy, Jinan University, Guangzhou 510632, China

3. Zhongshan Experimental Center, Guangdong College of Pharmacy, Zhongshan 528458, China

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ABSTRACT

Objective To study the chemical constituents from the roots of *Rubus parvifolius*. **Methods** The chemical constituents were extracted and purified by silica gel column chromatography. NMR spectra were used for structural identification. **Results** Phytochemical study on the roots of *R. parvifolius* led to the isolation of one ceramide (**1**), two anthraquinones (**2** and **3**), four triterpenoids (**4**–**7**), two flavonoids (**8** and **9**), one fatty acid ester (**10**), and two sterols (**11** and **12**). **Conclusion** Compound **1** is isolated from the plants of family Rosaceae for the first time, and compounds **2**–**5** are isolated from genus *Rubus* for the first time. Though *R. parvifolius* shares the same major chemical types (triterpenoid, flavonoid, and anthraquinone) with those of *R. alceaefolius*, a substituent of *R. parvifolius*, their individual constituents are different. In addition, *R. parvifolius* contains ceramide (**1**) with high concentration, while caffeoylquinic acid reported in *R. alceaefolius* has not been found in *R. parvifolius*. Furthermore, the results from our phytochemical study are consistent with the DNA phylogenic relationship between *R. parvifolius* and *R. alceaefolius* (two separated subgenera), suggesting that the substitution of the former species with the latter one in folk medicine might not be suitable.

Key words

chemical constituents; *Rubus alceaefolius*; *Rubus parvifolius*

1. Introduction

Rubus L. in the family Rosaceae is a large genus comprising 700 species worldwide, of which 210 are distributed in China (Meng et al, 2011). Some species of the genus have juicy fruits; And the fruits, roots, and leaves of some species can be used as traditional medicines. *Rubus parvifolius* L. is a shrub growing in many areas of Asia and is used as a traditional Chinese herbal medicine for the treatment of osteoma, nasopharyngeal carcinoma, chronic pyelonephritis, and laryngopharyngitis (Mei et al, 2009; GDFDA, 2011; Mei, 2011). *R. parvifolius* is a native species

*Corresponding author: **Mei QX** **Jiang RW** Tel: +86-20-8522 1016 Fax: +86-20-8522 1559 E-mail: trwjiang@jnu.edu.cn meiquanxi@163.com

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of Guangdong, China. So far, a number of triterpenes, diterpenes, flavonoids, sterols, saponins, and volatile components were found from this plant. (Liang et al, 2005; Gao, 2011; Chen and Mei, 2013; Hu, 2013; Chen et al, 2014). Modern pharmacological studies showed that this herb has properties of hepatoprotective, antioxidant (Gao et al, 2011), anti-tumor (Zheng et al, 2007), and cerebral ischemia protection (Wang et al, 2006). Interestingly, the total saponins isolated from this plant were found to show strong anti-fatigue capacity (Gao et al, 2011) in forced swimming mice model. Due to the pronounced pharmaceutical activities and large demand of *R. parvifolius* in folk medicine, *R. alceaefolius* is often used as the substituent of *R. parvifolius* (Mei, 2011).

Till now, the chemical constituent of *R. parvifolius* is still not fully investigated and whether it is proper to use *R. alceaefolius* as a substitute is still not clear. We reported herein the phytochemical studies on the roots of *R. parvifolius* collected in Guangdong province, and the chemotaxonomic significance of the isolated compounds was discussed.

2. Materials and methods

The roots of *R. parvifolius* were collected in Guangdong province of China in November 2012. It was identified through the morphological comparison with the standard herb by Prof. Zhi-jian Fang, Guangdong College of Pharmacy, and a voucher specimen (No. 20120102) of the plant was deposited in the laboratory of Guangdong College of Pharmacy. The materials were then air-dried and coarsely powdered.

The air dried and pulverized roots of *R. parvifolius* (20 kg) were extracted with 20-fold 95% ethanol under reflux conditions. After removing the solvent under reduced pressure, an aliquot of the crude extract (1.412 kg) was suspended in H_2O, and the aqueous suspension was extracted with petroleum ether, chloroform, ethyl acetate, and *n*-butanol, successively.

The petroleum ether extract was concentrated under reduced pressure to afford blackish green syrup (54.8 g). This syrup was subjected to silica gel column chromatography (100−200 mesh, 1.00 kg) eluted with petroleum ether-EtOAc gradient (100:0→60:40) to afford 14 fractions (Frs. A−N). Further purification was submitted to silica gel and Sephadex LH-20 chromatographies and preparative TLC. Chromatography (silica gel) of Fr. B yielded compounds **2** (20 mg), **3** (18 mg), and **4** (8 mg). Chromatography of Fr. E by Sephadex LH-20 yielded compound **11** (12 mg). Crystallization of Fr. I at room temperature yielded compound **5** (9 mg). Purification of Fr. K by preparative TLC yielded compound **1** (800 mg). Similar to Fr. K, preparative TLC of Fr. L yielded compound **10** (28 mg).

The EtOAc extract upon concentration under reduced pressure afforded blackish syrup (120.7 g). This syrup was subjected to silica gel chromatography (100−200 mesh, 1.50 kg) eluted with a CH_2Cl_2-CH_3OH gradient (100:0→65:35) to afford Frs. A′−K′. Compound **6** (18 mg) was obtained by preparative TLC on Fr. A′. Crystallization of Fr. D′ by slow evaporation at room temperature afforded compound **7** (1.0 g). Chromatography of Fr. F′ by Sephadex LH-20 yielded compound **8** (135 mg) and **9** (115 mg); While chromatography of Fr. K′ by silica gel afford compound **12** (23 mg). The structural identification of the above compounds was achieved by comparison of their spectroscopic data (IR, ESI-MS, ^{1}H-NMR, ^{13}C-NMR, and 2D NMR) with those reported in the literature, and the structures of these compounds are shown in Figure 1.

3. Results and discussion

3.1 Structure identification

We studied the chemical components of the roots of *R. parvifolius* collected in Guangdong province. Twelve compounds were isolated and identified by spectroscopic methods as aurantiamide acetate (**1**), chrysophanol (**2**), physcion (**3**), lupan-3-one (**4**), oleanolic acid (**5**), pomolic acid (**6**), tormentic acid (**7**), (+)-catechin (**8**), (−)-epi-catechin (**9**), monononadecanoin (**10**), stigmastane-3, 6-dione (**11**), and daucosterol (**12**). Among them, compound **1** was isolated from the plants of family Rosaceae for the first time, and compounds **2**−**5** were isolated from the plants of genus *Rubus* for the first time.

Compound **1**: colorless needle-like crystal; molecular formula: $C_{27}H_{28}N_2O_4$, EI-MS (*m/z*): 444 $[M]^+$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 2.0 (3H, s, H-1), 2.82 (2H, dd, J = 7.8, 6.8 Hz, H-11), 3.00 (1H, dd, J = 13.7, 8.3 Hz, H-10b), 3.14 (1H, dd, 13.7, 6.8 Hz, H-10a), 3.88 (1H, dd, J = 11.2, 6.0 Hz, H-3b), 3.99 (1H, dd, J = 11.2, 4.5 Hz, H-3a), 4.29 (1H, m, H-4), 4.79 (1H, m, H-7), 6.71 (1H, d, J = 8.6 Hz, H-Benzoyl- NH), 7.15 (2H, d, J = 2.2 Hz, H-2″, 6″), 7.19 (3H, t, H-3‴, 4‴, 5‴), 7.22 (3H, t, H-3″, 4″, 5″), 7.26 (2H, t, H-2″, 6″), 7.42 (2H, t, H-3′, 5′), 7.53(1H, t, H-4′), 7.71 (2H, dd, J = 8.5, 1.4 Hz, H-2′, 6′). ^{13}C-NMR (400 Hz, CD_3OD) δ: 20.8 (C-1), 38.1 (C-11), 39.0 (C-10), 51.1 (C-4), 56.6 (C-5), 66.1 (C-3), 127.5 (C-4‴), 127.8 (C-3′, 5′), 128.4 (C-2‴, 6‴), 128.6 (C-2′, 6′), 129.5 (C-2″,6″), 129.5 (C-4″), 130.3 (C-3‴, 5‴) 130.3 (C-3″, 5″), 132.8 (C-4′), 135.3 (C-1′), 138.5 (C-1‴), 138.9 (C-1″), 169.9 (C-9), 172.5 (C-6), 173.2 (C-2). The ^{1}H-NMR and ^{13}C-NMR data were in agreement with those given in Nwodo et al (2014), and thus compound **1** was identified as aurantiamide acetate.

Compound **2** was obtained as yellow amorphous powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.46 (3H, s, H-CH_3), 7.10 (1H, s, H-2), 7.27 (1H, t, H-7), 7.65 (1H, s, H-4), 7.67 (1H, d, J = 8.5 Hz, H-5), 7.81 (1H, s, J = 7.6 Hz, H-6), 12.00 (1H, s, 1-OH), 12.1 (1H, s, 8-OH). ^{13}C-NMR (400 MHz, $CDCl_3$) δ: 22.2 (C-CH_3), 113.8 (C-9a), 115.9 (C-8a), 119.9 (C-5), 121.4 (C-4), 124.4 (C-7), 124.6 (C-2), 133.3 (C-10a), 133.7 (C-4a), 136.9 (C-6), 149.3 (C-3), 162.5 (C-1), 162.8 (C-8), 182.0 (C-10), 192.6 (C-9). The ^{1}H-NMR and ^{13}C-NMR data were in agreement with those given in literature (Sanchez et al, 2011), and compound **2** was identified as chrysophanol.