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PHENOLIC CONSTITUENTS WITH INHIBITORY ACTIVITIES ON ACETYLCHOLINESTERASE FROM THE RHIZOMES OF *Gastrodia elata*

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Gastrodia elata Blume (Orchidaceae) is a famous traditional Chinese medicine (TCM) used for the treatment of convulsive diseases such as epilepsy [1]. This drug (Chinese name "Tianma") also has numerous beneficial properties: tranquilizing and allaying excitement; analgesia; expelling toxins; enhancing strength and virility; improving circulation and memory [2]. It is prescribed for headaches, migraines, dizziness, epilepsy, rheumatism, neuralgia, paralysis, and other neuralgic and nervous disorders.

Phytochemical studies on the rhizome of *G. elata* have reported the composition of volatile compounds [3] and main phenolic compounds [4–6]. *G. elata* has obvious pharmacologic actions on the nervous system, such as tranquilizing and allaying excitement [7], and neuroprotective properties [8].

Acetylcholinesterase (AChE) is a serine protease that hydrolyzes the neurotransmitter acetylcholine, and its activity serves to terminate synaptic transmission. The loss of synapses is the major correlate of cognitive impairment and Alzheimer′s disease [9]. Acetylcholinesterase inhibitors (AChEIs) stop AChE from breaking down acetylcholine, thereby increasing the level and duration of action of acetylcholine. Phenolic compounds from *G. elata* have been thought to be the major active components in this plant [10]. We investigated the inhibitory effects on AChE of phenolic compounds from *G. elata* to understand their pharmacologic mechanisms of neuroprotection.

The chemical investigation on the ethanol extract of *Gastrodia elata* led to the isolation of ten phenolic compounds (**1–10**).

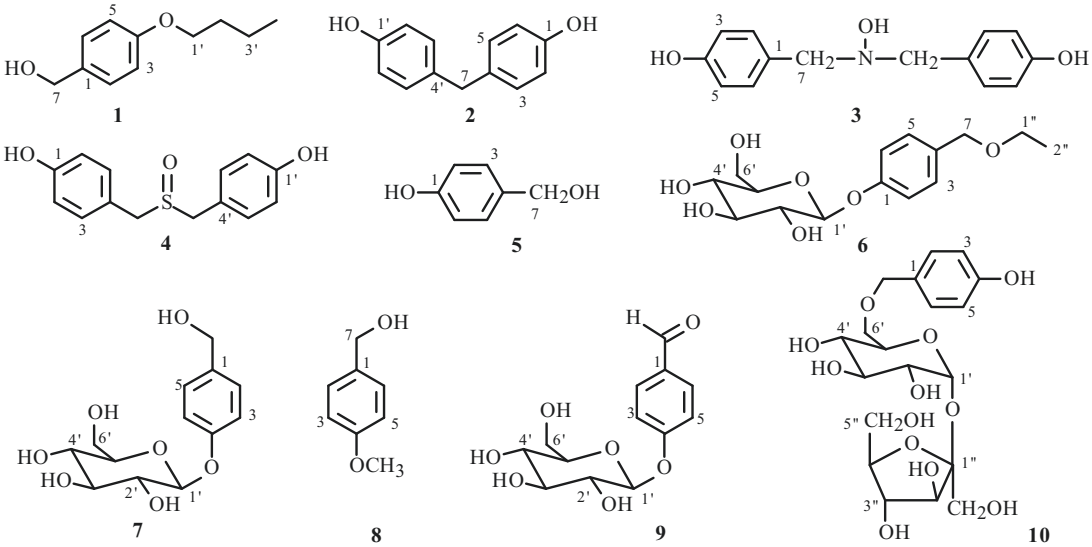

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TABLE 1. Inhibitory Activity of Phenolic Compounds from *G. elata* against Acetylcholinesterase

Compound	%	Compound	%
1	51.5	**7**	< 10
2	16.1	**8**	< 10
3	< 10	**9**	29.7
4	< 10	**10**	< 10
5	23.4	Tacrine*	57.7
6	< 10		

*Positive control.

The rhizome of *G. elata* was collected from Zhaotong City (Yunan Province, China) in September 2008 and identified by Prof. Jun Zhou from the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (ZTM200801) is deposited. The air-dried rhizome of *G. elata* (2 kg) was powdered and extracted thrice with 95% EtOH under reflux. The combined extracted EtOH solution was evaporated under reduced pressure, then suspended in water and partitioned with EtOAc and *n*-BuOH sequentially to yield EtOAc-soluble (15 g) and *n*-BuOH-soluble (19 g) fractions. The EtOAc-soluble fraction was separated by silica gel column chromatography (CC) and eluted with a $CHCl_3$–MeOH gradient solvent system to give five fractions (1–5). Fraction 2 (2.8 g) was subjected to CC over silica gel and eluted with petroleum ether–EtOAc to give four subfractions, 2a–2d. Subfraction 2b (0.8 g) was chromatographed repeatedly and eluted with petroleum ether–EtOAc to yield compounds **1** (10 mg), **5** (28 mg), **3** (18 mg), and **4** (33 mg). Subfraction 2c (0.5 g) was chromatographed and eluted with petroleum ether–EtOAc to give **8** (8 mg). Fraction 3 (1.5 g) was subjected to CC over silica gel and eluted with petroleum ether–EtOAc to give three subfractions, 3a–3c. Subfraction 3b was chromatographed repeatedly and eluted with petroleum ether–EtOAc to yield **2** (33 mg) and **4** (8 mg). Fraction 4 (1.1 g) was chromatographed repeatedly over a silica gel column with a petroleum ether–EtOAc solvent system and then chromatographed over a Sephadex LH-20 column to give **3** (5 mg). The *n*-BuOH-soluble fraction was separated on a Sephadex LH-20 column with chloroform–MeOH as the eluent, and then purified further on an RP-18 column eluted with a MeOH–H_2O gradient system to yield **6** (12 mg), **7** (22 mg), **9** (10 mg), and **10** (5 mg).

These isolates (**1–10**) were characterized as 4-butoxyphenylmethanol (**1**) [11], 4,4′-methylenediphenol (**2**) [12], gastrodamine (**3**) [13], 4,4′-sulfinylbis(methylene)diphenol (**4**) [14], 4-(hydroxymethyl)phenol (**5**) [12], 4-(ethoxymethyl)-glucopyranosyl-phenol (**6**), gastrodin (**7**) [12], (4-methoxyphenyl)methanol (**8**) [11], 4-*O*-glucopyranosyl-benzaldehyde (**9**) [15], and gastrodin A (**10**) [16]. Structures of compounds **1–10** were based on comparison of their NMR data with those reported in the literature [11–16]. All these isolates were evaluated for their inhibitory activities against AChE using a spectrophotometric method [17].

Results (Table 1) showed that four compounds, 4-butoxyphenylmethanol (**1**), 4,4′-methylenediphenol (**2**), 4-(hydroxymethyl)phenol (**5**), and 4-*O*-glucopyranosyl-benzaldehyde (**9**), possessed inhibitory activity with values of 51.5, 16.1, 23.4, and 29.7%, respectively, at 50 μM. Tacrine was used as a positive control and showed 57.7% inhibition. The other six phenolic compounds were inactive. In accordance with the function of AChE in the nervous system, the discovery of bioactive AChEIs from *G. elata* could help us to understand their mechanism of neuroprotective action.

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