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α-GLUCOSIDASE INHIBITORY COMPOUNDS FROM SEEDS OF *Cassia obtusifolia*

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Cassia obtusifolia L. belongs to the Leguminosae family and is found in the region of south of Changjiang River, China [1]. The dried seed of *C. obtusifolia* is a traditional Chinese medicine used to protect the liver and improve eyesight. Phytochemical research showed that anthraquinone and naphthopyrone are the main compounds in the seed of *C. obtusifolia.* Pharmacological investigations showed that anthraquinones in the seed of *C. obtusifolia* have antibacterial activity, regulate blood lipids and blood pressure, and possess antimutant activity [2–5]. However, no research has so far been conducted concerning the α-glucosidase inhibitory compounds from this plant. In order to investigate α-glucosidase inhibitory compounds from the seed of *C. obtusifolia*, six anthraquinones were isolated by column chromatography and identified by MS and NMR spectroscopy, and the α-glucosidase inhibitory activity of six compounds and extraction of *C. obtusifolia* were assayed. Results are presented here.

The seeds of *C. obtusifolia* were collected in September, 2008 from the medical herb garden in Henan University, Kaifeng, China. The sample was identified by Prof. Changqin Li. A voucher specimen was deposited in the Institute of Chinese Materia Medica, Henan University.

Dried seeds of *C. obtusifolia* (1 kg) were extracted three times with methanol under reflux. After evaporation of solvent *in vacuo*, the concentrated extract was suspended in water and extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The solution was concentrated under reduced pressure to yield the petroleum ether extract (5 g), the EtOAc extract (15 g), and the *n*-BuOH extract (21 g). Based on the α-glucosidase inhibition assay (Table 1), the activity of EtOAc extract was higher than that of acarbose as positive control. The EtOAC extract was subjected to CC over silica gel (200–300 mesh) developing with $CHCl_3$–MeOH (100:1 to 8:2) to yield three fractions. Fraction 1 was separated on silica gel H with petroleum ether–EtOAC (50:1 to 8:2) and further chromatographed on Sephadex-LH 20 ($CHCl_3$–MeOH 1:1) to yield compounds **1** (1.2 mg) and **2** (3.4 mg). Fraction 3 was separated on silica gel H with petroleum ether–actone (7:3) and further chromatographed on Sephadex-LH 20 (acetone) to yield compounds **3** (18.2 mg), **4** (121.1 mg), **5** (1.6 mg), and **6** (2.1 mg). The structure of compounds **1–6** was identified using spectral methods and mass spectrometry.

Chrysoobtusin (**1**) [6], obtusifolin (**2**) [7], 8-*O*-methylchrysophanol (**3**) [8], physcion (**4**) [9], aurantioobtusin (**5**) [10], and 1-*O*-methylemodin (**6**) [11] were identified by ^1H and ^{13}C NMR and mass spectroscopic techniques according to previously published spectral data.

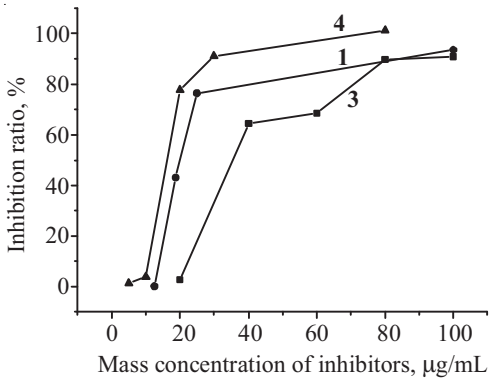

Fig. 1. Effect of mass concentration of compounds **1**, **3**, and **4** from *C. obtusifolia* against α-glucosidase.

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TABLE 1. Inhibitory Effect of Extracts and Compounds **1–6** from *C. obtusifolia* against α-Glucosidase

Sample	Prescreening concentration, $\mu g \cdot mL^{-1}$	α-Glucosidase inhibition, I (%)	IC_{50}, $\mu g \cdot mL^{-1}$
Total methanol extract	1500	54.97	1384.2
Petroleum ether extract	1500	61.75	1310.2
EtOAc extract	1500	77.07	932.8
n-BuOH extract	1500	61.08	1114.3
1	100	87.40	16.20
2	100	30.78	Nt.
3	100	66.19	20.05
4	100	90.89	35.39
5	100	0	Nt.
6	100	14.24	Nt.
Acarbose	1500	68.43	1081.2

Nt.: not available because of low activity (I% < 50).

Biological Assay. Compounds **1–6** were tested for their α-glucosidase inhibitory activity according to the literature [12]. All reactions were carried out with three replications. Acarbose was used as positive control. The α-glucosidase inhibitory activity of compounds **1**, **3**, and **4** was higher than that of acarbose (Table 1), and the activity of **1**, **3**, and **4** showed a dependence on concentration (Fig. 1). The activity of compounds **1** and **4** was higher than that of acarbose.

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