Acetylcholinesterase Activity of Alkaloids from the Leaves of *Waltheria brachypetala*

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Abstract

Chemical investigation of the leaves of *Waltheria brachypetala* Turks (Sterculiaceae) resulted in the isolation of quinolinone alkaloids, waltherione-A, waltherione-B (*N*-methylwaltherione-A), 8-methoxyflindersine, and the cyclic peptide alkaloid waltherine. The inhibition of activity of acetylcholinesterase (AChE) by the alkaloids was evaluated. Waltherione-A, waltherione-B and waltherine showed significant activity.

Key words

Waltheria brachypetala · Sterculiaceae · quinolinone alkaloids · acetylcholinesterase inhibitors

Abbreviations

AChE: acetylcholinesterase
DTNB: 5,5′-dithio-bis(2-nitrobenzoic acid)

Species of Sterculiaceae are used in Brazilian traditional medicine in the treatment of bronchitis, laryngitis, throat inflammation and as tumor agents [1]. The Hermannieae tribe of this family is composed of only two genera, *Waltheria* and *Melochia*. *W. douradinha* [1], and *W. americana* [2], *M. corchorifolia* [3] and *M. tomentosa* [4] are the most studied species. Plants belonging to this tribe are known to contain cyclic peptides, quinolinone and isatine alkaloids [5], [1]. The occurrence of quinolinone alkaloids in the Sterculiaceae was first reported from *Melochia tomentosa* which afforded a 4-quinolinone alkaloid, named melochinone, with a 7-membered ring fused to the quinolinone system and an open chain analogue derivative named melovinone [4]. More recently, the waltherione-A analogue of melochinone was isolated from *Waltheria douradinha* [1], *M. chamaedrys* [5] and *M. odorata* [6].

Waltheria brachypetala Turks grows in Brazilian semiarid regions, mainly in Bahia State (Brazil) and is known as "malva" by the local population. The leaves are traditionally used to treat tooth ache and other diseases. To date, there are no reports dealing with its chemical composition or biological activities. The present paper describes evaluation of the AChE inhibitory potential of waltherione-A (**1**), waltherione-B (**2**), 8-methoxyflindersine (**3**), and the cyclic peptide alkaloid waltherine-A (**4**) isolated from the leaves of *W. brachypetala* (**Fig. 1**).

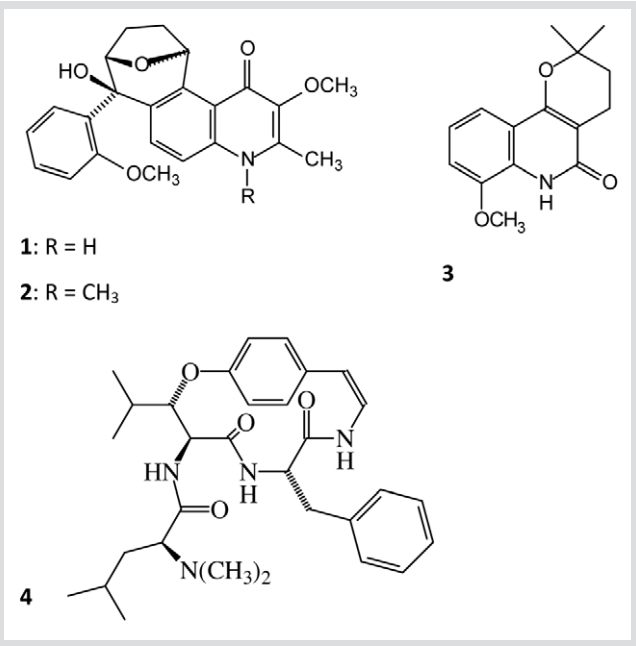

Fig. 1 Alkaloid constituents isolated from *W. brachypetala*.

The quinolinone alkaloids **1**, **2**, **3** and the cyclic peptide alkaloid **4** were identified by analysis and comparison of their spectral data [^{1}H- and ^{13}C-NMR (1 D and 2 D)] with literature values [1]. The presence of these compounds in *W. brachypetala* confirms that quinoline alkaloids are common in the Hermannieae, especially derivatives of melochinone [4] such as waltherione-A (**1**). The occurrence of 8-methoxyflindersine (**3**) was unexpected since this compound is common in Rutaceae [7], [8]. However, 2-quinolinone alkaloids were found in *Sterculia lychnophora* Hance (Sterculiaceae) [9].

The effects of **1**–**4** on the inhibition of AChE were assessed using different concentrations of these compounds. The results showed that only three compounds had inhibitory activity (▶ **Fig. 2**). Waltherione-A (**1**), *N*-methylwaltherione (**2**), and waltherine (**4**) inhibited the enzyme activity with IC_{50} values of 134.1 ± 20.9 μg/mL, 122.7 ± 19.7 μg/mL, and 113.4 ± 35.2 μg/mL, respectively, in a response dose-dependent manner. Furthermore, **4** at a concentration of 100 μg/mL displayed the best inhibitory potency (51.4%) compared to the standard physostigmine, which exhibited 71.2% inhibition at the same concentration. These data suggest a good potential of these compounds as AChE inhibitors.

Materials and Methods

Leaves of *Waltheria brachypetala* Turks (Stecurlariaceae) were collected on 07–05–2003 in Casa Nova (Bahia), near the entrance of "Balneário Dunas do Velho Chico", 09°22′57"S, 41°08′24"W, and were identified by Prof. Dr. L. P. de Queiróz. A voucher specimen (HUEFS 72654) was deposited in the Herbarium of the Universidade Estadual de Feira de Santana for reference.

The dried ground leaves of *W. brachypetala* (2000 g) were extracted by maceration with MeOH (4 × 4 L, 24 hours each, room temperature) furnishing the MeOH extract (390 g). This extract was partitioned with MeOH : H_2O (9 : 1) and hexane (3 × 300 mL) to provide the hexane phase (18.50 g), and the hydroalcoholic phase was sequentially partitioned with $CHCl_3$/MeOH : H_2O (6 : 4) to provide the $CHCl_3$ phase (16.27 g). The hexane phase was subjected to silica gel (0.04–0.2 mm) CC (42 × 4 cm) eluted with hexane:EtOAc to obtain 21 fractions (25 mL). The fractions from the main CC eluted with hexane : EtOAc 1 : 1 were evaluated by TLC, which showed positive spots using Dragendorff reagent. These fractions (123 mg) were then submitted to silica (0.04–0.073 mm) CC (22 × 2 cm) eluted with $CHCl_3$: MeOH (97 : 3) to afford **4** (30 mg). The $CHCl_3$ phase was subjected to SiO_2 (0.04–0.2 mm) CC (44 × 4 cm) eluted with $CHCl_3$: EtOAc. The Dragendorff positive fractions eluted with 50% EtOAc (1172.5 mg) were acidified by adding 300 mL of 1 N HCl, and then the material was extracted with dichloromethane, affording an organic (DCM1) and an acid phase (AP1), respectively. In sequence, the acid phase was basified through pH = 9 by addition of NH_4OH (3 N) and extracted with dichloromethane. The organic phase (353 mg) was purified through flash CC (22 × 2 cm) on SiO_2 (0.04–0.073 mm) using dichloromethane:acetone (9 : 1, 8 : 2

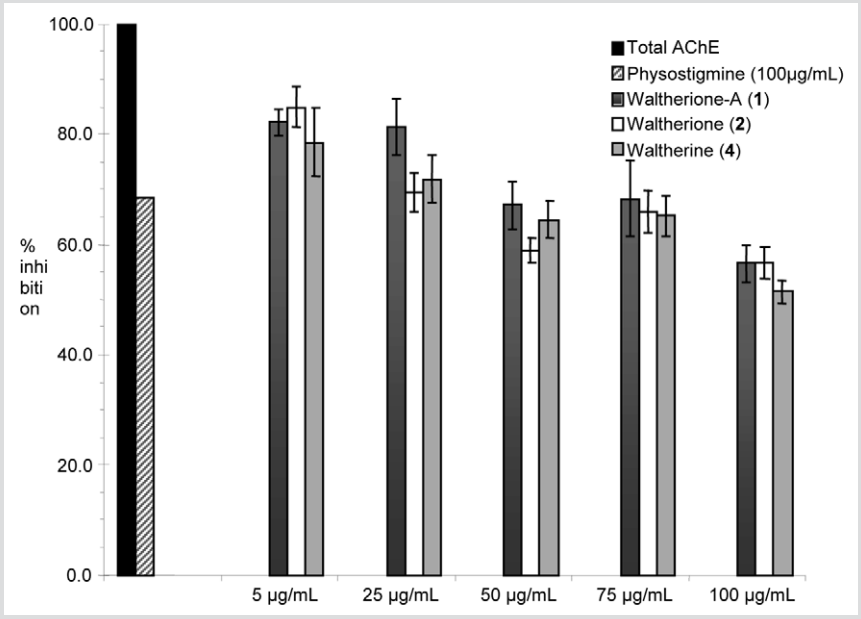

Fig. 2 Inhibition effect *in vitro* of waltherione-A, *N*-methylwaltherione, and waltherine on acetylcholinesterase activity. Data are expressed as mean ± SEM.

and 7 : 3, respectively) as eluents, which furnished compounds **3** (30 mg), **1** (245 mg) and **2** (50 mg), respectively.

The *in vitro* inhibition of AChE was determined by spectrophotometry using a colorimetric method [10]. Briefly, 300 μL of red blood cells, as the source of AChE, were incubated with 5 μL of pure compounds (5 – 100 μg/mL), physostigmine (100 μg/mL) (positive control), or buffer for 15 min prior to the addition of 10 μL of 20 mM DTNB in a 96-well microplate. The reaction was then started by adding 5 μL of 60 mM acetylthiocholine. The change in absorbance was recorded at 405 nm on a microplate reader (Stat Fax – 2600). DTNB, AChE and the substrate were dissolved in 0.1 M of sodium phosphate buffer (pH 7.4). All samples were analyzed in triplicate. Blood was obtained from researcher donors. In the measurements of the activities, the results were calculated using IC_{50} values with 95% confidence intervals determined using the probit analysis method with Stats Direct Statistical software.

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