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ANTIOXIDANT CONSTITUENTS FROM LEAVES OF *Engelhardtia roxburghiana*

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Engelhardtia roxburghiana Wall. (Juglandaceae) is a subtropical tree grown in Guangdong, Guangxi, and Fujian Provinces of China. *Engelhardtia chrysolepis* Hance is a synonym of *E. roxburghiana* Wall. As a sweet tea, the dried leaves of the plant have been used in Chinese folk medicine for the treatment of obesity, fever, and pain. The leaves of *E. roxburghiana* have been reported to show multiple physiological activities, including anticoagulant, hypolipidemic, hypoglycemic [1], aldose reductase inhibitory activities [2,3], protection against oxidative damage [4], and protective effects on bladder function [5]. Phytochemical studies on the leaves of the plant resulted in the isolation of eucryphin, quercitrin, astilbin, isoastilbin, neoastilbin, and neoisoastilbin [6, 7].

In the course of screening for bioactive metabolites, we noticed significant antioxidant activity responsible for protective effects on bladder function [5] in the crude extract of the leaves of *E. roxburghiana.* Guided by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging monitoring [8, 9], five compounds were isolated. Most of them showed potent antioxidant activity.

In the present study, *E. roxburghiana* growing in Zhaoqing of Guangdong Province was examined. Samples of the plant were collected in August 2007, and a voucher specimen was deposited at the Drug Research Center, Guangzhou Medical College. The dried leaves of *E. roxburghiana* (2.5 kg) were extracted with ethanol (70%) at room temperature. The crude EtOH extract displayed potent DPPH radical-scavenging activity (IC_{50}, 6.19 μg/mL). The crude EtOH extract was suspended in water and extracted successively with petroleum ether (IC_{50}, 23.53 μg/mL), ethyl acetate (IC_{50}, 3.72 μg/mL), and *n*-butanol (IC_{50}, 4.59 μg/mL). Guided by the assay [8, 9], the ethyl acetate extract (150 g) was subsequently subjected to a series of chromatographic techniques using silica gel columns (mesh 200–300) and Sephadex LH-20 to afforded compounds **1**–**5**. The five compounds were found to be identical with *β*-sitosterol (**1**) [10], gallic acid (**2**) [11], quercetin (**3**) [12], astilbin (**4**) [6], and dihydroquercetin (**5**) [13], based on a comparison of the physical, NMR, and MS spectral data with those of authentic compounds. Compounds **2**, **3**, and **5** were isolated from *E. roxburghiana* for the first time.

***β*-Sitosterol (1)**: $C_{29}H_{50}O$, colorless needles. ESI-MS *m/z* 415 $[M + H]^+$. The compound is identical to an authentic sample [10].

Gallic acid (2): $C_7H_6O_5$, yellow powder. ESI-MS *m/z* 171 $[M + H]^+$ [11].

Quercetin (3): $C_{15}H_{10}O_7$, yellow needle crystals, ESI-MS *m/z* 303 $[M + H]^+$ [12].

Astilbin (4): $C_{21}H_{22}O_{11}$, white amorphous powder. ESI-MS *m/z* 449 $[M - H]^+$. ^1H and ^{13}C NMR spectral data for **4** agree fully with those published [6].

Dihydroquercetin (5): $C_{15}H_{12}O_7$, white amorphous powder. ESI-MS *m/z* 305 $[M + H]^+$ [13].

The antioxidant activity of the crude extract, fractions, and the isolated compounds was determined by way of the radical scavenging activity [8, 9] using DPPH. Each test sample (0.1 mL) of various concentrations (0.11–12.0 μg/mL in MeOH) was added to 3.9 mL of freshly prepared DPPH solution (40 μg/ mL in MeOH), and the mixture vortexed for 15 s. The blank sample consisted of 0.1 mL of methanol added to 3.9 mL DPPH. After a 90 min incubation period at room temperature in the dark, the absorbance was measured at 517 nm. The tests were carried out in triplicate. The inhibition percentage (%) of radical-scavenging activity was calculated as follows: $I\% = (1 - A_S/A_0) \times 100$, where A_0 is the absorbance of the blank and A_s is the absorbance in the presence of the test sample at different concentrations. The IC_{50} (concentration providing 50% inhibition) was calculated graphically using a calibration curve in the linear range by plotting the sample concentration vs the corresponding scavenging effect. The crude EtOH extract displayed DPPH scavenging activity (IC_{50}, 6.19 μg/mL). The petroleum ether,

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ethyl acetate, and *n*-butanol fractions were evaluated for DPPH activity. The ethyl acetate fraction was found to be more active (IC_{50} values for petroleum ether, ethyl acetate, and *n*-butanol were 23.53, 3.72, and 4.59 μg/mL, respectively). Compounds **2**–**5** were proved to possess pronounced scavenging activity with IC_{50} values of 1.46, 2.48, 3.65, and 6.85 μg/mL, respectively.

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