DOI 10.1007/s10600-017-2167-0
Chemistry of Natural Compounds, Vol. 53, No. 5, September, 2017

FOUR FLAVONOLS WITH ANTIOXIDANT ACTIVITY FROM THE BARK OF *Cajanus cajan*

Xiao-Yu Xu,[1] Qing-Fei Fan,[2] Rui Zhan,[3] Ai-Ping Li,[1*] Zhi-Ming Kang,[1] Qi-Shi Song,[2*] and Kai-Bin Zheng[1,4*]

Cajanus cajan, namely pigeonpea, is an edible bean belonging to the genus *Cajanus*, of the family Leguminosae, which is widely distributed in tropical and subtropical regions. Pigeonpea is used as a traditional medicine in the folk medicine of many countries [1–3].

Pigeonpea has been affirmed to possess a number of bioactivities such as antimicrobial [4], antioxidant [5], hypolipidemic [6], anticancer [3], and anti-osteoporosis [7, 8], characterized by flavonoids and stilbenoids. In the present study, bioassay-guided investigation of the pigeonpea bark led to the isolation of four flavonols.

The air-dried bark (1.0 kg) of *Cajanus cajan* was collected, then shattered and refluxed with 70% ethanol (20 L) for 2 h three times. The extracts were combined and concentrated in vacuum to yield the residue, then suspended in water and successively partitioned three times with petroleum ether, dichloromethane, and *n*-butanol. These three fractions were screened by DPPH radical scavenging activity, and the *n*-butanol fraction was chosen for further fractionation. The *n*-butanol fraction was subjected to DM-101 macroporous resin with gradient eluent by MeOH–H_2O, then repeatedly chromatographed over silica gel column with an eluent of CH_2Cl_2–MeOH (95:5–75:25) and purified by Sephadex LH-20 to afford compounds **1**–**4**. Their structures were identified by ESI-MS, 1D NMR, and 2D NMR.

Quercetin (1). Yellow crystals (MeOH). ESI-MS *m/z* 301 $[M - H]^-$. Based on ^1H and ^{13}C NMR spectral data and comparison with those reported in the literature [9].

Isoquercitrin (2). Yellow crystals (MeOH). ESI-MS *m/z* 463 $[M - H]^-$. D-Glucose was identified after acid hydrolysis of **2**. Based on ^1H and ^{13}C NMR spectral data and comparison with those reported in literature [10].

Quercetin 3-*O*-β-D-Xylofuranosyl(1→2)-β-D-galactopyranoside (3). Yellow powder (MeOH). ESI-MS *m/z* 619 $[M + Na]^+$. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.75 (1H, dd, J = 8.5, 2.2, H-6′), 7.51 (1H, d, J = 2.2, H-2′), 6.81 (1H, d, J = 8.5, H-5′), 6.38 (1H, d, J = 1.9, H-8), 6.17 (1H, d, J = 2.0, H-6), 5.69 (1H, d, J = 7.7, H-1″), 4.54 (1H, d, J = 7.4, H-1‴), 3.75 (1H, m, H-2″), 3.67 (1H, m, H-4″), 3.61 (1H, m, H-3″), 3.34 (1H, m, H-5″), 3.26 (2H, m, H-6″, 4‴), 3.12 (1H, m, H-3‴), 3.05 (1H, m, H-2‴), 3.04 (1H, m, H-5‴). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 177.38 (C-4), 164.07 (C-7), 161.25 (C-5), 156.17 (C-2), 155.23 (C-9), 148.49 (C-4′), 144.94 (C-3′), 133.05 (C-3), 122.27 (C-6′), 121.15 (C-1′), 115.74 (C-2′), 115.19 (C-5′), 104.66 (C-1‴), 103.81 (C-10), 98.59 (C-6), 98.29 (C-1″), 93.37 (C-8), 79.84 (C-2″), 76.20 (C-3‴), 75.88 (C-5″), 73.94 (C-2‴), 73.62 (C-3″), 69.39 (C-4‴), 67.72 (C-4″), 65.65 (C-5‴), 59.94 (C-6″). D-Galactose and D-xylose were identified in the acid hydrolysis of **3**. The two sugars were (1→2) linked as confirmed by the HMBC correlation of H-1‴ at δ 4.54 with C-2″ at δ 79.84. The sugar moiety connected to the C-3 of quercetin was confirmed by the HMBC correlation of H-1″ at δ 5.69 with C-3 at δ 133.05. Thus, **3** was identified as quercetin 3-*O*-β-D-xylofuranosyl(1→2)-β-D-galactopyranoside [11].

1) Institute of Crop Sciences, Fujian Academy of Agricultural Sciences, No. 100, Pudang Rd., 350013, Fuzhou, P. R. China; 2) Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, No. 88, Xuefu Rd., 650223, Kunming, P. R. China, e-mail: songqs@xtbg.ac.cn; 3) Institute of Quality Standards & Testing Technology for Agro-Products, Xinjiang Academy of Agricultural Sciences, No. 403, Nanchang Rd., 830091, Urumqi, P. R. China; 4) Institute of Subtropical Agriculture, Fujian Academy of Agricultural Sciences, Dengke, 363005, Zhangzhou, P. R. China, e-mail: k03163@163.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 5, September–October, 2017, pp. 815–816. Original article submitted January 10, 2016.

 0009-3130/17/5305-0956

Quercetin 3-*O*-β-D-Glucuronopyranoside (4). Yellow plates (MeOH). ESI-MS *m/z* 477 $[M - H]^-$. D-Glucuronic acid was identified after acid hydrolysis of **4**. Based on ^1H and ^{13}C NMR spectral data and comparison with those reported in literature [12].

Compounds **3** and **4** were isolated for the first time from the family Leguminosae, while **3** has been reported only once from *Nelumbo nucifera* [11].

Antioxidant Activity. The ferric reducing antioxidant power (FRAP) of compounds was determined as reported by [13]. The DPPH radical scavenging activity of fractions and compounds was performed as reported by [14]. $ABTS^{\cdot+}$ radical scavenging capacity of fractions and compounds was carried out as described by [15]. Ascorbic acid was used as positive control. The rank order for FRAP of the four compounds was **1** > **2** > **4** > **3**. The DPPH radical scavenging activity of the *n*-butanol fraction (EC_{50} 33.8 ± 0.7 $\mu g \cdot mL^{-1}$) was higher than the dichloromethane and petroleum ether fractions (EC_{50} 280 ± 6.4, > 400 $\mu g \cdot mL^{-1}$). Compounds **1**, **2**, and **4** exhibited good DPPH scavenging activities (EC_{50} 11.8 ± 0.4, 18.2 ± 0.9, 18.3 ± 1.6 $\mu g \cdot mL^{-1}$), while compound **3** (EC_{50} 33.2 ± 1.0 $\mu g \cdot mL^{-1}$) exhibited moderate activity compared with ascorbic acid (EC_{50} 14.9 ± 0.4 $\mu g \cdot mL^{-1}$). The *n*-butanol fraction displayed the highest $ABTS^{\cdot+}$ scavenging activity with EC_{50} of 221.2 ± 5.9 $\mu g \cdot mL^{-1}$ compared with the other two fractions (EC_{50} > 800, 800 $\mu g \cdot mL^{-1}$). Compound **1** exhibited good $ABTS^{\cdot+}$ scavenging activity (EC_{50} 67.3 ± 0.8 $\mu g \cdot mL^{-1}$), while compounds **2–4** (EC_{50} 229.1 ± 3.6, 361.5 ± 15.4, 241.8 ± 4.9 $\mu g \cdot mL^{-1}$) exhibited moderate activity compared with ascorbic acid (EC_{50} 82.8 ± 0.4 $\mu g \cdot mL^{-1}$).

Structure–Antioxidant Activity Relationship. The glycosylation of the hydroxyl group at C-3 of the C-ring reduced the antioxidant activity, as shown in compound **2** compared with **1**. The antioxidant activity of compound **4** was lower than **2**, showing that the type of glycosyl at C-3 affects antioxidant activity. A disaccharide unit led to a greater reduction of antioxidant activity than a monosaccharide unit at C-3 of the aglycone, as shown in compound **3** compared with **2** and **4** [16]. These results demonstrated that the existence, type, and number of glycosyl affected the antioxidant activity of the flavonol.

ACKNOWLEDGMENT

We thank the Key Projects of Fujian Province (No. 2014N0024) and the Special Funds of Fujian Province (No. CXTD-1-1301) for financial support.

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